

Communication

Sulfur, Phosphorus and Metals in the Stoichiometric Estimation of Biomethane and Biohydrogen Yields

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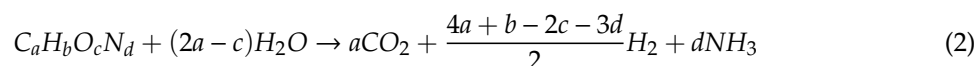


Abstract: The estimation of biomethane or biohydrogen yield is used to evaluate energy recovery during the process of the anaerobic treatment of waste and wastewater. Mathematically calculated theoretical values can also be used in biomethane or biohydrogen potential tests as reference points to calculate which fraction of substrate is decomposed, when the substrate degradation stopped and when the sample's self-digestion begins. This study suggests expanded forms of equations for anaerobic processes leading to either biomethane or biohydrogen. The traditional equations describing the conversion of a substrate with known carbon, hydrogen, oxygen and nitrogen composition were expanded to account for the composition of sulfur (for biohydrogen yields) and phosphorus (both biohydrogen and biomethane yields). As an optional part, one metal cation was also incorporated into the chemical formula of the evaluated wastewater composition in case the compound of biodegradable interest exists as a salt. The equations derived here can be useful for researchers estimating energy recovery based on the elemental analysis of samples, such as algal biomass harvested during harmful algal blooms (HABs). Examples of biomethane and biohydrogen yield estimations from sulfur- and phosphorus-containing compounds are also provided.

Keywords: anaerobic digestion; methane production; hydrogen fermentation; gross yield estimation

1. Introduction

Biomethane and biohydrogen are two main products considered in energy recovery from waste and wastewater [1–4], which are considered to be a replacement for conventional fossil fuels [5–8]. The anaerobic digestion process leading to those products is described by equations [9–11]:

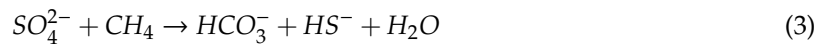


Even though these equations are quite helpful and widely used, they do not consider two extra widespread elements: sulfur (S) and phosphorus (P).

Sulfur compounds exist in municipal wastewater streams as surfactants (like soaps, shampoos, laundry detergents, etc.), but can be also present in industrial wastewater [12]. Organosulfur compounds like proteins, specifically those comprised of methionine and cysteine, are present in animal manure, crop farming wastes and dairy factories, etc. Biologically, sulfur is present in antioxidant systems and is part of iron capturing systems [13,14], and is thus present in slaughterhouse wastewater streams.

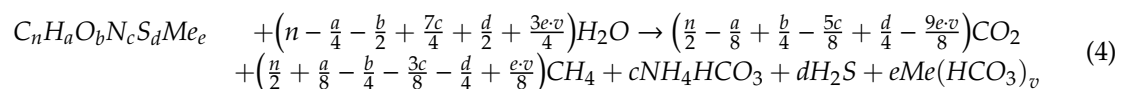
The decomposition of sulfur compounds under anaerobic conditions produces hydrogen sulfide (H₂S) [15], and this reaction competes with the formation of methane [16] and molecular hydrogen. Despite the fact that sulfur has an oxidation state of S²⁻ in most organic compounds that will not

require any reducing reaction, sulfur also exists as part of sulfate groups (in compounds like sodium dodecyl sulfate), and the conversion of sulfate groups will consume the reducing agent needed for the formation of CH₄ or H₂ [17]. Another reason to account for sulfur content in a substrate is that the microbial consortia involved in anaerobic digestion processes can also have representatives that are able to carry out the anaerobic oxidation of methane (AOM) [18] based on the following equation:



Phosphorus is a part of proteins (like casein in milk), phospho-lipids and nucleic acids, etc. [19], is very abundant in microalgal and plant cells [20], and is released as orthophosphate [21,22]. Also, in most organic compounds, phosphorus is chemically bound directly to oxygen (as in phosphates) and detaches from compounds together with it. That phosphorus-bound oxygen, which is included in Equation (1) as total oxygen, is counted as such which oxidizes carbon into CO₂. However, the phosphorus-bound oxygen remains bound as orthophosphate product, not as an evolved CO₂. The result is an overestimation of the CO₂ yield by Equation (1), while a portion of it does not evolve. In addition to this, the yield of CH₄ is underestimated. Similarly, reaction Equation (2) underestimates the hydrogen yield. Accounting for phosphorus would be more important for projects related to algal biomass conversion, since algae are utilized more often for carbon capture, and they require phosphorus for growth.

Some studies attempted to incorporate the sulfur in an equation for methanogenesis [23,24] whilst also paying attention to metals [25], resulting in the equation:



where v denotes the valence of a metal cation Me . However, no correction in a hydrogen production equation for sulfur content was found, and no studies considered both sulfur and phosphorus simultaneously for either methane or hydrogen yield. Both S and P should be considered in an anaerobic process, especially when treating a mixed waste stream.

With the introduction of H₂S and H₃PO₄, as final products of S and P transformation under anaerobic conditions, into Equations (1) and (2), and mineralization half-reactions [26], an 'expanded equation' of anaerobic fermentation, leading to the formation of either biomethane or biohydrogen, were attempted to be derived. These expanded equations add both the quality and novelty to previous representations of the stoichiometry of the anaerobic digestion process, where it is important to account for sulfur- and phosphorus-containing compounds occurring in municipal and industrial wastes, specifically with regard to evaluating the bioenergy potential for resource recovery operations.

2. Results and Discussion

To derive an expanded equation for the evolution of methane and hydrogen, equations were balanced as follows:

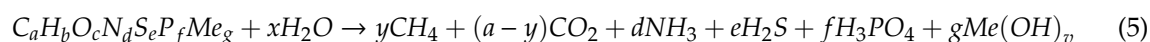
- Sulfur (S) is fully converted into hydrogen sulfide (H_2S).
- Phosphorus (P) is completely converted into ortho-phosphoric acid (H_3PO_4).
- Nitrogen (N) is fully transformed into ammonia (NH_3).
- Any possible metal (Me) is converted into its hydroxide ($Me(OH)_v$) form.

Even though ammonia and metal hydroxide should instantly react with acidic products like aqueous carbon dioxide, hydrogen sulfide or orthophosphoric acid, it is not that clear, since the exact product depends on the strength of the acidic product, and solubility. Other options could be to either expect the formation of bicarbonate salts, as in [25], since the carbon dioxide will be always a sub-product, or hydroxide. Since the exact form (hydroxide or bicarbonate) of a product doesn't change the actual yield of either biomethane or biohydrogen, the hydroxide product was selected. Later,

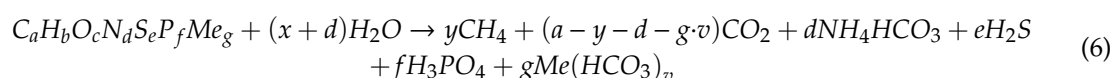
it can be easily re-calculated into any other salt, and will affect only the water balance of the equation. However, equations with bicarbonate as a product are also derived. In each case, the equilibration of the reactions produced a system of linear algebraic equations, which were solved in a way similar to the method of Gaussian elimination [27].

2.1. Derivation of the Equation for Methanogenic Digestion

The equation for methanogenesis including sulfur, phosphorus and one metal (in case a compound exists as a salt) with defined products is:



Or, assuming the bicarbonate as a final product of a metal compound:



The elemental balance for Equation (5) is shown in Table 1.

Table 1. Elemental Balance in Methanogenesis Equation.

Reagents Side	Element	Products Side
a	C	$y + (a - y) = a$
$b + 2x$	H	$4y + 3d + 2e + 3f + g \cdot v$
$c + x$	O	$2a - 2y + 4f + g \cdot v$
d	N	d
e	S	e
f	P	f
g	Me	g

Determination of coefficients x and y results from solving the system of two equations:

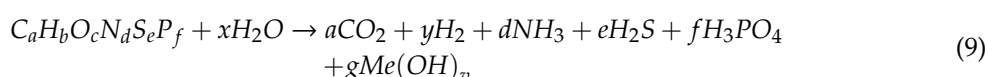
$$\begin{cases} b + 2x = 4y + 3d + 2e + 3f + g \cdot v \\ c + x = 2a - 2y + 4f + g \cdot v \end{cases} \quad (7)$$

where the first equation is system is taken from the hydrogen balance of Table 1, and the second one represents the oxygen balance from the same table. Solving the system of Equation (7) results in following system of equations:

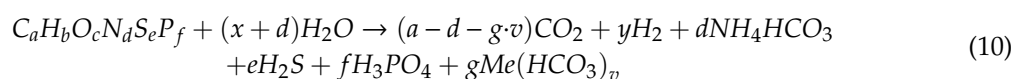
$$\begin{cases} x = \frac{4a - b - 2c + 3d + 2e + 11f + 3g \cdot v}{4} \\ y = \frac{4a + b - 2c - 3d - 2e + 5f + g \cdot v}{8} \end{cases} \quad (8)$$

2.2. Derivation of Equation for Hydrogen Fermentation

Changing the equation from methane to hydrogen formation is represented as follows:



Or, assuming the bicarbonates as metal and ammonia final compounds:



The elemental balance for Equation (9) is represented in Table 2.

Table 2. Elemental Balance in Hydrogen Formation Equation.

Reagents Side	Element	Products Side
a	C	a
$b + 2x$	H	$2y + 3d + 2e + 3f + g \cdot v$
$c + x$	O	$2a + 4f + g \cdot v$
d	N	d
e	S	e
f	P	f

This leads to solution of a system of equations:

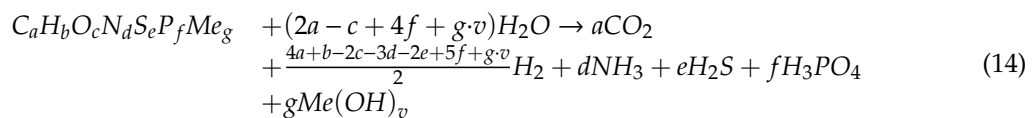
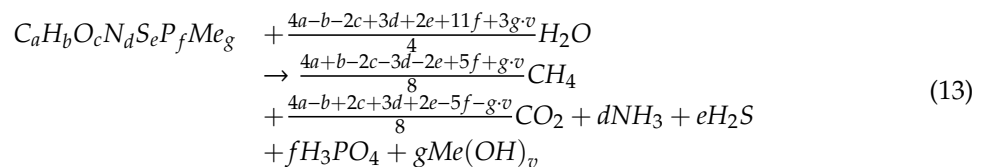
$$\begin{cases} b + 2x = 2y + 3d + 2e + 3f + g \cdot v \\ c + x = 2a + 4f + g \cdot v \end{cases} \quad (11)$$

Thus, Equations (9) and (10) are solved by the system of equations:

$$\begin{cases} x = 2a - c + 4f + g \cdot v \\ y = \frac{4a + b - 2c - 3d - 2e + 5f + g \cdot v}{2} \end{cases} \quad (12)$$

2.3. Yield Estimation

Introducing the calculated solutions into Equations (5) and (9), we obtain the finalized equations:



Reactions (13) and (14) allow the estimation of methane and hydrogen recovery from an arbitrary substrate with the formula $C_a H_b O_c N_d S_e P_f M e_g$, based on the following equations:

$$Y_{CH_4} = \frac{V_M \times (4a + b - 2c - 3d - 2e + 5f + g \cdot v)}{8 \times M(C_a H_b O_c N_d S_e P_f M e_g)} \quad (15)$$

$$Y_{H_2} = \frac{V_M \times (4a + b - 2c - 3d - 2e + 5f + g \cdot v)}{2 \times M_r(C_a H_b O_c N_d S_e P_f M e_g)} \quad (16)$$

where:

- V_M : molar volume of gas
- $M(C_a H_b O_c N_d S_e P_f M e_g)$: molar mass of substrate, calculated on formula

$$M_r(C_a H_b O_c N_d S_e P_f M e_g) = aA_r(C) + bA_r(H) + cA_r(O) + dA_r(N) + eA_r(S) + fA_r(P) + g v A_r(Me) \quad (17)$$

where $A_r(X)$ is the atomic mass of X^{th} element with required precision. In this study, the IUPAC (International Union of Pure and Applied Chemistry) Periodic table of the Elements with a precision to the 3rd decimal digit was used, which yields:

$$\begin{array}{lll} A_r(C) = 12.011 & A_r(H) = 1.008 & A_r(O) = 15.999 \\ A_r(N) = 14.007 & A_r(S) = 32.060 & A_r(P) = 30.974 \end{array}$$

As a side note, the molar volume of the gas depends on its own conditions (temperature and pressure), and may have several values:

- 22.711 $\frac{\text{L}}{\text{mole}}$ under conditions of 0 °C (273.15 K) and 1 bar (10⁵ Pa) according to IUPAC [28]
- 22.407 $\frac{\text{L}}{\text{mole}}$ under conditions of 273 K and 1 013 hPa, according to technical rules VDI 4630 [29]

Even though the conditions defined in VDI 4630 may seem to be more relevant when reporting results of anaerobic digestion process, it is not yet an international standard with an assigned ISO number. Therefore, IUPAC definitions may be preferable when reporting data for international use. However, since the purpose of anaerobic treatment is energy recovery as gaseous products with further combustion, it may be reasonable to report those at 1 atm and 25 °C, since those are the conditions referenced for the heat energy of combustion for gases [30]. However, further discussion of such a controversy is not a point of this study.

Equations (13) and (14) are simplified using the following assumptions:

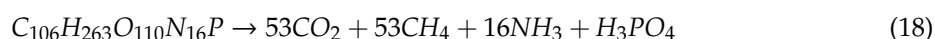
- The microbial decomposers have representatives of all metabolic pathways with the needed enzymatic systems.
- Reactions do not include ‘substrate losses’ due to the growth of cells and their consumption of material to increase biomass production (no biomass formation from the substrate).
- No toxicity effect takes place (i.e. ammonia toxicity for the methanogenic consortia).
- The exact species of a reaction product (molecular, ionized, dissociated) is not considered.
- No interaction occurs between reaction products; for example, the binding of ammonia with hydrogen sulfide or ortho-phosphoric acid.
- Complete decomposition is assumed, where sulfur is converted to hydrogen sulfide; however, it may generate volatile organic sulfur compounds (VOSC) [31,32].

Some examples of calculations according to the obtained equations are collected in Table 3.

Table 3. Some examples of methane or hydrogen gas yield calculations from various sources. Gas yield is normalized to 0 °C, 1 bar.

Substrate.	Molecular Formula	Biomethane Yield, $\frac{\text{mL}}{\text{g}}$	Biohydrogen Yield, $\frac{\text{mL}}{\text{g}}$	Reference for Formula
Casein	$C_{81}H_{125}N_{22}O_{39}P$	426.798	1707.193	[33]
Microalgae biomass	$C_{106}H_{263}O_{110}N_{16}P$	338.755	1355.021	[34,35]
Chlorella biomass	$CH_{1.719}O_{0.404}N_{0.175}P_{0.0105}$	548.226	2192.905	[36]
Methionine	$C_5H_{11}O_2NS$	418.578	1674.310	[31,32]
Cysteine	$C_3H_7O_2NS$	234.319	937.276	[31,32]
Cystine	$C_6H_{12}O_4N_2S_2$	212.656	850.626	[37]
Marine phytoplankton	$C_{106}H_{175}O_{42}N_{16}P$	563.806	2255.223	[35,38]

It is worth mentioning that the derived equation for the methanogenic digestion of general microalgal biomass formula correlates with the equation available in literature [39]:



2.4. Correction for the Species of Reaction Product

All products in reactions defined by Equations (13) and (14), except H₂ and CH₄, can dissociate and exist in various forms depending on concentrations, the solution’s pH partial pressure and temperature, etc. [40–44]. Thus, the exact form could be corrected due to the conditions for the certain product formation process. The methanogenic conditions of pH (optimal in the range of 6.8–7.5, fully eliminated outside of 5–8.5 [45–47]) and redox potential (optimal in the range from –400 to –200 mV, can exist in range –420 . . . +100 mV if no oxygen is present, with some exceptions [48–51]) define the

following species of dissociation that should be in the solution: HS^- , H_2PO_4^- , NH_4^+ ; and a mixture of CO_2 and HCO_3^- . In such forms, they should be consumed for building anaerobic microbial biomass, with a composition usually expressed as $\text{C}_5\text{H}_7\text{O}_2\text{NP}_{0.06}\text{S}_{0.1}$ according to [52–55]. For metal cations, which can exist in multiple oxidation states, the exact oxidation state is defined by the redox potential of the digestion media compared to the electro-chemical potential of the oxidation state change of the metal cation.

Thus, if any salt compounds will be formed in solution, they will be: NH_4HCO_3 , NH_4HS and $\text{NH}_4\text{H}_2\text{PO}_4$. However, this may be not true for some metal cations present in the substrate or solution.

3. Conclusions

The equations derived and presented here can be used for the stoichiometric estimation of biomethane or biohydrogen yields during the anaerobic treatment of a substrate with an arbitrary elemental composition expressed as $\text{C}_a\text{H}_b\text{O}_c\text{N}_d\text{S}_e\text{P}_f\text{M}_g$. These equations are not recommended for the exact estimation of total biogas yield, since they do not account for the solubility of gaseous products and the chemical interaction between reaction products. Moreover, the values obtained for gas yields might need to be corrected for the energy costs of biomass growth according to an existing thermodynamic framework, as well as the toxic or inhibitory effects of the substrate's composition and concentration.

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