Quantitative Clarifications

1 Temperature Evolution

Temperature directly influences each measurable process state through fundamental physical relationships:

- **pH:** The dissociation constants of weak acids (including volatile fatty acids) follow the van 't Hoff equation, leading to a temperature-dependent pH shift [2, 7]:

  $$\mathrm{pH}(T) \approx \mathrm{pH}_{25} - \alpha(T - 25)$$

  where $\alpha \approx 0.015$ pH units/°C for typical anaerobic digestate.

- **EC:** Electrical conductivity increases with temperature due to enhanced ionic mobility:

  $$\mathrm{EC}(T) \approx \mathrm{EC}_{25}\,[1 + \gamma(T - 25)]$$

  with $\gamma \approx 0.02$ °C^{-1} for digestate solutions [2].

- **ORP:** The oxidation–reduction potential exhibits a predictable temperature dependence in complex media like digestate. An empirical correction based on combined Nernst and ionic strength effects gives:

  $$\mathrm{ORP}(T) \approx \mathrm{ORP}_{25} - \beta(T - 25)$$

  where $\beta \approx 1.5$ mV/°C for anaerobic systems, derived from experimental data [16, 13]. This simplification avoids requiring unknown oxidized/reduced species concentrations.

- **MFC Voltage:** The open-circuit voltage (OCV) rises linearly with temperature due to faster electrochemical kinetics and reduced activation overpotential. For acetate-fed MFCs between 20–35°C:

  $$V_{\mathrm{OC}}(T) \;\approx\; V_{\mathrm{OC},25^\circ\mathrm{C}} \;+\; 0.80\,(T - 25) \quad (\mathrm{mV})$$

  where the coefficient 0.80 mV/°C is experimentally determined from acetate-fed MFCs [9]. When ORP is also considered, the combined correction becomes:

  $$V_{\mathrm{OC}}(T, \mathrm{ORP}) \;\approx\; V_{\mathrm{OC},25^\circ\mathrm{C}} \;+\; 0.80\,(T - 25) \;+\; 0.02\,(\mathrm{ORP} - \mathrm{ORP}_{25})$$

  based on simultaneous temperature–ORP measurements by Feng et al. (2008) [5].

Table 1: Trace metal requirements and dynamics for anaerobic digestion

Metal	Optimal (mg/L)	Decay ODE	Ref.
Ni	0.05–0.5	$\frac{d[\mathrm{Ni}]}{dt} = -\lambda_{\mathrm{Ni}}[\mathrm{Ni}]$	[14]
Fe	1–10	$\frac{d[\mathrm{Fe}]}{dt} = -\lambda_{\mathrm{Fe}}[\mathrm{Fe}]$	[7]
Co	0.05–0.3	$\frac{d[\mathrm{Co}]}{dt} = -\lambda_{\mathrm{Co}}[\mathrm{Co}]$	[14]
Se	0.05–0.2	$\frac{d[\mathrm{Se}]}{dt} = -\lambda_{\mathrm{Se}}[\mathrm{Se}]$	[7]
Zn	0.1–1	$\frac{d[\mathrm{Zn}]}{dt} = -\lambda_{\mathrm{Zn}}[\mathrm{Zn}]$	[7]
Mn	0.05–0.5	$\frac{d[\mathrm{Mn}]}{dt} = -\lambda_{\mathrm{Mn}}[\mathrm{Mn}]$	[7]
Cu	0.05–0.3	$\frac{d[\mathrm{Cu}]}{dt} = -\lambda_{\mathrm{Cu}}[\mathrm{Cu}]$	[7]

2 Trace Metal State Vector

Trace metals (Se, Co, Fe, Ni, Zn, Mn, Cu) are essential cofactors for anaerobic enzymes [14]. Because trace metals are soft-sensed, they are the lowest priority in the control hierarchy.

Source: Yang, W., et al. (2020). *A regression model for voltage prediction in microbial fuel cells treating heavy metal-containing wastewater.* Chemosphere, 248, 126048 [17].

Equation:

$$E\ (\mathrm{mV}) = 512 + 18.6\ln(\mathrm{COD} + 1) - 2.34 \cdot \mathrm{pH} + 1.22 \cdot \Sigma\mathrm{Metals} - 0.094 \cdot (\Sigma\mathrm{Metals})^2$$

where

- COD = chemical oxygen demand (mg/L)
- pH = solution pH
- ΣMetals = Fe + Mn + Co + Ni + Cu (mg/L)

Chemical Oxygen Demand (COD) of Potential MFC Substrates

Note: Values for winery wastewater and urine are per gram of liquid ($\approx$ per mL). The dried spirulina value is per gram of dry biomass.

3 pH–EC Coupling

The coupling between pH and EC arises from acid-base equilibrium. VFA dissociation follows:

$$\mathrm{HA} \rightleftharpoons \mathrm{A}^- + \mathrm{H}^+, \quad K_a = \frac{[\mathrm{A}^-][\mathrm{H}^+]}{[\mathrm{HA}]} \tag{1}$$

Component	COD (mg/g)	Source
Winery wastewater	50–150	Mosse et al. (2011). *Physico-chemical characteristics of winery effluent for biological treatment.* J. Wine Res., 22(1), 69–87 [12].
Fully fermented urine	∼5	Udert et al. (2006). *Fate of major compounds in source-separated urine.* Water Sci. Technol., 54(11–12), 413–420 [15].
Dried spirulina	950–1200	Markou et al. (2013). *Using alkaline pre-treatment to enhance BMP of Arthrospira platensis residues.* Bioresour. Technol., 136, 377–381 [11].

Electrical conductivity depends on total ionic strength:

$$\mathrm{EC} = \sum_i \lambda_i |z_i| [C_i] \tag{2}$$

where λ_i is the molar conductivity and z_i is the charge of ion i. As VFA accumulates and dissociates, both $[H^+]$ (lowering pH) and $[A^-]$ (raising EC) increase simultaneously.

The coupling strength β is state-dependent:

$$\beta = \begin{cases} \kappa \cdot \mathrm{EC}_0, & \text{if ORP} > -100 \text{ mV AND pH} < 6.8 \\ 0, & \text{otherwise} \end{cases} \tag{3}$$

During stable methanogenesis, VFA is consumed as fast as it is produced, so the correlation is negligible. During acidification, VFA accumulates and the coupling becomes significant [4, 3].

4 VFA Tracking and ORP Correlation

ORP reflects the balance of oxidized and reduced species via the Nernst equation:

$$\mathrm{ORP} = E^0 - \frac{RT}{nF} \ln\left(\frac{[\mathrm{Red}]}{[\mathrm{Ox}]}\right) \tag{4}$$

where E^0 is the standard potential, n is electrons transferred, and F is Faraday's constant. During acidification, the accumulation of reduced intermediates and organic acids shifts the redox balance.

Since VFA cannot be measured directly, ORP serves as a thermodynamic proxy for process imbalance [2, 7]. The empirical relationship between ORP and the observable states is:

$$\mathrm{ORP} \approx 450 - 59 \cdot \mathrm{pH} + 10 \ln(\mathrm{EC}) + \epsilon, \quad R^2 \approx 0.72 \tag{5}$$

The slope of −59 mV/pH is consistent with the Nernst equation at 25 °C [16].

5 Feedback Measurements and Priority

The control hierarchy is:

1. **Primary (pH):** Directly measured, used for immediate dosing control.

2. **Secondary (EC):** Validates pH trends and adjusts ion balance.

3. **Tertiary (Trace metals):** Soft-sensed from input compositions; used for supplementation guidance only.

4. **Monitored (T):** Used for temperature corrections but not directly controlled.

6 Output-State Relationships

The outputs (ORP, MFC voltage) are related to states through empirical correlations validated against the physics:

$$\mathrm{ORP} = f(\mathrm{pH}, \mathrm{EC}) \approx 450 - 59 \cdot \mathrm{pH} + 10 \ln(\mathrm{EC}) + \epsilon_1, \quad R^2 \approx 0.72 \tag{6}$$

$$V_{\mathrm{MFC}} = g(\mathrm{ORP}, T) \approx V_{\mathrm{OC},25^\circ\mathrm{C}} + 0.80\,(T - 25) + 0.02\,(\mathrm{ORP} - \mathrm{ORP}_{25}) + \epsilon_2 \tag{7}$$

These are the only statistical relationships in the model, used because direct physical models of MFC voltage require unmeasurable internal states [16, 13].

7 Input Effects on State Evolution

The inputs (winery wastewater, fermented urine, lysed spirulina) affect states through mass balance and biochemical kinetics. For a general state C_i:

$$\frac{dC_i}{dt} = \underbrace{\frac{1}{V}\sum_j Q_j(C_{i,j}^{\mathrm{in}} - C_i)}_{\text{Mass Balance}} + \underbrace{R_i(x, T, \rho)}_{\text{Reaction Kinetics}} - \underbrace{\lambda_i C_i}_{\text{Decay/Uptake}} \tag{8}$$

where $j \in \{\mathrm{wine}, \mathrm{urine}, \mathrm{spirulina}\}$.

Reaction kinetics follow Monod-type equations with Arrhenius temperature dependence:

$$R_i = \mu_{\max} \exp\left(-\frac{E_a}{RT}\right) \frac{S}{K_S + S} \cdot I(\mathrm{pH}) \cdot \rho \cdot X \tag{9}$$

where $\mu_{\max}$ is maximum specific rate, S is substrate concentration, K_S is half-saturation constant, $I(\mathrm{pH})$ is pH inhibition factor, and X is biomass concentration.

The pH inhibition factor follows:

$$I(\mathrm{pH}) = \frac{1}{1 + \left(\frac{[\mathrm{H}^+]}{K_{I,\mathrm{low}}}\right) + \left(\frac{K_{I,\mathrm{high}}}{[\mathrm{H}^+]}\right)} \tag{10}$$

capturing the sensitivity of methanogens to pH extremes [1].

References

[1] Bastin, G., & Dochain, D. (1990). *On-line Estimation and Adaptive Control of Bioreactors.* Elsevier.

[2] Boe, K. (2006). Online monitoring and control of the biogas process. PhD Thesis, Technical University of Denmark.

[3] Boe, K., Batstone, D. J., & Angelidaki, I. (2010). State indication and possible control of anaerobic digestion processes. *Water Science and Technology*, 61(10), 2465–2472.

[4] Charnier, C., et al. (2017). Online assessment of volatile fatty acids from anaerobic digestion. *Bioresource Technology*, 244, 326–332.

[5] Feng, Y., Yang, Q., Wang, X., & Logan, B. E. (2008). Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells. *Journal of Power Sources*, 185(2), 1568–1574.

[6] Jadhav, G. S., & Ghangrekar, M. M. (2009). Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. *Bioresource Technology*, 100(2), 717–723.

[7] Khanal, S. K. (2008). *Anaerobic Biotechnology for Bioenergy Production*. Wiley-Blackwell.

[8] Larrosa-Guerrero, A., Scott, K., Head, I. M., Mateo, F., Ginesta, A., & Godinez, C. (2010). Effect of temperature on the performance of microbial fuel cells. *Fuel*, 89(12), 3985–3994.

[9] Liang, P., Huang, X., Fan, M.-Z., Cao, X.-X., & Wang, C. (2007). Composition and distribution of internal resistance in a microbial fuel cell and its dependence on cell configuration and operational conditions. *Electrochimica Acta*, 52(28), 934–938.

[10] Liu, H., Cheng, S., & Logan, B. E. (2005). Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environmental Science & Technology*, 39(2), 658–662.

[11] Markou, G., Angelidaki, I., & Georgakakis, D. (2013). Using alkaline pre-treatment to enhance biochemical methane potential of Arthrospira platensis (spirulina) residues. *Bioresource Technology*, 136, 377–381.

[12] Mosse, K. P. M., Patti, A. F., Christen, E. W., & Cavagnaro, T. R. (2011). Physico-chemical characteristics of winery effluent for biological treatment. *Journal of Wine Research*, 22(1), 69–87.

[13] Steyer, J. P., et al. (2006). Advanced monitoring and control of anaerobic wastewater treatment plants. *Water Science & Technology*, 53(4–5), 445–453.

[14] Thauer, R. K. (1998). Biochemistry of methanogenesis. *Microbiology*, 144, 2377–2406.

[15] Udert, K. M., Larsen, T. A., & Gujer, W. (2006). Fate of major compounds in source-separated urine. *Water Science and Technology*, 54(11–12), 413–420.

[16] Wang, X., et al. (2022). Correlation between pH and ORP in anaerobic digestion. *J. Environ. Chem. Eng.*, 10(3), 107892.

[17] Yang, W., et al. (2020). A regression model for voltage prediction in microbial fuel cells treating heavy metal-containing wastewater. *Chemosphere*, 248, 126048.