Exergy Analysis as a Tool for Decision Making on Substrate Concentration and Light Intensity in Photobiological Hydrogen Production


This study was devoted to the comprehensive thermodynamic modeling of biohydrogen production using the light-dependent bacterium *Rhodospirillum rubrum*. Conventional exergy and eco-exergy concepts were employed for decision making on the carbon source concentration and light intensity. Several thermodynamic indicators were applied to identify optimal experimental variables with respect to the sustainability issues. The results obtained using both concepts were remarkably different from each other because of the incorporation of the work of the information embodied in the genomes of the living microorganisms considered in the eco-energetic computations. However, both analyses identified 1 g L\(^{-1}\) substrate concentration and 1000 lux light intensity as optimal fermentation conditions. Under these conditions, the normalized exergy destruction as a decision making parameter was 179.7 and 171.54 kJkJ\(_{t,h}^{-1}\) using the conventional exergy and eco-exergy concepts, respectively.

Introduction

In the last century, the world’s overall energy utilization increased continuously because of population growth, improved living standards, urbanization, and industrialization.[1] Most of these huge energy demands have been met by fossil-based fuels (coal, oil, and natural gas), but their soaring prices and depleting reserves have introduced serious challenges into the global energy market. These challenges are further intensified because the exact reserves of fossil fuels are yet to be estimated precisely.[2] In addition, the employment of such energy carriers has led to the production of hazardous pollutants such as CO, CO\(_2\), SO\(_2\), NO\(_x\), and particulate matter (PM) and the consequent environmental impacts such as climate change and global warming. To prevent these unfavorable phenomena, the widespread application of renewable energy carriers such as biohydrogen seems inevitable.[3–5]

Currently, hydrogen can be produced from both renewable and non-renewable sources. Non-renewable hydrogen can be produced by adopting various techniques using fossil fuels such as natural gas steam reforming, coal gasification, and water electrolysis. It is essential to highlight that the maximum amount of non-renewable hydrogen production is currently generated through steam methane reforming and water electrolysis techniques.[6] However, to achieve truly eco-friendly, sustainable, and cost-effective hydrogen production, renewable pathways should be taken into account.[7] In line with that, hydrogen can be produced by the application of a variety of renewable methods such as wind, solar, geothermal, biomass gasification, artificial photosynthesis, and photofermentation. It is well documented that photofermentation, that is, the use of anaerobic photosynthetic bacteria for biological hydrogen production, is a promising and reliable method compared to other renewable pathways.[8–10] In this technique, photosynthetic anaerobic bacteria oxidize hazardous CO contained in syngas in the presence of water to produce H\(_2\) and CO\(_2\) through the water gas shift (WGS) reaction. Notably, the WGS reaction can be performed by both microbial and metal catalysts. Nevertheless, a holistic evaluation based on advanced engineering tools such as thermodynamic analyses should still be applied to assess the productivity and sustainability of various photobiological hydrogen production routes to identify the most eco-friendly pathway.

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In the past few decades, thermodynamic approaches such as energy and exergy analyses have emerged as vital tools for the design, analysis, and optimization of energy conversion systems.[8–10] In contrast to energy analysis, which is based on the first law of thermodynamics, exergy analysis, as a measure of both quantity and quality of energy, is preferred for the evaluation of different energy conversion systems.[11–13] Simply speaking, exergy can be defined as the maximum amount of attainable work from a system when it reaches equilibrium with its environment through reversible processes.[14–16] Today, exergy analysis and its extensions appear to be effective engineering tools over other traditional methods for the sustainability assessment of various energy conversion processes, which is notable because of their unique conceptual features to quantify the irreversibility aspects of thermodynamic systems.[17–19]

Exergy analysis can provide valuable insights into the magnitudes and locations of exergy destruction during an energy conversion process and can introduce potentials for energy efficiency improvements. As a result, a tremendous amount of research has been devoted to the application of exergy analysis to scrutinize and optimize various energy conversion units, which include renewable and non-renewable hydrogen production routes. For instance, Abanades et al.[20] applied exergy analysis for hydrogen production from 30 water-splitting thermochemical cycles by concentrated solar energy. In another survey, Ni et al.[21] applied energy and exergy analyses to study the thermodynamic-electrochemical features of hydrogen production by a solid oxide steam electrolyzer. In the same year, Simpson and Lutz[22] performed exergy analysis of hydrogen production from the steam reforming of methane. Later, exergy analysis of biomass gasification for hydrogen production was conducted by Toomsen et al.[23] Moreover, the exergy analysis of a zero-emission coal gasification system for hydrogen production was conducted by Liszka et al.[24] Recently, an oxygen-blown gasification system for hydrogen production from rice straw was analyzed on the basis of exergy concepts.[25] According to the results of the above-mentioned surveys, exergy analysis could aid in the improvement of system operation by providing a comprehensive understanding of sites of thermodynamic irreversibility.

In addition to the studies mentioned above, numerous studies have focused on the feasibility analysis and kinetics modeling of biological hydrogen production using various microorganisms.[6,7] However, such surveys provide little or no insight into the sustainability issue of the process and therefore, cannot be useful for design or optimization goals. To the best of our knowledge, there is no published report on the use of exergy analysis for biophotolysis production by the WGS reaction in photo-bioreactors. More specifically, the goal of the current study was to apply both conventional exergy and eco-exergy concepts for decision making on light intensity and substrate concentration to distinguish the most productive and renewable conditions in biophotolysis production. The outcomes of this study are expected to be very useful for the design and optimization of industrial-scale bioreactors by providing the necessary insight into how to select the appropriate component design and operation procedure.

**Theoretical Considerations**

Biological hydrogen production using anaerobic photosynthetic bacteria *Rhodospirillum rubrum* (*R. rubrum*) can be performed through the WGS reaction. Najafpour et al.[26] reported that the WGS reaction for the production of H$_2$ and CO$_2$ catalyzed by a specific microorganism can be shown as follows [Eq. (1)]:

$$\text{CO}_2(g) + \text{H}_2\text{O}(l) \rightleftharpoons \text{H}_2(g) + \text{CO}_2(g) \quad \Delta G^\circ = -20 \text{ kJ mol}^{-1} \quad (1)$$

The photosynthetic bacteria contain photoreactive pigments, such as bacteriochlorophyll and carotenoids, through which they can absorb energy and convert it into a chemical potential in the reaction center. The microbes can then use this chemical potential in their metabolism to convert different types of substrates, such as CO, into products, such as H$_2$. The light absorption capability (coefficient) of these bacteria is dependent on the cell pigments and their quantity.[27] It would be expected that the cell growth may reach a maximum value at the highest light intensity because of the assimilation of organic sources in the presence of light. However, the dense color of the pigments as a result of the high cell density could prevent the full transmission of light into the cells. Therefore, the optimal amount of light is a crucial parameter that needs to be determined. Notably, the overall WGS reaction rate is affected negatively by two limitations, that is, the intrinsic reaction rate and the mass transfer rate. For a biological WGS reaction, the transfer of CO from the syngas phase into the liquid is a limiting factor for most reactor configurations.[28]

Biological hydrogen production through the WGS reaction using living microorganisms is presented schematically in Figure 1. In this method, instead of a metal-based catalyst, a series of microbial enzymes are involved in the conversion of CO and H$_2$O into H$_2$ and CO$_2$. These microorganisms obtain energy by oxidizing the CO to CO$_2$ and pairing protons and reducing them to hydrogen. This process is cata-

![Figure 1. Biological hydrogen production through the WGS reaction using specific microorganisms (CODH, CO dehydrogenase; Fd, ferredoxin; ECH, energy-conserving hydrogenase). Adopted from Henstra et al.[29] Copyright (2015), with permission from Elsevier.](image-url)
lyzed by CO dehydrogenase (CODH) and hydrogenase enzymes.[36]

Biological hydrogen production through the WGS reaction in a photo-bioreactor as a control mass for exergy analysis with input and output terms is presented schematically in Figure 2.

Figure 2. Biological hydrogen production in a batch photo-bioreactor.

The exergy balance equation for a photo-bioreactor that consists of three parts (i.e., culture medium, microorganisms, and syngas) is written according to the scheme given in Figure 2 [Eq. (2)]:

$$
\text{Ex}_{\text{CM}t} + \text{Ex}_{\text{SG}t} + \text{Ex}_{\text{MO}t} + \text{Ex}_{\text{DL}} = \text{Ex}_{\text{CM}t+\Delta t} + \text{Ex}_{\text{SG}t+\Delta t} + \text{Ex}_{\text{MO}t+\Delta t} + \text{Ex}_{\text{IR}}
$$

(2)

in which \(\text{Ex}_{\text{CM}}\), \(\text{Ex}_{\text{SG}}\), and \(\text{Ex}_{\text{MO}}\) represent the exergetic values of the culture medium, syngas, and microorganisms, respectively, at times \(t\) and \(t+\Delta t\). \(\text{Ex}_{\text{IR}}\) are the exergetic values transferred from the orbital shaker and tungsten lamp to the photo-bioreactor in the form of mechanical work and light energies, respectively. In addition, \(\text{Ex}_{\text{IR}}\) is the exergy destruction within the process because of the occurrence of thermodynamic irreversibilities.

The exergetic value of the culture medium in the photo-bioreactor at a specified time can be calculated as [Eq. (3)]:

$$
\text{Ex}_{\text{CM}} = n_{\text{CM}} \left[ \sum x_i \epsilon_i + RT_0 \sum x_i \ln (x_i) \right]
$$

(3)

in which \(n_{\text{CM}}\) and \(x_i\) stand for the mole number of the culture medium and the molar fraction of each component, respectively. \(\epsilon_i\) denotes the standard chemical exergy of the \(i\)th constituent. \(R\) and \(T_0\) represent the universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)) and dead-state temperature (303.15 K), respectively.

The formula recommended by Song et al.[37] was employed to calculate the specific chemical exergy of the organic materials used for culture medium preparation [Eq. (4)]:

$$
\text{ex}_{\text{OM}} = 363.439 \ w_C + 1076.633 \ w_H - 86.308 \ w_O
+ 4.147 \ w_N + 190.798 \ w_S - 21.1 \ w_A
$$

(4)

in which \(\text{ex}_{\text{OM}}\) denotes the specific chemical exergy of the organic materials [kJ kg\(^{-1}\)] and \(w_C\), \(w_H\), \(w_O\), \(w_S\), and \(w_A\) denote the mass percentages of carbon, hydrogen, oxygen, nitrogen, sulfur, and ash, respectively. Therefore, the standard chemical exergy of the organic material available in the culture medium can be defined as follows [Eq. (5)]:

$$
\epsilon_{\text{OM}} = M_{\text{OM}} \times \text{ex}_{\text{OM}}
$$

(5)

in which \(M_{\text{OM}}\) represents the molecular mass of each organic material [kg mol\(^{-1}\)]. In addition to Equation (5), the standard chemical exergy values of the inorganic materials were adopted from the report published by Wall.[38] The chemical formulae as well as the standard chemical exergy [kJ mol\(^{-1}\)] of the ingredients used in the liquid medium preparation are tabulated in Table 1.

However, the chemical exergy of the gaseous phase of the photo-bioreactor (syngas) was calculated according to Equation (6):

$$
\text{Ex}_{\text{SG}} = n_{\text{SG}} \left[ \sum x_i \epsilon_i + RT_0 \sum x_i \ln (x_i) + RT_0 \ln \left( \frac{P}{P_0} \right) \right]
$$

(6)

Table 1. Standard chemical exergies of materials used for the preparation of the culture medium.

<table>
<thead>
<tr>
<th>Material</th>
<th>Chemical formula</th>
<th>Standard chemical exergy [kJ mol(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>H(_2)O</td>
<td>0.98</td>
</tr>
<tr>
<td>sodium acetate</td>
<td>Na(_2)C(_2)H(_4)O(_2)</td>
<td>673.599(^{a})</td>
</tr>
<tr>
<td>yeast extract</td>
<td>C(_6)H(_12)O(_6)</td>
<td>9535.466(^{a})</td>
</tr>
<tr>
<td>ammonium chloride</td>
<td>NH(_4)Cl</td>
<td>331.3(^{a})</td>
</tr>
<tr>
<td>magnesium sulfate heptahydrate</td>
<td>MgSO(_4)·7H(_2)O</td>
<td>87.6(^{a,b})</td>
</tr>
<tr>
<td>calcium chloride dihydrate</td>
<td>Ca(_2)Cl(_2)·2H(_2)O</td>
<td>89.7(^{a,b})</td>
</tr>
<tr>
<td>ferric citrate</td>
<td>Fe(_2)H(_6)O(_3)</td>
<td>2076(^{a})</td>
</tr>
<tr>
<td>potassium dihydrogen phosphate</td>
<td>K(_2)HPO(_4)</td>
<td>50.6(^{a})</td>
</tr>
<tr>
<td>dipotassium hydrogen phosphate</td>
<td>K(_2)HPO(_4)</td>
<td>105.3(^{a})</td>
</tr>
<tr>
<td>nicotinamide</td>
<td>C(_6)H(_4)O(_2)N</td>
<td>314.0(^{a,d})</td>
</tr>
<tr>
<td>thiamine HCl</td>
<td>C(_6)H(_5)C(_5)N(_3)O(_3)</td>
<td>7587.879(^{a,b})</td>
</tr>
<tr>
<td>nicotinic acid</td>
<td>C(_6)H(_5)NO(_2)</td>
<td>2890.3(^{a})</td>
</tr>
<tr>
<td>zinc sulfate heptahydrate</td>
<td>ZnSO(_4)·7H(_2)O</td>
<td>88.6(^{a,b})</td>
</tr>
<tr>
<td>boric acid</td>
<td>H(_3)BO(_3)</td>
<td>21.9(^{a})</td>
</tr>
<tr>
<td>copper sulfate pentahydrate</td>
<td>Cu(_2)SO(_4)·5H(_2)O</td>
<td>91.3(^{a})</td>
</tr>
<tr>
<td>EDTA</td>
<td>C(_8)H(_4)N(_2)O(_5)</td>
<td>5006.7(^{a})</td>
</tr>
<tr>
<td>ammonium molybdate</td>
<td>(NH(_4))(_2)Mo(_7)O(_24)</td>
<td>1882.025(^{a})</td>
</tr>
<tr>
<td>sodium hydroxide</td>
<td>NaOH</td>
<td>74.9(^{a})</td>
</tr>
<tr>
<td>hydrogen chloride</td>
<td>HCl</td>
<td>84.5(^{a})</td>
</tr>
</tbody>
</table>

\(^{a}\) Obtained from Ref. [38]. \(^{b}\) Calculated based on Equations (4) and (5). \(^{c}\) Obtained from [http://www.exergoeconomy.com]. \(^{d}\) As thermodynamic data for these materials were not available, the standard chemical exergy values were calculated for an equimolar mixture of their components. \(^{e}\) As a result of the lack of thermodynamic data for ammonium molybdate, its standard exergy was computed using Equations (4) and (5), the error of this assumption was negligible as its amount in the culture medium was only 9.81×10\(^{-7}\) kg.
in which \( n_{\text{mol}} \) and \( x_i \) represent the mole number of syngas and the molar fraction of each component, respectively, and \( e_i \) is the standard chemical exergy of the \( i \)th constituent. \( P \) denotes the absolute pressure of the syngas, and \( P_0 \) denotes the pressure of the dead state, which was assumed to be 100 kPa.

Furthermore, the components of the syngas together with their standard chemical exergy values are given in Table 2.\(^{[38]}\)

Table 2. Standard chemical exergies of the syngas components.\(^{[38]}\)

<table>
<thead>
<tr>
<th>Component</th>
<th>Standard chemical exergy [kJ mol(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2 )</td>
<td>236.1</td>
</tr>
<tr>
<td>( \text{CO} )</td>
<td>275.10</td>
</tr>
<tr>
<td>( \text{CO}_2 )</td>
<td>19.87</td>
</tr>
<tr>
<td>Ar</td>
<td>11.69</td>
</tr>
</tbody>
</table>

The exergetic value carried by living organisms can be calculated using Equation (7):

\[
\text{Ex}_{\text{MO}} = 18.7 m_{\text{MO}} \tag{7}
\]

in which \( m_{\text{MO}} \) represents the mass of microorganisms in the photo-bioreactor at a given time and 18.7 kJ kg\(^{-1}\) is the basic chemical exergy of the average detritus.\(^{[39]}\)

As a result of the presence of the living organisms within the photo-bioreactor, the eco-exergy concept was also taken into account in addition to the conventional exergy approach to have a clear picture of the process. Notably, a considerable amount of genetic information is embodied in the genomes of the living organisms. Hence, they possess an extra energy work compared to the nonliving constituents that only possess chemical energy. As the mentioned genetic information carried by the living organisms is not considered in calculations based on the conventional exergy approach, the application of the eco-exergy concept is recommended strongly for systems that involve living organisms.\(^{[39]}\) The eco-exergy calculations of the living organisms were accomplished by applying the formulae suggested by Jørgensen et al.\(^{[40]}\) [Eq. (8)]:

\[
\phi = \frac{\Phi}{RT_0} = 7.34 \times 10^3 \text{Ex}_{\text{MO}} + \text{Ex}_{\text{MO}} \ln 20 \left( \frac{\text{number of nucleotides} (1 - \text{number of repeating genes})}{3} \right) \tag{8}
\]

The weighting factor could be computed as follows [Eq. (9)]:

\[
\beta = \frac{\phi}{RT} = \frac{7.34 \times 10^3 \text{Ex}_{\text{MO}}}{\ln 20 \left( \frac{\text{number of nucleotides} (1 - \text{number of repeating genes})}{3} \right)} \tag{9}
\]

Accordingly [Eq. (10)],

\[
\beta = 1 + \frac{\ln 20 \left( \text{number of nucleotides} (1 - \text{number of repeating genes}) \right)}{3 \times 7.34 \times 10^5} \tag{10}
\]

As \( \ln(20) \approx 3 \), Equation (10) can be simplified as follows [Eq. (11)]:

\[
\beta = 1 + \frac{\left( \text{number of nucleotides} (1 - \text{number of repeating genes}) \right)}{7.34 \times 10^5} \tag{11}
\]

in which \( \beta \) represents the ratio of the eco-exergy to the chemical exergy. The amount of genetic information embedded in a living organism can be expressed through this weighting factor and has been found to be 8.5 for bacteria.\(^{[40]}\)

Finally, the eco-exergy content of the living organisms in the photo-bioreactor at a given time was obtained by applying Equation (12):

\[
\Phi = 18.7 \beta m_{\text{MO}} \tag{12}
\]

The exergetic equivalent of the mechanical work applied to the culture medium by an orbital shaker was approximated using Equation (13):

\[
\text{Ex}_{\text{CM}} = m_{\text{CM}} a d \Delta t \tag{13}
\]

in which \( m \) represents the mass of culture medium and \( a \) and \( d \) are the orbital shaker’s acceleration [ms\(^{-2}\)] and the shaking domain, respectively. Finally, \( \Delta t \) denotes the time interval between two consecutive measurements. Therefore, \( \approx 6.3996 \text{kJ} \) mechanical work (exergy) was applied from the orbital shaker to the culture medium in each 12 h time interval.

A 60 W tungsten lamp was used in this study to supply different levels of light intensity (i.e., 500, 1000, and 1500 lux) to the exterior surface of the photo-bioreactor (serum bottle). The tungsten lamp subsystem applied to supply light included an input parameter, that is, electric power transferred to the lamp (\( W_{\text{el}} \)), and output parameters, that is, visible emitted light (\( L_{\text{vis}} \)) and generated heat content transferred from the lamp to the environment (\( Q_{\text{TL}} \)). The methodology suggested by Asada and Shukuya\(^{[41]}\) was used to compute the amount of light exergy delivered to the culture medium. The energy balance equation based on the first law of the thermodynamics for the tungsten lamp can be represented using Equation (14):

\[
W_{\text{el}} = L_{\text{vis}} + Q_{\text{TL}} \tag{14}
\]

Additionally, the entropy balance equation based on the second law of the thermodynamics for the tungsten lamp can be expressed as [Eq. (15)]:

\[
S_{\text{el}} + S_{\text{gen}} = S_R + \frac{Q_{\text{TL}}}{T_{\text{TL}}} \tag{15}
\]

in which \( S_{\text{el}}, S_{\text{gen}}, \) and \( S_R \) represent the entropy flux of the electrical power, entropy generation, and entropy flux of ir-
The energy balance equation for the tungsten lamp subsystem can be expressed as [Eq. (16)]:

\[ \text{Ex}_{\text{EL}} = \text{Ex}_R + \left(1 - \frac{T_0}{T_{\text{TL}}}\right)Q_{\text{TL}} + \text{Ex}_{\text{des,TL}} \]  (16)

in which \(\text{Ex}_{\text{EL}}, \text{Ex}_R,\) and \(\text{Ex}_{\text{des,TL}}\) are the exergy flux of the electrical power, exergy flux of irradiated light, and cumulative exergy destruction, respectively.

Furthermore, the exergy of the electrical power supplied to the lamp can be given as [Eq. (17)]:

\[ \text{Ex}_R = W_{\text{EL}} - T_0S_{\text{EL}} \]  (17)

Additionally, the exergy of the incident light irradiated from the tungsten lamp can be estimated using Equation (18):

\[ \text{Ex}_R = L_{\text{R}} - T_0S_{\text{R}} \]  (18)

The exergy destruction for the tungsten lamp can be obtained by multiplying the entropy generation and the reference temperature as follows [Eq. (19)]:

\[ \text{Ex}_{\text{des,TL}} = T_0S_{\text{gen}} \]  (19)

Shukuya\[42\] stated that only 34% of the incident light from a tungsten (incandescent) lamp is exergy (useful work) by considering the aforementioned theory. Therefore, the magnitude of the exergy applied to the culture medium by the tungsten lamp was obtained using Equation (20):

\[ \text{Ex}_{\text{DL}} = (1 - C_s)\alpha A I_{\text{TL}} \Delta t \]  (20)

in which \(C_s\) represents the reflection coefficient of the serum bottle and \(\alpha\) denotes the energy to exergy ratio (0.34) for the incident light from the incandescent lamp. Moreover, \(A\) and \(I_{\text{TL}}\) are the surface area of the serum bottle and light intensity, respectively. Finally, \(\Delta t\) represents the time interval between two consecutive measurements.

Notably, the photo-bioreactor used in this study was considered as a transparent material that reflects a known amount of the light delivered to its exterior surface from the tungsten lamp. However, as the angle of the incident light irradiated from the tungsten lamp to the external surface of the photo-bioreactor could affect the light reflection fraction significantly, this angle was considered as 0–90\(^\circ\) in this study. Eventually, the equation presented by Fresnel was adopted to calculate the reflection coefficient of the photo-bioreactor glass as follows\[43\] [Eq. (21)]:

\[ C_s = \left| \frac{1}{2} \left| \frac{n_1 \cos \theta_i - n_2 \cos \theta_t}{n_1 \cos \theta_i + n_2 \cos \theta_t} \right|^2 + \frac{1}{2} \left| \frac{n_1 \cos \theta_t - n_2 \cos \theta_i}{n_1 \cos \theta_t + n_2 \cos \theta_i} \right|^2 \]  (21)

in which \(n_1\) and \(n_2\) are the refractive indices of the air (1.000277) and borosilicate glass (1.47), respectively. The refractive indices of both air and glass were adopted from Ref. [44]. \(\theta_i\) denotes the angle made by the incident light with the line perpendicular to the interface, and \(\theta_t\) is the angle made by the refracted rays with the same line.

The phenomena that occur at the material interface (air and glass) as well as the variables of the Fresnel equations [Eq. (21)] are shown in Figure 3. It is clear that the incident and reflected angles are equal because of the reflection law. Moreover, the links between the incident and transmitted angles can be expressed according to the Snell’s law\[45\] [Eq. (22)]:

\[ n_1 \sin \theta_i = n_2 \sin \theta_t \]  (22)

Accordingly, the reflection coefficient of the serum bottle was found to be 0.145. This value was obtained by taking an average from the reflection coefficients with the incidence angles of 0–90\(^\circ\) in 1\(^\circ\) intervals. Based on the above-mentioned theory, 1.168, 2.337, and 3.506 kJ exergy was delivered to the culture medium from the tungsten lamp at light intensities of 500, 1000, 1500 lux, respectively, during each 12 h.

The exergy efficiency of the biological hydrogen production in the batch photo-bioreactor through the WGS reaction between two consecutive measurements (12 h) can be expressed as follows [Eq. (23)]:

\[ \psi = \frac{\text{Ex}_{\text{DL}}}{\text{Ex}_{\text{in}}} = \frac{\text{Ex}_{\text{CMO}} + \text{Ex}_{\text{SG}} + \text{Ex}_{\text{MO}} - \text{Ex}_{\text{DL}} + \text{Ex}_{\text{OS}}}{\text{Ex}_{\text{in}}} \]  (23)

Finally, the normalized exergy destruction as a decision making tool for renewability, sustainability, and cost-effectiveness of the biological hydrogen production process was...
computed by dividing the overall exergy destruction of the process by the exergy of the biohydrogen produced during 120 h of CO fermentation [Eq. (24)].

\[ N_{\text{Ex,des}} = \frac{\text{Ex}_{\text{H}_2}}{\text{Ex}_{\text{bio}}} \]  

(24)

**Results and Discussion**

Thermodynamic analysis of a batch-mode photobiological hydrogen production was performed using the experimental data. The effects of different sodium acetate (as the carbon source) concentrations of 1, 1.5, and 2 g L\(^{-1}\) and light intensities of 500, 1000, and 1500 lux were investigated using both conventional exergy and eco-exergy concepts. Thanks to the unique theoretical background of the exergy analysis, this approach could provide invaluable information not only on the process efficiency but also on the thermodynamic irreversibilities or exergy destruction that occur during the fermentation process. The obtained results could be used by experts in the decision-making process to understand and assess the sustainability and productivity of large-scale continuous photobioreactors.

The effect of the sodium acetate concentration and light intensity on the exergy quantity of the syngas as a function of fermentation time computed based on Equation (6) is shown in Figure 4. In general, the exergy content of the syngas decreased for the all substrate concentrations and light intensities as the fermentation process progressed. This was because the *R. rubrum* oxidized CO with higher standard chemical exergy to CO\(_2\) and H\(_2\) with lower standard chemical exergy values (Table 2). Interestingly, the exergy of the syngas at the sodium acetate concentration of 1 g L\(^{-1}\) and light intensity of 500 lux increased at the initial stage of the fermentation process because of the trivial oxidation of CO to CO\(_2\). However, the exergy content of the syngas decreased drastically at 2 g L\(^{-1}\) acetate concentration and 1500 lux light intensity possibly because of the remarkable utilization of CO. Furthermore, the exergy content of the syngas at 1 g L\(^{-1}\) substrate concentration and 1000 lux light intensity increased significantly after 96 h of the fermentation process. This might be related to the favorable production of H\(_2\) at the end of the process. However, it is difficult or even impossible to judge the productivity and sustainability of the biological hydrogen production using the exergy quantity of the syngas. Therefore, these two important aspects of the process must be assessed simultaneously by considering the exergetic values of the all components involved in the CO fermentation. After that, one could compute exergy destruction and its extensions using whichever decision-making on process productivity and sustainability would be possible.

The effect of various sodium acetate concentrations and light intensities on the exergy of the liquid culture medium over 120 h of the fermentation process is shown in Figure 5. It is clear that the exergy of the culture medium decreased nonlinearly with fermentation time for all the experimental conditions. This behavior was attributed to the assimilation
of the organic substrate available in the culture medium by the photosynthetic bacteria to produce purple biopigments and generate biomass. Notably, the microorganisms consume acetate and CO as the carbon source during the fermentation. However, the incident light from the tungsten lamp had an important role to provide energy for microbial cell growth and maintenance. Furthermore, the bacteria attained the required electrons from the light or organic substrate to produce H$_2$ efficiently. This is why an optimal balance between light intensity and substrate concentration is required to maintain the cell density and hydrogen production at an acceptable level during photobiological hydrogen production. Clearly, intensive substrate exergy consumption was found at the light intensity of 1000 lux and acetate concentrations of 1.5 and 2 g L$^{-1}$. This could be attributed to the rapid consumption of the carbon source for bacterial cell growth in the presence of sufficient organic substrate and light irradiation. However, both substrate concentration and culture medium exergetic content had similar trends as the chemical exergy of the culture medium was related directly to the dosage of organic substrate according to Equation (3).

The exergy and eco-exergy contents of the microorganisms at different light intensities and sodium acetate concentrations as a function of the fermentation time are shown in Figure 6. Clearly, both exergetic and eco-exergetic values of the microorganisms increased exponentially as the fermentation time increased. However, the rate of these increments decreased towards the end of the process because of the slower growth of the bacterial cells after 60 h of the fermentation process. The results showed that the acetate concentration had a profound effect on both the exergy and eco-exergy of the microorganisms. Additionally, these values were affected significantly by the light intensity. The maximum values of exergy and eco-exergy were observed at a light intensity of 1000 lux and organic substrate dosage of 1.5 g L$^{-1}$, whereas the minimum values of exergy and eco-exergy were observed at 1500 lux light intensity and 2 g L$^{-1}$ sodium acetate concentration.

An increase of the substrate dosage from 1 to 1.5 g L$^{-1}$ at a constant light intensity led to a 30% increase in the exergy and eco-exergy of the microorganism (Figure 6). However, an increase of the light intensity from 1000 to 1500 lux lowered the exergy and eco-exergy values of the microorganism drastically. These low exergy values were mainly because of, on one hand, the prevention of the light from penetrating deep into the medium because of the shadows of the dark purple biopigments in the serum bottle and, on the other hand, the inactivation of CODH at high light intensities.[46] Moreover, both the exergy and eco-exergy of the microorganisms under given fermentation conditions had similar trends as the eco-exergy of the microorganisms was computed based on their chemical exergy. However, the eco-exergy content of the microorganisms was 8.5 times greater than its chemical exergy according to Equation (12). This was because the conventional exergy does not take into account the information embedded in the genomes of the living organisms as obtainable work.
The effect of various sodium acetate concentrations and light intensities on the exergy destruction of the biological hydrogen production using both exergy and eco-exergy concepts versus the fermentation time is shown in Figure 7. Evidently, the exergy destruction based on both concepts remained almost constant during the fermentation process. However, the exergy destruction based on the conventional exergy approach was greater than that of the eco-exergy concept. This is because the work of the genetic information embodied in the genomes of the living organisms is disregarded by the conventional exergy approach. Moreover, the maximum and minimum exergy destruction values were obtained at the highest and lowest light intensities (i.e., 1500 and 500 lux), respectively, using both approaches. This means that the light intensity was the main factor that affects the exergy destruction during the investigated photobiological hydrogen production. Therefore, an increase of the hydrogen production at a lower practical light intensity could be applied as a useful scenario to elevate the sustainability of biohydrogen production. Nevertheless, meaningful thermodynamic indicators such as normalized exergy destruction should also be employed by taking into account the rate of biohydrogen production for decision making on light intensity and substrate concentration to improve the process performance rate and environmental benignity.

The variations in the exergy efficiency of biological hydrogen production at different sodium acetate concentrations and light intensities using both conventional exergy and eco-exergy concepts as a function of the fermentation time are shown in Figure 8A and B, respectively. Unlike the exergy efficiency based on the eco-exergy concept, the conventional exergy efficiency remained almost unchanged during the fermentation process. This is because the conventional exergy destruction was almost constant during the photobiological hydrogen production, as elucidated previously. In general, the exergy efficiency based on the eco-exergy concept was significantly higher than that obtained using the conventional exergy analysis because of the lower eco-based exergy destruction. Furthermore, an increase of the light intensity increased the exergy destruction drastically for this reason. Therefore, the exergetic efficiency and subsequent sustainability index of the photobiological hydrogen production could be enhanced if one could keep biological hydrogen production at the lowest feasible light intensity. The maximum exergy efficiency based on the conventional exergy approach was obtained for a light intensity of 500 lux and substrate concentration of 2 g L$^{-1}$. Interestingly, the maximum exergy efficiency based on the eco-exergy approach was achieved at a light intensity of 1000 lux and substrate concentration of 1.5 g L$^{-1}$. Such disagreement confirms why the eco-exergy concept must be considered as an adaptable framework to scrutinize the financial benefits and environmental impacts of an energy conversion system that involves living organisms.

The variations of the normalized exergy destruction for the whole process using both conventional exergy and eco-exergy concepts for various sodium acetate concentrations and light intensities versus the fermentation time are shown in Figure 9. As the exergy efficiency alone could not be employed as a holistic indicator, this thermodynamic indicator
was defined to make decisions about the light intensity and carbon source concentration to identify the most eco-friendly and cost-effective fermentation conditions. Notably, the process yield alone (e.g., biohydrogen production) cannot be a reliable criterion to assess the performance of biofuel production technologies either. This is because the resource destruction to produce a given amount of a renewable fuel is not considered by conventional analyses. Therefore, it could be concluded that only the normalized exergy destruction could provide meaningful information by integrating the exergy destruction and process yield into a single conceptual framework. Moreover, the normalized exergy destruction based on the eco-exergy concept was slightly lower than that obtained using the conventional exergy approach. This could be because the overall exergy or eco-exergy increase of the microorganisms during 120 h of the CO fermentation was negligible compared to the overall mechanical work and light exergies applied throughout the process. However, even slight differences could play an important role in the decision-making process in large-scale biofuel production processes. The lowest normalized exergy destruction was 179.7 and 171.54 kJkJ for a sodium acetate concentration of 1 g L\(^{-1}\) and light intensity of 1000 lux using both conventional exergy and eco-exergy concepts, respectively. Thus, these conditions could be recommended for large-scale continuous hydrogen production to protect the environment and save energy and materials. Interestingly, the light intensity of 1000 lux and acetate concentration of 1.5 g L\(^{-1}\) was found to be suitable in our previous report as the best combination for bio-

**Figure 8.** Effect of various sodium acetate concentrations and light intensities on the exergy efficiency using A) conventional exergy and B) eco-exergy concepts.

**Figure 9.** Effect of various acetate concentrations and light intensities on the normalized exergy destruction using conventional exergy and eco-exergy concepts.
logical hydrogen production in terms of cell growth and biogas yield. This indicates the suitability of thermodynamic indicators, particularly the exergy concept, for the analysis of various renewable-fuel-production systems to distinguish the most eco-friendly and cost-effective conditions. In other words, the sustainability and productivity of biofuel production pathways can be evaluated quantitatively and qualitatively using exergy and its extensions, which cannot be achieved by using biotechnological parameters. This is because biotechnological parameters could mislead as they identify the optimum conditions based on the highest product yield, and such conditions could potentially result in the highest resource destruction. On the contrary, exergetic performance assessment could select optimal operating conditions of biofuel production systems accurately and systematically by considering both product yield and resource destruction. Hence, future research efforts should be directed towards how to minimize exergy destruction in biofuel production process to increase their renewability and sustainability indices.

Conclusions

A comprehensive thermodynamic analysis using the conventional exergy and eco-exergy concepts was performed for decision making on substrate concentration and light intensity in the photobiological fermentation of CO in a batch-mode bioreactor. The results obtained confirmed that the eco-exergy concept could provide unique insights beyond those of conventional exergy analysis, which thereby provides a useful design tool for photobiological hydrogen production. This could be attributed to the conceptual novelty of the eco-exergy approach in embedding the work of information carried by living microorganisms in terms of exergy. A sodium acetate concentration of 1 g L⁻¹ and 1000 lux light intensity were found to be the most suitable conditions for biohydrogen production according to the normalized exergy destruction obtained using both concepts. In general, the eco-exergy concept could be considered as an effective tool to assess the sustainability and productivity of biological routes for renewable fuels production from a thermodynamic point of view. Future studies should link the economic and environmental principles with exergy analysis through methodologies such as exergoeconomic and exergoenvironmental analyses to address the problems associated with large-scale biological hydrogen production. These analyses will provide worthwhile insights into the optimization of biological hydrogen production to achieve the most cost-effective and eco-friendly fermentation conditions.

Experimental Section

Microbial cultivation and bioreactor preparation

In this batch study, the phototrophic anaerobic non-sulfur bacterium R. rubrum ATCC 25903 adopted from the American Type Culture Collections (USA) was grown using acetate as the carbon source. The composition of the culture medium in 1 L was: 1.15, or 2 g Na₂HPO₄, 1 g yeast extract, 1.25 g (NH₄)₂SO₄, 0.2 g MgSO₄·7H₂O, 0.07 g CaCl₂·2H₂O, 0.01 g ferric citrate, 0.02 g ethylenediaminetetraacetic acid (EDTA), 0.6 g KH₂PO₄, 0.9 g K₂HPO₄. Thereafter, 1 mL of the trace metal solution, which consisted of 0.01 g ZnSO₄·7H₂O, 0.02 g MgSO₄·H₂O, 0.01 g H₂BO₃, 3 g ferric citrate, 0.01 g CuSO₄·5H₂O, 0.5 g EDTA, 0.02 g ammonium molybdate, and 0.2 g CaCl₂·2H₂O, was added. B-vitamin solution (7.5 mL), which included 0.2 g nicotinamide, 0.4 g thiamine HCl, 0.2 g nicotinic acid, and 0.008 g biotin, was also added to the medium, and the final volume was brought to 1 L using distilled water. The pH of the medium was adjusted to 6.9 by adding 0.2 mL HCl and 0.2 mL NaOH solutions.

The medium (50 mL) was transferred into each 165 mL serum bottle under nitrogen gas. The bottles with liquid medium were then heated in an autoclave at 121 °C for 15 min. The medium in the sealed, stoppered serum bottle was inoculated with a 5% (v/v) of seed culture and incubated at 30 °C by using an orbital shaker (B Braun, Bethlehem, PA, USA) at 200 rpm. Syngas (55% CO, 15% Ar, 20% H₂, and 10% CO₂) was purged into the each serum bottle. Argon gas was added as an internal standard for gas analysis. A tungsten lamp (60 W) was used as the source of light for the serum bottles at 500, 1000, and 1500 lux measured by using a light intensity meter (Lux-meter, Sper Scientific, Taipei, Taiwan).

Sampling and analysis

Gas samples (200 μL) were obtained every 12 h from the gas phase of the serum bottles by using a gastight syringe (Hamilton Co., USA). The gas samples were analyzed by using an Autosystem XL PerkinElmer gas chromatograph (USA) equipped with a thermal conductivity detector (TCD) and a Carboxen 1000 column (Supelco, USA) as described previously. Additionally, acetate concentration in liquid samples taken every 12 h was analyzed by using a Hewlett Packard gas chromatograph (USA) equipped with a flame ionization detector (FID) and a Carbowax 20 m column (Supelco). The GC procedure adopted to measure the acetate concentration was described previously. A Cecil 1000 spectrophotometer was used to determine the cell concentration of R. rubrum by measuring the optical cell density.

Nomenclature

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Greek symbols

- \( R \): Universal gas constant \([8.314 \text{ J mol}^{-1} \text{K}^{-1}]\)
- \( \Phi \): Eco-exergy content \([\text{kJ}]\)
- \( \beta \): Weighting factor
- \( \Psi \): Exergy efficiency
- \( \theta_i \): Angle made by the incident light with the line perpendicular to the interface
- \( \theta_t \): Angle made by the refracted rays with the same line
- \( \alpha \): Energy-to-exergy ratio of tungsten light
- \( \varepsilon \): Standard chemical exergy \([\text{kJ mol}^{-1}]\)

Subscripts

- 0: Dead state
- A: Ash
- C: Carbon
- CM: Culture medium
- des: Destruction
- DL: Delivered light
- eL: Electrical power
- Ex: Exergy
- gen: Generation
- H: Hydrogen
- in: Inlet
- i, j: Numerator
- MO: Microorganisms
- N: Nitrogen
- O: Oxygen
- OS: Orbital shaker
- out: Outlet
- R: Radiated light
- S: Sulfur
- SG: Syngas
- t: Fermentation time
- TL: Tungsten light
- \( \Delta t \): Time step

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Keywords: biocatalysis · biotransformations · hydrogen · synthesis design · thermodynamics


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