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Exploring Dark Fermentation Effluent in Two-Stage Anaerobic Digestion

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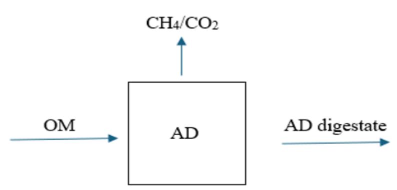
Abstract

Dark fermentation and anaerobic digestion are anaerobic fermentation processes that use organic matter as feedstocks under anaerobic microbial conditions. Buswell's equation can represent anaerobic fermentation in accordance with the elemental composition of any organic matter. Compared to 1-stage anaerobic digestion, 2-stage anaerobic digestion can better enhance biohydrogen and biomethane production. In a 2-stage anaerobic digestion, dark fermentation effluent is considered an intermediate between the first dark fermentation stage and the second anaerobic digestion stage. However, currently, there is no established study on the general elemental composition of a dark fermentation effluent. The purpose of this research is to use Buswell's equation to explore this process. This research developed an empirical Buswell's equation for biohydrogen as a model through the integration of theoretical Buswell's concept and experimental biohydrogen potential. This model identifies the elemental composition and characteristics of a dark fermentation effluent and improves the understanding of the correlation between an organic matter and its dark fermentation effluent. When the theoretical biomethane potential of dark fermentation effluent is found, the valid biodegradability index of 2-stage anaerobic digestion can be elucidated.

Keywords: dark fermentation (DF), anaerobic digestion (AD), Buswell's equation for biohydrogen (BEqH), Buswell's equation for biomethane (BEq), empirical Buswell's equation for biohydrogen (empirical BEqH), dark fermentation effluent (DF effluent), two-stage anaerobic digestion

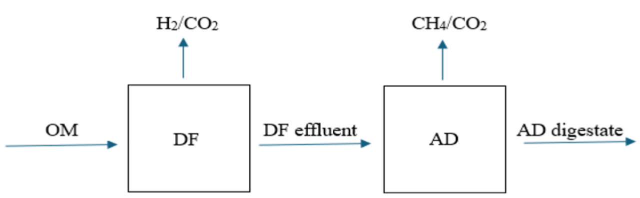
Graphical Abstract

1-stage AD



1-stage AD BEq: $C_xH_yO_z$ (OM) + nH_2O H_2O → nCH_4 CH_4 + nCO_2 CO_2

2-stage AD



1st DF empirical BEqH: $C_xH_yO_z$ (OM) → Exp-nH_2 H_2 + Exp-nCO_2 CO_2 + $C_aH_bO_c$ (DF effluent)

2nd AD BEq: $C_aH_bO_c$ (DF effluent) + nH_2O H_2O → nCH_4 CH_4 + nCO_2 CO_2

1. Introduction

Anaerobic fermentation (AF) plays a vital role in circular carbon economy (Sun, 2023; Wu et al., 2022). Dark fermentation (DF) and anaerobic digestion (AD) are sustainable AF processes which use organic matters (OM) as feedstocks under anaerobic microbial conditions. As an industrial biochemical technology (Meegoda et al., 2018; Náthia-Neves et al., 2018), AD facilitates biomethane production, waste reduction, and biofertilizer preparation. DF promotes biohydrogen generation, biomass/organic waste degradability, and dark fermentation effluent (DF effluent) formation in the absence of light. Figure 1 shows 1-stage AD, 1-stage DF, and 2-stage AD. Compared to the single-stage AD, the two-stage AD leads to an improvement in biohydrogen and biomethane generation (Bull et al., 1984; Azbar et al, 2001; Liu et al., 2006; Schievano et al., 2012).

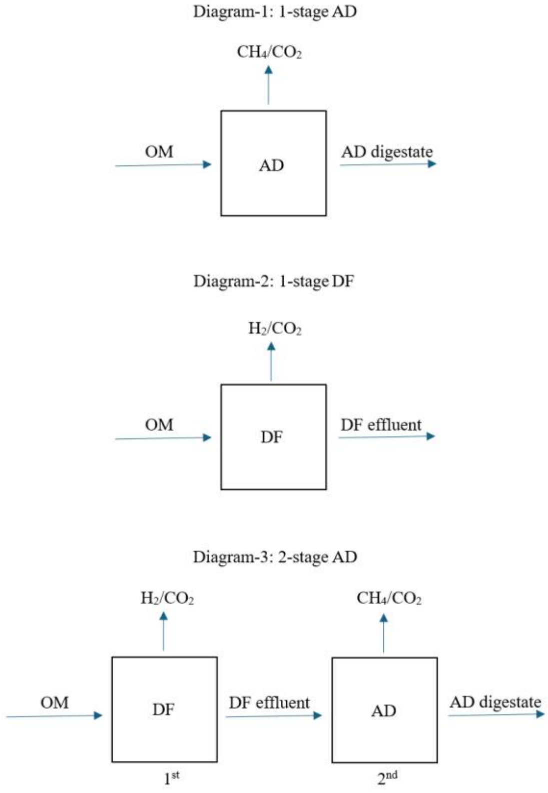


Figure 1. Anaerobic fermentation diagrams: 1-stage AD, 1-stage DF, and 2-stage AD

When an empirical formula of OM is identified, the stoichiometric Buswell's equation for biomethane (BEq) (Buswell & Mueller, 1952; Boyle, 1977; Yuen & Lau, 2023) and Buswell's equation for biohydrogen (BEqH) (Yuen & Lau, 2024a) can be known, and they are shown as follows:

$$C_xH_yO_zX_wN_vS_uP_t + \frac{4x-y-2z+w+3v+2u+11t}{4}H_2O \rightarrow \frac{4x+y-2z-w-3v-2u+5t}{8}CH_4 + \frac{4x-y+2z+w+3v+2u-5t}{8}CO_2 + wHX + vNH_3 + uH_2S + tH_3PO_4$$

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

BEq and BEqH can represent AD and DF respectively. BEq determines the quantitative amount of biomethane (nCH_4) and theoretical biomethane potential (TBMP) (Angelidaki & Sanders, 2004; Yuen et al., 2024b). BEqH, meanwhile, determines the quantitative amount of biohydrogen (nH_2) and theoretical biohydrogen potential (TBHP) (Yuen & Lau, 2024a). Experimental biomethane potential (EBMP) and experimental biohydrogen potential (EBHP) can be measured by the biomethane potential (BMP) test (Raposo et al., 2011) and biohydrogen potential (BHP) test (Carrillo-Reyes et al, 2019). The biodegradability index (BDI) is applied to evaluate the biodegradable performance of OM. The biodegradability index for biomethane (BDI-M%) (Nielfa et al., 2015) is defined by the percentage ratio of EBMP to TBMP, and the biodegradability index for biohydrogen (BDI-H%) (Yuen & Lau, 2024a) is defined by the percentage ratio of EBHP to TBHP.

$$\text{BDI-M\% (for biomethane)} = \frac{\text{EBMP}}{\text{TBMP}} \times 100\%$$

$$\text{BDI-M (for biomethane)} = \frac{\text{EBMP}}{\text{TBMP}}$$

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

$$\text{BDI-H (for biohydrogen)} = \frac{\text{EBHP}}{\text{TBHP}}$$

Biohydrogen, biogenic carbon dioxide, and the DF effluent are generated in the 1-stage DF or the 1st DF reactor of the 2-stage AD process. A DF effluent mainly contains volatile fatty acids and alcohol, which are then converted to biomethane and digestate in the 2nd AD reactor. According to Buswell's concept, when the general elemental composition of DF effluent is identified, its corresponding Buswell's parameters can be determined. However, no research has been done in this area. Furthermore, there is a lack of information about TBMP for DF effluent (TBMP-DF effluent). The prevalent approach to calculate both BDI-M% of 1-stage AD (BDI-M1%) and 2-stage AD (BDI-M2%) is TBMP for organic matter (TBMP-OM) of 1-stage AD.

Our research argues that the validity of BDI-M1% and BDI-M2% depends on TBMP-OM and TBMP-DF effluent respectively. It aims to (i) establish an empirical BEqH model, (ii) identify the general elemental composition of DF effluent, (iii) evaluate the characteristics of DF effluent, (iv) determine TBMP-DF effluent, and (v) elucidate the difference between BDI-M1% and BDI-M2%.

2. Background to Empirical Buswell's Equation for Biohydrogen

This research establishes a new stoichiometric chemical equation named empirical Buswell's equation for biohydrogen (empirical BEqH), in which the Buswell's concept and the experimental quantity amount of biohydrogen are integrated to identify the theoretical elemental composition of DF effluent.

2.1 Buswell's Concept for Pure or Mixed Organic Matter, $C_xH_yO_zX_wN_vS_uP_t$

The nature of BEq is a biochemical redox reaction (Yuen & Lau, 2024c; 2024d). When the chemical formula of an OM is identified, the BEq's parameters for AD and BEqH's parameters for DF can be determined under anaerobic conditions. When the mean oxidation number of organic carbons (ONc) of OM acts as a critical metric, the BEq's parameters (Yuen & Lau, 2024b; Yuen et al., 2024e) and BEqH's parameters (Yuen et al., 2024a) can be quantified and represented by simple mathematical equations.

2.2 Empirical BEqH

When the empirical BEqH is established, the OM (reactant), H_2, CO_2, and DF effluent (products) are identified. They are shown in the following chemical reaction:

$$C_xH_yO_zX_wN_vS_uP_t \rightarrow \text{Exp-nH}_2\ H_2 + \text{Exp-nCO}_2\ CO_2 + C_aH_bO_cX_wN_vS_uP_t$$

$C_xH_yO_zX_wN_vS_uP_t$ denotes empirical formula or molecular formula of OM

$C_aH_bO_cX_wN_vS_uP_t$ denotes empirical formula of DF effluent

Exp-nH_2 denotes experimental quantity amount of biohydrogen

Exp-nCO_2 denotes calculated quantity amount of carbon dioxide

The empirical BEqH is set up to bridge OM ($C_xH_yO_zX_wN_vS_uP_t$) and DF effluent ($C_aH_bO_cX_wN_vS_uP_t$) using experimental Exp-nH_2 and calculated Exp-nCO_2. Exp-nH_2 can be counted by the measured EBHP, and then the calculated quantity amount of Exp-nCO_2 can be determined by Exp-nH_2. Consequently, the elemental composition of DF effluent can be identified.

2.3 BEqH and Empirical BEqH

The theoretical BEqH and the empirical BEqH for $C_aH_bO_cX_wN_vS_uP_t$ are shown in the following:

$$C_xH_yO_zX_wN_vS_uP_t + \text{nH}_2\text{O}\ H_2O \rightarrow \text{Theo-nH}_2\ H_2 + \text{Theo-nCO}_2\ CO_2 + wHX + vNH_3 + uH_2S + tH_3PO_4$$

$$C_xH_yO_zX_wN_vS_uP_t \rightarrow \text{Exp-nH}_2\ H_2 + \text{Exp-nCO}_2\ CO_2 + C_aH_bO_cX_wN_vS_uP_t$$

Both the ratios, $\frac{\text{Theo}-\text{nH}_2}{\text{Theo}-\text{nCO}_2}$ and $\frac{\text{Exp}-\text{nH}_2}{\text{Exp}-\text{nCO}_2}$, are equal to $\frac{(4-\text{ONc})}{2}$ (Yuen et al., 2024a). When the Exp-nH_2 is found, the Exp-nCO_2 can then be counted.

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

$$\therefore \text{Exp-nCO}_2 = \text{Exp-H}_2 \times \frac{\text{Theo}-\text{nCO}_2}{\text{Theo}-\text{nH}_2} = \text{Exp-H}_2 \times \frac{2}{(4-\text{ONc})}$$

2.4 Identification of the General Elemental Composition of DF Effluent

Based on the balanced empirical BEqH, the elemental composition of DF effluent ($C_aH_bO_cX_wN_vS_uP_t$) can be identified using experimental Exp-nH_2, calculated Exp-nCO_2, and calculated atomic coefficients (a, b, c).

$C_xH_yO_zX_wN_vS_uP_t \rightarrow$ Exp-nH_2 H_2 + Exp-nCO_2 CO_2 + $C_aH_bO_cX_wN_vS_uP_t$

Balance C: x = Exp-nCO_2 + a

Balance H: y = 2(Exp-nH_2) + b

Balance O: z = 2(Exp-nCO_2) + c

Find a = x – Exp-nCO_2

b = y – 2(Exp-nH_2)

c = z – 2(Exp-nCO_2)

2.5 Correlation between $C_xH_yO_zX_wN_vS_uP_t$ (OM) and $C_aH_bO_cX_wN_vS_uP_t$ (DF effluent)

When the elemental composition of $C_aH_bO_cX_wN_vS_uP_t$ is identified, its corresponding BEq's parameters, such as nCH_4 and TBMP can be determined by Buswell's concept. The correlation and discrepancy between OM and DF effluent can also be revealed by the working procedures later shown.

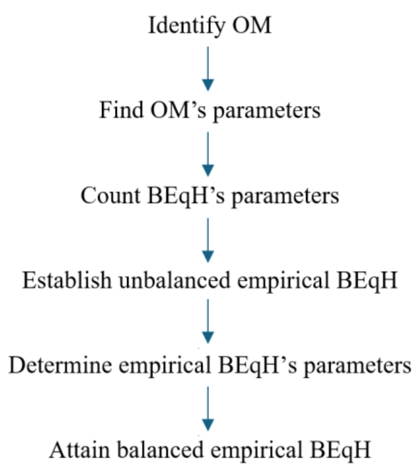
3. Experimental Procedure

Secondary data are used for analysis and step-by-step procedures are shown in this section.

3.1 Flowchart for Establishing Empirical BEqH

The flow chart for attaining balanced empirical BEqH is shown in Figure 2. When the balanced empirical BEqH is attained, a general elemental composition of DF effluent ($C_aH_bO_cX_wN_vS_uP_t$) can be identified.

Figure 2. Flowchart for establishing empirical BEqH



3.2 Selection of Literature Data: Mass Percentages of Elements, EBMP, and EBHP

The data of (i) mass percentages of elements (mass%), (ii) EBMP of 1-stage AD, and (iii) EBHP and EBMP of 2-stage

AD are retrieved from literature (Deng et al., 2019). The selected mass% are organized in Table 1a, and the experimental EBHP and EBMP of the silage are organized in Table 1b.

Table 1a. The mass percentages of elements: the silage

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

Table 1b. The measured EBHP and EBMP of 1-stage AD and 2-stage AD

OM	Process	EBHP (mL/g, at STP)	EBMP (mL/g, at STP)
pre-treated silage	1-stage AD	-	237.10
	2-stage AD	68.26	304.39
untreated silage	1-stage AD	-	261.00
	2-stage AD	17.47	392.84

3.3 Working Procedures for 1-stage DF or 1st DF of 2-stage AD (BDI-H%)

Example 1. Identify the empirical BEqH using the measured EBHP, the value of which is 68.26 mL/g of the pretreated silage as OM in the DF process.

Step 1. Find OM's empirical formula and empirical mass (μ_{OM}) of the silage (Yuen et al., 2024e)

OM	C%	H%	O%	N%	$\mu_{OM} = \sum$mass%	Empirical formula
silage	50.5	6.5	41.3	1.7	100.00	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$

Step 2. Determine OM's parameters: ONc and x

$C_xH_yO_zN_v$	ONc = $\frac{-y+2z+3v}{x}$	x
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	–0.220	4.204

Step 3. Set up unbalanced empirical BEqH

$C_xH_yO_zN_v$ (OM) → Exp-nH_2 H_2 + Exp-nCO_2 CO_2 + $C_aH_bO_cN_v$ (DF effluent)
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ → Exp-nH_2 H_2 + Exp-nCO_2 CO_2 + $C_aH_bO_cN_{0.121}$

Step 4. Identify BEqH's parameters: $\frac{nH_2}{nCO_2}$, TBHP, and BDI-H%

$\frac{nH_2}{nCO_2} = \frac{(4-ONc)}{2}$	TBHP = $\frac{11200\,x(4-ONc)}{\mu OM}$	Measured EBHP	BDI-H% = $\frac{EBHP}{TBHP} \times 100\%$
$= \frac{4-(-0.220)}{2}$ = 2.11	$= \frac{11200\,(4.204)[4-(-0.220)]}{100.00}$ = 1986.978	= (68.26)	$= \frac{68.26}{1986.978} \times 100\%$ = 3.44%

Step 5. Determine stoichiometric coefficients of empirical BEqH: Exp-nH_2 and Exp-CO_2

Exp-nH_2 = $\frac{\mu\,(EBHP)}{22400}$	$\frac{nH_2}{nCO_2} = \frac{(4-ONc)}{2}$	$\frac{nCO_2}{nH_2} = \frac{2}{(4-ONc)}$	Exp-nCO_2 = Exp-nH_2 ($\frac{nCO_2}{nH_2}$)
$= \frac{100.00\,(68.26)}{22400}$ = 0.305	$= \frac{4-(-0.220)}{2}$ = 2.110	$= \frac{1}{2.110}$	= 0.305 ($\frac{1}{2.110}$) = 0.145

Step 6. Identify elemental composition of $C_aH_bO_cN_v$: by conservation of atoms

Balanced general empirical BEqH:

$C_xH_yO_zX_wN_vS_uP_t \rightarrow$ Exp-nH$_2$ H_2 + Exp-nCO$_2$ CO_2 + $C_aH_bO_cX_wN_vS_uP_t$

Balance C: x = Exp-nCO$_2$ + a

Balance H: y = 2(Exp-nH$_2$) + b

Balance O: z = 2(Exp-nCO$_2$) + c

Find a = x – Exp-nCO$_2$, b = y – 2(Exp-nH$_2$), and c = z – 2(Exp-nCO$_2$)

Balanced empirical BEqH: $C_{4.204}H_{6.448}O_{2.581}N_{0.121} \rightarrow 0.305\ H_2 + 0.145\ CO_2 + C_aH_bO_cN_{0.121}$

Balance C: 4.204 = 0.145 + a

Balance H: 6.448 = 2(0.305) + b

Balance O: 2.581 = 2(0.145) + c

Find a = 4.204 – 0.145 = 4.059, b = 6.448 – 2(0.305) = 5.838, and c = 2.581 – 2(0.145) = 2.291

The elemental composition of DF effluent is identified as $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$

Step 7. Attain the balanced empirical BEqH

$C_{4.204}H_{6.448}O_{2.581}N_{0.121} \rightarrow 0.305\ H_2 + 0.145\ CO_2 + C_{4.059}H_{5.838}O_{2.291}N_{0.121}$

3.4 Working Procedures for 1-stage AD (BDI-M1%)

Example 2. Based on the given mass% and the measured EBMP, the value of which is 237.10 mL/g of the pretreated silage, (i) find the empirical formula and the empirical formula mass (μ) of the silage; (ii) determine OM's parameters: ONc, and x, (iii) find BEq's parameters: nCH$_4$, TBMP, and BDI-M1%, and (iv) attain the balanced BEq

With reference to Example 1, steps 1 and 2 can be found.

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

OM	C%	H%	O%	N%	$\mu_{OM} = \sum$mass%	Empirical formula
silage	50.5	6.5	41.3	1.7	100.0	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$

Step 2. Determine OM's parameters: ONc and x

$C_xH_yO_zN_v = C_{4.204}H_{6.448}O_{2.581}N_{0.121}$

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

Step 3. Determine BEq's parameters: nCH$_4$, TBMP, and BDI-M1%

BEq: $C_xH_yO_zN_v$ + nH$_2$O $H_2O \rightarrow$ Theo-nCH$_4$ CH_4 + Theo-nCO$_2$ CO_2 + vNH_3

nCH$_4$ = $\frac{x(4-ONc)}{8}$	nCO$_2$ = x – nCH$_4$	TBMP = $\frac{22400\ (nCH_4)}{\mu OM}$	Measured EBMP	BDI-M1% = $\frac{EBMP}{TMBP} \times 100\%$
$= \frac{4.204[4-(-0.220)]}{8}$ = 2.218	= 4.204 – 2.218 = 1.986	$= \frac{22400\ (2.218)}{100.00}$ = 496.832	(237.10)	$= \frac{237.10}{496.832} \times 100\%$ = 47.72%

Step 4. Attain the balanced BEq

$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + nH$_2$O $H_2O \rightarrow 2.218CH_4 + 1.986CO_2 + 0.121NH_3$

Balance O: 2.581 + nH$_2$O = 2(1.986)

nH$_2$O = 1.391

The balanced BEq of 1-stage AD is $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + 1.391 nH_2O H_2O → 2.218CH_4 + 1.986CO_2 + 0.121NH_3

3.5 Working Procedures for 2-stage AD (BDI-H% and BDI-M2%)

Example 3. Based on the measured EBHP, the value of which is 68.26 mL/g and EBMP, the value of which is 237.10 mL/g of the pretreated silage in the 2-stage AD process, (i) find empirical BEqH's stoichiometric coefficients: Exp-nH_2 and Exp-nCO_2, (ii) identify BEqH's parameters: nH_2, TBHP, and BDI-H%, (iii) determine the balanced empirical BEqH, and (iv) find BEq's parameters: nCH_4, TBMP, and BDI-M2%

With reference to Example 2, the OM's parameters of the silage and empirical BEqH's parameters are found.

Step 1. Establish unbalanced empirical BEqH (from OM to DF effluent)

$$C_{4.204}H_{6.448}O_{2.581}N_{0.121} \rightarrow \text{Exp-}nH_2\ H_2 + \text{Exp-}nCO_2\ CO_2 + C_aH_bO_cN_{0.121}$$

Step 2. Identify stoichiometric coefficients of empirical BEqH: Exp-nH_2 and Exp-nCO_2

Exp-nH_2 = $\frac{\mu\ (EBHP)}{22400}$	$\frac{nH_2}{nCO_2} = \frac{(4-ONc)}{2}$	$\frac{nCO_2}{nH_2} = \frac{2}{(4-ONc)}$	Exp-nCO_2 = Exp-nH_2 ($\frac{nCO_2}{nH_2}$)
$= \frac{100.00\ (68.26)}{22400}$ = 0.305	$= \frac{4-(-0.220)}{2}$ = 2.110	$= \frac{1}{2.110}$	= 0.305 ($\frac{1}{2.110}$) = 0.145

Step 3. Identify BEqH's parameters of OM: $\frac{nH_2}{nCO_2}$, TBHP, and BDI-H%

$\frac{nH_2}{nCO_2} = \frac{(4-ONc)}{2}$	TBHP = $\frac{11200\ x(4-ONc)}{\mu OM}$	Measured EBHP	BDI-H (%) = $\frac{EBHP}{TBHP}$ × 100%
$= \frac{4-(-0.220)}{2}$ = 2.110	$= \frac{11200\ (4.204)[4-(-0.220)]}{100.00}$ = 1986.978	= (68.26)	$= \frac{68.26}{1986.978}$ × 100% = 3.44%

Step 4. Determine elemental composition of $C_aH_bO_cN_v$

Unbalanced empirical BEqH: $C_{4.204}H_{6.448}O_{2.581}N_{0.121} \rightarrow 0.305\ H_2 + 0.145\ CO_2 + C_aH_bO_cN_{0.121}$

a = x – Exp-nCO_2	b = y – 2(Exp-nH_2)	c = z – 2(Exp-nCO_2)	DF effluent
= 4.204 – 0.145 = 4.059	= 6.448 – 2(0.305) = 5.838	= 2.581 – 2(0.145) = 2.291	$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$

Step 5. Attain the balanced empirical BEqH of the 1st DF of the 2-stage AD

$$C_{4.204}H_{6.448}O_{2.581}N_{0.121}\ (OM) \rightarrow 0.305\ H_2 + 0.145\ CO_2 + C_{4.059}H_{5.838}O_{2.291}N_{0.121}\ (DF\ effluent)$$

Step 6. Determine DF effluent's parameters: ONc, x, and $\mu_{DF\ effluent}$

$C_xH_yO_zN_v = C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent)

ONc = $\frac{-y+2z+3v}{x}$	x	$\mu_{DF\ effluent}$
$= \frac{-5.838+2(2.291)+3(0.121)}{4.059}$ = –0.220	4.059	92.99

Step 7. Determine BEq's parameters of DF effluent: nCH_4, TBMP, and BDI-M2%

Unbalanced BEq: $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF Effluent) → nCH_4 CH_4 + nCO_2 CO_2 + 0.121NH_3

$nCH_4 = \frac{x(4-ONc)}{8}$	$nCO_2 = x - nCH_4$	$TBMP = \frac{22400\,(nCH_4)}{\mu DF\ effluent}$	Measured EBMP	$BDI\text{-}M2\% = \frac{EBMP}{TMBP} \times 100\%$
$= \frac{4.059[4-(-0.220)]}{8}$ = 2.141	= 4.059 – 2.141 = 1.918	$= \frac{22400\,(2.141)}{92.99}$ = 515.737	(304.39)	$= \frac{304.39}{515.737} \times 100\%$ = 59.02%

Step 8. Attain the balanced BEq of the 2nd AD in the 2-stage AD (from DF effluent to biogas)

$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent) + nH_2O H_2O → $2.141CH_4 + 1.918CO_2 + 0.121NH_3$

Balance O: $2.291 + nH_2O = 2(1.918)$

$nH_2O = 1.545$

The balanced BEq of the 2nd AD is $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent) + $1.545H_2O$ → $2.141CH_4 + 1.918CO_2 + 0.121NH_3$

4. Compare 1-stage AD and 2-stage AD

With reference to Examples 2 and 3 using the pretreated silage as OM, the calculated data of 1-stage AD and 2-stage AD are summarized and compared.

4.1 BEq for 1-stage AD: the Process from OM to Biomethane

OM	ONc	x	μ_{OM}	nCH_4	TBMP	Measured EBMP	BDI-M1%
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	–0.220	4.204	100.00	2.218	496.832	(237.10)	47.72%

The balanced BEq is $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + $1.391H_2O$ → $2.218CH_4 + 1.986CO_2 + 0.121NH_3$

4.2 Empirical BEqH for 1st DF of 2-stage AD: the Process from OM to Biohydrogen and DF Effluent

OM	Measured EBHP	Exp-nH_2	Exp-nCO_2	DF effluent	TBHP	BDI-H%
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	(68.26)	0.305	0.145	$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$	1986.978	3.44%

The balanced empirical BEqH is

$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) → $0.305\ H_2 + 0.145\ CO_2$ + $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent)

4.3 BEq for 2nd AD of 2-stage AD: the Process from DF Effluent to Biomethane

DF effluent	ONc	x	$\mu_{DF\ Effluent}$	nCH_4	TBMP	Measured EBMP	BDI-M2%
$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$	–0.220	4.059	92.99	2.141	515.737	(304.39)	59.02%

The balanced BEq for DF effluent is

$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF Effluent) + $1.545H_2O$ → $2.141CH_4 + 1.918CO_2 + 0.121NH_3$

5. Stoichiometric Buswell's Equations and BDI%

5.1 Buswell's Equations and BDI% for Pretreated Silage

With reference to Examples 2 and 3, the Buswell's equations and BDI% for pretreated silage are summarized in Table 2a and Table 2b respectively.

Table 2a. Compare 1-stage AD (1 step: BEq only) and 2-stage AD (2 steps: empirical BEqH and BEq) for pretreated silage

Process-pretreated silage		Buswell's equation	BDI% and its corresponding chemical equation
1-stage AD		BEq from OM to biomethane	BDI-M1% $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + $1.391H_2O$ → $2.218CH_4 + 1.986CO_2 + 0.121NH_3$
2-stage AD	1st DF	Empirical BEqH from OM to biohydrogen + DF effluent	BDI-H% $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) → $0.305\ H_2 + 0.145\ CO_2 + C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent)
	2nd AD	BEq from DF effluent to biomethane	BDI-M2% $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent) + $1.545H_2O$ → $2.141CH_4 + 1.918CO_2 + 0.121NH_3$

Table 2b. Compare 1-stage AD (BDI-M1%) and 2-stage AD (BDI-H% and BDI-M2%) for pretreated silage

Process-pretreated silage	Measured EBHP	TBHP	BDI-H%	Measured EBMP	TBMP	BDI-M%
1-stage AD	-	-	-	(237.10)	496.832	47.72%
2-stage AD	(68.26)	1986.978	3.44%	(304.39)	515.737	59.02%

5.2 Buswell's Equations and BDI% for Untreated Silage

By the same, previous working procedures, the calculated data of 1-stage AD and 2-stage AD for untreated silage are determined and summarized in Table 3a and Table 3b.

Table 3a. Compare 1-stage AD (1 step: BEq only) and 2-stage AD (2 steps: empirical BEqH and BEq) for untreated silage

Process-untreated silage		Buswell's equation	BDI% and its corresponding chemical equation
1-stage AD		BEq from OM to biomethane	BDI-M1% $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + $1.391H_2O$ → $2.218CH_4 + 1.986CO_2 + 0.121NH_3$
2-stage AD	1st DF	Empirical BEqH from OM to biohydrogen + DF effluent	BDI-H% $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) → $0.078\ H_2 + 0.037CO_2 + C_{4.167}H_{6.292}O_{2.507}N_{0.121}$ (DF Effluent)
	2nd AD	BEq from DF effluent to biomethane	BDI-M2% $C_{4.167}H_{6.292}O_{2.507}N_{0.121}$ (DF Effluent) + $1.431H_2O$ → $2.198CH_4 + 1.969CO_2 + 0.121NH_3$

Table 3b. Compare 1-stage AD (BDI-M1%) and 2-stage AD (BDI-H% and BDI-M2%) for untreated silage

Process-untreated silage	Measured EBHP	TBHP	BDI-H%	Measured EBMP	TBMP	BDI-M%
1-stage AD	-	-	-	(261.00)	496.832	52.53%
2-stage AD	(17.47)	1986.978	0.88%	(392.84)	501.377	78.35%

6. Compare BDI-M1% and BDI-M2%

6.1 1-stage AD for BDI-M1%

With reference to Table 2a and Example 2 (1-stage AD), the calculation of BDI-M1% is based on TMBP-OM and its corresponding BEq is shown as follows: $C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) + $1.391H_2O \rightarrow 2.218CH_4 + 1.986CO_2 + 0.121NH_3$

Empirical formula of OM	TBMP-OM = $\frac{22400\ (nCH_4)}{\mu OM}$	Measured EBMP	BDI-M1% = $\frac{EBMP}{TBMP-OM} \times 100\%$
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	= $\frac{22400\ (2.218)}{100.00}$ = 496.832	(237.10)	= $\frac{237.10}{496.832} \times 100\%$ = 47.72%

6.2 2-stage AD for BDI-H% and BDI-M2%

With reference to Table 3a and Example 3 (2-stage AD), the calculation of BDI-H% is based on TBHP-OM and the corresponding empirical BEqH is shown as follows:

$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$ (OM) $\rightarrow 0.305\ H_2 + 0.145\ CO_2 + C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent)

Empirical formula of OM	TBHP-OM = $\frac{22400\ (nH_2)}{\mu OM}$	Measured EBHP	BDI-H% = $\frac{EBHP}{TBHP-OM} \times 100\%$
$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	= $\frac{22400\ (8.870)}{100.00}$ = 1986.978	(68.26)	= $\frac{68.26}{1986.978} \times 100\%$ = 3.44%

The calculation of BDI-M2% is based on TBMP-DF effluent and its corresponding BEq is shown as follows: $C_{4.059}H_{5.838}O_{2.291}N_{0.121}$ (DF effluent) + $1.545H_2O \rightarrow 2.141CH_4 + 1.918CO_2 + 0.121NH_3$

Empirical formula of DF effluent	TBMP-DF effluent = $\frac{22400\ (nCH_4)}{\mu DF\ effluent}$	Measured EBMP	BDI-M2% = $\frac{EBMP}{TBMP-DF\ effluent} \times 100\%$
$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$	= $\frac{22400\ (2.141)}{92.99}$ = 515.737	(304.39)	= $\frac{304.39}{515.737} \times 100\%$ = 59.02%

6.3 BDI% of 1-stage DF, 1-stage AD, and 2-stage AD

An accurate and comprehensive understanding of TBMP-DF effluent contributes to the correct calculation of BDI-M2% in 2-stage AD. It is critical to be aware that the value of BDI-M1% depends on the relationship between OM and CH_4 in 1-stage AD (Figure 3), and the value of BDI-M2% depends on the relationship between DF effluent and CH_4 in 2-stage AD (Figure 4). Therefore, if the value of BDI-M2% is attained according to the relationship between OM and CH_4 in 2-stage AD (Figure 4), then the calculation will be erroneous because DF effluent has been overlooked.

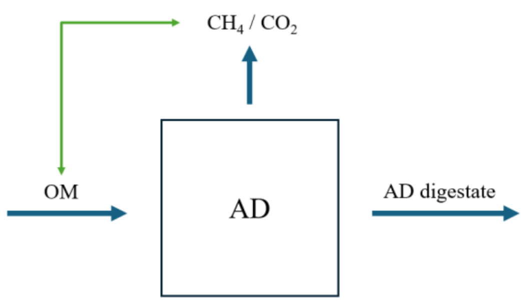


Figure 3. Relationship of OM and CH_4 in 1-stage AD

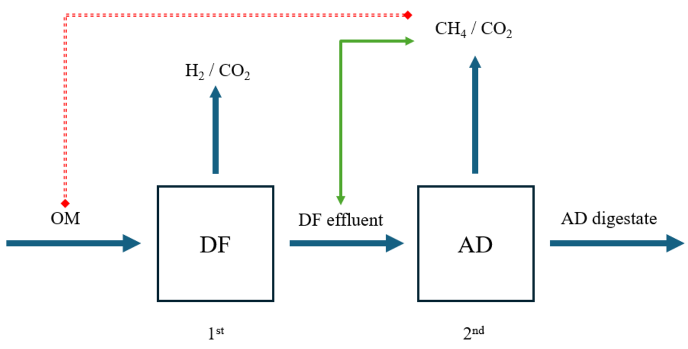


Figure 4. Relationships of OM and CH_4, DF effluent and CH_4 in 2-stage AD

The BDI% in 1-stage DF, 1-stage AD, and 2-stage AD are summarized in Table 4. Mathematical representations of BDI-M1% and BDI-M2% are related to TBMP-OM and TBMP-DF effluent respectively.

Table 4. Summarized BDI% in 1-stage DF, 1-stage AD, and 2-stage AD

Process	BDI-H%	BDI-M%
1-stage DF	$\text{BDI-H\%} = \frac{\text{EBHP}}{\text{TBHP-OM}} \times 100\%$	-
1-stage AD	-	$\text{BDI-M1\%} = \frac{\text{EBMP}}{\text{TBMP-OM}} \times 100\%$
2-stage AD	$\text{BDI-H\%} = \frac{\text{EBHP}}{\text{TBHP-OM}} \times 100\%$	$\text{BDI-M2\%} = \frac{\text{EBMP}}{\text{TBMP-DF effluent}} \times 100\%$

6.4 Compare the Calculation of BDI-M2% from Existing Publications and this Study

Due to the lack of information for TMBP-DF effluent in current publications, both calculations of 1-stage AD and 2-stage AD on BDI-M1% and BDI-M2% are based on TBMP-OM. Using the data of sections 6.1 and 6.2, the calculation in the publication is demonstrated as follows:

$$\text{BDI-M1\%} = \frac{\text{EBMP-M1}}{\text{TMBP-OM}} \times 100\% = \frac{237.10}{496.832} \times 100\% = 47.72\%$$

$$\text{BDI-M2\%} = \frac{\text{EBMP-M2}}{\text{TMBP-OM}} \times 100\% = \frac{304.39}{496.832} \times 100\% = 61.02\%$$

The TBMP-DF effluent is determined by the established empirical BEqH in this study. The calculations of BDI-M1% and BDI-M2% are based on TBMP-OM and TBMP-DF effluent respectively and are shown as:

$$\text{BDI-M1\%} = \frac{\text{EBMP-M1}}{\text{TMBP-OM}} \times 100\% = \frac{237.10}{496.832} \times 100\% = 47.72\%$$

$$\text{BDI-M2\%} = \frac{\text{EBMP-M2}}{\text{TMBP-DF effluent}} \times 100\% = \frac{304.39}{515.737} \times 100\% = 59.02\%$$

Table 5 organized the calculated BDI-M2% from this study and existing publications. Since TBMP-DF effluent (equals 515.737) is greater than its corresponding TBMP-OM (equals 496.832), the inaccurate BDI-M2% value of 61.02% using TMBP-OM is higher than that of 59.02% using TBMP-DF effluent.

Table 5. Compare 1-stage AD (BDI-M1%) and 2-stage AD (BDI-H% and BDI-M2%) from this study and existing publications

Process	Measured EBMP	This study		Existing publications	
		TBMP	BDI-M%	TBMP	BDI-M%
1-stage AD	(237.10)	496.832	47.72%	496.832	47.72%
2-stage AD	(304.39)	515.737	59.02%	496.832	61.02%

7. Importance of DF Effluent

The elemental composition of DF effluent can be identified by the empirical BEqH model. Consequently, the relationship between OM and DF effluent can be revealed.

7.1 Compare OM and DF Effluent

Using BEq concept, nCH_4 and TBMP of OM ($C_xH_yO_zN_v$), pretreated silage DF effluent ($C_aH_bO_cN_v$), and untreated silage ($C_aH_bO_cN_v$) are determined. The quantitative characteristics of the silage (OM) and its DF effluents of the pretreated and untreated silage are calculated and summarized in Table 6.

Table 6. OM and DF effluent's characteristics of the treated and untreated silage

Substrate	OM ($C_xH_yO_zN_v$) silage	DF effluent ($C_aH_bO_cN_v$) pretreated silage	DF effluent ($C_aH_bO_cN_v$) untreated silage
Empirical formula	$C_{4.204}H_{6.448}O_{2.581}N_{0.121}$	$C_{4.059}H_{5.838}O_{2.291}N_{0.121}$	$C_{4.167}H_{6.292}O_{2.507}N_{0.121}$
ONc	-0.220	-0.220	-0.220
nCH_4	2.218	2.141	2.198
nc	4.204	4.059	4.167
μ	100.00	92.99	98.20
$\frac{nc}{\mu}$	0.04204	0.04365	0.04243
TBMP	496.832	515.737	501.377

7.2 Characteristics of DF effluent

According to the empirical BEqH model, the relationship between any OM and its DF effluent can be revealed. With reference to Table 6, Table 7 shows the general characteristics of an OM and its DF effluent.

Table 7. Characteristics of OM and its DF effluent

Parameter	OM	vs	DF effluent
	$C_xH_yO_zX_wN_vS_uP_t$		$C_aH_bO_cX_wN_vS_uP_t$
ONc	ONc-OM	=	ONc-DF effluent
nCH_4	nCH_4-OM	>	nCH_4-DF effluent
nc	nc-OM = x	>	nc-DF effluent = a
μ	μ_{OM}	>	$\mu_{DF\ effluent}$
$\frac{nc}{\mu}$	$\frac{x}{\mu OM}$	<	$\frac{a}{\mu DF\ effluent}$
TBMP	TBMP-OM	<	TBMP-DF effluent

The fixed molar ratio of H_2 and CO_2 are removed from OM and DF effluent is produced, meaning that the OM and its corresponding DF effluent will have the same ONc. The atomic coefficients (a, b, c) and formula mass of DF effluent are smaller than the atomic coefficients (x, y, z) and formula mass of OM.

Compare $nCH_4 = \frac{x(4-ONc)}{8}$ for OM and $nCH_4 = \frac{a(4-ONc)}{8}$ for DF effluent:

∵ ONc-OM = ONc-DF effluent,
nc-OM = x > nc-DF effluent = a,
nCH_4 is proportional to nc
∴ nCH_4-OM > nCH_4-DF effluent

The nCH_4 of OM has a greater value than that of DF effluent.

When the DF effluent's parameters (a, ONc, and μ) are determined, TBMP-DF effluent can be calculated by the mathematical equation of TBMP-DF effluent = $\frac{2800\ a(4-ONc)}{\mu DF\ effluent}$.

Compare TBMP = $\frac{2800\ x(4-ONc)}{\mu OM}$ for OM and TBMP = $\frac{2800\ a(4-ONc)}{\mu DF\ effluent}$ for DF effluent:

∵ ONc-OM = ONc-DF effluent,
nc-OM = x > nc-DF effluent = a,
μOM >> μDF effluent,
$\frac{nc}{\mu}$-OM < $\frac{nc}{\mu}$-DF effluent,

TBMP is proportional to $\frac{nc}{\mu}$

∴ TBMP-OM < TBMP-DF effluent

The TBMP-OM of OM has a smaller value than that of TBMP-DF effluent.

7.3 Significance of DF Effluent

When the elemental composition of DF effluent is identified, its quantitative characteristics and theoretical Buswell's parameters can be determined. Compared to that of TBMP-OM, the theoretical value of TBMP-DF effluent is higher. Hence, any DF effluent can be further utilized as a precursor for 2nd of AD or in subsequent processes for facilitating the concept of circular carbon economy.

Figure 5 shows DF Process, DF Effluent, and subsequent processes. Using OM as feedstock, DF plays a significant role in producing a DF effluent in the 1st stage and then DF Effluent can be utilized in the following 2nd stage and further multi-stage.

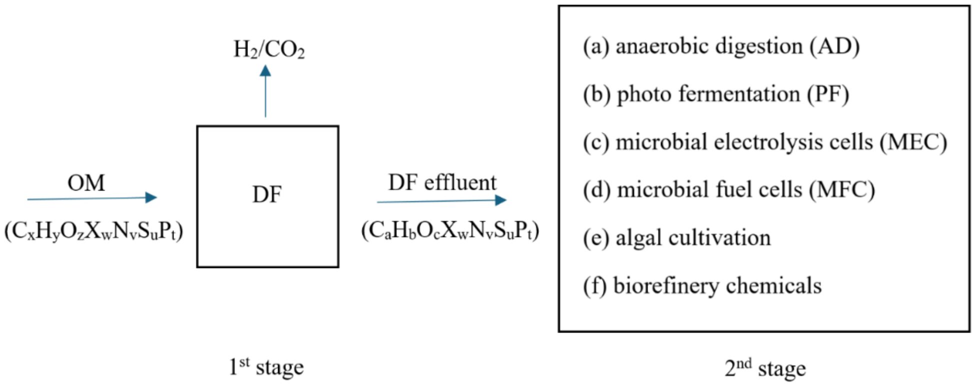


Figure 5. DF Process, DF Effluent, and subsequent processes

8. Conclusion

The empirical BEqH model is established in this research through the integration of theoretical Buswell's concept and experimental EBHP. When this model is used to study OM feedstocks, the following purposes are met: (1) the elemental composition of a DF effluent is identified, (2) the characteristics of a couple of any OM and its DF effluent are revealed,

(3) the BEq's parameters of a DF effluent are quantified, and (4) the TBMP-DF effluent is determined as a valid parameter to quantify BDI-M2% in a 2-stage AD. When compared to 1-stage AD and 2-stage AD, this research demonstrates that the TBMP-OM and TBMP-DF effluent are critical parameters that can determine BDI-M1% of 1-stage AD and BDI-M2% of 2-stage AD respectively.

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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 the final manuscript.

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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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