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Anaerobic Digestion as an Electron Economy: An Objective-Oriented Electron Allocation Framework

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Abstract

Anaerobic systems are increasingly operated for objectives beyond methane production, such as volatile fatty acid production, chain elongation, biogas upgrading, and reductive treatment. Mechanistically, these systems largely share the same reaction network; operationally, the same pathway can be the target in one system and a competing pathway in another. Electron balances quantify allocation at the mechanistic level but leave its operational meaning implicit, making low performance difficult to diagnose. This study proposes an objective-oriented electron allocation framework (E-framework) that adds an operational level to electron balances by assigning roles to electron-consuming pathways, here termed electron sinks, according to the objective. Low target recovery has three possible causes, each requiring a different intervention point and approach: electrons not reaching the terminal sinks, electrons allocated to competing sinks, and an allocation opportunity limited by influent conditions. The first two are separated by decomposing target recovery into sink expression and target alignment. Across 104 literature cases from seven system types, the two indicators varied within system families, and identical target recovery arose from opposite combinations. The third is identified against a nominal benchmark defined by thermodynamic priority and electron-accepting capacity. Applied to sulfate-reducing methanogenic systems (52 cases), the benchmark showed that sulfate reduction typically received fewer electrons than allowed, and separated interventions that helped systems reach the benchmark from those that changed it. Tracking across successive analysis windows revealed whether low target recovery was transient, persistent, or reversible, indicating whether to accelerate realization, act on the single-window diagnosis, or restore operating conditions. The E-framework turns electron balances into a diagnostic basis for intervention.

Keywords. Anaerobic digestion; E-framework; Electron sink; Sink expression; Target alignment; Nominal allocation benchmark; Diagnosis-guided intervention

Nomenclature

Symbol	Description	Unit
Latin symbols		
A_{target}	Target alignment: fraction of electrons recorded at the quantified sinks that is allocated to the target sink	–
C_i	Amount of electron acceptor available to bounded sink i within the analysis window	mol or mol/L
C_{SR}	Amount of sulfate supplied	mol/L
COD_{in}	Biodegradable COD of the electron input	mg/L
E_{in}	Total electron input considered in the analysis	mol e [–]
$E_{\text{window input}}$	Electron equivalents supplied at the start of or during the analysis window	mol e [–]
$E_{\text{pool,start}}$	Electrons in quantified pool components carried over from operation preceding the analysis window	mol e [–]
$E_{\text{pool,end}}$	Electrons remaining in the electron pool at the end of the analysis window	mol e [–]
$E_{\text{pool,out}}$	Electrons leaving the system boundary in components still classified as part of the pool	mol e [–]
E_{target}	Electrons allocated to the target sink	mol e [–]
$E_{\text{competing}}$	Electrons allocated to the quantified competing sinks	mol e [–]
$E_{\text{unresolved}}$	Residual electrons that cannot be assigned to the quantified terms	mol e [–]
E_x	Electron equivalents of component x	mol e [–]
E_i, E_r	Electrons allocated to bounded sink i and residual sink r (superscript nom or eff)	mol e [–]
$E_i^{\text{nom,limit}}$	Upper bound of the nominal allocation of bounded sink i while it holds thermodynamic priority	mol e [–]
f_i, f_r	Fraction of the electron input allocated to bounded sink i and residual sink r (superscript nom or eff)	–
f_{target}	Target recovery: fraction of the electron input allocated to the target sink	–
$f_{\text{competing}}$	Fraction of the electron input allocated to the competing sinks	–
$f_{\text{expressed}}$	Sink expression: fraction of the electron input recorded at the quantified sinks	–
f_s^0	Fraction of donor electrons used for cell synthesis	–
m	Number of bounded sinks holding thermodynamic priority	–
n	Number of electrons transferred per mole of reaction	mol e [–] /mol
n_x	Electron stoichiometry of component x	mol e [–] /mol
N_x	Amount of component x consumed, formed, or transported within the analysis basis	mol
R	Universal gas constant (8.314)	J/(mol·K)
T	Absolute temperature	K
y_i	Amount of electron acceptor reduced by bounded sink i	mol or mol/L
y_i^*	Crossover point: amount of acceptor reduced at which the per-electron Gibbs energy of sink i equals that of the residual sink	mol or mol/L
y, y^*	Amount of sulfate reduced, and its value at the SR–MG crossover point	mol/L
Greek symbols		
ΔA_{target}	Deviation of effective from nominal target alignment, $A_{\text{target}}^{\text{eff}} - A_{\text{target}}^{\text{nom}}$	–

Symbol	Description	Unit
$\Delta f_i, \Delta f_r$	Deviation of effective from nominal allocation of bounded sink i and residual sink r, e.g. $\Delta f_i = f_i^{\text{eff}} - f_i^{\text{nom}}$	–
$\Delta f_{\text{SR}}, \Delta f_{\text{MG}}$	Deviation of effective from nominal allocation of sulfate reduction and methanogenesis	–
ΔG	Gibbs free energy change of a reaction	kJ/mol
$\Delta G/e^-$	Gibbs free energy change per electron transferred	kJ/mol e^-
$\Delta G_{\text{SR}}^0, \Delta G_{\text{MG}}^0$	Standard Gibbs free energy change of acetate-driven sulfate reduction and acetoclastic methanogenesis	kJ/mol
v_i	Electron equivalents accepted per mole of electron acceptor of bounded sink i	mol e^- /mol
Subscripts		
competing	Competing sink(s)	
expressed	Quantified sinks (sink expression)	
i	Bounded sink i	
in	Input	
j	Bounded sink with higher priority than sink i	
MG	Methanogenesis	
pool	Electron pool	
r	Residual sink	
SR	Sulfate reduction	
target	Target sink	
x	Component x	
Superscripts		
eff	Effective: derived from measured data	
nom	Nominal: defined by the allocation benchmark	
limit	Upper bound of the nominal allocation	
*	Crossover point	
0	Standard conditions	

1. Introduction

1.1. Anaerobic digestion as a mature but evolving technology

Anaerobic digestion (AD) is a mature technology for treating organic wastes and wastewaters [1], [2]. Historically, its primary objectives have been organic matter stabilization and energy recovery in the form of methane [3]. Methane production has therefore served as the central performance outcome, and stable operation has been equated with the sustained conversion of intermediates to methane. When methanogenic microorganisms are inhibited, volatile acids accumulate alongside shifts in pH, biogas composition, and methane yield [4], [5], and these changes are conventionally interpreted as an indicator of unbalanced treatment [4].

Modern anaerobic systems, however, increasingly pursue objectives beyond waste stabilization and methane recovery. Major examples include volatile fatty acids (VFA) production, chain elongation, biological biogas upgrading, and bioelectricity generation [6], in which compounds that are intermediates under conventional AD can become the desired products. For instance, the volatile acids whose accumulation signals instability in methane-oriented AD are the target products in VFA production. These systems also treat increasingly complex streams containing reducible inorganic species, such as sulfate [7], nitrate [8], and metals [9], and reducible organic compounds, such as azo dyes [10] and nitro- or halogenated organics [11]; these constituents introduce additional reduction pathways alongside methanogenesis. Stress-inducing constituents, including high ammonia concentrations [12], [13] and elevated salinity [14], [15], can further alter anaerobic conversion and pathway activity. In parallel, a growing number of intervention strategies are used to steer process performance, including cosubstrate addition [16], supplementation of conductive [17], [18] or mineral materials [19], operational condition adjustment [20], integrating microbial electrochemical technologies [21], nutrient and trace element supplementation [22], and bioaugmentation [23].

This diversification of objectives, feedstocks, and interventions means that methane yield alone is no longer a universal indicator of success: a decline may reflect desired diversion toward another pathway, whereas enhanced methanogenesis may be undesirable when a different pathway is the operational target. Performance must therefore be evaluated against the intended functional outcome, of which methane production is one among several. Likewise, because similar overall performance can arise from different limitations, diagnosing which aspect of the existing system state limits the intended outcome is an essential step in determining what an intervention should change.

1.2.Process-based descriptions and the need for an objective-oriented interpretive layer

Over the past several decades, AD has been predominantly described through process-oriented frameworks that link substrate degradation, intermediate accumulation, product formation, and process stability through biochemical conversions, physicochemical interactions, and changes in state variables over time. A canonical quantitative representation of this approach is Anaerobic Digestion Model No. 1 (ADM1), first published by the International Water Association in 2002, which integrates hydrolysis, acidogenesis, acetogenesis, and methanogenesis into a unified

kinetic and stoichiometric framework [24]. Subsequent extensions have broadened ADM1 to additional substrates and intermediates, electron-accepting processes involving nitrate, sulfate, and iron, phosphorus transformations, alternative pathways such as syntrophic acetate oxidation, and inhibition functions related to ammonia, sulfide, and VFA [25]. Process-oriented models are designed to describe and predict system behavior. An objective-oriented interpretive layer, applied to modeled outcomes, can connect these outcomes to the operational objective and provide a consistent basis for identifying which changes would support it. Such an interpretation requires a common basis that is independent of any particular product and allows alternative metabolic destinations to be compared consistently.

1.3. Anaerobic digestion as an electron-driven energy-conserving system

AD can be seen from two complementary perspectives: carbon transformation and energy conservation. Within the redox steps of carbon transformation, organic compounds carry reducing equivalents corresponding to their degree of reduction; as these compounds are oxidized or reduced, reducing equivalents are transferred and redistributed among species within the reaction network. From a bioenergetic perspective, microorganisms conserve energy from the transfer of electrons between electron donors and acceptors [26]. The energy available from this transfer depends on the redox potential difference between the donor-acceptor pair, as described by $\Delta G = -nF\Delta E$, where ΔG is the Gibbs free energy change of the reaction (kJ/mol), n is the number of electrons transferred per mole of reaction (mol e^- /mol), F is the Faraday constant (96.485 kJ/(V·mol)), and ΔE is the difference in redox potential between the electron-acceptor and electron-donor couples (V) [27], [28]. The conserved energy supports cellular maintenance and growth [27], [29]. Carbon transformation and microbial energy conservation therefore converge at electron transfer: the same electron-transfer processes that redistribute reducing equivalents among compounds also provide the energetic basis for microbial activity. Expressing these processes on an electron-equivalent basis consequently allows different substrates, intermediates, and products to be compared within a common quantitative framework.

1.4. Electron allocation as the basis of functional outcomes in anaerobic systems

If reducing equivalents are taken as a common quantitative basis, the functional outcome of an anaerobic system can be interpreted through the question: where are the electrons ultimately allocated? During anaerobic conversion, reducing equivalents are transferred through

intermediate compounds before reaching downstream electron-consuming pathways, which can be regarded as electron sinks. The term electron sink has mainly been used to describe where electrons go in specific ecosystems such as the rumen [30]. In the present framework, an electron sink is treated more generally as an electron-consuming pathway; methanogenesis is one such sink among several. The electron-sink concept provides a neutral description of where reducing equivalents are ultimately consumed, while allowing the functional significance of each pathway to depend on the system objective. This distinction is particularly relevant to multi-objective anaerobic systems, in which the same pathway may have different functional meanings under different operating purposes.

The realization of these electron-transfer pathways is constrained by both thermodynamic and system-specific conditions. Thermodynamics determines whether a pathway is energetically feasible and how much energy the corresponding reaction can provide [31], [32]. Many anaerobic reactions operate close to thermodynamic limits: growth-equilibrium Gibbs free energies estimated for acetoclastic methanogens, propionate-oxidizing syntrophs, and hydrogenotrophic methanogens range from only about -3 to -18 kJ per mole of substrate [33]. Consequently, relatively small changes in environmental conditions can alter pathway feasibility [31], [34]. Kinetic, ecological, and physicochemical constraints, including reaction rates, electron-carrier availability, mass transfer, and microbial interactions, further govern whether a feasible pathway is effectively expressed [32], [35]. In this framework, these constraints are collectively referred to as electron accessibility.

Taken together, these concepts allow AD to be viewed as an electron economy, in which reducing equivalents provide a common quantitative basis for describing allocation across pathways. Electron-based accounting is well established in environmental biotechnology, where it is used, for example, to quantify product selectivity in fermentation and chain elongation [26], [36], and to partition substrate electrons among current, biomass, and unidentified processes in bioelectrochemical systems [37]. However, such accounting describes electron allocation without interpreting its significance for the operational objective.

Building on this accounting, the proposed objective-oriented electron-allocation framework (E-framework) introduces an objective-oriented diagnostic layer through three linked distinctions. First, it distinguishes the biochemical reaction network from the operational roles assigned to its

pathways, allowing the same process to be interpreted as a target or a competing pathway depending on the intended outcome. Second, it decomposes target electron recovery into the fraction of input electrons recorded at the quantified sinks (sink expression) and the fraction of those electrons allocated to the target (target alignment), distinguishing incomplete sink expression from unfavorable allocation among sinks. Third, for systems containing electron-acceptor-limited sinks (hereafter, bounded sinks), it establishes a nominal allocation benchmark based on thermodynamic priority and electron-accepting capacity, allowing observed allocation to be evaluated against the opportunity defined by these constraints. Together, these distinctions allow systems with different objectives to be compared on a common basis and link the diagnosed limitation to an appropriate intervention.

To develop and demonstrate this framework, this study aims to (i) formulate the E-framework and its allocation indicators; (ii) apply the indicators to literature-derived cases across different system types; (iii) develop a nominal allocation benchmark for systems containing bounded sinks and apply it to sulfate-reducing methanogenic systems as a case study; and (iv) examine how electron allocation evolves across successive analysis windows.

2. An electron-based framework (E-framework) for objective-oriented analysis

Figure 1 outlines the E-framework in three steps: defining the scope of analysis (**Section 2.1**), constructing and interpreting the electron map (**Section 2.2**), and quantifying electron allocation through representative indicators (**Section 2.3**).

2.1. Defining the scope of analysis

Before constructing the electron map, the operational objective and the analysis domain are defined. The operational objective defines the outcome that the system is designed or operated to achieve. The analysis domain defines the scope within which electron allocation is evaluated and comprises two components: the system boundary and the analysis window. The system boundary determines which electron inputs, components, and pathways are included in the analysis, as well as the analytical endpoint at which electrons are considered to have reached their final destination within the defined scope. The analysis window defines the time period or operational state over which electron allocation is evaluated. This is important because, at different time points, electrons may still be carried by intermediate compounds or may already have been transferred to downstream pathways; consequently, the observed electron-allocation state

depends on the selected analysis window. For batch systems, the analysis may be performed at specified reaction times or over a complete reaction cycle; for continuous systems, at steady state or over a representative stable period; and for semi-continuous systems, over individual feed-withdrawal cycles or across multiple operational cycles. Electron allocation evaluated within a single analysis window therefore represents a snapshot of the system state. Repeating the same analysis across successive windows can provide a time-resolved trajectory of electron allocation, as discussed later in **Section 3.3**.

2.2. Constructing the electron map

2.2.1. Mechanistic and operational levels of interpretation

As illustrated in **Figure 1**, the E-framework distinguishes between two levels of interpretation: the mechanistic level and the operational level. The mechanistic level represents the electron-transfer network that occurs under the biochemical conditions of the system, including the pathways through which electrons are released, transferred, and ultimately consumed. The operational level interprets the same network according to the operational objective and analysis domain defined in **Section 2.1**. At this level, components and pathways are assigned analytical roles for evaluating electron allocation within the selected scope.

2.2.2. Mapping the network onto the donor-pool-sink structure

After the scope of analysis has been defined, the mechanistic network is mapped onto three analytical roles: electron donor, electron pool, and electron sinks. These roles represent, respectively, the source of electrons, the electron-carrying forms that remain within the network, and the pathways at which electrons are considered to have reached the analytical endpoint. The electron donor is the source that supplies reducing equivalents to the system. Electrons may be released through donor oxidation or introduced from an external electron source. The electron pool comprises intermediates and electron carriers that remain capable of transferring electrons to downstream pathways. It includes electron-carrying forms whose electrons have not yet reached the analytical endpoint within the selected analysis window. Electron sinks are electron-consuming pathways at which electrons are considered to have reached the analysis endpoint. In the E-framework, an electron sink is interpreted and named according to the corresponding electron-consuming pathway. For example, methane production may be used to quantify electron allocation to methanogenesis, while the sink itself is defined as the methanogenesis pathway.

The resolution at which a sink is represented depends on the operational objective and the available data. Methanogenesis may be treated as a single sink or further distinguished into acetoclastic and hydrogenotrophic methanogenesis when pathway-specific resolution is relevant to the analysis and supported by the available data.

Based on the operational objective, the identified sinks are further assigned functional roles. The target sink represents the electron-consuming pathway consistent with the intended system outcome, while competing sinks represent other electron-consuming pathways that receive electrons without contributing to the primary objective.

2.3. Electron allocation and representative indicators

After the electron map has been constructed according to the operational objective, electron allocation is quantified within the selected system boundary and analysis window. All quantities are expressed on a common electron-equivalent basis and on the same temporal basis.

For an analysis window in which a pre-existing electron pool can contribute to subsequent allocation, the total electron input considered in the analysis is defined as:

$$E_{\text{in}} = E_{\text{window input}} + E_{\text{pool, start}}$$

where $E_{\text{window input}}$ represents electron equivalents supplied at the start of or during the selected analysis window. Depending on the system, this term may include the primary electron donor as well as other electron-bearing inputs that contribute to the quantified products.

$E_{\text{pool, start}}$ represents quantified electron-bearing components carried over from operation preceding the selected analysis window and remaining available for subsequent allocation. The corresponding electron balance is:

$$E_{\text{in}} = E_{\text{target}} + E_{\text{competing}} + E_{\text{pool, end}} + E_{\text{pool, out}} + E_{\text{unresolved}}$$

where E_{target} is the amount of electrons allocated to the target sink, $E_{\text{competing}}$ is the total amount allocated to the quantified competing sinks, $E_{\text{pool, end}}$ represents electrons remaining in the electron pool at the end of the analysis window, and $E_{\text{pool, out}}$ represents electrons leaving the system boundary in components that are still classified as part of the pool. $E_{\text{unresolved}}$ represents the residual electron fraction that cannot be assigned to the quantified terms.

The relevance of the pool terms depends on the selected analysis basis. In a batch system without material withdrawal, $E_{\text{pool,out}} = 0$, while $E_{\text{pool,end}}$ may remain at the selected endpoint. At steady state, pool components are continuously produced and consumed, while their inventory in the reactor remains constant. Over a steady-state analysis period, $E_{\text{pool,start}}$ and $E_{\text{pool,end}}$ are therefore equal and have no net effect on the balance, and only the pool components leaving the reactor with the effluent need to be represented by $E_{\text{pool,out}}$. In semi-continuous systems evaluated over individual feed-withdrawal cycles, both changes in the retained pool and electron outflow through withdrawal may need to be considered.

Each component x is converted to electron equivalents as: $E_x = n_x N_x$ where N_x is the amount of x consumed, formed, or transported within the analysis basis, and n_x is its electron stoichiometry (mol e⁻/mol). Calculations for individual donors and sinks are given in **Section 3.1**.

The unresolved term is the difference between the defined electron input and the sum of the quantified terms:

$$E_{\text{unresolved}} = E_{\text{in}} - (E_{\text{target}} + E_{\text{competing}} + E_{\text{pool,end}} + E_{\text{pool,out}})$$

$E_{\text{unresolved}}$ represents the deviation of the measured electron balance from complete closure ($E_{\text{unresolved}} = 0$). A balance with $E_{\text{unresolved}} < 0$ is referred to as over-closed. Because the fraction of donor electrons used for cell synthesis is small under anaerobic conditions (e.g., $f_s^0 \approx 0.06$ for methanogenesis and 0.10 for sulfate reduction with acetate as the electron donor [38]), electron assimilation into microbial biomass is not included as a separate term and is contained in $E_{\text{unresolved}}$. For denitrification ($f_s^0 \approx 0.6$) [38], sink expression may be underestimated.

Three indicators describe the resulting electron-allocation state. Target recovery (f_{target}) is the fraction of the electron input allocated to the target sink:

$$f_{\text{target}} = \frac{E_{\text{target}}}{E_{\text{in}}}$$

Sink expression ($f_{\text{expressed}}$) is the fraction of the electron input recorded at the quantified sinks within the analysis window; the remainder is retained in the pool, leaves the boundary in pool components, or remains unresolved:

$$f_{\text{expressed}} = \frac{E_{\text{target}} + E_{\text{competing}}}{E_{\text{in}}}$$

Target alignment (A_{target}) is the fraction of these electrons allocated to the target sink:

$$A_{\text{target}} = \frac{E_{\text{target}}}{E_{\text{target}} + E_{\text{competing}}}$$

A value approaching 1 indicates allocation predominantly aligned with the operational objective. Because A_{target} is calculated only from quantified sinks, unmeasured competing sinks may lead to its overestimation. The three indicators are related by:

$$f_{\text{target}} = f_{\text{expressed}} \times A_{\text{target}}$$

so that similar target recoveries can be distinguished by their combinations of sink expression and target alignment.

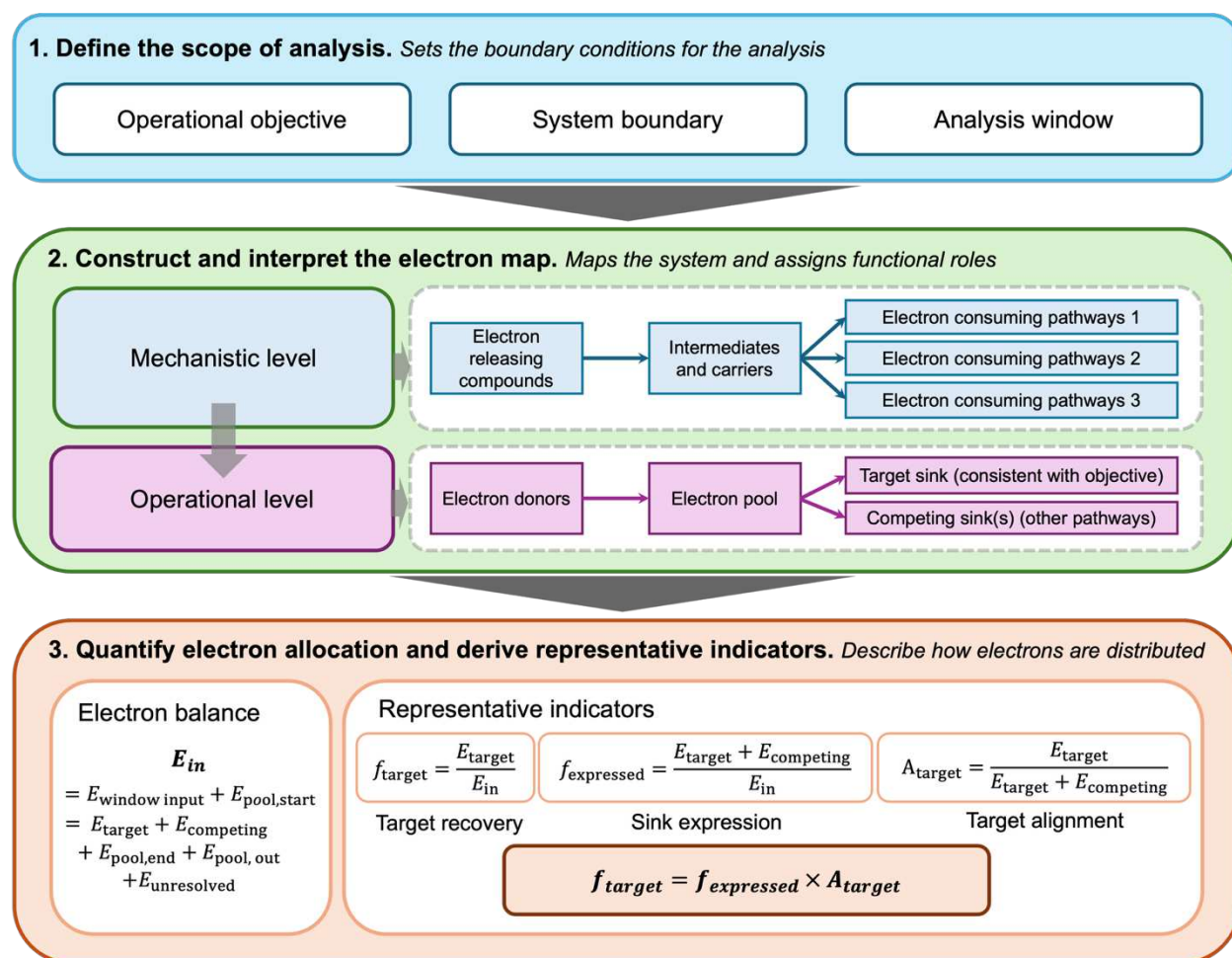


Figure 1. Conceptual structure and analytical workflow of the E-framework.

3. Applications of the E-framework

3.1. General application across representative anaerobic systems

3.1.1. Qualitative mapping of representative systems

Figure 2 illustrates the transition from the mechanistic to the operational level of the E-framework. **Figure 2a** integrates the reactions relevant to VFA production, chain elongation, biological biogas upgrading, and reductive treatment into a single electron-transfer network, in which single arrows denote predominantly one-way reactions and opposing arrows denote reversible or interconvertible ones. **Figure 2b** applies the operational level (Section 2.2) to this network for each objective. The four systems thus share the same mechanistic network and become distinct analytical cases only when roles are assigned according to the objective.

In VFA-oriented AD systems (**Figure 2b1**), the operational objective is to produce, accumulate and recover VFAs, primarily acetate, propionate and butyrate, as the target products [39] because they serve as platform intermediates for a range of downstream bioprocesses [39], [40]. At the mechanistic level, organic substrates are converted through hydrolytic and fermentative pathways to VFAs, while these VFAs and shared intermediates such as H_2 /formate may continue to be consumed by downstream pathways including methanogenesis and chain elongation [41], [42], [43]. Mapped onto the E-framework, the organic substrate is assigned as the electron donor; transferable intermediates, including lactate, ethanol and H_2 /formate, constitute the electron pool; VFA-forming pathways are assigned as the target sink; and downstream electron-consuming pathways, particularly methanogenesis, are treated as competing sinks [41], [44], [45]. In chain-elongating systems (**Figure 2b2**), the operational objective is to convert short-chain fatty acids (SCFAs) into higher-value medium-chain fatty acids (MCFAs) [46], whose higher energy density and lower water solubility facilitate recovery by phase separation or in-line extraction [36]. At the mechanistic level, reducing equivalents from ethanol or lactate are coupled with SCFA acceptors such as acetate, propionate, and butyrate through reverse β -oxidation, extending the carbon chain in successive C_2 units [36], [46]. When mapped onto the E-framework, ethanol and lactate are identified as electron donors; SCFAs and electron-carrying intermediates that remain available for further elongation are retained within the electron pool; and pathways forming the selected chain-elongated products are defined as target sinks, while methanogenesis represents a typical competing sink. Additional competing sinks may be defined depending on the selected target and analysis window. When caproate or longer-chain products are designated

as the target, premature termination at butyrate or diversion to butanol may be treated as competing allocation, depending on the selected target and analysis window [47], [48]. In lactate-fed systems, conversion of lactate to propionate through the acrylate pathway may constitute a competing pathway when propionate is a terminal product [49]. In biological biogas upgrading systems (**Figure 2b3**), the operational objective is to enrich the CH₄ content of biogas, primarily by supplying external H₂ to convert CO₂ into CH₄ [50], [51]. At the mechanistic level, H₂ can be used to reduce CO₂ to CH₄ through hydrogenotrophic methanogenesis ($4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) or to acetate through homoacetogenesis ($4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{acetate} + 2\text{H}_2\text{O}$) [50], [52]. When mapped onto the E-framework, external H₂ is identified as the electron donor; H₂/formate and other electron-carrying intermediates that remain available for further conversion are retained within the electron pool; hydrogenotrophic methanogenesis is defined as the target sink, while homoacetogenesis is the principal competing sink [53]. Unreacted H₂ leaving the system boundary is treated as electron-pool outflow because its electrons have not yet reached a terminal sink [54], [55]. In reductive treatment systems (**Figure 2b4**), the operational objective is to remove or transform oxidized electron acceptors present together with organic matter in the wastewater, such as sulfate, nitrate, Fe(III), or azo dyes. At the mechanistic level, electrons derived from the organic substrate are transferred through reduced intermediates and electron carriers before being allocated to terminal reduction pathways or methanogenesis [7], [56]. When mapped onto the E-framework, the organic substrate is identified as the electron donor; electron-carrying intermediates such as acetate, propionate, butyrate, lactate, ethanol, and H₂/formate are retained within the electron pool; the reduction pathway is defined as the target sink, while methanogenesis commonly acts as a competing sink. In this case, the electron-accepting capacity of these reductive target sinks is finite and is determined by the amount of electron acceptor available together with the stoichiometry of the corresponding reaction [57], [58].

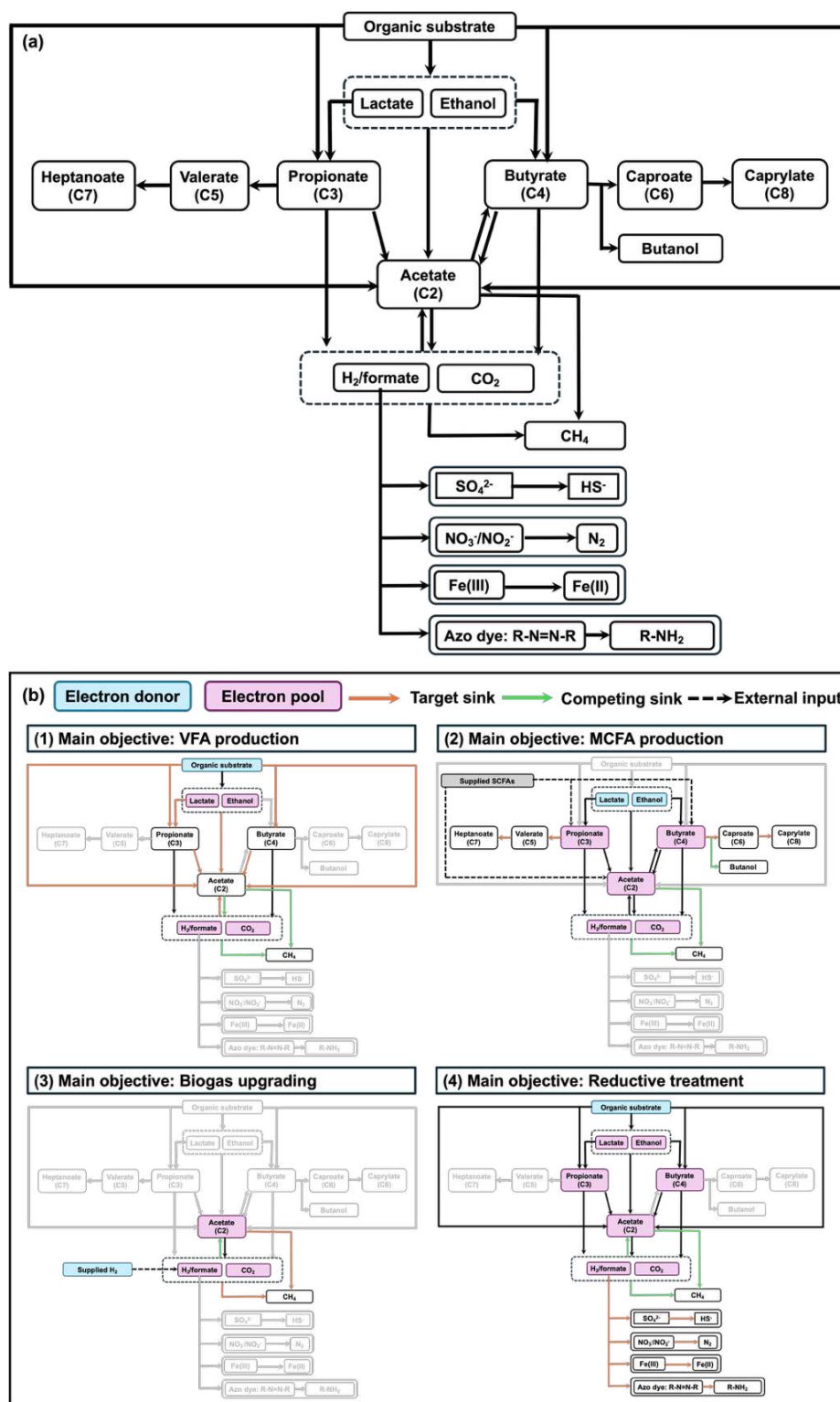


Figure 2. (a) Mechanistic level: integrated electron-transfer network and (b) operational level: objective-dependent interpretation of the same network. External inputs are shown in (b) only where they define the analytical case.

When the operational level is added to the mechanistic network, **Figure 2** provides an operational map identifying where electron allocation to the target can be limited. Target recovery is low when electrons do not progress sufficiently from the donor or pool to the sinks, or when electrons that reach the sinks are diverted to competing sinks; the former corresponds to low sink expression and the latter to low target alignment (**Section 2.3**). Reported interventions can be grouped accordingly. Interventions acting on sink expression aim to improve electron delivery to the sinks, for example by enhancing hydrolysis of particulate substrates in VFA production [45], or by relieving product inhibition, such as sulfide toxicity in sulfate-containing systems [7]. Interventions acting on target alignment operate at branch points between target and competing pathways, by improving access to the target sink, suppressing competing sinks, or changing the balance between electron supply and acceptor availability. Examples include the suppression of methanogenesis by 2-bromoethanesulfonate (2-BES), low pH, short hydraulic retention time (HRT), or thermophilic operation in VFA production and chain elongation [45], [36], [47], [48], [59], and adjustment of the substrate-to-acceptor ratio, such as the ethanol/acetate ratio in chain elongation [36], [48] or the chemical oxygen demand (COD)/sulfate ratio in sulfate-containing systems [57], [60]. Conductive materials are often applied to improve access to a target sink through extracellular electron transfer [18], but their effects on sink expression and target alignment have rarely been distinguished.

The reversibility of reactions in **Figure 2** further indicates how far along the network allocation can still be adjusted. The H_2 /formate- CO_2 region is shared across multiple objectives and is interconvertible, so electrons reaching it can still be redistributed among downstream pathways. In biological biogas upgrading, for example, with the same inoculum and gas mixture, inhibition of methanogenesis by 2-BES directed approximately 34% of CO_2 toward acetate, whereas in thermally pretreated inocula, in which methanogens were not suppressed, the converted CO_2 was directed entirely to CH_4 [52]. By contrast, once electrons pass through a predominantly one-way branch toward an analytical endpoint within the timescale considered, allocation becomes effectively committed and intervention must occur before that commitment point.

3.1.2. Basic electron-allocation descriptors across system types

Building on the qualitative mapping in **Section 3.1.1**, this section quantitatively evaluates the two electron-allocation dimensions defined in **Section 2.3**, sink expression and target alignment,

across literature-derived cases. Because reported measurements and pathway resolution differed among systems, system-specific calculation procedures were applied; case IDs, equations, assumptions, worked examples, and case-level calculations are provided in **Supplementary Note 1** and **Supplementary Data 1**. For consistency across studies, $E_{\text{pool,end}}$ and $E_{\text{pool,out}}$ were not assigned separately, and the corresponding electrons are contained in $E_{\text{unresolved}}$; where pool components were reported, their contribution to $E_{\text{unresolved}}$ is described in Supplementary Note 1. For cases with real wastes or wastewaters, E_{in} was based on total COD without biodegradability correction, so f_{target} and $f_{\text{expressed}}$ represent lower bounds. Of the 134 cases compiled, 26 were excluded because the required electron-allocation descriptors could not be determined from the reported data, and a further 4 were excluded because $f_{\text{expressed}}$ fell outside the physically valid range [0, 1]. Excluded cases and the corresponding reasons for exclusion are documented in Supplementary Data 1. The remaining 104 cases were used to construct **Figure 3**: VFA production (n = 15), chain elongation (n = 26), biogas upgrading (n = 17), and systems with a reductive sink (n = 46), comprising azo dye reduction (n = 18) and denitrification (n = 13), in which the reduction pathway was the target, and sulfate reduction (n = 10) and Fe(III) reduction (n = 5), in which methane was the target.

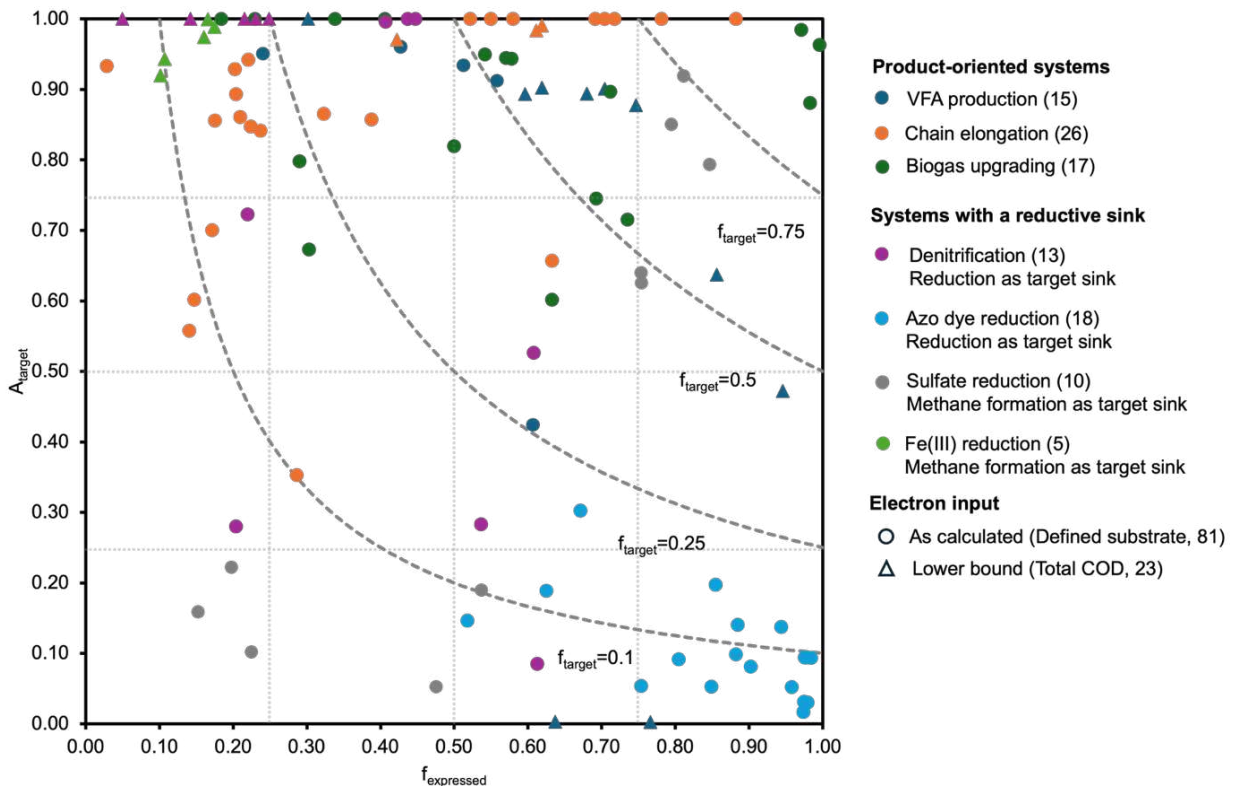


Figure 3. Cross-system expression–alignment plane for target electron recovery. Each point represents a literature-derived case ($n = 104$). Dashed contours indicate constant f_{target} (0.10, 0.25, 0.50, and 0.75). Circles denote cases with a defined substrate; triangles denote cases with real wastes or wastewaters, for which f_{target} and $f_{\text{expressed}}$ are lower bounds. Data were compiled from [41], [42], [43], [46], [47], [48], [49], [51], [52], [53], [54], [55], [56], [57], [59], [60], [61], [62], [63], [64], [65], [66], [67], [68], [69], [72].

Figure 3 places each case on an expression–alignment plane, with $f_{\text{expressed}}$ on the horizontal axis and A_{target} on the vertical axis. Moving to the right indicates that a larger fraction of the input electrons reached the quantified sinks, whereas moving upward indicates that a larger fraction of these electrons was allocated to the target sink. Because $f_{\text{target}} = f_{\text{expressed}} \times A_{\text{target}}$ (Section 2.3), cases with the same target recovery lie on the same dashed contour. Across the 104 cases, $f_{\text{expressed}}$ ranges from approximately 0.03 to 1.00 and A_{target} ranges from near 0 to 1.00. Using the midpoint of each axis (0.5) as a descriptive partition, the cases were distributed across all four regions: low $f_{\text{expressed}}$ /low A_{target} ($n = 6$), low $f_{\text{expressed}}$ /high A_{target} ($n = 38$), high $f_{\text{expressed}}$ /low A_{target} ($n = 25$), and high $f_{\text{expressed}}$ /high A_{target} ($n = 35$).

Across the dataset, $f_{\text{expressed}}$ and A_{target} were negatively correlated ($r = -0.41$, $p < 0.001$). This association, however, arose from differences between system families, mainly the azo dye cases combining high $f_{\text{expressed}}$ with low A_{target} ; it was not significant when azo dye cases were excluded ($r = -0.03$, $p = 0.78$) or when both descriptors were centered on their system-family means ($r = 0.06$, $p = 0.58$). Within system families, the two descriptors therefore varied independently, consistent with their representing distinct dimensions of the electron-allocation state. This distinction is directly visible from the iso- f_{target} contours, i.e., lines of constant target recovery, because similar target recovery can arise from markedly different combinations of $f_{\text{expressed}}$ and A_{target} . For example, CE_Fu_02 [47] and RedAzo_Dai_09 [56] had similar f_{target} values of approximately 0.20, but CE_Fu_02 combined low $f_{\text{expressed}}$ (0.22) with high A_{target} (0.94), whereas RedAzo_Dai_09 showed higher $f_{\text{expressed}}$ (0.67) but lower A_{target} (0.30). Similarly, RedDen_Xie_05 [58] and RedAzo_Dai_06 [56] both had $f_{\text{target}} = 0.05$, but RedDen_Xie_05 had $f_{\text{expressed}} = 0.05$ and $A_{\text{target}} = 1.00$, whereas RedAzo_Dai_06 had $f_{\text{expressed}} = 0.96$ and $A_{\text{target}} = 0.05$. Target recovery (f_{target}), a commonly used overall

performance metric often reported as removal efficiency or process efficiency in anaerobic systems, is therefore insufficient on its own for diagnosing system performance. For more accurate diagnostic interpretation, f_{target} therefore needs to be decomposed into A_{target} , target alignment, and $f_{expressed}$, sink expression, as mentioned in **Section 2.3**.

In **Figure 3**, because the compiled cases include both baseline and intervention conditions, the position of each system family reflects both its typical behavior and which dimension limits its target recovery. Chain elongation and biogas upgrading maintained generally high A_{target} (mean = 0.87 and 0.88, respectively) across a wide range of $f_{expressed}$ (0.03–0.88 and 0.18–1.00, respectively), consistent with their operation around a well-defined target, in which competing pathways are suppressed by design (e.g., methanogenesis inhibition by 2-BES [47], [48], [52], or H_2 for hydrogenotrophic methanogenesis [54], [55] and homoacetogenesis [52]); their recovery was therefore limited mainly by sink expression. VFA production also occurred predominantly at high A_{target} but showed greater variability and included several cases with low values (range 0.00–1.00, mean = 0.72), consistent with VFAs being both target products and intermediates that can be consumed by downstream pathways. These agreements with known operating behavior support the interpretation of the two descriptors.

At a broader level, systems with a reductive sink separated from product-oriented systems mainly along the A_{target} axis. Product-oriented systems ($n = 58$) clustered at high A_{target} (mean = 0.83; 9% of cases below 0.5), whereas systems in which the reduction pathway was the target (denitrification and azo dye reduction, $n = 31$) generally occupied lower values (mean = 0.38; 68% below 0.5), despite comparable mean $f_{expressed}$ values in the two groups (mean = 0.51 and 0.64, respectively). Systems in which methane formation was the target but a reductive sink was also present (sulfate and Fe(III) reduction, $n = 15$) fell in between (mean $A_{target} = 0.64$). What these systems share is a reductive sink whose capacity is bounded by the amount of electron acceptor supplied (**Section 3.1.1**). This bound makes A_{target} ambiguous: a given value may reflect genuine competition among sinks or simply the limited capacity of the bounded sink. Resolving this requires incorporating the system's stoichiometric constraints into the framework.

3.2. Applying the E-framework to systems with a bounded sink

To resolve the ambiguity identified in **Section 3.1.2**, this section introduces a nominal allocation benchmark, defined as the allocation expected from the thermodynamic priority of the sinks and the electron-accepting capacity of each bounded sink relative to the electron input. The benchmark is first formulated in general terms (**Section 3.2.1**) and then applied to sulfate reduction-methanogenesis systems using literature-derived data (**Section 3.2.2**).

3.2.1. Formulation of the nominal allocation benchmark

3.2.1.1. Mechanistic level: thermodynamic priority and acceptor constraints

The nominal allocation of each sink is the fraction of the electron input that it would receive if allocation were determined solely by thermodynamic priority and by the electron-accepting capacity of the bounded sinks, and is denoted by the superscript “nom”. Two types of sinks are distinguished. A bounded sink i has a finite capacity set by the amount of electron acceptor available, $\nu_i C_i$, where C_i is the amount of electron acceptor available within the analysis window and ν_i is the number of electron equivalents accepted per mole of acceptor. The residual sink r receives the electrons not allocated to the bounded sinks and is assumed to have sufficient capacity to receive this remainder. The electron input, E_{in} , is defined as in **Section 2.3**; when reported as biodegradable COD, $E_{in} = \frac{COD_{in}}{8}$.

Thermodynamic priority is established by comparing each bounded sink with the residual sink on a per-electron basis ($\Delta G/e^-$). A bounded sink with a less negative $\Delta G/e^-$ than the residual sink holds no priority, and its nominal allocation is zero. A bounded sink with a more negative $\Delta G/e^-$ holds priority, but only until one of two conditions is reached: depletion, when its acceptor has been fully consumed ($y_i = C_i$, where y_i is the amount of acceptor reduced), or crossover, when its decreasing thermodynamic driving force becomes equal to that of the residual sink. The crossover point, y_i^* , is the amount of acceptor reduced at which $\Delta G_i(y_i^*)/e^- = \Delta G_r(y_i^*)/e^-$. The maximum electron amount that sink i can receive while holding priority is $E_i^{nom,limit} = \nu_i \min(C_i, y_i^*)$, where the superscript “limit” indicates the upper bound of the nominal allocation of sink i . The bounded sink is therefore capacity-limited when crossover does not occur before depletion ($y_i^* \geq C_i$), in which case this limit equals the full capacity $\nu_i C_i$, and crossover-limited when $y_i^* < C_i$.

For a system with m bounded sinks holding priority, ranked from the most to the least favorable, electrons are allocated sequentially. Each bounded sink receives the smaller of its nominal limit and the electron input remaining after allocation to higher-priority sinks. The residual sink receives the remainder:

$$E_i^{nom} = \min(E_i^{nom,limit}, E_{in} - \sum_{j<i} E_j^{nom}); E_r^{nom} = E_{in} - \sum_{i=1}^m E_i^{nom}$$

Here, $\sum_{j<i} E_j^{nom}$ is the total electron amount assigned to bounded sinks with higher priority than sink i . The nominal allocation fractions are $f_i^{nom} = \frac{E_i^{nom}}{E_{in}}$ and $f_r^{nom} = \frac{E_r^{nom}}{E_{in}}$. This rule assumes that the ordering among bounded sinks remains fixed within the analysis window. For the two-sink system ($m = 1$):

$$f_i^{nom} = \frac{\min(E_i^{nom,limit}, E_{in})}{E_{in}}; f_r^{nom} = 1 - f_i^{nom}$$

To distinguish them from the nominal allocation, quantities derived from measured data are referred to as effective and are denoted by the superscript “eff”. The effective electron amounts captured by each sink, E_i^{eff} and E_r^{eff} , are obtained by converting measured product formation or acceptor consumption into electron equivalents (**Section 2.3**). The corresponding effective allocation fractions are $f_i^{eff} = \frac{E_i^{eff}}{E_{in}}$ and $f_r^{eff} = \frac{E_r^{eff}}{E_{in}}$. The sum of the effective allocation fractions across all quantified sinks equals $f_{expressed}$ (**Section 2.3**): $f_{expressed} = \sum_{i=1}^m f_i^{eff} + f_r^{eff}$. The deviations between effective and nominal allocation, $\Delta f_i = f_i^{eff} - f_i^{nom}$ and $\Delta f_r = f_r^{eff} - f_r^{nom}$, indicate whether each sink received more or less of the electron input than specified by the benchmark.

3.2.1.2. Operational level: interpreting observed allocation against the benchmark

At the operational level, the benchmark is referenced to the target defined by the operational objective. Depending on the objective, the target may be a bounded sink or the residual sink, and the nominal allocations of the target and competing sinks, f_{target}^{nom} and $f_{competing}^{nom}$, follow directly from **Section 3.2.1.1** by summing the allocations within each group. Because the benchmark allocates the entire electron input among the sinks, $f_{expressed}^{nom} = f_{target}^{nom} + f_{competing}^{nom} = 1$, so that

the nominal target alignment equals the nominal target allocation, $A_{\text{target}}^{\text{nom}} = f_{\text{target}}^{\text{nom}}$. The effective indicators, $f_{\text{target}}^{\text{eff}}$, $f_{\text{expressed}}^{\text{eff}}$, and $A_{\text{target}}^{\text{eff}}$, are calculated from measured data as in **Section 2.3**.

The deviation $\Delta A_{\text{target}} = A_{\text{target}}^{\text{eff}} - A_{\text{target}}^{\text{nom}}$ shows whether the electrons reaching the sinks were allocated toward the target more or less than the benchmark specifies, whereas $f_{\text{expressed}}^{\text{eff}}$ shows how much of the input reached the sinks. When ΔA_{target} deviates from zero, the sink-level deviations, Δf_i and Δf_r (**Section 3.2.1.1**) identify which sink is responsible for the deviation.

3.2.2. Application to sulfate reduction-methanogenesis systems

Sulfate-containing anaerobic systems are used here as a case study. Electrons from organic substrates pass through a shared intermediate pool before being allocated between sulfate reduction (SR), treated as the bounded sink i , and methanogenesis (MG), treated as the residual sink r ; the deviations Δf_i and Δf_r defined in **Section 3.2.1.1** are therefore denoted Δf_{SR} and Δf_{MG} . The dataset comprises 61 cases from 10 studies (Table 1; Supplementary Data 2), covering batch ($n = 36$), continuous ($n = 23$), and semi-continuous ($n = 2$) operation, temperatures of 25–37 °C, pH 6.2–9.0, and influent SO_4^{2-} /COD ratios of 0.04–2.00. Interventions included the addition of conductive or iron-based materials (powdered activated carbon (PAC), Fe_3O_4 , biochar, stainless steel, and zero-valent iron (ZVI)), applied voltage, and N-acetyl-homoserine lactones, as well as systematic variation of the SO_4^{2-} /COD ratio. For batch cases, allocation was evaluated cumulatively at the end of the incubation, whereas for continuous cases it was evaluated at steady state.

Table 1. Operating conditions of the sulfate reduction-methanogenesis studies in **Section 3.2.2**

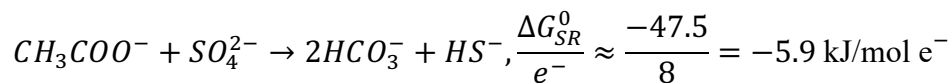
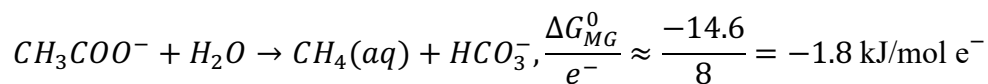
No	Study	Mode (reactor)	Substrate	Temp. (°C)	Baseline SO_4^{2-} /COD	Intervention	Target sink	n
1	Lu et al. [57]	Continuous (UASB)	Starch	35	0.12	Varying SO_4^{2-} /COD (0.12–2.02)	MG	6 (6)
2	Paepatung et al. [70]	Semi-continuous (fill-and-draw)	Glucose	28–30	0.50	ZVI	MG	2 (2)
3	Li et al. [71]	Continuous (UASB)	Lactate	37	0.81	Stainless steel	MG	2 (2)
4	Chen et al. [73]	Batch	Glucose	35	0.25	AHLs (2, 20 μM)	MG	3 (3)

5	Shu and Wu [74]	Continuous (CFR)	Ethanol	35	0.50	Fe ₃ O ₄ , PAC	MG	3 (3)
6	Cetecio glu et al. [75]	Batch	Acetate, propionate, butyrate	35	0.20	Varying SO ₄ ²⁻ /COD (0.20–2.00)	MG	9 (2)
7	Mahesh et al. [60]	Batch	Post-tanning wastewater	-	0.83	Varying SO ₄ ²⁻ /COD (0.83–1.60)	SR	3 (3)
8	Prakash et al. [76]	Continuous (UASB)	Glucose	37	0.10	Applied voltage; Varying SO ₄ ²⁻ /COD (0.10–1.00)	MG	12 (12)
9	Sarti and Zaiat [77]	Batch (AnSBR)	Butanol + sulfonation wastewater	25	0.27	Varying SO ₄ ²⁻ /COD (0.27–0.38)	SR	5 (5)
10	Xu et al. [78]	Batch	Synthetic wastewater	35	0.04	Biochar; Varying SO ₄ ²⁻ /COD (0.04–0.83)	MG	16 (14)

Note. *n*: number of compiled cases (cases retained after exclusion). MG: methanogenesis; SR: sulfate reduction; UASB: upflow anaerobic sludge blanket; CFR: continuous-flow reactor; AnSBR: anaerobic sequencing batch reactor; ZVI: zero-valent iron; PAC: powdered activated carbon; AHLs: N-acyl homoserine lactones.

3.2.2.1. Mechanistic level: crossover and realization of the nominal allocation

For each case, $E_{in} = COD_{in}/8$, and the amount of sulfate supplied, $C_{SR} = [SO_4^{2-}]/96$ (mol/L), gives an electron-accepting capacity of $8C_{SR}$. The SR-MG competition is evaluated at the acetate node, a shared intermediate and the main methanogenic substrate, so that both reactions transfer $8 e^-$ per acetate:



where the value for methanogenesis refers to dissolved CH₄ (**Supplementary Note 2, Section S2.1**). Sulfate reduction therefore initially holds priority. Because both reactions transfer the same number of electrons, the crossover condition of **Section 3.2.1.1** becomes:

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

Expressing each concentration as a function of the amount of sulfate reduced, y , yields a quadratic equation in y with a single positive root, y^* (**Supplementary Note 2, Section S2.1**). This root always satisfies $0 < y^* < C_{SR}$, so the SR-MG pair is formally crossover-limited under all operating conditions. The analytical solution was used because it proves this bound for all operating conditions and shows how each variable shifts y^* , which case-by-case numerical solution cannot establish. It relies on the simplifications described in Supplementary Note 2; for more complex reaction sets, the same crossover condition can be solved numerically. Whether this formal crossover has practical consequences for the nominal allocation depends on how far y^* lies below C_{SR} , as quantified by y^*/C_{SR} .

Across the 61 cases, y^*/C_{SR} ranged from 0.989 to 1.000 (**Supplementary Note 2, Section S2.1, Supplementary Data 2, Sheet Mechanistic level**), so crossover occurred only after at least 98.9% of the initial sulfate had been reduced; the lowest value was obtained for the only case with a methane fraction of 2%. Because several inputs required estimation or standardized assumptions, this result was tested over a factorial grid of sulfate and bicarbonate concentrations, pH, methane fraction, and temperature, with levels extending beyond the observed ranges (**13,824 combinations; Supplementary Note 2, Section S2.4, Table S1**). In the 3,600 combinations in which all variables were within the observed ranges, none gave $y^*/C_{SR} < 0.95$; the lowest value (0.960) required extremes from different studies that did not co-occur in any compiled case. Over the full grid, approximately 19% of the combinations gave $y^*/C_{SR} < 0.95$, almost all below 5% methane (**Supplementary Note 2, Section S2.4, Figure S1**). Low methane fractions favor MG through its reaction quotient and therefore move the crossover earlier, but such conditions are self-limiting: an active MG would raise the methane fraction, whereas an inactive MG no longer functions as the residual sink. Although the SR-MG pair is formally crossover-limited, its nominal SR allocation is therefore effectively capacity-limited under literature-relevant conditions, and f_{SR}^{nom} was calculated from sulfate capacity as $\min(8C_{SR}, E_{in})/E_{in}$, equivalent to the stoichiometric COD/SO₄²⁻ ratio of 0.67 [79], [80].

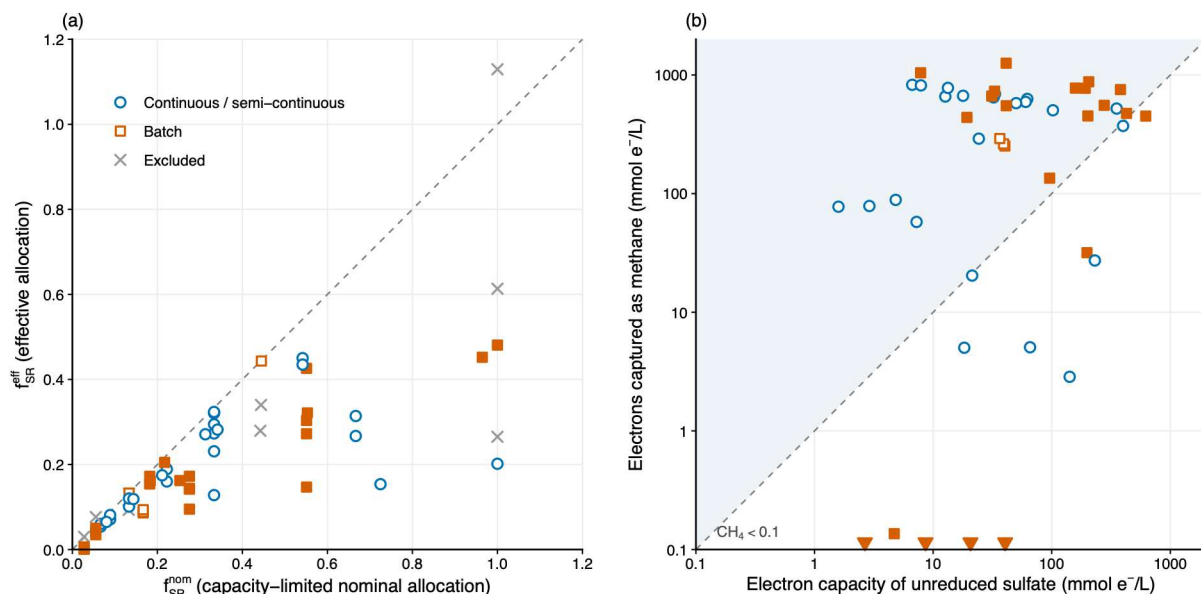


Figure 4. Mechanistic evaluation of nominal-effective SR allocation in SR-MG systems. (a) Effective versus nominal SR allocation. (b) Electrons recovered as methane versus the electron-accepting capacity of the unreduced sulfate; downward triangles indicate cases with negligible methane (< 0.1 mmol e^-/L), plotted at the lower axis limit. Filled symbols indicate lower-bound cases (E_{in} based on total COD). Dashed lines indicate the 1:1 relationship. Data sources are listed in **Table 1**.

Figure 4a compares the effective SR allocation, f_{SR}^{eff} , with the corresponding nominal allocation, f_{SR}^{nom} . The 1:1 line represents complete realization of the nominal SR allocation. Nine cases were excluded: seven with over-closed electron balances and two in which the reported sulfate reduction exceeded the sulfate supplied (**Supplementary Note 2**). In the 52 retained cases, the median $f_{SR}^{eff}/f_{SR}^{nom}$ was 0.77, and the median Δf_{SR} was -0.051 , indicating that SR typically received less of the electron input than its nominal allocation. **Figure 4b** examines whether the negative deviation of SR can be accounted for by electron allocation to MG, by comparing the electrons recovered as methane with the electron-accepting capacity of the sulfate that remained unreduced. Of the 47 cases in which less than 95% of the supplied sulfate was reduced, 34 lay above the 1:1 line, consistent with electron diversion toward MG while sulfate capacity remained available. In the other 13 cases, methane did not account for the deviation, which reflects incomplete electron expression at the terminal sinks. Comparison with the

nominal benchmark therefore quantifies the unrealized part of the allocation opportunity and indicates where it went.

3.2.2.2. Operational level: allocation diagnosis and intervention

At the operational level, target and competing sinks were assigned according to the objective of each study (**Supplementary Data 2, Sheet Operational level**): MG was the target sink in 44 of the 52 retained cases, and SR in the remaining 8. **Figure 5** applies the indicators of **Section 3.2.1.2** in three steps: the observed allocation state is identified in the expression-alignment plane (**Figure 5a**), positioned relative to the nominal benchmark (**Figure 5b**), and followed through interventions (**Figures 5c and 5d**). Nominal-fixed interventions, including conductive materials (**Figure 5c-1**), functional additives (**Figure 5c-2**), and applied voltage (**Figure 5c-3**), left the nominal allocation unchanged and were compared with the untreated control. Nominal-changing interventions altered the allocation opportunity; because the nominal SR allocation is effectively capacity-limited (**Section 3.2.2.1**), these were represented by changes in the influent $\text{SO}_4^{2-}/\text{COD}$ ratio and compared with the lowest-ratio condition within each study (**Figure 5d**). In Figures 5c and 5d, changes are shown in the plane of Δf_{MG} (horizontal axis) and Δf_{SR} (vertical axis).

Because the nominal allocations sum to one, $\Delta f_{MG} + \Delta f_{SR} = f_{expressed}^{eff} - 1$, so the dashed lines of slope -1 are iso- $f_{expressed}^{eff}$ lines, and the line through the origin corresponds to the nominal state ($f_{expressed}^{eff} = 1$). Movement toward the upper right, crossing these lines, indicates increasing $f_{expressed}^{eff}$, meaning that more of the input electrons reached the quantified sinks. Movement along the lines indicates reallocation between the two sinks, from SR to MG when moving toward the lower right.

In **Figure 5a**, cases occupied all four regions of the expression-alignment plane but were concentrated in the high-expression/high-alignment region (33/52). The distribution differed with target identity: 32 of the 44 MG-target cases fell in this region, whereas 6 of the 8 SR-target cases combined low expression with high alignment. **Figure 5b** positions each case relative to the nominal benchmark by plotting $f_{expressed}^{eff}$ against ΔA_{target} . In 40 of the 52 cases, allocation between the target and competing sinks remained close to the benchmark ($|\Delta A_{target}| \leq 0.15$). For these cases, the remaining question is how much of the nominal opportunity was realized at the terminal sinks, as indicated by $f_{expressed}^{eff}$. When $f_{expressed}^{eff} \approx 1$, as in Prakash_09 [76], the

system is close to its nominal state. When $f_{\text{expressed}}^{\text{eff}} < 1$, two routes are available. The first increases terminal-sink realization while keeping $\Delta A_{\text{target}} \approx 0$. In the Chen cases (Chen_01–Chen_03) [73], where MG was the target, increasing the N-acyl-homoserine lactone (AHL) dose from 0 to 2 and 20 μM raised $f_{\text{expressed}}^{\text{eff}}$ from 0.590 to 0.613 and 0.676, while ΔA_{target} changed only slightly from 0.021 to 0.023 and 0.028 (**Figure 5b**); in **Figure 5c-2**, Δf_{MG} increased from -0.329 to -0.251 , and the arrows crossed the iso- $f_{\text{expressed}}$ lines. Fe_3O_4 and PAC addition in the Shu cases (Shu_01–Shu_03) [74] followed the same route, raising $f_{\text{expressed}}^{\text{eff}}$ from 0.759 to 0.859 and 0.825 with ΔA_{target} remaining between -0.059 and -0.040 (**Figure 5c-1**). The second route changes the allocation opportunity itself, and its outcome depends on whether the target is the residual or the bounded sink. In the Lu cases (Lu_01–Lu_06) [57], where MG was the target, increasing $\text{SO}_4^{2-}/\text{COD}$ from 0.12 to 0.51 reduced the nominal opportunity of MG, but the system continued to track the benchmark (ΔA_{target} between -0.032 and $+0.005$ and $f_{\text{expressed}}^{\text{eff}} = 0.755$ – 0.848). When $\text{SO}_4^{2-}/\text{COD} = 1.09$ – 2.02 , however, $f_{\text{expressed}}^{\text{eff}}$ fell to approximately 0.200 – 0.230 , and the trajectory crossed the iso- $f_{\text{expressed}}$ lines with increasingly negative Δf_{SR} (**Figure 5d-2**), consistent with the sulfide inhibition and VFA accumulation reported in the original study [57]. In the Mahesh cases [60], where SR (the bounded sink) was the target, increasing $\text{SO}_4^{2-}/\text{COD}$ from 0.83 to 1.61 raised $A_{\text{target}}^{\text{eff}}$ from 0.497 to 1.000 while the cases remained close to the benchmark, but $f_{\text{expressed}}^{\text{eff}}$ decreased from 0.646 to 0.481 and Δf_{SR} from -0.232 to -0.519 (**Figure 5d-3**): the nominal opportunity of the target expanded faster than the system could realize it. Thus, for cases close to the benchmark but with $f_{\text{expressed}}^{\text{eff}} < 1$, nominal-fixed interventions improve realization of the existing opportunity, whereas the effect of nominal-changing interventions depends on target identity, reducing the opportunity of a residual target but expanding that of a bounded target.

When ΔA_{target} deviated clearly from zero, allocation departed from the benchmark, and the sink-level deviations Δf_{SR} and Δf_{MG} identify the sink responsible. In the Li cases (Li_01–Li_02) [71], where MG was the target, ΔA_{target} was -0.405 without and -0.268 with stainless steel (**Figure 5b**): both sinks were below nominal, but the deficit was much larger for MG ($\Delta f_{\text{MG}} = -0.433$ and -0.356) than for SR ($\Delta f_{\text{SR}} = -0.092$ and -0.107) (**Figure 5c-1**), and stainless steel increased

$f_{\text{expressed}}^{\text{eff}}$ from 0.475 to 0.537. In the Paepatung cases [70], ZVI addition raised Δf_{MG} from -0.642 to -0.205 and brought ΔA_{target} from -0.508 to -0.047 , while $f_{\text{expressed}}^{\text{eff}}$ increased from 0.152 to 0.754 (Figure 5c-2). Thus, correcting a deviation against MG requires preferential improvement of MG realization. For a residual target, intervention should therefore target MG directly or, if the nominal opportunity of MG is itself insufficient, reduce the capacity or accessibility of the bounded SR sink. When the bounded sink is the target, the logic differs. In the Sarti and Zaiat cases [77], $A_{\text{target}}^{\text{eff}} \approx 1$ (**Figure 5a**) and $\Delta A_{\text{target}} = 0.745\text{--}0.818$ while $f_{\text{expressed}}^{\text{eff}}$ remained at 0.154–0.205. Δf_{SR} was close to zero (-0.091 to -0.010), whereas Δf_{MG} was strongly negative (-0.747 to -0.818): the large positive ΔA_{target} arose from under-realization of the competing MG sink. Thus, when the bounded sink is the target, the objective is to realize the nominal opportunity of the target as fully as possible, i.e., to bring Δf_{SR} toward zero. If SR is already close to nominal, as in these cases, further intervention toward MG is only needed when the objective also includes increasing overall terminal-sink realization.

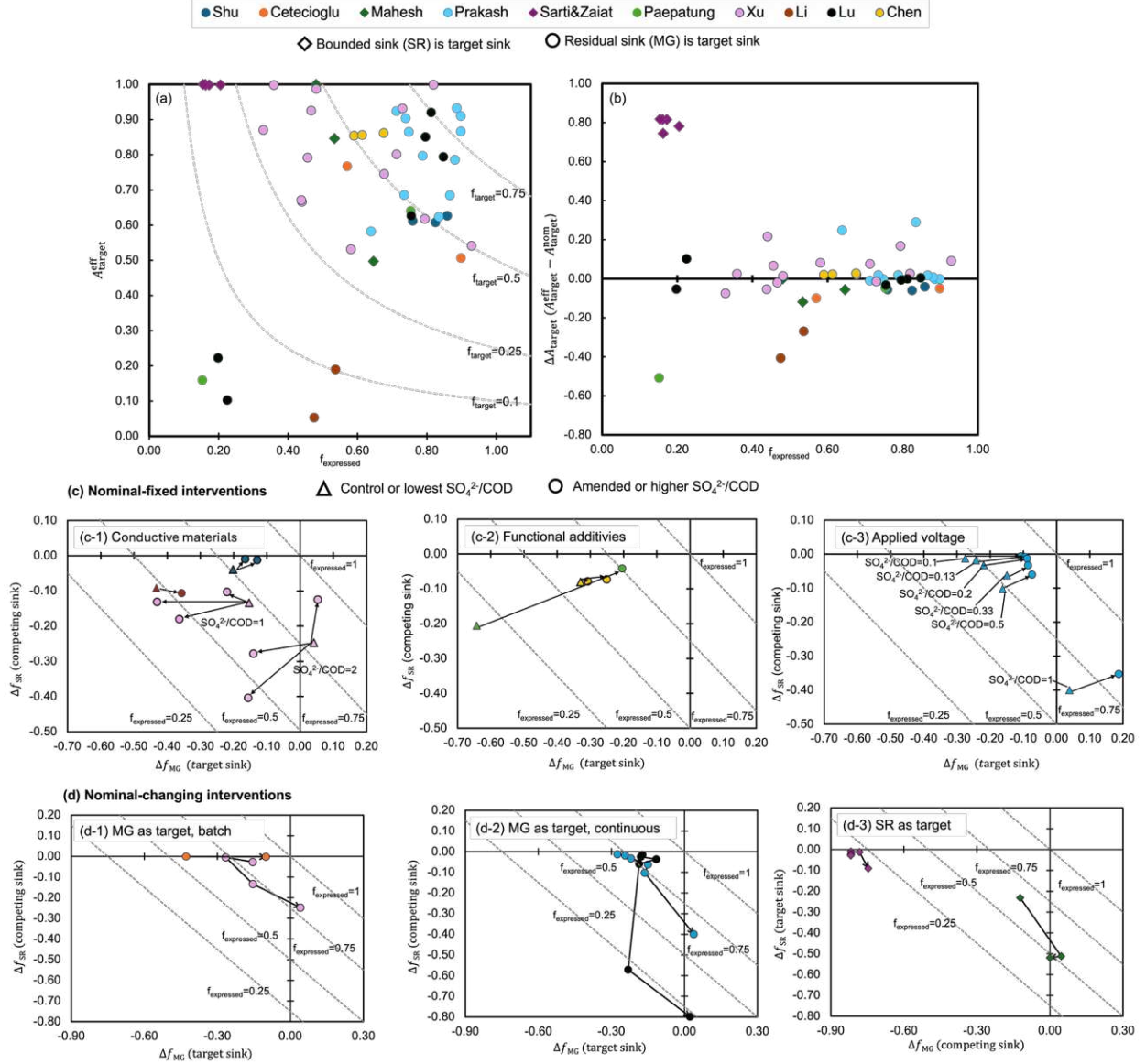


Figure 5. Operational mapping and intervention trajectories in SR-MG systems: (a) effective operational state; (b) deviation of target alignment from its nominal value; (c) nominal-fixed interventions; (d) nominal-changing interventions. In (c) and (d), arrows indicate the direction of change, and dashed lines indicate constant $f_{\text{expressed}}$. Cases from Lu et al., Li et al., and Paepatung et al. also appear in Figure 3.

3.3. Time-resolved interpretation of electron allocation

The allocation state evaluated in Sections 3.1.2 and 3.2 is a snapshot, taken at the end of a batch incubation or at steady state in continuous operation. Because electrons pass through successive commitment points before reaching terminal sinks (Figure 2, Section 3.1), the observed state

depends on when it is observed, and a single snapshot cannot always explain low target recovery. Three situations can be distinguished: electrons may not yet have reached the terminal sinks; electrons that have reached the sinks may be allocated unfavorably relative to the target; or the system may have shifted to a different allocation state. Repeating the analysis across successive analysis windows yields an electron-allocation trajectory, in which terminal-sink realization and allocation direction are tracked together over time, allowing these situations to be separated.

Figure 6 illustrates this approach for SR-MG systems using two studies, with MG as the target sink and SR as the competing sink, consistent with the focus of both studies on methane production [74], [81]. In the cycle experiments of Shu et al. [74], sludge from continuously fed reactors without amendment or with PAC or Fe₃O₄ was incubated for 24 h with ethanol-containing synthetic wastewater (2,315–2,420 mg COD/L; 1,455–1,660 mg SO₄²⁻/L). Because the SO₄²⁻/COD ratio was the same in all three conditions, the conductive materials acted as nominal-fixed interventions. In the study of Jing et al. [81], a mesophilic UASB reactor (35 °C) was fed with synthetic wastewater containing ethanol and acetate (approximately 3,000 mg COD/L), and approximately 3,000 mg SO₄²⁻/L. The reactor was operated for more than 180 days, during which the HRT was progressively reduced from 48 to 2 h and then restored to 6 h. Calculation details and case-level data are provided in **Supplementary Data 3, Sheet Note**.

Figure 6a-1 shows the batch trajectories of $f_{expressed}^{eff}$ and A_{target}^{eff} . All three conditions progressed toward greater terminal-sink realization, but at different rates. At 4 h, $f_{expressed}^{eff}$ was 0.27 in the control, 0.45 with PAC, and 0.52 with Fe₃O₄, with corresponding A_{target}^{eff} values of 0.28, 0.35 and 0.39. By 7 h, $f_{expressed}^{eff}$ had reached 0.48, 0.68, and 0.79 in the same order. A_{target}^{eff} was not high from the beginning: SR was realized first, and alignment toward MG increased progressively as methanogenesis caught up, from approximately 0.15–0.33 at 1–2h to 0.62 at 24 h. By 24h, the three trajectories converged toward similar target alignment (A_{target}^{eff} = 0.616–0.619), with $f_{expressed}^{eff}$ of 0.92 in the control and 0.95–0.98 in the amended conditions, and final target recovery was similar (0.57, 0.59, and 0.61). Although ethanol was depleted by 4 h in the amended conditions and by 7 h in the control, $f_{expressed}^{eff}$ continued to increase until approximately 12 h and 24 h, respectively, as electrons continued to pass through intermediate

pools. A low $f_{expressed}^{eff}$ observed early in these trajectories therefore reflected electrons that had not yet reached the terminal sinks, and donor depletion did not mark the completion of terminal allocation. PAC and Fe_3O_4 accelerated realization of the existing allocation opportunity without altering the final allocation direction, and are therefore most useful when the objective is to shorten the time required to reach terminal allocation.

Figure 6a-2 shows the corresponding deviations from the benchmark. ΔA_{target} increased from approximately -0.37 to -0.28 at 1 h to between -0.01 and $+0.08$ at 24 h. Δf_{MG} increased from -0.53 , -0.61 , and -0.58 at the start to $+0.02$, -0.04 , and $+0.01$ at 24 h for the control, PAC, and Fe_3O_4 conditions, respectively, whereas Δf_{SR} remained slightly negative (-0.11 , -0.01 , and -0.02). The early allocation against MG was therefore transient. In the amended conditions, both sinks approached their nominal allocations, and the trajectories ended close to the benchmark. In the control, SR remained below its nominal allocation, and the corresponding electrons were left to the residual MG sink, giving a small positive final ΔA_{target} ($+0.08$) and f_{target}^{eff} above f_{target}^{nom} (0.57 vs. 0.54). This suggests that, when the residual sink is the target, f_{target}^{eff} can exceed f_{target}^{nom} without any change in the influent-defined opportunity, provided that the bounded sink remains below its nominal allocation throughout the analysis window. Such a sustained departure from the benchmark in favor of the target underlies the route of limiting the competing bounded sink identified in **Section 3.2.2.2**.

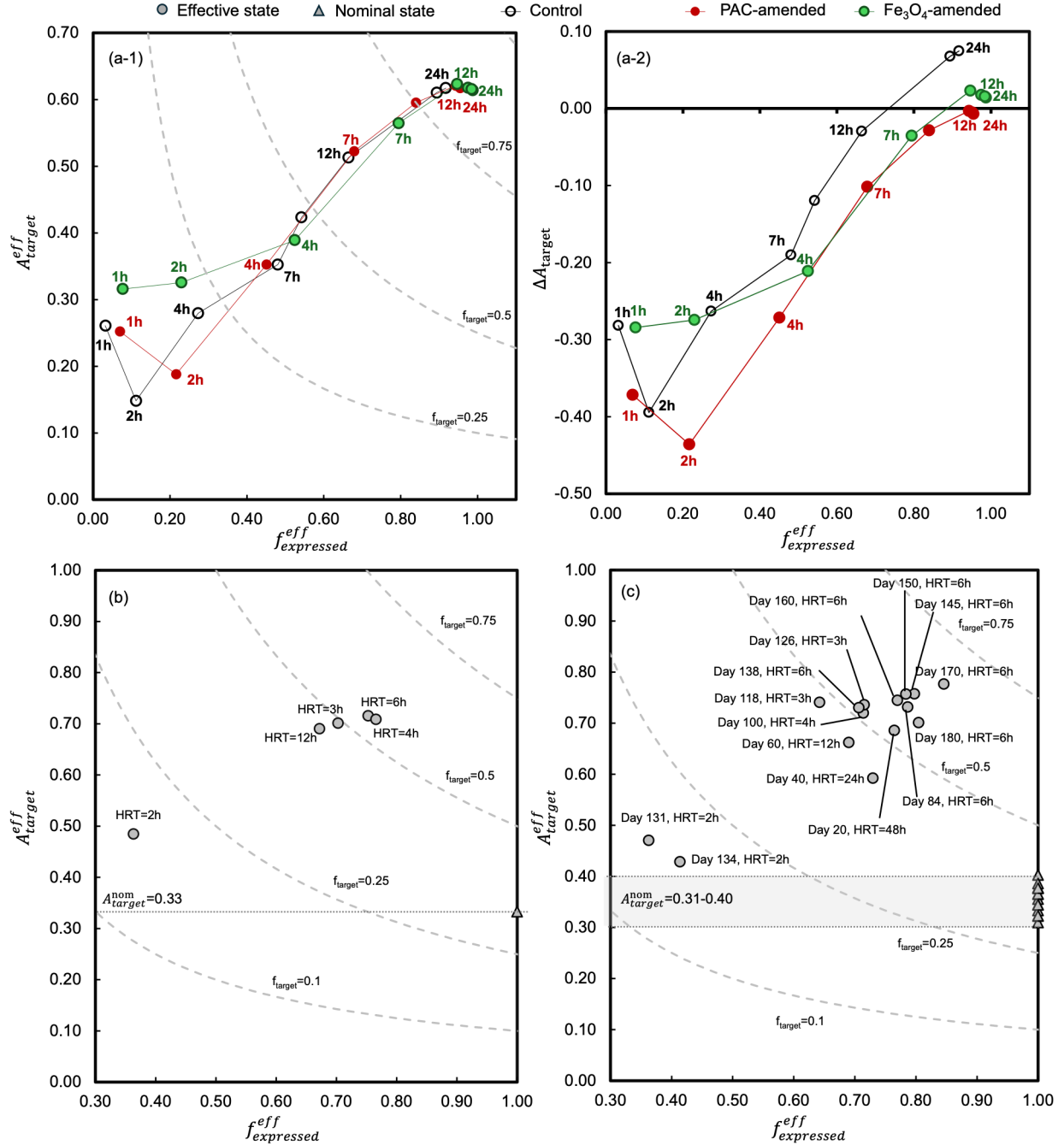


Figure 6. Time-resolved electron-allocation trajectories relative to nominal allocation benchmarks. (a) Batch evolution of $f_{expressed}$ and A_{target} under control, PAC, and Fe_3O_4 conditions (Data were compiled from [74]); (b) allocation-state shifts across HRT conditions; and (c) operational-time evolution of the continuous reactor relative to the corresponding nominal target-alignment range (Data were compiled from [81]).

Figure 6b and **6c** show the continuous reactor of Jing et al. under changing HRT. At HRTs of 12–3 h, $f_{expressed}^{eff}$ remained within 0.67–0.77 and A_{target}^{eff} within 0.69–0.72, corresponding to $f_{target}^{eff} \approx 0.46$ –0.54 (**Figure 6b**). When HRT was reduced from 3 to 2 h, $f_{expressed}^{eff}$ fell from 0.70 to 0.36, A_{target}^{eff} from 0.70 to 0.48, and f_{target}^{eff} from 0.49 to 0.18. Over operational time (**Figure 6c**), these values fell from 0.72 and 0.74 on Day 126 (HRT 3 h) to 0.36 and 0.47 on Day 131 (HRT 2 h), and by Day 134, $f_{expressed}^{eff}$ had recovered only to 0.41 while A_{target}^{eff} declined further to 0.43. After HRT was restored to 6 h, the trajectory returned to the previous allocation region by Day 138 ($f_{expressed}^{eff} = 0.71$, $A_{target}^{eff} = 0.73$), and subsequent states remained within $f_{expressed}^{eff} \approx 0.77$ –0.85 and $A_{target}^{eff} \approx 0.70$ –0.78. The deterioration at HRT 2 h therefore shifted the system to a different allocation state, involving both a loss of terminal-sink realization and a shift away from the MG target (although A_{target}^{eff} remained above its nominal range of 0.31–0.40), but this shift was reversible once the operating condition was restored.

Tracking the allocation state over time therefore adds a dynamic layer to the framework. It separates the rate at which electrons reach the terminal sinks from the direction in which they are allocated, identifies when terminal allocation is actually complete, and reveals whether a deviation from the benchmark is transient, persistent, or reversible. These distinctions indicate whether intervention should accelerate realization, act on the single-window diagnosis, or restore the operating condition.

4. Implications, limitations and future directions

4.1. Implications

For research, the E-framework provides a common electron-allocation space for anaerobic systems such as VFA production, chain elongation, biogas upgrading, and reductive treatment, which are conventionally evaluated with process-specific metrics. Normalization through shared allocation descriptors can reveal system-level patterns that remain obscured within individual process classes, and allows data from different studies to be accumulated, reinterpreted, and compared. Beyond the reinterpretation of existing data, this common structure may also guide experimental design and reporting so that datasets from different anaerobic systems can be integrated on a consistent basis.

For operation, the E-framework provides a diagnostic step before intervention (**Figure 7**). Because similar target recovery can arise from different limitations, low target recovery is first classified by whether the system contains a bounded sink, which determines whether a nominal benchmark is available. Where a benchmark is available, comparison of observed and nominal allocation separates electron diversion, incomplete realization, and an opportunity limited by influent conditions, and identifies whether a nominal-fixed or nominal-changing intervention is appropriate. Where no benchmark is available, sink expression and target alignment separate incomplete expression from competing allocation. Because a single analysis window may misrepresent the allocation state, the diagnosis is then verified across successive windows before intervention.

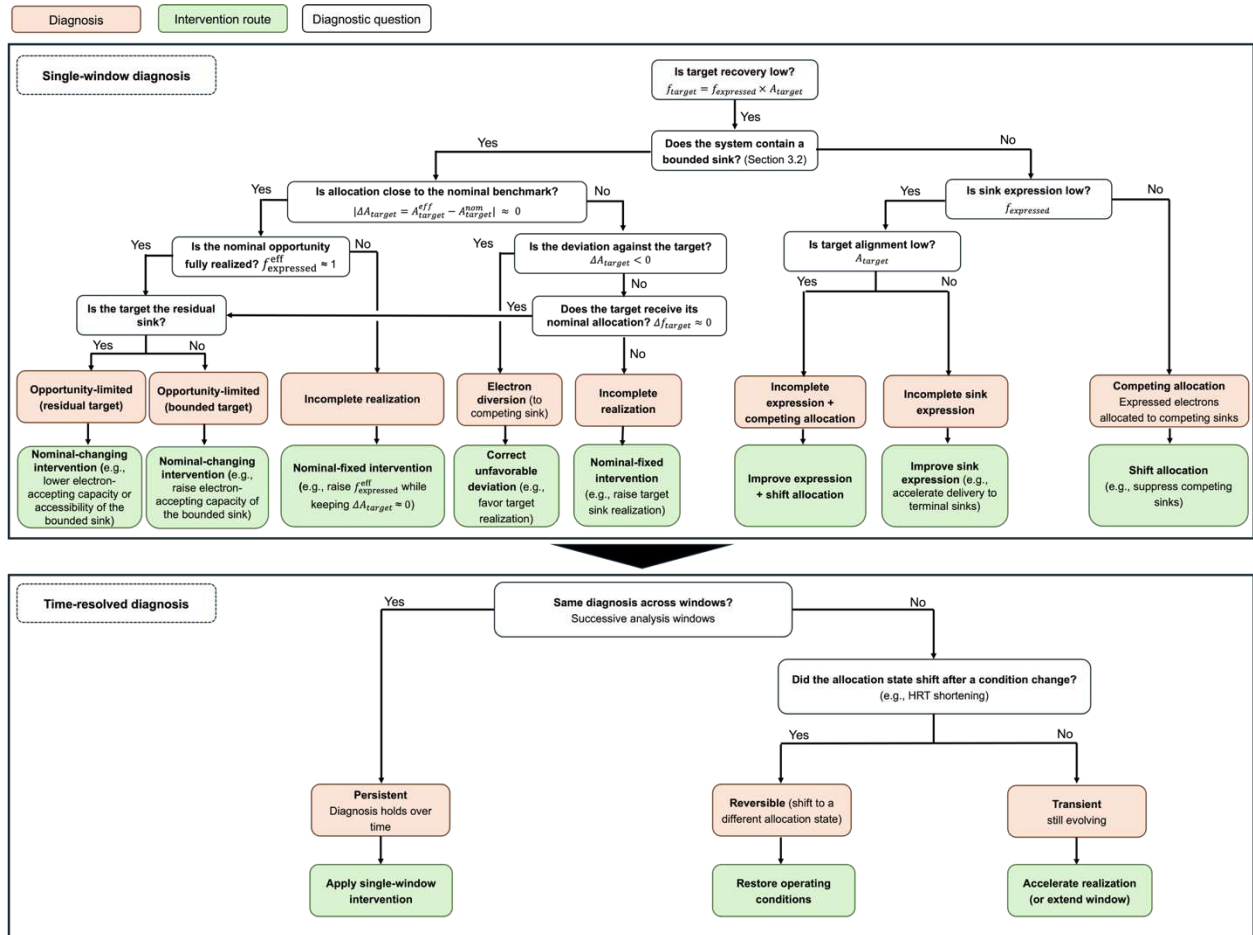


Figure 7. Diagnosis-guided intervention in the E-framework. (Top) Single-window diagnosis: systems with a bounded sink are evaluated against the nominal benchmark, whereas systems

without one are diagnosed directly from sink expression and target alignment. (Bottom) Time-resolved diagnosis across successive analysis windows, applied to any single-window diagnosis.

4.2.Limitations and future directions

The present analysis has several limitations that also indicate directions for further development. First, the literature-derived datasets differ in experimental conditions and reporting completeness, which limits the generalization of the observed cross-system patterns. Electrons in unmeasured pools, electron-bearing outputs, and biomass synthesis are contained in the unresolved term, so that sink expression may be underestimated where cell yield is substantial [38], whereas unmeasured competing sinks may inflate target alignment. Cases in which the electron balance exceeded closure were excluded, which may affect the representativeness of the retained datasets. More complete electron-equivalent accounting of inputs, major pools, biomass, sinks, and outflows, together with consistent definitions of the operational objective, system boundary, and analysis window, would support further evaluation across existing and prospective datasets. Second, the nominal benchmark serves as a reference defined by thermodynamic priority and electron-accepting capacity, against which observed allocation is evaluated. Competition between pathways is also governed by kinetic and ecological factors, such as substrate affinity and growth rate, so deviations from the benchmark reflect electron accessibility as well as operational performance. The benchmark further assumes a fixed priority order among bounded sinks and a single residual sink with sufficient capacity, and its application here was limited to SR-MG systems evaluated at the acetate node, where nominal allocation was effectively capacity-limited. Further development should address changing thermodynamic priorities, multiple residual sinks, interacting capacity constraints, and conditions with operationally relevant crossover. Third, the intervention routes and time-resolved diagnosis (**Sections 3.2.2.2 and 3.3**) were illustrated with a limited number of studies. A systematic intervention map could be developed by quantifying, for each intervention type, its effect on sink expression, target alignment, and the nominal benchmark across different initial allocation states and operational objectives. Experiments designed with complete electron accounting across successive analysis windows would provide the data required for such mapping. Combining the E-framework with dynamic process models could support this effort: the models would predict how electron allocation responds to an intervention, and the E-framework would evaluate

whether that response moves the system toward its objective, providing a basis for future prediction and control.

5. Conclusion

This study proposed the E-framework, an objective-oriented diagnostic layer built on electron balances. Whereas electron balances describe where electrons are allocated, the E-framework interprets that allocation against the operational objective and identifies what limits the intended outcome. Three main conclusions emerge. First, the functional role of a pathway depends on the operational objective: methanogenesis can act as either the target or a competing sink, so performance evaluation begins with defining the objective. Second, decomposing target electron recovery into sink expression and target alignment places systems with different objectives in a common allocation space. Across 104 literature-derived cases, this decomposition showed that systems with similar recovery can be limited by entirely different causes. Third, for systems containing bounded sinks, comparison with the nominal benchmark distinguishes incomplete realization of the available opportunity from an opportunity already limited by influent conditions. In sulfate-reducing methanogenic systems, sulfate reduction consistently received less than its nominal allocation, owing either to diversion toward methanogenesis or to incomplete sink expression. Tracked across successive analysis windows, these indicators further reveal whether a deviation is transient, persistent, or reversible. These analyses were synthesized into a diagnostic decision tree that links each identified limitation to a corresponding intervention route. Intervention selection can therefore be grounded in the diagnosed electron-allocation state in addition to overall performance. Coupled with process-oriented models that predict allocation responses to operating changes, the E-framework could evaluate these predictions against the objective, moving from diagnosis to prediction and control.

Author Contributions

Thu Huong Nguyen: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Software, Visualization, Project administration, Writing – original draft.

Taira Hidaka: Validation, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Claude Opus 5.5 (Anthropic) to improve the language, clarity, and structure of the manuscript text and to check the consistency of reported data. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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