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Exploring Electron Conversion Efficiency for Biohydrogen in Dark Fermentation

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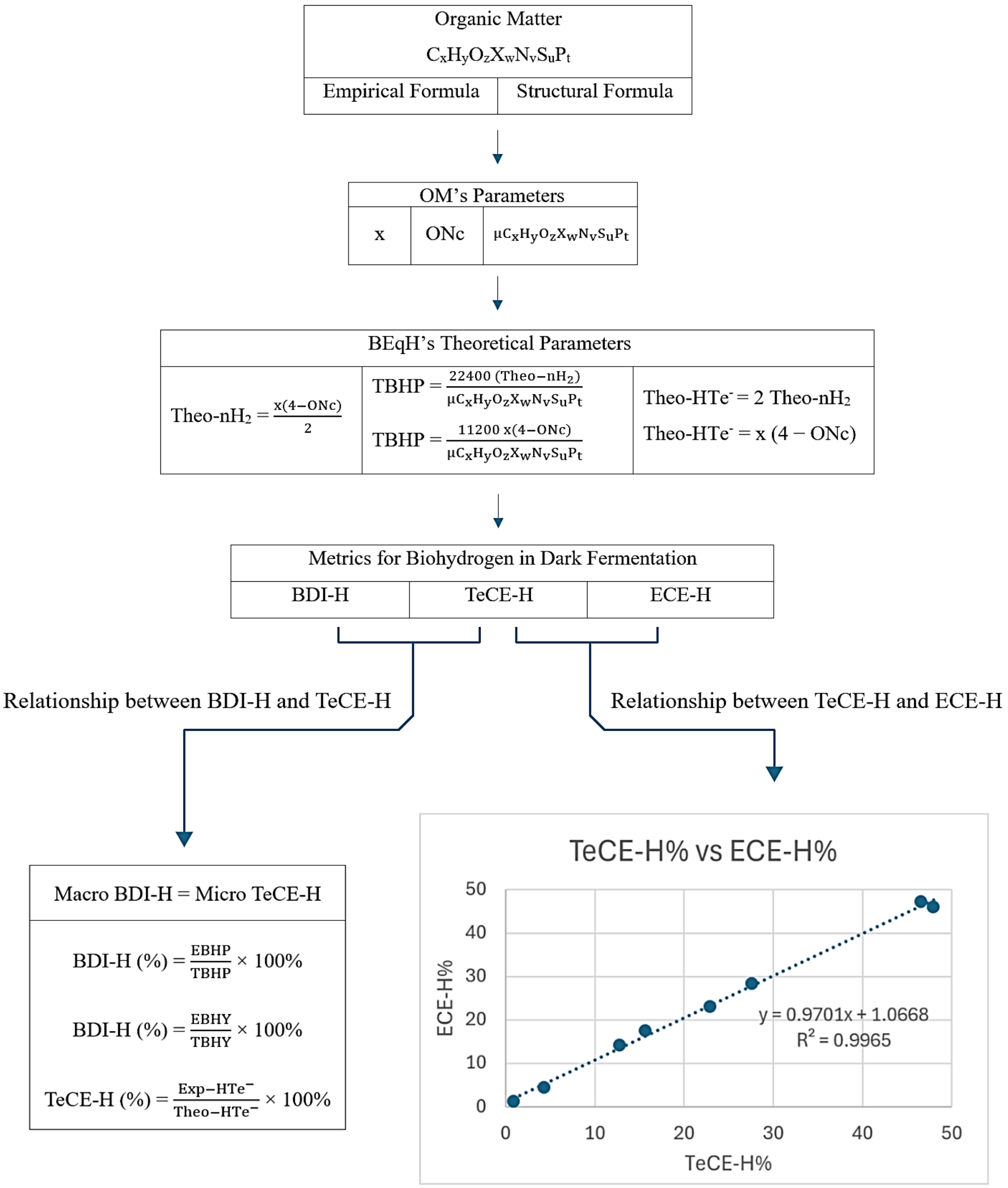
Abstract

Buswell's equation for biohydrogen can represent dark fermentation in accordance with the elemental composition of any organic matter. When the empirical formula or structural formula of an organic matter is identified, the theoretical amount of biohydrogen, theoretical biohydrogen potential, theoretical biohydrogen yield, and theoretical number of transferred electrons can be determined. Currently the metrics that measure the biodegradability performance of organic matters in dark fermentation are biodegradability index and energy conversion efficiency. However, the concept of electron conversion efficiency has not been well investigated. The aims of this article are to develop the electron conversion efficiency for biohydrogen to be a metric and explore the relationships between the biodegradability index and the energy conversion efficiency. The article shows that among the three metrics, electron conversion efficiency and biodegradability index are numerically identical, and that there is a strong positive correlation between electron conversion efficiency and energy conversion efficiency. In addition, the established electron conversion efficiency functions as a cross-reference for dark fermentation, dark fermentation coupled photochemical system, and electrochemical system under anaerobic conditions.

Keywords: dark fermentation (DF), Buswell's equation for biohydrogen (BEqH), theoretical biohydrogen potential (TBHP), biodegradability index (BDI), energy conversion efficiency (ECE), electron conversion efficiency (TeCE)

Graphical Abstract



$$\text{Theo-nH}_2 = \frac{x(4-\text{ONc})}{2}$$

$$\text{TBHP} = \frac{22400\,(\text{Theo}-\text{nH}_2)}{\mu C_xH_yO_zX_wN_vS_uP_t}$$

$$\text{TBHP} = \frac{11200\,x(4-\text{ONc})}{\mu C_xH_yO_zX_wN_vS_uP_t}$$

$$\text{Theo-HTe}^- = 2\ \text{Theo-nH}_2$$

$$\text{Theo-HTe}^- = x\,(4-\text{ONc})$$

$$\text{BDI-H}\ (\%) = \frac{\text{EBHP}}{\text{TBHP}} \times 100\%$$

$$\text{BDI-H}\ (\%) = \frac{\text{EBHY}}{\text{TBHY}} \times 100\%$$

$$\text{TeCE-H}\ (\%) = \frac{\text{Exp}-\text{HTe}^-}{\text{Theo}-\text{HTe}^-} \times 100\%$$

1. Introduction

Electron transfer is the essence of redox reaction, involving chemistry and biology. Redox reaction is an important process for biological functions, energy production, and electrochemical technologies. It facilitates electron transfer between substances, dominates primary energy transfer in living organisms, and powers essential biological processes. The proton-coupled electron transfer is the root for chemical and biological energy conversions (Nocera, 2022) and plays an essential role in electrocatalytic processes such as hydrogen production, carbon dioxide reduction, and oxygen reduction (Warburton et al. 2022). The half redox reaction for proton and hydrogen atom is shown as:

$$H^+ + e^- \rightarrow H$$

Molecular hydrogen (H_2) is a non-carbon source which has high energy density. It is used significantly in the fields of non-fossil energy, transportation, industries, and laboratories. H_2 can be generated by chemical, thermochemical, photochemical, electrochemical, or biochemical methods. The chemical equation for hydrogen production is represented as:

$$2H^+ + 2e^- \rightarrow H_2$$

Anaerobic fermentation (AF) is a biochemical technology mediated by microbials in the absence of molecular oxygen (O_2). AF consists mainly of dark fermentation (DF), one-stage anaerobic digestion (1-stage AD), and two-stage anaerobic digestion (2-stage AD). Using biomass, organic waste, and wastewater as feedstocks, DF facilitates biomass treatment, waste management, pollution and environmental control, and biohydrogen generation in the absence of light (D' Silva et al., 2023; Albuquerque et al., 2024, Ghimire et al., 2015; Moussa et al., 2022). DF can be utilized to convert organic waste to biohydrogen, biogenic carbon dioxide, and dark fermentation effluent in the carbon-based circular economy (Yuen et al., 2025).

Buswell's equation for biomethane (BEq) (Buswell & Boruff, 1932; Buswell & Mueller, 1952; Boyle, 1977; Yuen & Lau, 2023) and Buswell's equation for biohydrogen (BEqH) (Yuen & Lau, 2024a) can represent AD and DF, respectively. When an empirical formula of OM is identified, the stoichiometric BEq and BEqH can be found and are shown in the following:

$$C_xH_yO_zX_wN_vS_uP_t+\frac{4x-y-2z+w+3v+2u+11t}{4}H_2O\rightarrow\frac{4x+y-2z-w-3v-2u+5t}{8}CH_4+\frac{4x-y+2z+w+3v+2u-5t}{8}CO_2+wHX+vNH_3$$

$$+uH_2S+tH_3PO_4$$

$$C_xH_yO_zX_wN_vS_uP_t+(2x-z+4t)H_2O\rightarrow\frac{(4x+y-2z-w-3v-2u+5t)}{2}H_2+xCO_2+wHX+vNH_3+uH_2S+tH_3PO_4$$

Using the mean oxidation number of organic carbons (ONc) of OM as a metric, the mathematical relationships between BEqH's parameters, such as theoretical quantity of biohydrogen (nH_2), theoretical biohydrogen potential (TBHP), theoretical biohydrogen yield (TBHY), and theoretical number of transferred electrons can be established (Yuen et al., 2024b). The biodegradability index for biohydrogen (BDI-H) and the energy conversion efficiency for biohydrogen (ECE-H) are used to evaluate the biodegradability performance of OM.

With reference to the biodegradability index for biomethane (BDI-M) (Nielfa et al., 2015), BDI-H (Yuen & Lau, 2024a) is defined as the ratio of experimental biohydrogen potential (EBHP) to theoretical biohydrogen potential (TBHP). BDI-M and BDI-H are represented below:

$$\text{BDI-M} = \frac{\text{Experimental biomethane potential}}{\text{Theoretical biomethane potential}} = \frac{\text{EBMP}}{\text{TBMP}}$$

$$\text{BDI-H} = \frac{\text{Experimental biohydrogen potential}}{\text{Theoretical biohydrogen potential}} = \frac{\text{EBHP}}{\text{TBHP}}$$

Energy conversion efficiency (ECE) is a metric which evaluates the biodegradability of OM (Zhang et al., 2017; Xia et al., 2013). The energy conversion efficiency for H_2 (ECE-H%), the energy conversion efficiency for CH_4 (ECE-M%), and the energy conversion efficiency for H_2 and CH_4 (ECE-HM%) are shown in the following:

$$\text{Energy conversion efficiency for } H_2 \text{ (ECE-H\%)} = \frac{\text{Heating value of } H_2}{\text{Heating value of OM}} \times 100\%$$

$$\text{Energy conversion efficiency for } CH_4 \text{ (ECE-M\%)} = \frac{\text{Heating value of } CH_4}{\text{Heating value of OM}} \times 100\%$$

$$\text{Energy conversion efficiency for } H_2 \text{ and } CH_4 \text{ (ECE-HM\%)} = \text{ECE-H\%} + \text{ECE-M\%}$$

$$= \frac{\text{Heating value of } H_2 + \text{Heating value of } CH_4}{\text{Heating value of OM}} \times 100\%$$

AF is a biochemical redox system in which the number of transferred electrons (Te^-) for the generation of biohydrogen (Yuen et al., 2024b) and biomethane (Yuen & Lau, 2024c) can be counted. Electron transfer and energy conversion are two critical aspects in the study of AF (He, 2013; Cheng et al. 2020; Liu et al., 2021). The relationship between electron transfer mechanism and energy efficiency is established in microbial fuel cells (Schröder, 2007). Electron conversion efficiency (TeCE) is a metric which measures the performance of electronic or photoelectronic devices (Iwasaki et al., 2024), however, it has not been used in AF. Currently, due to the lack of electron-based metric, it is difficult to understand the nature of biodegradability and energy conversion of OM, and elucidate the relationships between electron transfer, biodegradability performance, and energy conversion.

Compared to DF and 2-stage AD, a series of promising technologies such as photo fermentation (PF), direct biophotolysis, indirect biophotolysis, microbial electrolysis cell (MEC), microbial fuel cell (MFC) (Vasiliadou et al., 2018; Ieropoulos & Greenman, 2023), and their coupling with DF are employed for biohydrogen production (Lee et al., 2022). H_2% yield, coulombic efficiency, current density, power density, and energy recovery% are used to measure the biohydrogen production efficiency in the electrochemical system (Cheng & Logan, 2007; He, 2017). Between AF system and electrochemical system, as well as within the coupling system, there is no knowledge of an electron-based metric as a cross-reference.

The purposes of this research are (i) to develop the application of TeCE-H as a metric for biohydrogen generation, (ii) to establish the relationship between TeCE-H and BDI-H, (iii) to find the correlation between TeCE-H and ECE-H, (iv) to integrate the relationships between TeCE-H, BDI-H, and ECE-H, and (v) to develop TeCE-H as a cross-reference between AF system and electrochemical system. To quantify the new metric, i.e., TeCE-H, three factors are needed: (1) the BEqH model, (2) the ONc of OM, and (3) the mathematical relationships between BEqH's parameters.

2. Methods and Materials

2.1 OM's Parameters

OM is composed of pure or mixed organic compounds, such as carbohydrate, protein, lipid, biomass, biowaste, food waste, and wastewater, and they are often used as feedstocks. When the empirical formula (EF) or structural formula (SF) of OM is found, its numerical OM's parameters can be quantified.

OM: EF or SF → OM's parameters: ONc, x, μ_{OM}

2.2 BEqH's Parameters

The flowchart below shows how parameters of OM are developed into parameters of BEqH:

OM's parameters: ONc, x, μ_{OM} → BEqH's parameters: nH_2, TBHP, Te^-

Using ONc of OM as a redox metric, the mathematical relationships of BEqH's parameters (Yuen et al., 2024b) are shown in Table 1.

Table 1. BEqH's parameters and mathematical relationships

Chemical formula	$C_xH_yO_zX_wN_vS_uP_t$
ONc of OM	$\text{ONc} = \frac{-\sum \text{ON}_{\text{inc}}}{x}$ $\text{ONc} = \frac{-(y\ \text{ON}_H + z\ \text{ON}_O + w\ \text{ON}_X + v\ \text{ON}_N + u\ \text{ON}_S + t\ \text{ON}_P)}{x}$ (for SF) $\text{ONc} = \frac{-y+2z+w+3v+2u-5t}{x}$ (for EF)
Mathematical Relationship	Theoretical nH_2 = Theo-nH_2 = $\frac{x(4-\text{ONc})}{2}$
	$\text{TBHP} = \frac{22400\ (\text{Theo}-nH_2)}{\mu C_xH_yO_zX_wN_vS_uP_t}$ $\text{TBHP} = \frac{11200\ x(4-\text{ONc})}{\mu C_xH_yO_zX_wN_vS_uP_t}$
	TBHY = Theoretical nH_2 = Theo-nH_2
	Theoretical HTe^- = Theo-HTe^- = 2 Theo-nH_2 Theoretical HTe^- = Theo-HTe^- = x (4 − ONc)

2.3 BDI-M and TBMP

The theoretical TBMP can be determined by BEq (Angelidaki & Sanders, 2004; Yuen et al., 2024d). After integrating with the experimental EBMP (Owen et al., 1979; Angelidaki et al., 2009; Raposo et al., 2011; 2012), the metric BDI-M can consequently be found to evaluate the biodegradability of OM. The mathematical representations of TBMP, EBMP, and BDI-M are shown in the following:

$$\text{TBMP (at STP, mL/g)} = \frac{22400\ (\text{mole of biomethane})}{\mu_{OM}}$$

$$= \frac{22400\ (nCH_4)}{\mu_{OM}}$$

$$= \frac{2800\ x(4-ONc)}{\mu_{OM}}$$

$$\text{EBMP (at STP, mL/g)} = \frac{\text{maximum amount of produced volumetric methane}}{\text{mass of added volatile solid}}$$

$$= \frac{VCH_4}{VS_{added}}$$

$$\text{BDI-M\%} = \frac{\text{EBMP}}{\text{TBMP}} \times 100\%$$

$$\text{BDI-M} = \frac{\text{EBMP}}{\text{TBMP}}$$

2.4 BDI-H and TBHP

Although the BHP protocol (Carrillo-Reyes et al., 2019; 2020) was implemented, due to the lack of theoretical TBHP, the usage of BDI-H as a metric has been obstructed. Recently, the establishment of the BEqH model provides a key to determine TBHP (Yuen & Lau, 2024a) and it is shown in the following:

$$\text{TBHP (at STP, mL/g)} = \frac{22400\ (nH_2)}{\mu_{OM}}$$

$$= \frac{11200\ x(4-ONc)}{\mu_{OM}}$$

EBHP is defined as the ratio of the maximum amount of produced volumetric hydrogen (VH_2) to the mass of added volatile solid (VS_{added}), and the equation is shown in the following:

$$\text{EBHP (at STP, mL/g)} = \frac{\text{maximum amount of produced volumetric hydrogen}}{\text{mass of added volatile solid}}$$

$$= \frac{V_{H2}}{VS_{added}}$$

EBHP can be measured in the processes of DF, PF, and 2-stage AD. BDI-H is defined as the ratio of experimental EBHP to theoretical TBHP. It is shown as:

$$\text{BDI-H\%} = \frac{\text{EBHP}}{\text{TBHP}} \times 100\%$$

$$\text{BDI-H} = \frac{\text{EBHP}}{\text{TBHP}}$$

2.5 Biohydrogen Percent Yield

Experimental biohydrogen yield (EBHY) is commonly used for measuring biohydrogen production. It is expressed as the amount of biohydrogen per amount of OM ($\frac{\text{amount of biohydrogen}}{\text{amount of OM}}$). The unit will be either mL/g, mol/g, or mol/mol. Due to the lack of stoichiometric chemical equations for biohydrogen production, theoretical biohydrogen yield (TBHY) cannot be found. If the OM's chemical formula is determined, its stoichiometric BEqH can be established. When the experimental EBHY is measured, TBHY and molar ratio-H ($\frac{\text{EBHY}}{\text{TBHY}}$) can also be found. Biohydrogen percent yield (HBPY%) is a stoichiometric term, which is defined as the ratio of EBHY (Exp-nH_2, mole) to TBHY (Theo-nH_2, mole) (Yuen & Lau, 2024a).

$$\text{BHPY\%} = \frac{\text{Experimental biohydrogen yield}}{\text{Theoretical biohydrogen yield}} \times 100\%$$

$$= \frac{\text{EBHY}}{\text{TBHY}} \times 100\%$$

$$= \frac{\text{Exp−nH}_2}{\text{Theo−nH}_2} \times 100\%$$

$$\frac{\text{EBHY}}{\text{TBHY}} = \frac{\text{Exp−nH}_2}{\text{Theo−nH}_2} = \text{Molar ratio-H}$$

At standard temperature and pressure (STP), the unit for EBHY, EBHP, TBHY, and TBHP is mL/g. EBHY equals EBHP and TBHY equals TBHP.

$$\text{BHPY\%} = \frac{\text{Experimental biohydrogen yield}}{\text{Theoretical biohydrogen yield}} \times 100\%$$

$$= \frac{\text{EBHY}}{\text{TBHY}} \times 100\%$$

$$= \frac{\text{EBHP}}{\text{TBHP}} \times 100\%$$

$$\frac{\text{EBHY}}{\text{TBHY}} = \frac{\text{EBHP}}{\text{TBHP}} = \text{BDI-H}$$

2.6 Theoretical and Experimental Number of Transferred Electrons

The mathematical equations of the theoretical number of transferred electrons for biomethane (Theo-MTe$^-$) in AD (Yuen & Lau, 2024c) and the theoretical number of transferred electrons for biohydrogen (Theo-HTe$^-$) in DF (Yuen et al., 2024b) are shown in the following:

Theoretical MTe$^-$ = Theo-MTe$^-$ = Theo-nCH_4 (x + ONc)

Theoretical HTe$^-$ = Theo-HTe$^-$ = 2 Theo-nH_2

The experimental number of transferred electrons for biomethane (Exp-MTe$^-$) and the experimental number of transferred electrons for biohydrogen (Exp-HTe$^-$) are calculated from Exp-nCH_4 and Exp-nH_2 respectively.

Experimental MTe$^-$ = Exp-MTe$^-$ = Exp-nCH_4 (x + ONc)

Experimental HTe$^-$ = Exp-HTe$^-$ = 2 Exp-nH_2

2.7 Retrieving Experimental and Calculated Data from Literature

The mass percentages of elements (mass%), EBHP, and ECE-H% of OM are retrieved from published works. The selected mass% are used to identify EF of OM. The selected EBHP are used to calculate BDI-H% and TeCE-H%. The selected ECE-H% are used to compare the calculated TeCE-H%.

3. Counting the Electron Conversion Efficiency of Dark Fermentation

3.1 Electron Conversion Efficiency (TeCE)

TeCE is defined as the ratio of the experimental number of transferred electrons (Exp-Te$^-$) to the theoretical number of transferred electrons (Theo-Te$^-$) and is shown as:

$$\text{TeCE} = \frac{\text{Exp−Te}^-}{\text{Theo−Te}^-}$$

The electron conversion efficiency for H_2 (TeCE-H%) and the electron conversion efficiency for CH_4 (TeCE-M%) are demonstrated as follows:

$$\text{Electron conversion efficiency for H}_2\text{ (TeCE-H\%)} = \frac{\text{Exp−HTe}^-}{\text{Theo−HTe}^-} \times 100\%$$

$$\text{Electron conversion efficiency for CH}_4\text{ (TeCE-M\%)} = \frac{\text{Exp−MTe}^-}{\text{Theo−MTe}^-} \times 100\%$$

3.2 OM and BEqH

The general BEqH is shown in the following:

$$C_xH_yO_zX_wN_vS_uP_t+(2x-z+4t)H_2O \rightarrow \frac{x(4-\text{ONc})}{2}H_2+xCO_2+wHX+vNH_3+uH_2S+tH_3PO_4$$

When the EF of an OM is identified, the parameters of OM can be calculated. Consequently, the parameters of BEqH can be determined. Example 1 shows two processes: how EF is calculated according to the mass% (Yuen & Lau, 2024d), and how parameters of OM are calculated according to the EF (Yuen & Lau, 2024c).

3.3 Step-by-step Procedures

Table 2 shows the retrieved data of the mass% (Deng et al., 2019). Example 1 illustrates the working procedures for calculations of OM's parameters and BEqH's parameters.

Table 2. Selected data: mass% of OM

OM	Atom	C	H	O	N
silage	Mass%	50.5	6.5	41.3	1.7

Example 1. Based on the selected mass% of the silage, (i) find OM's parameters: empirical formula, μ_{OM}, ONc, and x, (ii) find BEqH's parameters: Theo-nH$_2$, TBHP, and Theo-HTe$^-$.

Step 1. Find the empirical formula and the empirical mass (μ_{OM}) of the silage

OM	Atom	C	H	O	N	$\mu_{OM} = \sum$mass%
silage	Mass%	50.5	6.5	41.3	1.7	100.0

$\sum$mass% = summation of mass percentages of elements

$\sum$mass% = C% + H% + O% + N%

= (50.5%+6.5%+41.3%+1.7%)

= 100.0%

Empirical mass (μ_{OM}, g/mol) = $\sum$mass% = 100.0

Empirical formula: $C_xH_yO_zN_v$

AC = atomic coefficients = x, y, z, v

$$\text{nC} : \text{nH} : \text{nO} : \text{nN} = \frac{\text{C\%}}{\mu\text{C}} : \frac{\text{H\%}}{\mu\text{H}} : \frac{\text{O\%}}{\mu\text{O}} : \frac{\text{N\%}}{\mu\text{N}} = x : y : z : v$$

$$x : y : z : v = \frac{50.5}{12.011} : \frac{6.5}{1.008} : \frac{41.3}{15.999} : \frac{1.7}{14.007}$$

AC	x	y	z	v	Empirical formula
silage	4.204	6.448	2.581	0.121	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$

Step 2. Count ONc, x, and μ_{OM}

$\mu_{OM} = \mu C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ = $\sum$mass% = 100.0

OM	Empirical formula	ONc = $\frac{-y+2z+3v}{x}$	x	μ_{OM}
silage	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	–0.220	4.204	100.00

Step 3. Determine nH$_2$, TBHP, and HTe$^-$

Empirical formula	Theo-nH$_2$ = $\frac{x(4-\text{ONc})}{2}$	TBHP = $\frac{22400\,(\text{Theo-nH}_2)}{\mu\text{OM}}$	Theo-HTe$^-$ = 2 Theo-nH$_2$
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	8.870	1986.880	17.740

3.4 Electron Conversion Efficiency for Biohydrogen

The electron conversion efficiency for biohydrogen (TeCE-H) is defined as the ratio of the experimental number of transferred electrons (Exp-HTe$^-$) to the theoretical number of transferred electrons (Theo-HTe$^-$). The mathematical relationships are demonstrated as follows:

$$\text{TeCE-H\%} = \frac{\text{Exp-HTe}^-}{\text{Theo-HTe}^-} \times 100\%$$

$$\text{TeCE-H} = \frac{\text{Exp-HTe}^-}{\text{Theo-HTe}^-}$$

The measured and calculated data of the 1st DF of 2-stage AD (Deng et al., 2019) are summarized in Table 3.

Table 3. Selected data: EBHP, and ECE-H%

OM	Process	EBHP	ECE-H%
silage	1st DF of 2-stage AD	17.47	1.2%

Based on the retrieved experimental and calculated data, the working procedures for calculations of TeCE-H% are demonstrated in Example 2.

Example 2. The experimental data of EBHP that equals 17.47 (mL/g, at STP) is found in 1st DF of 2-stage AD. Determine the TeCE-H% of the silage.

With reference to Example 1, Step 1 and Step 2 are used to determine OM's parameters and BEqH's parameters. Through Step 3, Exp-nH_2, Exp-HTe^-, and TeCE-H% of the silage can be found.

Step 1. Identify OM's parameters

OM	Empirical formula	ONc = $\frac{-y+2z+3v+2u}{x}$	x	μ_{OM}
silage	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	–0.220	4.204	100.00

Step 2. Determine Theo-nH_2, TBHP, and HTe^-

Empirical formula	Theo-nH_2 = $\frac{x(4-ONc)}{2}$	TBHP = $\frac{22400\ (Theo-nH_2)}{\mu OM}$	Theo-HTe^- = 2 Theo-nH_2
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	8.870	1986.880	17.740

Step 3. Count Theo-nH_2, Exp-HTe^-, and TeCE-H%

Based on the relationships between Theo-nH_2, TBHP, and Theo-HTe^-, the relationships between Exp-HTe^-, EBHP, and Exp-HTe^- are found.

$$\because \text{EBHP} = \frac{22400\ (\text{Exp}-\text{nH}_2)}{\mu_{OM}} \qquad \therefore \text{Exp-nH}_2 = \frac{\text{EBHP}\ (\mu_{OM})}{22400}$$

$$\because \text{Exp-HTe}^- = 2\ \text{Exp-nH}_2 \qquad \therefore \text{Exp-HTe}^- = \frac{(\text{EBHP})\ (\mu_{OM})}{11200}$$

Count Theo-HTe^-	Count Exp-HTe^-	Find TeCE-H%
Theo-HTe^- = 2 Theo-nH_2 = 2 (8.870) = 17.740	Exp-HTe^- = $\frac{(EBHP)\ (\mu_{OM})}{11200}$ = $\frac{(17.47)(100.0)}{11200}$ = 0.156	TeCE-H% = $\frac{Exp-HTe^-}{Theo-HTe^-}$ × 100% = $\frac{0.156}{17.740}$ × 100% = 0.88%

3.5 Relationships between BDI-H, Molar ratio-H, and TeCE-H

The triangular mathematical relationships between theoretical TBHP, Theo-nH_2, and Theo-HTe^- are shown in Figure 1.

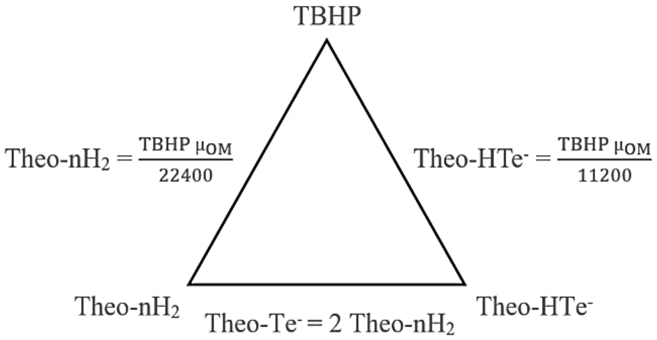


Figure 1. Triangular mathematical relationships between TBHP, Theo-nH_2, and Theo-HTe^-

Example 3. Given EBHP equals 17.47 (mL/g, at STP). Find BDI-H and Molar ratio-H.

With reference to Example 2 and the triangular relationship in Figure 1, the working procedures are shown in the following:

Step 1. Count BDI-H	Step 2. Count Exp-nH$_2$	Step 3. Count Theo-nH$_2$	Step 4. Count Molar ratio-H
BDI-H = $\frac{\text{EBHP}}{\text{TBHP}}$	Exp-nH$_2$ = $\frac{\text{EBHP}\ \mu_{\text{OM}}}{22400}$	Theo-nH$_2$ = $\frac{\text{TBHP}\ (\mu_{\text{OM}})}{22400}$	Molar ratio-H = $\frac{\text{Exp}-\text{nH}_2}{\text{Theo}-\text{nH}_2}$
EBHP = 17.47	EBHP = 17.47	TBHP = 1986.880	Theo-nH$_2$ = 8.870
TBHP = 1986.880	μ_{OM} = 100.00	μ_{OM} = 100.00	Exp-nH$_2$ = 0.0792
$\frac{\text{EBHP}}{\text{TBHP}} = \frac{17.47}{1986.880}$ = 0.0088	Exp-nH$_2$ = $\frac{(17.74)(100.00)}{22400}$ = 0.0792	Theo-nH$_2$ = $\frac{(1986.880)\ (100.00)}{22400}$ = 8.870	$\frac{\text{Exp}-\text{nH}_2}{\text{Theo}-\text{nH}_2} = \frac{0.0792}{8.870}$ = 0.0089

In Example 3, the difference between $\frac{\text{EBHP}}{\text{TBHP}} = 0.0088$ and $\frac{\text{Exp}-\text{nH}_2}{\text{Theo}-\text{nH}_2} = 0.0089$ is caused by the act of rounding numbers. The calculations confirm that $\frac{\text{EBHP}}{\text{TBHP}}$ and $\frac{\text{Exp}-\text{nH}_2}{\text{Theo}-\text{nH}_2}$ are identical. In addition, through the integration of the theoretical set and the experimental set, three sets of ratios, i.e., $\frac{\text{EBHP}}{\text{TBHP}}$, $\frac{\text{Exp}-\text{nH}_2}{\text{Theo}-\text{nH}_2}$, and $\frac{\text{Exp}-\text{HTe}^-}{\text{Theo}-\text{HTe}^-}$, are proven to be equal. Their relationships are summarized in Table 4.

Table 4. BEqH's parameters: relationships between BHP, nH$_2$ and HTe$^-$

Theoretical relationships TBHP, Theo-nH$_2$, and Theo-HTe$^-$	Experimental relationships EBHP, Exp-nH$_2$, and Exp-HTe$^-$
TBHP = $\frac{\mathbf{22400\ (Theo-nH_2)}}{\mu_{\text{OM}}}$	EBHP = $\frac{22400\ (\text{Exp}-\text{nH}_2)}{\mu_{\text{OM}}}$
Theo-nH$_2$ = $\frac{\mathbf{TBHP}\ \mu_{\text{OM}}}{\mathbf{22400}}$	Exp-nH$_2$ = $\frac{\text{EBHP}\ \mu_{\text{OM}}}{22400}$
Theo-HTe$^-$ = 2 Theo-nH$_2$	Exp-HTe$^-$ = 2 Exp-nH$_2$
Theo-HTe$^-$ = $\frac{\mathbf{TBHP}\ \mu_{\text{OM}}}{\mathbf{11200}}$	Exp-HTe$^-$ = $\frac{\text{EBHP}\ \mu_{\text{OM}}}{11200}$
$\frac{\mathbf{EBHP}}{\mathbf{TBHP}} = \frac{\mathbf{Exp-nH_2}}{\mathbf{Theo-nH_2}} = \frac{\mathbf{Exp-HTe^-}}{\mathbf{Theo-HTe^-}}$	
BDI-H% = Molar ratio-H% = TeCE-H% BDI-H% = $\frac{\mathbf{EBHP}}{\mathbf{TBHP}} \times 100\%$ Molar ratio-H% = $\frac{\mathbf{Exp-nH_2}}{\mathbf{Theo-nH_2}} \times 100\%$ TeCE-H% = $\frac{\mathbf{Exp-HTe^-}}{\mathbf{Theo-HTe^-}} \times 100\%$	

DF is a biochemical redox reaction, in which organic carbons (from OM) release electrons and protons (from OM and/or H$_2$O) accept electrons (Yuen & Lau, 2024c). The unbalanced half redox reactions are shown in the following.

Oxidation: $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (ONc = –0.220) + $H_2O \rightarrow CO_2$ (ONc = +4) + NH_3 + H^+ + ne$^-$

Reduction: H^+ (ON_H = +1) + ne$^-$ → H_2 (ON_H = 0)

TeCE-H metric is developed to measure biohydrogen generation efficiency. The biodegradability of OM is dominated by Te$^-$, hence microscopic TeCE-H determines macroscopic BDI-H. The selected data EBHP, ECE-H% and the calculated data TBHP, BDI-H%, TeCE-H% are summarized in Table 5.

Table 5. Selected data of EBHP, ECE-H% and calculated data of TBHP, BDI-H%, TeCE-H%

OM	Process	EBHP	TBHP*	BDI-H%*	TeCE-H%*	ECE-H%
silage	1st DF of 2-stage AD	17.47	1986.880	0.88%	0.88%	1.2%

* Data calculated by the authors

4. Relationship Between TeCE-H and ECE-H

The new TeCE-H metric elucidates the efficiency of electron transfer in biohydrogen generation. Both arithmetic and graphical methods are used to understand the correlation between electron-based TeCE-H and energy-based ECE-H.

4.1 Comparing TeCE-H and ECE-H: Using a Relative Percentage (%)

TeCE-H% = $\frac{\mathbf{Exp-HTe^{-}}}{\mathbf{Theo-HTe^{-}}} \times 100\%$	ECE-H% = $\frac{\textbf{Heating value of } \mathbf{H_2}}{\textbf{Heating value of OM}} \times 100\%$
TeCE-H = $\frac{\mathbf{Exp-HTe^{-}}}{\mathbf{Theo-HTe^{-}}}$	ECE-H = $\frac{\text{Heating value of } H_2}{\text{Heating value of OM}}$

Relative percentage (%) compares the discrepancy between TeCE-H% and ECE-H%, or between TeCE-H and ECE-H. The mathematical formula is shown in the following:

$$\text{Relative percentage (\%) of ECE-H\%} = \frac{(\text{TeCE}-\text{H\%})-(\text{ECE}-\text{H\%})}{(\text{TeCE}-\text{H\%})} \times 100\%$$

$$\text{Relative percentage (\%) of ECE-H} = \frac{(\text{TeCE}-\text{H})-(\text{ECE}-\text{H})}{(\text{TeCE}-\text{H})} \times 100\%$$

Selected parameters of EBHP and ECE-H% are retrieved from Table 3 and literature (Xia et al., 2013). Theoretical parameters are calculated by the authors, and they are organized in Table 6. The relative percentage of ECE-H% shows that the deviations lie between –36.36% and +4.13%.

Table 6. Selected and calculated parameters: TeCE-H% and ECE-H%

OM	μ*	TBHP*	EBHP	BDI-H%*	TeCE-H%*	ECE-H%	Relative Percentage (%) of ECE-H%*
[a] Silage, *$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	100.000	1986.880	17.47	0.88	0.88	1.2	-36.36
[b] Glucose, $C_6H_{12}O_6$	180.156	1492.040	715.9 [c]	47.98	47.98	46.0	+4.13
[b] Glucose, $C_6H_{12}O_6$	180.156	1492.040	341.9	22.91	22.91	23.0	-0.39
[b] Xylose, $C_5H_{10}O_5$	150.130	1492.040	190.6	12.77	12.77	14.2	-11.20
[b] Sucrose, $C_{12}H_{22}O_{11}$	342.297	1570.566	433.9 [c]	27.63	27.63	28.4	-2.79
[b] Cellulose, $C_6H_{10}O_5$	162.141	1657.816	259.9	15.68	15.68	17.5	-11.61
[b] Trehalose, $C_{12}H_{22}O_{11}$	342.297	1570.566	67.5	4.30	4.30	4.4	-2.33
[b] Trehalose, $C_{12}H_{22}O_{11}$	342.297	1570.566	731.3 [c]	46.56	46.56	47.2	-1.37

* Data calculated by the authors

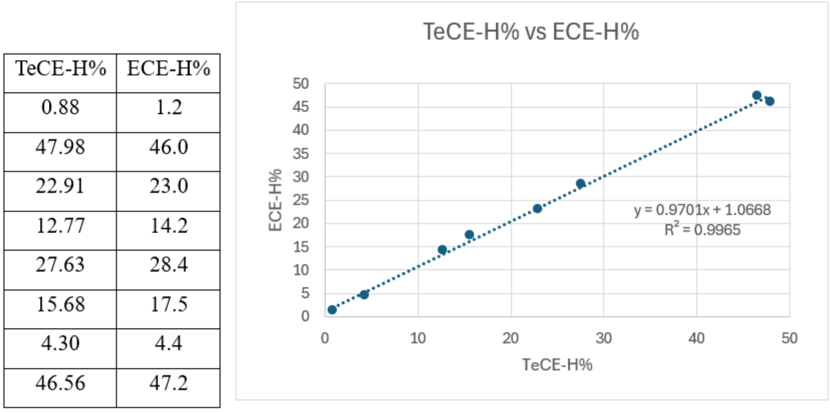
a Data of EBHP and ECE-H% is retrieved from Deng et al., 2019

b Data of EBHP and ECE-H% is retrieved from Xia et al., 2013

c EBHP = EBHP (DF) + EBHP (PF)

4.2 Correlation between TeCE-H% and ECE-H%: Using a Graphical Scatter Plot

Using the data of TeCE-H% and ECE-H% in Table 6, Figure 2 displays a strong positive correlation between TeCE-H% and ECE-H%. The correlation demonstrates that the electron transfer is a dominating driving force, which produces redox energy of BEqH.



TeCE-H%	ECE-H%
0.88	1.2
47.98	46.0
22.91	23.0
12.77	14.2
27.63	28.4
15.68	17.5
4.30	4.4
46.56	47.2

Figure 2. Graphical correlation between TeCE-H% and ECE-H% (DF and DF plus PF processes)

4.3 Correlation between EBHP and Exp-HTe⁻: Using a Graphical Scatter Plot

$\text{Theo-HTe}^- = \frac{\text{TBHP}\ \mu_{\text{OM}}}{11200}$	$\text{Exp-HTe}^- = \frac{\text{EBHP}\ \mu_{\text{OM}}}{11200}$	$\text{TeCE-H\%} = \frac{\text{Exp}-\text{HTe}^-}{\text{Theo}-\text{HTe}^-} \times 100\%$

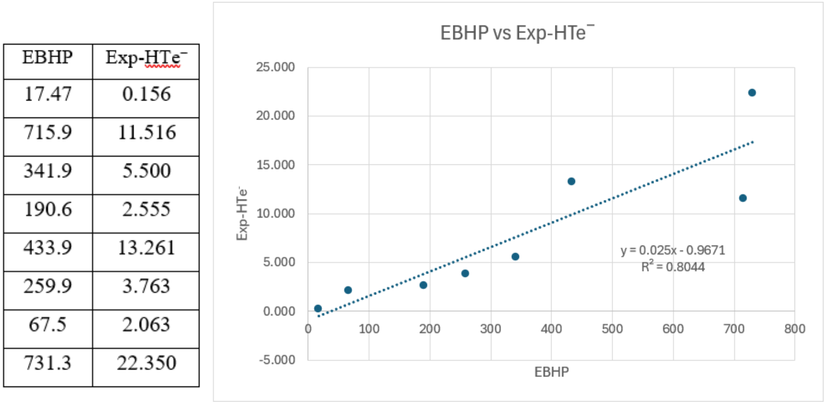
Referring to Table 6, the parameters are calculated by the above mathematical formulas, and they are organized in Table 7.

Table 7. Calculated parameters: μ, TBHP, Theo-HTe⁻, Exp-HTe⁻, and TeCE-H%

OM	μ*	TBHP*	Theo-HTe⁻*	EBHP	Exp-HTe⁻*	TeCE-H%*
[a] Silage, $^*C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	100.000	1986.880	17.740	17.47	0.156	0.88
[b] Glucose, $C_6H_{12}O_6$	180.156	1492.040	24.000	715.9	11.516	47.98
[b] Glucose, $C_6H_{12}O_6$	180.156	1492.040	24.000	341.9	5.500	22.91
[b] Xylose, $C_5H_{10}O_5$	150.130	1492.040	20.000	190.6	2.555	12.77
[b] Sucrose, $C_{12}H_{22}O_{11}$	342.297	1570.566	48.000	433.9	13.261	27.63
[b] Cellulose, $C_6H_{10}O_5$	162.141	1657.816	24.000	259.9	3.763	15.68
[b] Trehalose, $C_{12}H_{22}O_{11}$	342.297	1570.566	48.000	67.5	2.063	4.30
[b] Trehalose, $C_{12}H_{22}O_{11}$	342.297	1570.566	48.000	731.3	22.350	46.56

* Data calculated by the authors

Using the retrieved EBHP and calculated Exp-HTe⁻ data in Table 7, Figure 3 shows a positive correlation between EBHP and Exp-HTe⁻.



EBHP	Exp-HTe⁻
17.47	0.156
715.9	11.516
341.9	5.500
190.6	2.555
433.9	13.261
259.9	3.763
67.5	2.063
731.3	22.350

Figure 3. Graphical correlation between EBHP and Exp-HTe⁻ (DF and DF plus PF processes)

5. Metrics: AF System and Electrochemical System

DF, 2-stage AD, and DF plus PF are promising processes which generate biohydrogen. EBHP and BDI-H% are well used in the AF system. In addition, biophotolysis, MEC, MFC, and their coupling with DF are employed as sustainable technologies for biohydrogen production. YH_2 and RH_2% in MEC, and Exp-nH_2 and molar-H% in DF are compared in this section.

5.1 Relationships between DF's Metrics and Electrochemical System's Metrics

When the electron-based metric of DF is developed, the usage of the integrated parameters of macroscopic mass, macroscopic energy, and microscopic electron transfer can be extended to measure the biodegradability performance of OM in the electrochemical processes. The hydrogen production data of MEC (Table 8a) is retrieved from literature (Cheng & Logan, 2007).

Table 8a. Collected data of Exp-nH_2, H_2% yield, and energy recovery% at MEC

OM	YH_2, mol of H_2/mol of OM	RH_2%	Energy recovery% (η%)
Glucose	8.55	71	64
Cellulose	8.20	68	63
Acetic acid	3.65	91	82
Butyric acid	8.01	80	77
Lactic acid	5.45	91	82
Propionic acid	6.25	89	79
Valeric acid	8.77	67	66

A comparison between DF's metrics and MEC's metrics is shown in the following:

DF's parameters and metrics	MEC parameters and metrics
Exp-nH_2 BDI-H% = Molar-H% = TeCE-H% ECE-H%	$YH_2 = \frac{\text{mol of } H_2}{\text{mol of OM}}$ RH_2% = H_2% yield Energy recovery%

With reference to DF Examples 1 and 2, OM's parameters and BEqH's parameters are calculated and summarized in Table 8b.

Table 8b. Calculated OM's parameters and BEqH's parameters

OM	μ	nc = x	ONc	Theo-nH_2	TBHP	Theo-HTe^-
Glucose, $C_6H_{12}O_6$	180.156	6	0.000	12.000	1492.040	24.000
Cellulose, $(C_6H_{10}O_5)_n$	162.141	6	0.000	12.000	1657.816	24.000
Acetic acid, $C_2H_4O_2$	60.052	2	0.000	4.000	1492.040	8.000
Butyric acid, $C_4H_8O_2$	88.106	4	-1.000	10.000	2542.392	20.000
Lactic acid, $C_3H_6O_3$	90.078	3	0.000	6.000	1492.040	12.000
Propionic acid, $C_3H_6O_2$	74.079	3	-0.667	7.000	2116.659	14.000
Valeric acid, $C_5H_{10}O_2$	102.133	5	-1.200	13.000	2851.184	26.000

Using parameters and mathematical relationships below, the calculated molar-H%, BDI-H%, TeCE-H% of DF and the collected RH_2%, energy recovery% of MEC are organized in Table 8c.

For DF	For DF and MEC
Molar-ratio-H% = BDI-H% = TeCE-H% Molar-ratio-H% = $\frac{\text{Exp}-nH_2}{\text{Theo}-nH_2} \times 100\%$ TeCE-H% = $\frac{\text{Exp}-\text{HTe}^-}{\text{Theo}-\text{HTe}^-} \times 100\%$	Exp-nH_2 = YH_2 Exp-HTe^- = 2 Exp-nH_2 = 2 YH_2 Theo-HTe^- = 2 Theo-nH_2 BDI-H% = Molar-ratio-H% = RH_2% TeCE-H% = RH_2%

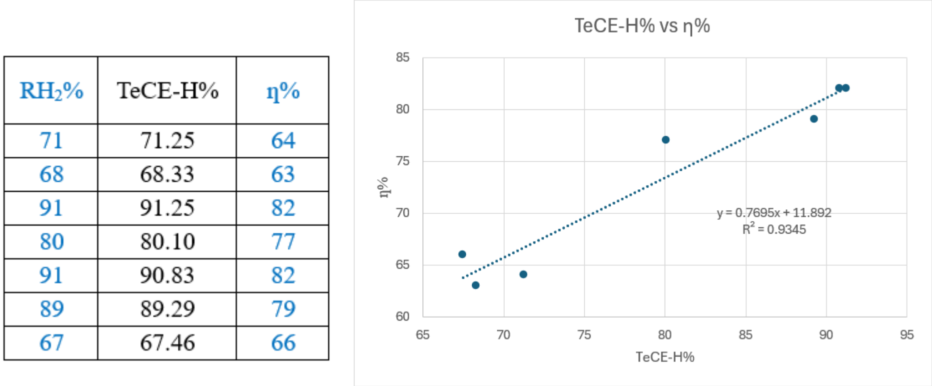
Table 8c. Calculated parameters and metrics of DF and MEC

OM	*Theo-nH_2	YH_2	*Molar-ratio H%	RH_2%	*BDI-H%	*TeCE-H%	η%
		Exp-nH_2					
Glucose, $C_6H_{12}O_6$	12.000	8.55	71.25	71	71.25	71.25	64
Cellulose, $(C_6H_{10}O_5)_n$	12.000	8.20	68.33	68	68.33	68.33	63
Acetic acid, $C_2H_4O_2$	4.000	3.65	91.25	91	91.25	91.25	82
Butyric acid, $C_4H_8O_2$	10.000	8.01	80.10	80	80.10	80.10	77
Lactic acid, $C_3H_6O_3$	6.000	5.45	90.83	91	90.83	90.83	82
Propionic acid, $C_3H_6O_2$	7.000	6.25	89.29	89	89.29	89.29	79
Valeric acid, $C_5H_{10}O_2$	13.000	8.77	67.46	67	67.46	67.46	66

* Data calculated by the authors

5.2 Correlation between TeCE-H% and η%: Using a Graphical Scatter Plot

Using the data of TeCE-H% (equal to RH_2%) and energy recovery% (η%) in Table 8c, Figure 4 demonstrates that there is a strong positive correlation between TeCE-H% and η% for the MEC process.



RH_2%	TeCE-H%	η%
71	71.25	64
68	68.33	63
91	91.25	82
80	80.10	77
91	90.83	82
89	89.29	79
67	67.46	66

Figure 4. Graphical correlation between TeCE-H% and η% (MEC)

5.3 Significance of TeCE-H Metric

The established TeCE-H metric for biohydrogen production is significant in the following areas:

(i) Through counting experimental and theoretical number of transferred electrons, the electron-based metric, i.e., TeCE-H, can be identified.

(ii) The biodegradability performance of OM can be understood by the relationship between microscopic TeCE-H and macroscopic BDI-H.

(iii) The thermal nature of OM and bioconversion energy can be understood by the relationship between microscopic TeCE-H and macroscopic ECE-H.

(iv) The integration of TeCE-H, BDI-H, and ECE-H reveals the relationships between the electron transfer, the biodegradability performance of OM, and the bioconversion energy of DF.

(v) TeCE-H is created as a cross-reference among DF, photochemical system, electrochemical system, and their hybrid systems under anaerobic conditions.

(vi) The establishment of a new TeCE-H opens the dialogue between theoretical Buswell’s model and industrial AF developments. It also builds connections among AF, photochemical system, and electrochemical system.

6. Conclusion

BEqH can represent DF in accordance with the elemental composition of any OM. Based on BEqH, the parameters of OM (ONc, x, and μ) and BEqH (Theo-nH_2, TBHP, and Theo-HTe^-) can be determined by an empirical formula or a structural formula. This research establishes BEqH as a redox model to develop electron-based metric. The research concludes that biohydrogen production is rooted in the process where electrons are transferred from organic carbons to proton during dark fermentation and the number of transferred electrons dominates the quantitative amount of biohydrogen. Through arithmetic calculation and mathematical deduction, electron conversion efficiency, i.e., TeCE-H, is established as an electron-based metric. This research further shows that microscopic electron-based TeCE-H and macroscopic mass-based BDI-H are identical, and it reveals that there is a strong positive correlation between electron-based TeCE-H and energy-based ECE-H. In addition, the established electron-based TeCE-H metric functions as a cross-reference for DF, DF coupled photochemical system, and DF coupled electrochemical system.

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Authors contributions

Dr. Pong Kau Yuen is responsible for designing the study, drafting, and revising the manuscript. Dr. Cheng Man Diana Lau is responsible for revising the manuscript. Kuok In Gabriel Yuen is responsible for data processing and figures drawing. All authors read and approved of the final manuscript.

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Competing interests

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.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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