Estimation of the biomass yield and stoichiometric coefficient during bioproduct formation through thermodynamic approach: a case study of biosurfactant production

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Abstract. Microbial growth occurs on a wide variety of compounds. One of the critical parameters in biotechnological processes is biomass yield. Biomass yield for many different microbial systems extensively studied due to its primary importance. Besides biomass yield, a stoichiometric coefficient determination is also important because it can predict the number of reactants and products needed. This paper aims to explain how to determine the biomass yield on electron donor (YDX) and stoichiometric coefficient during bioproducts formation through a thermodynamic approach. The case study focused on the biosurfactant production process. The calculation shows that the electron donor and incubation temperature affect the electron donor's biomass yield (YDX). For biosurfactant production, glucose obtains a higher value of YDX than propionate. By using the same electron donor, the higher the incubation temperature, the lower of YDX value. YDX value is useful for determining the stoichiometric coefficient of biomass growth during the biosurfactant production process through elemental mass balance. The type of electron donor and temperature affect the stoichiometric coefficient of biomass growth during the biosurfactant production process.

1. Introduction
Biotechnology has a positive role in agriculture, health, and the environment. In agriculture, biotechnology is useful for producing bioproducts that can help reduce food crises, improve food quality, and increase agricultural production. The relevant variable of biotechnological processes' economic evaluation is the biomass yield on C-source, Yx / s [1]. The biomass yield in cell growth is one of the critical parameters in various scientific studies involving microbial culture because it can determine the final biomass or cell concentration. Biomass yield (Yx/s) is a stoichiometric coefficient that characterizes a particular microbial strain's growth efficiency. It can indicate how much biomass can be grown per amount of carbon or substrate energy consumed. It is essential to optimize yield in various biotechnological processes to produce a reasonable amount of biological material. Optimization of
biomass yield is also a significant interest in the biotechnology industry for obtaining large quantities of products and synthesis rates, as they determine the economic viability of any bioprocess [2].

The biomass yield can vary from 0.01 to 1.0 C-mol biomass / C-mol substrate because it depends on microorganisms and their growth substrates [3]. The biomass yield can estimate oxygen consumption, carbon dioxide generation, and heat production. These parameters are of significant technological importance for large-scale bioreactors, particularly for O2 transport, CO2 removal, and cooling capacity [1].

One method to estimate biomass yield is a thermodynamic approach that expressed the biomass yield on electron donors (YDX). Estimating biomass yield from electron donors (YDX) is generally required in situations where the microorganism's biochemical properties are unknown or less well known concerning its biochemical properties. However, the carbon source, nitrogen source, donor electron, and acceptor electron are known. This information is generally referred to as a "black box" description. The biomass yield from electron donors (YDX) depends on several factors, namely: electron donors (through γD), electron acceptors (via ΔGA0V), N-source (via γx), and temperature, growth rate, carbon source (via Gibbs energy dissipation (through Ds01/ rx)) [4].

The parameter needed to estimate the C-source biomass yield must meet the requirements for black box information, general applications for chemotrophic growth systems, and their relationship with the Second Law of Thermodynamics. This parameter can be the Gibbs energy dissipation per C-mol produces measurable biomass from a well-established correlation [5]. The black box description provides a linear relationship between Gibbs principles of conservation and energy balance, which allows stoichiometric calculations and produces coefficients [3].

This paper attempts to explain how to estimate the biomass yield and stoichiometric coefficient during bioproducts formation through a thermodynamic approach. In this paper, a case study focused on the biosurfactant production process. Biosurfactant is a surfactant produced extracellularly by microorganisms such as bacteria, yeast, and fungi from various substrates such as sugars, oils, alkanes, and other substrates [6]. Biosurfactants have the potential to be applied commercially in the food and non-food industries. The advantages of biosurfactants are high biodegradability, effectiveness at extreme condition (pH, temperature, salinity), and can be able to produce using renewable substrates [7,8]

2. Methods

2.1. Research description used in this case study

The data used in this case study refers to the research conducted by Sakthipriya et al. [9] and Heryani and Putra [10]. Description regarding carbon sources, microorganisms species, reactor type, and working volume used in those research shown in Table 1.

| Research description | Sakthipriya et al.[11] | Heryani and Putra [10] |
|----------------------|------------------------|------------------------|
| Condition process    | Aerobic                | aerobic                |
| Carbon source        | waxy crude oil         | Glucose                |
| Nitrogen source      | Ammonium chloride (NH4CL) | Ammonium nitrate (NH4NO3) |
| Microorganism        | Bacillus substilis YB7  | Bacillus sp. BMN 14    |
| Reactor type         | Batch                  | batch                  |
| Working volume       | 50 mL in 250 mL Erlenmeyer flasks | 100 mL in 250 mL Erlenmeyer flasks |
| Temperature          | 35, 50, 75°C           | 37°C                   |
| Agitation            | 180 rpm                | 140 rpm                |
2.2. Black box description
Heijnen et al. [4] defined a general system of chemotrophic microbial growth, including a list of compounds relevant to the microbial growth system. The compounds that are always present in the growth system include biomass, H\(^+\), HCO\(_3^-\) and H\(_2\)O. N-sources, C-sources, electron donors, and electron acceptors vary widely but are easy to identify. C-source is generally the reduced organic compounds (for heterotrophic growth) or CO\(_2\) (for autotrophic growth). The electron donor is a reduced organic (for organotrophic) and inorganic (for lithotrophic) compounds. The electron acceptor is the oxidized compound.

The general system definition of microbial growth contains all the relevant compounds involved: electron donor, electron acceptor, N-source and C-source (always consumed); biomass, oxidized donor and reduced acceptor (always produced) and HCO\(_3^-\), H\(_2\)O and H\(^+\) (can be consumed or produced dependent on the specific system). The Gibbs energy dissipation is always positive, according to the Second Law of Thermodynamics (Figure 1.) The black box description’s basic feature represents the microbial growth by a macro chemical equation (Eq. 1)[4,5].

![Figure 1. Generalized system definition of microbial growth [4,5].](image)

\[
\begin{align*}
(... & C - \text{source} \pm (...)\text{donor} + (...)\text{acceptor} + (...)N - \text{source} + (...)H_20 + (...)HCO_3^- \\
+ (...)H^+ + 1CH_{1.8}O_{0.5}N_{0.2} + (...)\text{oxidized donor} \\
+ (...)\text{reduced acceptor}
\end{align*}
\]

2.3. Biomass yield estimation.
Chemotropic growth of microorganisms can occur on various chemicals within a wide range of temperatures and pH. The biomass yield (Y\(_{DX}\)) on electron donors can vary considerably. An alternative method used to predict biomass yield Y\(_{DX}\) is the Gibbs energy dissipation. In microbial growth, part of the substrate is used for biomass production and partly for maintenance, so the equation for the total Gibbs energy dissipation (D\(_s\)/rx) becomes [5]:

\[
\frac{D_s^{01}}{r_x} = \left[ \frac{D_s^{01}}{r_x} \right]_{\text{growth}} + \left[ \frac{m \in \mu}{\mu} \right]_{\text{maintenance}}
\]

(2)

For heterotrophic microbes, the above equation becomes:

\[
\frac{D_s^{01}}{r_x} = 200 + 18(6 - c)^{1.8} + \exp\left[\left(3.8 - \gamma s\right)^2\right]^{0.16} \times (3.6 + 0.4c)
\]

+ 4.5 \mu \exp\left[\frac{-70000}{R} \left(1 - \frac{1}{298}\right)\right]
\]

(3)
In macro chemical equations, a general definition for microbial growth written as follows:

\[-\frac{1}{Y_{DX}} \text{ electron donor} - (...) N - \text{ source} - \frac{1}{Y_{AX}} \text{ electron acceptor} + 1C - \text{ mol biomass} +

(...)H_{2}O + (...)HC\text{O}_{3}^{-} + (...) H^{+} + \frac{Q}{r_{x}} \text{ heat} + \frac{D_{s01}}{r_{x}} \text{ Gibbs energy} \tag{4}\]

From the macro chemical equations above (Eq. 3), there are three formulations of balance equations, i.e., the balance of degree of reduction, the Gibbs energy balance, and the enthalpy balance.

Balance of degree of reduction:

\[-\frac{Y_{D}}{Y_{DX}} - \frac{Y_{A}}{Y_{AX}} + Y_{X} = 0 \tag{5}\]

The Gibbs energy balance:

\[-\frac{Y_{D}}{Y_{DX}} \Delta G_{eD01}^{01} - \frac{Y_{A}}{Y_{AX}} \Delta G_{eA01}^{01} + Y_{X} \Delta G_{eX01}^{01} + \frac{D_{s01}}{r_{x}} = 0 \tag{6}\]

Enthalpy balance:

\[-\frac{Y_{D}}{Y_{DX}} \Delta H_{eD01}^{01} - \frac{Y_{A}}{Y_{AX}} \Delta H_{eA01}^{01} + Y_{X} \Delta H_{eX01}^{01} + \frac{Q}{r_{x}} = 0 \tag{7}\]

From the above balances, three equations can predict the biomass yield and heat of the reaction with the following equation [5]:

Biomass yield on electron donor

\[Y_{DX} = \frac{Y_{D} \Delta G_{av01}}{D_{s01}/r_{x} + Y_{X} \Delta G_{av01}} \tag{8}\]

Biomass yield on electron acceptor

\[Y_{AX} = \frac{(-Y_{A}) \Delta G_{av01}}{D_{s01}/r_{x}} \tag{9}\]

Mol acceptor/(C)-mol donor

\[Y_{DA} = -\frac{Y_{D}}{Y_{DX}} \times \frac{D_{s01}/r_{x}}{D_{s01}/r_{x} + Y_{X} \Delta G_{av01}} \tag{10}\]

The heat production per C-mol produced biomass

\[\frac{Q}{r_{x}} = \frac{\Delta H_{av01}^{01}}{\Delta G_{av01}^{01}} \times \frac{D_{s01}}{r_{x}} \tag{11}\]
The heat production per C-mol consumed substrate

\[ \frac{Q}{r_s} = \left( \frac{\gamma_D \Delta H_{av}^{01}}{D_s^{01} r_s} + \gamma_X \Delta G_{av}^{01} \right) \times D_s^{01} r_s \]  

(12)

2.4. Stoichiometric coefficient

The stoichiometric coefficient is useful for determining the ratio or ratio of moles between reactants and products. Determination of the stoichiometric coefficient of biomass growth during biosurfactant formation based on the \( Y_{DX} \) value through the mass balance of elements. The biomass stoichiometric coefficients are assumed to be +1. The other coefficients (...) and yields must be measured or calculated from elemental (C, H, O, N) and electric charge conservation relations [5].

3. Results and Discussions

The steps for calculating the biomass yield on electron donor (\( Y_{DX} \)) and stoichiometric coefficient through a thermodynamic approach include 1) identifying the electron donor and other relevant compounds in the biosurfactant production process; 2) determining the Gibbs energy dissipation, the yield of biomass to the donor/acceptor and the heat of the reaction; 3) determining stoichiometric coefficient through elemental mass balances.

3.1. Determining the electron donor and other relevant compounds

The biosurfactant production process carried out by Sakthipriya et al. [11] used waxy crude oil as a carbon source. Waxy crude oil is a heterogeneous mixture of hydrocarbons consisting of paraffin, aromatics, resins, and asphaltene with a paraffin composition of more than 50%, and its paraffin molecular structure ranges from \( C_{18}H_{38} \) to \( C_{55}H_{112} \) [12]. The main fraction (> 50%) in crude oil is alkanes depending on the oil source. Alkanes are saturated hydrocarbons that are chemically very inert as nonpolar molecules [13,14].

Biomass is built from C4-C5 metabolite molecules known as central building blocks with a range of reduction degrees \( (\gamma = 4) \). Suppose the carbon source is very different from the central building block, then several reactions (carbon-carbon coupling, oxidation, reduction) must be carried out in the primary pathway to convert the carbon source into a building block [4].

The substrate used in the research of the Sakthipriya et al. [11] was a long chain carbon (\( C_{18}H_{38} \) to \( C_{55}H_{112} \)). It needs degradation or hydrolysis process to convert it into building blocks (C4-C5). Kumari et al. [15] stated that the essential elements that affect the hydrocarbon biodegradation process are biomass concentration, the population of microbes, enzyme activity, and the environmental parameters, such as pressure and temperature. The enzymes’ high activity necessitates less energy to activate the hydrocarbon molecule to initiate biodegradation and speed up the process. Several enzymes responsible for the degradation of long-chain hydrocarbons are alkane monooxygenase, alcohol dehydrogenase, and aldehyde dehydrogenase. These enzymes cause various biochemical transformations of original oil substances and form a large number of intermediates.

The microorganism used in Sakthipriya et al. [11] is Bacillus subtilis YB7. This bacteria is one of the capable bacteria of producing enzymes that can help the biodegradation of crude oil under aerobic conditions. Aerobic degradation was initiated by the oxidation of a terminal methyl group to form primary alcohol. This activation has increased the solubility of hydrocarbon in water and introduced a reactive site for further reactions. The reaction's energy requirement was generated by the oxidation of a reduced biological intermediate NADH, which was re-oxidized by an electron acceptor. The primary alcohol was further oxidized by the enzyme (alcohol dehydrogenase) to form the corresponding aldehyde and converted into a fatty acid by aldehyde dehydrogenase [9].
The process of alkane degradation under aerobic conditions shown in more detail in Figure 3. The main pathway is the terminal oxidation of fatty acid. $\alpha$ - and $\omega$ - hydroxylation is catalyzed by the same set of enzymes.

![Chemical pathway diagram]

**Figure 2.** Peripheral aerobic pathways of n-alkane degradation [16].

Sakthipriya et al. [9] reported that the degradation rate of waxy crude oil reaches 70% within one day of incubation. The maximum extent of biodegradation (96%) was attained in about ten days. There was no significant increase in the percentage of degradation after ten days of incubation. Figure 4 shows the rate of degradation of waxy crude oil by *Bacillus subtilis* YB7.

The functional groups present in crude oil before and after degradation were confirmed using FTIR spectroscopy (Fig. 3). Figure 4(a) shows the FTIR spectrum of crude oil before and after microbial degradation. The peak obtained at 2984 cm$^{-1}$ was mainly due to the $\text{-C-H}$ stretching mode of CH$_3$ and CH$_2$ in the alkyl chain, which confirms paraffin's presence. The peak in spectrum at 1736 cm$^{-1}$ stands for the C=O group, conforming to carboxylic acid production. Weak bonds at 1446 cm$^{-1}$ and 1372 cm$^{-1}$ resulted from the $\text{-C-CH}_2$ and $\text{-C-CH}_3$ groups. The stretch at 1232 cm$^{-1}$ indicated a C-O bond, which might be alcohol or ester, the band at 1097 cm$^{-1}$ means a bond of C-O-C, and an indentation at 786 cm$^{-1}$ indicated an alkene bond.

From the explanation of the degradation process above, it was assumed that fatty acid produced from the degradation process of waxy crude oil is propionate (CH$_3$CH$_2$COOH, C$_3$H$_6$O$_2$). Therefore, the carbon source used for estimating the yield of biomass yield on electron donor/acceptor, the heat of the reaction, and the stoichiometric coefficient is propionate. Propionate is a type of volatile fatty acid, can be used...
as a single carbon source, especially in activated sludge systems, and is a suitable substrate for EBPR (enhanced biological phosphorus removal) performance.

Figure 3. a) percentage degradation of crude oil; b) Functional groups present before and after degradation using Bacillus subtilis at 50 °C and after 10 days

Meanwhile, Heryani and Putra [10] used glucose (C₆H₁₂O₆) as a carbon source in the biosurfactant production process, and the microorganism was Bacillus subtilis BMN 14. The biosurfactant production process also under aerobic conditions. With six C-atoms, glucose is readily substrate as a carbon source for forming biomass and metabolite products because the number of C-atoms is close to the central building block (C₄-C₅). Referring to the general definition of a microbial growth system that has been developed by Heijnen et al.[4] can then determine the relevant compounds involved in biomass growth during the biosurfactant production process as given in Table 2.

Table 2. Relevant compounds involved in biomass growth during the biosurfactant production*).

| Relevant compound | Sakthipriya et al.[11] Aerobic/Propionate | Heryani and Putra [10] Aerobic/Glucose |
|-------------------|-------------------------------------------|--------------------------------------|
| Biomassa          | CH₁₈O₅N₀₂                                  | CH₁₈O₅N₀₂                            |
| N-source          | NH₄⁺                                      | NH₄⁺                                 |
| H⁺                | H⁺                                        | H⁺                                   |
| HCO₃⁻             | HCO₃⁻                                     | HCO₃⁻                                |
| H₂O               | H₂O                                       | H₂O                                  |
| C-source          | C₃H₆O₂                                    | C₃H₁₂O₆                              |
| Electron donor    | C₃H₆O₂                                    | C₃H₁₂O₆                              |
| Oxidized donor    | HCO₃⁻                                     | HCO₃⁻                                |
| Electron acceptor | O₂                                        | O₂                                   |
| Reduced acceptor  | H₂O                                       | H₂O                                  |

*Referring to the general definition of the growth system developed by Heijnen et al., [4]

3.2. Determining the Gibbs energy dissipation and the biomass yield on electron donor (YDX)

According to Heijnen et al. [4], the substrate's growth process is used for biomass synthesis and maintenance. It means that Gibbs energy dissipation can distinguish between growth-related dissipation and maintenance related dissipation. Gibbs energy dissipation value can use to calculate the biomass yield and heat production that occurs during the biomass growth. The data required in calculating the Gibbs energy dissipation, biomass yield, and heat production shown in Table 3.
The data used for the calculation of the Gibbs energy dissipation, biomass recovery, and reaction heat production during biomass growth in the biosurfactant production process.

| Data | Sakthipriya et al. [11] | Heryani dan Putra [10] | Electron acceptor (O₂) |
|------|-------------------------|------------------------|------------------------|
| C-atoms | Propionate (C₃H₆O₂) | Glucose (C₆H₁₂O₆) | |
| γ | +4.67⁻ | +4⁻ | - |
| ΔGₑᶠ₀¹ (kJ/e-mol) | +26.939⁻ | +39.744⁻ | -78.719⁻ |
| ΔHₑᶠ₀¹ (kJ/e-mol) | -33.80⁻ | -25.75⁻ | -143⁻ |
| μ (jam⁻¹) | T : 35°C (308°K) = 0.075⁷ | T : 37°C (310°K) = 0.097⁷ | - |
| R (kJ/mol) | 8.314⁻ | 8.314⁻ | - |

Data were taken and processed from research data of Sakthipriya et al. [11]

Data were taken and processed from research data of Heryani dan Putra [10]

Table 3 shows that the carbon source/electron donor used in the biosurfactant production process affects the total Gibbs energy dissipation value. The production process using propionate produces a higher total Gibbs energy dissipation compared to a glucose substrate. In this case, one of the influencing factors was the degradation process of waxy crude oil. Bacillus subtilis YB7 performed substrate degradation process to form simple volatile fatty acids that can use as a carbon source; in this case, assume as propionate. The addition of a carbon-carbon coupling process or oxidation or reduction can increase energy dissipation [5]. Incubation temperature also affects the Gibbs energy dissipation value. Using the same carbon source (propionate), the Gibbs energy value of dissipation increases with increasing incubation temperature.

Table 4 shows that the carbon source/electron donor used in the biosurfactant production process affects the total Gibbs energy dissipation value. The production process using propionate produces a higher total Gibbs energy dissipation compared to a glucose substrate. In this case, one of the influencing factors was the degradation process of waxy crude oil. Bacillus subtilis YB7 performed substrate degradation process to form simple volatile fatty acids that can use as a carbon source; in this case, assume as propionate. The addition of a carbon-carbon coupling process or oxidation or reduction can increase energy dissipation [5]. Incubation temperature also affects the Gibbs energy dissipation value. Using the same carbon source (propionate), the Gibbs energy value of dissipation increases with increasing incubation temperature.

The values for biomass yield on electron donor (Yₐₓₜ), biomass yield on electron acceptor (Yₐₓₛₜ), mol acceptors to mol donors (Yₐₐₜ) shown in Table 5. Table 5 shows that the electron donor and incubation temperature affects the biomass gain against the electron donor (Yₐₓₜ). For biosurfactant production, glucose obtains a higher value of Yₐₓₜ than propionate. By using the same electron donor, the higher the incubation temperature, the lower of Yₐₓₜ value.
The black box description can estimate the energetic characteristics of the catabolic (donor/acceptor) redox pair. The value of heat production per biomass produced (Q/rx) and heat production per substrate consumed (Q/rs) shown in Table 6. Table 6 shows that the heat production per biomass (Q/rx) and the heat production per substrate consumed (Q/rs) of propionate as an electron donor is higher than glucose. The temperature also influences the value of Q/rx and Q/rs. The higher the temperature of the process, the higher the value of Q/rx and Q/rs. The high heat production is due to the maintenance process at a low specific growth rate (µ), which causes an increase in heat production per C-mol of biomass (Q/rx) [4]. The Q/rs value obtained for both processes (using propionate and glucose) is positive, which means that both processes are exothermic. It means that the process releases heat from the system to the environment.

Table 5. The value of biomass yield on electron donor (Y_DA), biomass yield on electron acceptor (Y_AX), and mol acceptor/C-mol donor (Y_DA).

| Electron donor | T (°K) | ΔG^01 (kJ/e-mol) | D^01/X (kJ/C-mol) | Y_DX (C-mol/C-mol) | Y_AX (C-mol/mol) | Y_DA (mol/C-mol) |
|----------------|--------|------------------|------------------|------------------|------------------|------------------|
| Propionate     | 308    | 105,658          | 578,771          | 0,483            | 0,730            | 0,661            |
|                | 323    | 105,658          | 905,760          | 0,366            | 0,466            | 0,783            |
|                | 348    | 105,658          | 4100,864         | 0,109            | 0,103            | 1,053            |
| Glucose        | 310    | 118,463          | 374,542          | 0,543            | 1,265            | 0,429            |

^a Taken and processed from research data of Sakthipriya et al. [11]  
^b Taken and processed from research data of Heryani and Putra [10]

The accuracy of the Gibbs energy dissipation approach has limitations because the calculations are based on the average microbiological behavior rather than the actual biochemistry of specific microorganisms. A more accurate value for biomass recovery obtains by calculation base on information about the generation/consumption of ATP or with the exact Y_DX measurement. However, a thermodynamic approach via the Gibbs energy dissipation can be considered to estimate the initial biomass yield value on the electron donor (Y_DX) [4].

According to Heijnen et al. [4], in a microbial growth system, there is a heat conversion process which expressed in Q/rx (heat production per C-mol produced biomass) and Q/rs (heat production per C -mol consumed substrate). Heat (Q) use as one of the energetic characteristic parameters and can calculate using ∆G^01 and ∆H^01. ∆G^01 and ∆H^01 represent the energetic qualities of the catabolic (donor/acceptor) redox pair. The value of heat production per biomass produced (Q/rx) and heat production per substrate consumed (Q/rs) shown in Table 6. Table 6 shows that the heat production per produced biomass (Q/rx) and the heat production per substrate consumed (Q/rs) of propionate as an electron donor is higher than glucose. The temperature also influences the value of Q/rx and Q/rs. The higher the temperature of biosurfactant production, the higher the value of Q/rx and Q/rs. The high heat production is due to the maintenance process at a low specific growth rate (µ), which causes an increase in heat production per C-mol of biomass (Q/rx) [4]. The Q/rs value obtained for both processes (using propionate and glucose) is positive, which means that both processes are exothermic. It means that the process releases heat from the system to the environment.

Table 6. The value of heat production per C-mol produced and heat production per C-mol consumed substrate.

| Electron Donor | T (°K) | ΔG^01 (kJ/e-mol) | ΔH^01 (kJ/e-mol) | Q/rx (kJ/C-mol X) | Q/rs (kJ/C-mol S) |
|----------------|--------|------------------|------------------|-------------------|-------------------|
| Propionate     | 308    | 105,658          | 578,771          | 109,2             | 598,173           | 50,915           |
|                | 323    | 105,658          | 905,760          | 109,2             | 939,1236          | 342,273          |
|                | 348    | 105,658          | 4100,864         | 109,2             | 4238,338          | 342,273          |
| Glucose        | 310    | 118,463          | 374,542          | 117,25            | 370,707           | 201,425          |

^a Taken and processed from research data of Sakthipriya et al. [11]  
^b Taken and processed from research data of Heryani and Putra. [10]

3.3. Determining the stoichiometric coefficients

The black box description can estimate the macro chemical equation's stoichiometric coefficients or the microbial growth description. The calculation is based on the elemental and electric charge conservation relations and an additional equation, which could be the experimental yield of biomass on C-source. The black box approach is beneficial when a preliminary estimation of the substrate's biomass yield is required or before knowing the microorganisms' properties.
Y_{DX} value is useful for determining the stoichiometric coefficient of biomass growth during biosurfactants formation through elemental mass balance. Y_{DX} use to assess the value of the coefficient 'a' (coefficient for electron donor). Determination of the electron donor coefficient (a) from the Y_{DX} value is shown in Table 7. The stoichiometric equation for aerobic biomass growth with propionate and glucose as carbon sources shows in equation 13 and equation 14, respectively.

\[ a \ C_3H_6O_2 + b \ O_2 + c \ NH_4^+ + d \ H_2O + e \ HCO_3^- + f \ H^+ + g \ CH_{1.8}O_{0.5}N_{0.2} \] (13)

Elemental mass balance for aerobic biomass growth with propionate as an electron donor as follow:

- C balance : 3a + e + g = 0
- H balance : 6a + 4c + 2d + e + f + 1.8 g = 0
- O balance : 2a + 2b + d + 3e + 0.5 g = 0
- N balance : c + 0.2g = 0
- Charge balance : c – e + f = 0

\[ a \ C_6H_{12}O_6 + b \ O_2 + c \ NH_4^+ + d \ H_2O + e \ HCO_3^- + f \ H^+ + g \ CH_{1.8}O_{0.5}N_{0.2} \] (14)

Elemental mass balance for aerobic biomass growth with glucose as an electron donor as follow:

- C balance : 6a + e + g = 0
- H balance : 12a + 4c + 2d + e + f + 1.8 g = 0
- O balance : 2a + 2b + d + 3e + 0.5 g = 0
- N balance : c + 0.2g = 0
- Charge balance : c – e + f = 0

| Electron Donor | T number | Y_{DX} (C-mol biomass/C-mol electron donor) | Y_{DX} (mol biomass/mol mol electron donor) | a = -1/Y_{DX} |
|---------------|---------|------------------------------------------|------------------------------------------|---------------|
| Propionate    | 35 3   | 0.483                                    | 1.449                                    | -0.690        |
|               | 50 3   | 0.366                                    | 1.098                                    | -0.911        |
|               | 75 3   | 0.109                                    | 0.327                                    | -3.058        |
| Glucose       | 37 6   | 0.543                                    | 3.258                                    | -0.307        |

The biomass stoichiometric coefficient is assumed to be +1. The other coefficients (...) and yields must be measured or calculated from elemental (C, H, O, N) and electric charge conservation relations [3]. Solving the equation 13 and 14 with the biomass stoichiometric coefficient (g) = 1 and the electron donor stoichiometric coefficient (a) is refer to Table 7, can then obtains other stoichiometric coefficients (b, c, d, e, and f) thus the stoichiometric equation can be formed.

The stoichiometric equation for aerobic biomass growth with propionate as an electron donor in biosurfactant production is as follows:

Propionate at T = 35°C

-0.690C_3H_6O_2 -1.365O_2 -0.2 NH_4^+ + 0.4 H_2O + 1.070 HCO_3^- + 1.270 H^+ + 1 CH_{1.8}O_{0.5}N_{0.2} (15)
Propionate at $T = 50^\circ C$

$$-0.911C_3H_4O_2 \cdot 2.138O_2 - 0.2 NH_4^+ + 0.4 H_2O + 1.733 HCO_3^- + 1.933H^+ + 1 CH_{1.8}O_{1.3}N_{0.2} \quad (16)$$

Propionate at $T = 75^\circ C$

$$-3.058C_3H_4O_2 \cdot 9.662O_2 - 0.2 NH_4^+ + 0.418 H_2O + 8.174 HCO_3^- + 8.374H^+ + 1 CH_{1.8}O_{1.3}N_{0.2} \quad (17)$$

The stoichiometric equation for aerobic biomass growth with glucose as an electron donor in biosurfactant production is as follows:

Glucose at $T = 37^\circ C$

$$-0.307 C_6H_{12}O_6 \cdot 1.406 O_2 - 0.2NH_4^+ + 0.4H_2O + 0.842HCO_3^- + 1.042 H^+ + 1CH_{1.8}O_{0.8}N_{0.2} \quad (18)$$

4. Conclusion

The accuracy of estimating the yield value of biomass to the electron donor ($Y_{DX}$) through the thermodynamic approach (the Gibbs energy dissipation) has limitations, especially for specific polymeric carbon sources. However, a thermodynamic approach via the Gibbs energy dissipation can be considered to estimate the initial biomass gain value for the electron donor ($Y_{DX}$). The result of the calculation shows that the electron donor and incubation temperature affect the electron donor's biomass yield ($Y_{DX}$). For biosurfactant production, glucose obtains a higher value of $Y_{DX}$ than propionate. By using the same electron donor, the higher the incubation temperature, the lower of $Y_{DX}$ value. $Y_{DX}$ value is useful for determining the stoichiometric coefficient of biomass growth during biosurfactants formation through elemental mass balance. The type of electron donor and temperature affect the stoichiometric coefficient of biomass growth during the biosurfactant production process.

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