Exergy-based sustainability assessment of continuous photobiological hydrogen production using anaerobic bacterium *Rhodospirillum rubrum*

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1. Introduction

Environmental crisis and depleting fossil fuel resources have pushed many investigators to seek eco-friendly and replenishing sources of energy (Jaber et al., 2015). That perfectly explains why renewable fuels production has become an attractive domain of research with an aim of partially or completely replacing the fossil fuels during the past few decades (Edama et al., 2014; Odetoeye et al., 2014; Aladetuyi et al., 2014). Furthermore, due to carbon neutral nature of biofuels, they are considered as promising solution to overcome the intensifying environmental impacts of fossil fuels combustion. Amongst various renewable energy carriers, biohydrogen has secured a unique place due to its high heating value and regeneration capability (Ozgür et al., 2010; Hsieh and Huang, 2013; Hay et al., 2013). Nevertheless, most of the hydrogen produced is supplied by non-renewable sources such as steam reforming or partial oxidation of natural gas, hydrocarbons, and coal as well as water splitting (Edwards et al., 2008). Hence, exploring and scaling up renewable and sustainable biohydrogen production routes in order to meet the increasing global energy demands and to warrant the future of global energy security seem vital.

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Up to date, various renewable routes for biohydrogen production such as biomass gasification, artificial photosynthesis, biophotolysis, and photo-fermentation have been developed with each having their own benefits and shortcomings. Among those, photobiological oxidation of harmful carbon monoxide through water-gas shift (WGS) reaction in a non-thermal process via phototrophic bacteria is considered industrially feasible and a very promising pathway for eco-friendly production of hydrogen (Najafpour et al., 2004; Younesi et al., 2008; Ismail et al., 2008). Nevertheless, it still essential to carry out a holistic assessment using advanced engineering tools to investigate the sustainability of such photobiological hydrogen production. In line with that, thermodynamic-based frameworks have been extensively used to evaluate and improve the sustainability of biological hydrogen production pathways (Toomssen et al., 2008; Modarresi et al., 2010).

Thermodynamic analyses, particularly exergy analysis could provide useful and meaningful information about the sustainability of a process due to their notable benefits in identifying the quality of various forms of energy. Nowadays, exergy analysis on the basis of the combination of the first and second law of thermodynamics is taken into account as a robust tool for measuring both quantity and quality of energy and material flows more conceptually and precisely than the conventional energy analysis. Simply speaking, exergy is defined as the maximum magnitude of theoretical work produced by a stream of matter, heat or work when brought to equilibrium with a dead state through reversible processes (Akbulut and Durmuş, 2010; Aghbashlo et al., 2012a, 2012b). Exergy analysis could also serve as a practical methodology for analysis and optimization of various energy conversion systems including biophotolysis with respect to their sustainability and renewability issues by diagnosing locations, types, and quantities of thermodynamic irreversibilities.

Up to now, a remarkable amount of research works have been successfully carried out to evaluate the sustainability of different hydrogen production procedures according to the exergy concept and its extensions. For instance, Hiraki and Akiyama (2009) applied exergetic life cycle analysis for waste aluminum treatment system with co-production of hydrogen and aluminum hydroxide. In the same year, energy and exergy analyses were applied for the oxygen production step of a copper–chlorine thermochemical water decomposition cycle for hydrogen production (Orhan et al., 2009). Later, Modarresi et al. (2010) exergetically analyzed the fermentative production of hydrogen in order to study the influence of the used feedstock, applied process parameters, as well as process and heat integration measures on process efficiency. Furthermore, Moniri et al. (2012) employed exergy analysis to compare five possible different pathways based on hydrogen sulfide splitting cycle for hydrogen production.

In addition, Ratlamwala and Dincer (2012) analyzed an integrated Cu–Cl thermochemical cycle for hydrogen production at various operating parameters such as mass fraction, pressure, and temperature. In another survey, Iribarren et al. (2014) evaluated environmental and thermodynamic performance of a base-case system for hydrogen production via biomass gasification using a life cycle assessment and an exergetic analysis. Furthermore, Zhang et al. (2015) presented a detailed energy and exergy of syngas containing hydrogen produced from rice husk gasification in an entrained flow reactor at various reactor temperatures and equivalence ratios. Recently, Fan et al. (2016) applied exergy analysis to investigate the beneficiary aspects of chemical looping combustion thermally coupled with steam methane reforming process over conventional steam methane reforming process.

Based on the successful published works mentioned above, there is no doubt that exergy analysis has an undeniable potential for evaluating various renewable production pathways. Although the scientific literature on biohydrogen production using batch and continuous bioreactors is abundant (Younesi et al., 2008; Gebicki et al., 2010; Boran et al., 2012), detailed exergetic performance assessment of continuous biological hydrogen production for decision making on process variables has been hardly performed so far. It is worth mentioning that exergetic formulation was recently developed and presented for a batch photo-bioreactor in our previous survey (Dadak et al., 2016). In the present survey, the exergetic formulation was extended to an industrial-like continuous bioreactor. More specifically, the present study was set to assess the sustainability of biohydrogen production in a continuous photo-bioreactor via Rhodospirillum rubrum from syngas through the use of both conventional and eco-exergy concepts at varying mass flow rates of the liquid media. The findings of such a holistic study could be very beneficial to engineers and researchers involved in design, simulation, optimization, and analysis of industrial-scale photo-bioreactors for achieving the most cost-effective and eco-friendly renewable hydrogen production routes.

It is worth mentioning that among all of the photosynthetic bacteria, Rhodospirillum rubrum is capable of producing biohydrogen at a close rate to the stoichiometric value. R. rubrum is a purple nonsulfur bacterium falling under the family Rhodospirillaceae and the Alpha subdivision of the kingdom Proteobacteria (Najafpour et al., 2006). Rhodovibrin and spirilloxanthin are the major carotenoids (pigments) giving the purple color to this microbe while also enabling it to absorb light energy for photosynthesis (Ogbonna et al., 1995). This spiral-shaped gram negative bacteria contains vesicular photosynthetic membranes with a cell width of 0.8–1 μm and a length of 3–10 μm. This organism also contains chlorophyll b (Brock et al., 2000). This organism is capable of growing both aerobically with oxygen or anaerobically using light as energy. It also grows in the dark without production of hydrogen and without cell pigments (Wakayama and Miyake, 2002). This bacterium reportedly carries out the CO oxidation to produce biohydrogen via the WGS pathway. The CO oxidation pathway contains CO dehydrogenase and hydrogenase enzymes, both of which are induced by the presence CO. The photosynthetic bacteria have four terminal enzymes mediating the H2 metabolism: (i) nitrogenase, (ii) a classical hydrogenase, (iii) a fermentative hydrogenase and (iv) a CO-linked hydrogenase (Koku et al., 2002).

2. Materials and methods

2.1. Microbial cultivation, culture media and syngas preparation

The anaerobic non-sulfur bacterium Rhodospirillum rubrum ATCC 10801 was obtained from the American Type Culture Collection (USA) and was used for continuous hydrogen fermentation from syngas in a continuous 2 L Biostat photobioreactor (B Braun, Germany). Purple non-sulfur bacteria such as R. rubrum, Rhodobacter sphaeroides, Rhodopseudomonas palustris and others are frequently used in photofermentation process to produce biohydrogen (Wu et al., 2012; Budiman et al., 2015; Hay et al., 2015). The microbes were grown in an enriched ATCC medium at 30 °C, under agitation and a light intensity provided by two tungsten lamps (40 W). The ingredients of the culture media included (in 1 L distilled water): 2.5 g acetic acid (neutralized by NaOH; pH 6.9), 1 g yeast extract, 1.25 g ammonium sulphate, 0.2 g magnesium sulphate heptahydrate, 0.07 g calcium chloride dihydrate, 0.01 g ferric citrate, 0.02 g EDTA, 0.6 g potassium dihydrogen phosphate, and 0.9 g dipotassium hydrogen phosphate. Moreover, the trace metal solution (1 mL) containing 0.01 g zinc sulphate heptahydrate, 0.02 g magnesium sulphate heptahydrate, 0.01 g boric acid, 3 g ferric citrate, 0.01 g copper sulphate pentahydrate, 0.5 g...
ethylenediaminetetraacetic acid, 0.02 g ammonium molybdate, and 0.2 g calcium chloride dihydrate was also added. Finally, B-vitamin solution (7.5 mL) including 0.2 g nicotinamide, 0.4 g thiamine HCl, 0.2 g nicotinic acid, and 0.008 g biotin was included in the culture media. The syngas composition was: 55% of carbon monoxide (CO), 20% of hydrogen (H2), 15% of carbon dioxide (CO2), and 10% of argon (Ar).

2.2. Experimental setup and working conditions of different parts

The experimental setup used for continuous photobiological hydrogen production via bacterial fermentation is depicted schematically in Fig. 1. A continuous flow of syngas and liquid media from separate reservoirs were supplied into the photobioreactor. Furthermore, pH, temperature, dissolved oxygen and volume level were automatically monitored in the reactor. Purified nitrogen was used in order to provide anaerobic conditions. A 5% inoculum was used and the experiments were conducted at 30 °C under a total pressure of 1 atm. In order to support the microbial growth, two tungsten lamps (40 W) were provided on both sides of the photobioreactor for supplying the average illumination of 1500 lux. A lux-meter (Sper Scientific, Taipei, Taiwan) was used for measuring the supplied light intensity. Various flow rates of the liquid media (0–0.85 mL/min) as well as different agitation speeds (150–500 rpm) were investigated over 540 h of continuous photobiological hydrogen production. A constant syngas flow rate of 12 mL/min was used throughout the experiments. The variations in the mass flow rate of the liquid media and agitation speed during the continuous fermentation of carbon monoxide at constant gas flow rate of 12 mL/min are tabulated in Table 1.

2.3. Analyses

The cell dry weight of the microorganisms was measured by reading the optical density at 400 nm using a spectrophotometer (Cecil Instrument, Cambridge, England). Then, the obtained data from the experiments were used for computing both exergy and eco-exergy content of the living organisms. Gas samples were analyzed by a gas chromatograph (Perkin Elmer, Autosystem XL, Boston, MA, USA) equipped with a thermal conductivity detector (TCD) and Carboxene 1000 column (Supelco, USA) as previously described by Najafpour et al. (2003). The residual acetate concentration in the liquid samples was also analyzed by a Hewlett Packard 5890 series II gas chromatograph (USA) equipped with a flame ionization detector (FID) and a 80/120 Carbopack B-DA/4% Carbowax 20 M column (Supelco, USA) as described elsewhere (Younesi et al., 2008; Najafpour and Younesi, 2007).

2.4. Theoretical considerations

A schematic representation of the bioreactor as a control volume including both input and output terms which was applied for exergy analysis is depicted in Fig. 2. According to this figure, the mass and exergy balance equations for the bioreactor could be written as:

\[
\frac{(M_{MO,t+\Delta t} - M_{MO,t}) + (M_{CM,t+\Delta t} - M_{CM,t})}{\Delta t} = (m_{LM,in} - m_{LM,out}) + (m_{SG,in} - m_{SG,out}) + (m_{CW,in} - m_{CW,out}) - m_{MO,out}
\]

where \(M_{MO}\) and \(M_{CM}\) are the masses of microorganisms and culture media in the bioreactor (kg), respectively, at times \(t\) and \(t+\Delta t\). In fact, these terms represent the variations in the mass of microorganisms as well as the variations in the mass of culture media in the

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>Agitation speed (rpm)</th>
<th>Liquid flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–60</td>
<td>150</td>
<td>0.00</td>
</tr>
<tr>
<td>60–132</td>
<td>500</td>
<td>0.00</td>
</tr>
<tr>
<td>132–192</td>
<td>500</td>
<td>0.25</td>
</tr>
<tr>
<td>192–288</td>
<td>500</td>
<td>0.40</td>
</tr>
<tr>
<td>288–360</td>
<td>500</td>
<td>0.55</td>
</tr>
<tr>
<td>360–432</td>
<td>500</td>
<td>0.65</td>
</tr>
<tr>
<td>432–492</td>
<td>500</td>
<td>0.25</td>
</tr>
<tr>
<td>492–540</td>
<td>500</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of the continuous bioreactor applied in this research for carbon monoxide fermentation using Rhodospirillum rubrum.
The exergy loss rate to the cooling water was determined as

\[
\dot{E}_{\text{des}} = n_{\text{LM}} \left( \sum_j x_j \epsilon_j + RT_0 \sum_j x_j \ln(x_j) \right) + m_{\text{LM}} C_{\text{LM}} \left( T_{\text{LM}} - T_0 \right) - T_0 \ln \left( \frac{T_{\text{LM}}}{T_0} \right)
\]

(4)

where \(n_{\text{LM}}\) and \(m_{\text{LM}}\) denote the mole (mol/s) and mass rates (kg/s) of the inflow and outflow liquid media, respectively, \(x_j\) is the molar fraction of each component within the inflow and outflow liquid media, \(\epsilon_j\) denotes the standard chemical exergy \((kJ/mol)\) of \(j\) th component of the inflow and outflow liquid media, \(C_{\text{LM}}\) is the specific heat capacity \((kJ/kg K)\) of the inflow and outflow liquid media, and \(T_{\text{LM}}\) is the temperature \((K)\) of inflow and outflow liquid media.

The specific chemical exergy of the organic materials used in the preparation of the fresh liquid media were specified using a semi-theoretical mathematical model developed by Song et al. (2012).

\[
\varepsilon_{\text{OM}} = 363.439C + 1075.633H - 86.308O + 4.14N + 190.798S - 21.1A
\]

(5)

\[
\varepsilon_{\text{OM}} = M_{\text{OM}} \varepsilon_{\text{OM}}
\]

(6)

where \(\varepsilon_{\text{OM}}\) and \(M_{\text{OM}}\) denote the specific chemical exergy \((kJ/kg)\) and molecular mass of an organic material \((kg/mol)\), respectively, while C, H, O, N, S and A stand for the percentages of carbon, hydrogen, oxygen, nitrogen, sulfur, and ash in an organic material, respectively.

Furthermore, the standard chemical exergy of the inorganic materials used in the liquid media preparation were compiled from the published literature (Wall, 1998) (Table 2).

The following equation was applied to obtain the exergy flow rates of the inflow and outflow syngas:

\[
\dot{E}_{\text{SG}} = n_{\text{SG}} \left( \sum_i x_i \epsilon_i + RT_0 \sum_i x_i \ln(x_i) \right) + m_{\text{SG}} C_{\text{SG}} \left( T_{\text{SG}} - T_0 \right) - T_0 \ln \left( \frac{T_{\text{SG}}}{T_0} \right)
\]

(7)

where \(n_{\text{SG}}\) and \(m_{\text{SG}}\) denote the mole (mol/s) and mass (kg/s) rates of the inflow and outflow gases, respectively, \(x_i\) is the molar fraction of each component, \(\epsilon_i\) stands for the standard chemical exergy \((kJ/mol)\) of \(m\) th component, \(P\) and \(P_0\) stand for the pressure \((kPa)\) of the syngas and the dead state, respectively, \(C_{\text{SG}}\) shows the specific heat capacity \((kJ/kg K)\) of inflow and outflow gases. The dead state pressure was assumed to be 100 kPa. The standard chemical exergy of the syngas components are given in Table 2.

The specific heat of the inflow and outflow gases was determined as follows:

\[
C_{\text{SG}} = \sum_n X_n C_n
\]

(8)

where \(X_n\) and \(C_n\) represent the mass fraction and specific heat of each syngas component, respectively. It is worth mentioning that the equations used for calculation of the specific heat capacity of the syngas components can be found elsewhere (Sonntag et al., 1998).

The exergy loss rate to the cooling water was determined as follows:
The standard chemical exergy of syngas components as well as materials used for the culture media preparation.

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical formula</th>
<th>Standard chemical exergy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$H_2O$</td>
<td>0.9$^a$</td>
</tr>
<tr>
<td>Acetate sodium</td>
<td>NaC$_2$H$_3$O$_2$</td>
<td>873.599$^b$</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>C$<em>6$H$</em>{12}$O$_2$</td>
<td>9535.466$^b$</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>660.6$^c$</td>
</tr>
<tr>
<td>Magnesium sulphate heptahydrate</td>
<td>MgSO$_4$·7H$_2$O</td>
<td>87.0$^d$</td>
</tr>
<tr>
<td>Calcium chloride dehydrate</td>
<td>CaCl$_2$·2H$_2$O</td>
<td>89.7$^d$</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>Fe$_3$O$_4$</td>
<td>2076.735$^b$</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>Na$_2$CO$_3$</td>
<td>1020.5$^c$</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>NaHCO$_3$</td>
<td>121.1$^c$</td>
</tr>
<tr>
<td>Glucose</td>
<td>C$<em>6$H$</em>{12}$O$_6$</td>
<td>183.18$^c$</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>C$_3$H$_6$O$_3$</td>
<td>123.14$^d$</td>
</tr>
<tr>
<td>Ethylene diamine tetraacetic acid (EDTA)</td>
<td>C$<em>{10}$H$</em>{16}$N$_2$O$_8$</td>
<td>5006.73$^c$</td>
</tr>
<tr>
<td>Ammonium molybdate</td>
<td>(NH$_4$)$_6$Mo$_7$O$_2$</td>
<td>1882.025$^e$</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H$_2$</td>
<td>236.1$^c$</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>CO</td>
<td>275.10$^c$</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>CO$_2$</td>
<td>19.87$^c$</td>
</tr>
<tr>
<td>Argon</td>
<td>Ar</td>
<td>11.69$^c$</td>
</tr>
</tbody>
</table>

$^a$ Obtained from Wall (1998).
$^b$ Calculated based on Equations (5) and (6).
$^c$ Obtained from http://www.exergoecology.com/.
$^d$ Since thermodynamic data for these materials were not available, their standard chemical exergy values were calculated for an equimolar mixture of their components.
$^e$ Due to the lack of thermodynamic data for ammonium molybdate, its standard exergy was computed using Equations (5) and (6). The error out of this assumption was negligible since its amount in culture media was only 9.81 × 10$^{-7}$ kg.

\[
\dot{E}_{x, CW} = m_{CW} C_{CW} \left[ T_{CW} - T_0 + \frac{T_{CW}}{T_0} \ln \frac{T_{CW}}{T_0} \right] \quad (9)
\]

where $m_{CW}$ is the mass flow rate (kg/s) of the cooling water, $C_{CW}$ is the specific heat capacity (kJ/kg K) of water, and $T_{CW}$ is the temperature (K) of the cooling water at the inlet and outlet sections.

The chemical exergy of the microorganisms accumulated in the photobioreactor at a given time was computed using the following equation:

\[
\dot{E}_{x, MO} = 18700 m_{MO} \quad (10)
\]

where $m_{MO}$ denotes the mass of microorganisms (kg) accumulated in the photobioreactor and 18,700 kJ/kg is the chemical exergy content of average detritus (Jørgensen et al., 2005; Draganovic et al., 2013).

Similarly, the chemical exergy of the outflow microorganisms from the photobioreactor was attained using the following equation:

\[
\dot{E}_{x, MO, out} = 18700 m_{MO, out} \quad (11)
\]

where $m_{MO, out}$ denotes the mass flow rate (kg/s) of outflow microorganisms.

Additionally, the eco-exergy concept was also employed to evaluate and analyze the biological hydrogen production process beyond the classical exergy analysis due to the presence of the living organisms in the continuous photobioreactor. In other words, the living organisms available in the bioreactor carry a considerable amount of genetic information. Unlike the classical exergy approach, such information are taken into account when the eco-exergy calculations are conducted (Draganovic et al., 2013). Moreover, it should be noted that such information is, in fact, a form of energy causing the microorganisms to possess more work energy compared to the non-living constituents which only carry chemical energy (Jørgensen et al., 2005). The following formula was used to calculate the eco-exergy ($\Phi$) of the living organisms:

\[
\frac{\Phi}{RT_0} = 7.34 \times 10^5 E_{x, MO} + E_{x, MO} \ln20^{1-N_{NRG}} \quad (12)
\]

\[
\beta = 1 + \frac{\ln 20(NN(1 - N_{NRG}))}{3 \times 7.34 \times 10^5} \quad (13)
\]

where $NN$ and $N_{NRG}$ are the number of nucleotides and number of repeating genes, respectively.

Since $\ln20 \approx 3$, therefore, the above equation could be rewritten as:

\[
\beta = 1 + \frac{NN(1 - N_{NRG})}{7.34 \times 10^5} \quad (14)
\]

where $\beta$ is the ratio of the eco-exergy to the chemical exergy accounting for how information is embodied in a given microorganism and was found to be 8.5 for bacteria (Jørgensen et al., 2005).

As mentioned above, the medium was agitated using a motor placed on top of the bioreactor and Reynolds number ($Re$) is required in order to calculate the magnitude of the mechanical work (exergy) transferred to the culture media. Therefore, the following formula was employed for this purpose:

\[
Re = \frac{\rho N D^2}{\mu} \quad (15)
\]

where $\rho$ and $N$ represent the fluid density (kg/m$^3$) and the impeller rotation speed (1/s), respectively. Moreover, $D$ and $\mu$ represent the diameter of impeller (m) and fluid viscosity (kg/m s), respectively. The magnitude of the exergy in the form of mechanical work delivered to the liquid media (which is proportional to the Reynolds
number) was obtained using the following equation (Ismail et al., 2008):

\[ P = N_p p R^3 D^5 \]  

(16)

where \( P \) and \( N_p \) represent the power of agitator (kW) and power number of the agitation rate, respectively. The power number was obtained using the standard curve provided by Perry et al. (1997).

Eventually, the exergy rate of the mechanical work transferred to the liquid media during the fermentation process was identical to the agitator power. The continuous photobioreactor used was made of a transparent glass reflecting a known fraction of the light delivered to the culture media. Moreover, the amount of the reflected light from the exterior surface of the photobioreactor was strongly affected by the angle of the incident light. In this study, this value was considered to be in the range of 0°–90° with 1° intervals. Additionally, Fig. 3 schematically represents the light reflection phenomenon occurring on the exterior surface of the photobioreactor. Obviously, a known fraction of the incident light was reflected and the rest would be supplied to the culture media. Accordingly, the following Fresnel equation was employed for computing the reflection coefficient of the continuous photobioreactor glass (Orfanidis, 2004):

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

(25)

where \( R \) represents the reflection coefficient of the photobioreactor, \( n_1 \) and \( n_2 \) are the refractive indices of the air and glass (clear Borosilicate glass), respectively. These values were considered at 1.00 and 1.47 for air and glass, respectively, using online refractive index website (Polyanskiy, 2015). \( \theta_i \) denotes the angle that incident light makes with the normal of the interface, and \( \theta_t \) is the angle that refractive rays make with the same line. Moreover, a quick glance at Fig. 3 reveals that the incident and reflected angles are identical considering the law of the reflection. Additionally, the Snell’s law was used in order to find the relation between the incident and refracted angles according to Hecht (2002) as follows:

\[ n_1 \sin \theta_i = n_2 \sin \theta_t \]  

(26)

The reflection coefficient of the continuous photobioreactor was found to be 0.145. According to the above-mentioned theory and assumptions, the exergetic value of the tungsten light delivered to the culture media was determined at 36.61 J/s.

\[ \hat{E}_{\text{ex}} = \hat{E}_\beta + \frac{1}{T_{TL}} Q_{TL} + \hat{E}_{\text{dest,TL}} \]  

(20)

\[ \hat{E}_{\text{ex}} = \hat{E}_\beta + \frac{1}{T_{TL}} Q_{TL} + \hat{E}_{\text{dest,TL}} \]  

(21)

\[ \hat{E}_{\text{ex}} = \hat{E}_\beta + \frac{1}{T_{TL}} Q_{TL} + \hat{E}_{\text{dest,TL}} \]  

(22)

\[ \hat{E}_{\text{ex}} = \hat{E}_\beta + \frac{1}{T_{TL}} Q_{TL} + \hat{E}_{\text{dest,TL}} \]  

(23)

where \( \hat{E}_{\text{ex}} \) denotes the exergy content (kJ) of the electrical energy, \( \hat{E}_\beta \) is the exergy (kJ) of the incident light transferred to the external surface of the photobioreactor, and \( \hat{E}_{\text{dest,TL}} \) represents the exergy destruction (kJ) within the lamp.

Shukuya (2012) reported that only 34% of the incident light from the tungsten lamp (incandescent lamp) could be as exergy or useful work according to the above-mentioned theory. Therefore, the magnitude of the exergy transferred to the culture media from the tungsten lamp could be computed using the following formula:

\[ \hat{E}_{\text{ex}} = (1 - \alpha) A I_{TL} \]  

(24)

where \( \alpha \) and \( A \) are the reflection coefficient and surface area (m²) of the photobioreactor that receives the incident light from the tungsten lamp, respectively. \( \alpha \) denotes the energy to exergy ratio of the incident light which was considered to be 34%. Finally, \( I_{TL} \) denotes the light intensity of the incident light (W/m²).

Fig. 3. Schematic representation of the incident light and the related angles.
The rational exergy efficiency related to the photobioreactor was achieved by applying the following equation:

\[ \psi = \frac{\Delta Ex_{out}}{\Delta Ex_{in}} = \frac{\Delta Ex_{LM,i} + \Delta Ex_{LM,i} + \Delta Ex_{MO,i} + \Delta Ex_{CW,i} + \Delta Ex_{DL} + \Delta Ex_{W}}{\Delta Ex_{LM,i} + \Delta Ex_{LM,i} + \Delta Ex_{MO,i} + \Delta Ex_{CW,i} + \Delta Ex_{DL} + \Delta Ex_{W}} \Delta t \]

In addition to the rational exergy efficiency, exergy efficiency of the process was also calculated using the following equation in order to select the optimum flow rate of the syngas as well as the best agitation speed by considering the exergy content of the biogas throughout the experiment.

\[ \phi = \frac{\Delta Ex_{H_2}}{\Delta Ex_{des}} \Delta t \]

where \( \Delta Ex_{H_2} \) represents the chemical exergy rate of the produced hydrogen.

Furthermore, the following formula was used in order to compute the energetic productivity index for decision making on the mass flow rate of the liquid media:

\[ \psi = \frac{\Delta Ex_{H_2}}{\Delta Ex_{des}} \]

where \( \Delta Ex_{H_2} \) and \( \Delta Ex_{des} \) denote the chemical exergy rate of the produced hydrogen and the destructed exergy rate during the fermentation, respectively.

The sustainability index (SI) of the fermentation process could be found as follows:

\[ SI = \frac{1}{1 - \psi} \]

Finally, the energetic improvement potential rate was achieved using the equation given below:

\[ IP = \frac{(1 - \psi)(\Delta Ex_{in} - \Delta Ex_{out})}{\Delta t} \]

### 3. Results and discussions

Fig. 4 presents the effect of fresh liquid media on the exergy rate of the inflow and outflow gases over 540 h of continuous carbon monoxide fermentation. The exergy rate of the outflow syngas varied between 1.12 and 1.57 kJ/s, whereas the exergy rate of the inflow syngas was fixed at 1.62 kJ/s. Clearly, the exergy rate of the outflow syngas was significantly lower than the exergy rate of the inflow syngas for all the liquid media flow rates. This occurred due to the oxidation of the carbon monoxide with higher standard chemical exergy for producing molecular hydrogen and carbon dioxide with lower standard chemical exergies through the WGS reaction. The process can be represented by the following reaction:

\[ H_2O + CO \rightarrow H_2 + CO_2 \]

Moreover, the exergy rate of the outflow syngas followed an approximately stable trend after the first 180 h of the fermentation process indicating satisfactory bio-upgrading of carbon monoxide to hydrogen.

The effect of fresh liquid media on exergy and eco-exergy of the microorganisms accumulated in the bioreactor over 540 h of continuous fermentation is depicted in Fig. 5. The maximum exergy...
and eco-exergy of the microorganisms in the photobioreactor at the liquid media flow rate of 0.65 ml/min were found to be 117.31 kJ and 997.18 kJ, respectively. Obviously, both the exergy and eco-exergy of the microorganisms amass in the photobioreactor exponentially increased at the beginning of the fermentation process due to the microbial growth. Nevertheless, an approximately stable trend with negligible fluctuations was observed towards the end of the fermentation process. These results agreed well with the cell dry weight reported in our previous publication (Najafpour et al., 2003). This could be attributed to the fact that the exergy and eco-exergy values of the microorganisms are strongly related to the cell weight based on Equations (10) and (12). However, both the exergy and eco-exergy of the microorganisms in the bioreactor decreased at the end of the process due to the wash-out phenomenon at higher liquid media flow rates. This could have unfavorably lowered the cell density and residual time in the bioreactor and, consequently, the CO uptake and H2 production.

Furthermore, a comparison between the exergy and eco-exergy of the microorganisms in the bioreactor confirmed that the eco-exergy value at a given time was considerably higher than its corresponding chemical exergy value. In fact and as mentioned earlier, the eco-exergy approach conceptually reflects the work of information embodied in the genomes of living organisms in the energetic computations. Nevertheless, both exergy and eco-exergy values of the microorganisms in the bioreactor had analogous tendencies since the eco-exergy of the microorganisms was determined by multiplying the weighting factor (β) and chemical exergy content of the microorganisms (Equation (12)).

Fig. 6 exhibits the effect of fresh liquid media on exergy and eco-exergy of the outflow microorganisms from the bioreactor during the 540 h of continuous fermentation. Obviously, both exergy and eco-exergy of the outflow microorganisms and liquid media flow rate possessed similar trends over the fermentation period. More specifically, there was a certain drop in exergy and eco-exergy rates between 400 and 500 h. This could be ascribed to the fact that the cell population and retention time were directly proportional to the liquid media flow rate.

The effect of liquid media flow rate on the exergetic values of the inflow and outflow liquid media as well as the exergetic content of the culture media over the 540 h of continuous fermentation are represented in Fig. 7. The exergetic quantities of the inflow and outflow liquid media were found to be in the range of 0.00–1.86 kJ/s and 0.00–1.41 kJ/s, respectively. Generally, a similar trend was observed between a given liquid media flow rate and its corresponding exergetic inflow rate. This occurred because of a direct relation between the liquid media mole rate and the exergy of the inflow liquid media according to Equation (4). Evidently, the exergy of the outflow liquid media was markedly lower than the exergy of the inflow liquid media for all the liquid media flow rates. This could be attributed to the consumption of the carbon source to support microbial survival and growth and water to produce molecular hydrogen through the WGS reaction (Chen et al., 2008; Pakpour et al., 2014). Furthermore, the exergy rate of the outflow liquid media followed a similar trend to that of the inflow liquid media. In better words, the exergy of the outflow liquid media was strongly influenced by the exergy of the inflow liquid media. On the other hand, the exergetic value of the culture media drastically decreased at the start of the fermentation process due to the rapid utilization of sodium acetate by the microorganisms for propagation, growth, and survival. In general, the microbial growth or exergy of the microorganism was the important factor affecting the exergy quantity of the culture media.

Fig. 8 shows the effect of liquid media flow rate on exergy destruction rate and normalized exergy destruction in the bioreactor during the fermentation period. The exergy destruction rate and normalized exergy destruction were found to be in the range of 0.26–1.18 kJ/s and 0.97–3.06 using the conventional exergy and eco-exergy approaches over 540 h, respectively. Generally, it could be roughly concluded that both exergy destruction rate and normalized exergy destruction had stable trends after 72 h of the fermentation process due to the adequate growth of the microorganisms in the culture media at the beginning of the process.
Several reasons could be considered for exergy destruction in a photobioreactor such as severe chemical and biochemical reactions, intensive heat and mass transfer phenomena, quick mixing process, light absorption and conversion by microorganisms, carbon source utilization by microorganisms, and microbial cell growth and death (Aghbashlo et al., 2016). Hence, it could be concluded that for cost-effective and environmentally-benign production of biohydrogen, the exergy destruction rate must be reduced during photobiological hydrogen production. Furthermore, the exergy destruction rate and normalized exergy destruction obtained in the present study using both concepts did not significantly vary from each other probably because of the sluggish growth of the microorganisms and low volume of the employed photobioreactor. Nevertheless, the eco-exergy approach is undoubtedly preferred in scrutinizing renewable energy conversion systems involving living organisms from the sustainability and productivity viewpoints.

The effect of liquid media flow rate on the rational and process exergy efficiencies over the 540 h of continuous fermentation is shown in Fig. 8. The rational exergy of the process ranged from 48.79% to 84.51% and 49.24%–84.54% using the conventional and eco-exergy concepts, respectively. Moreover, the process exergetic efficiency was found to be in the range of 14.71–22.90% and 14.71–22.84% using the conventional exergy and eco-exergy concepts, respectively. Both the rational and process exergy efficiency stabilized after 72 h of the fermentation process due to the same reason previously elucidated.

A comparison between the process exergy efficiency with the rational exergy efficiency showed that the process exergy efficiency was lower than the rational energy efficiency for all the liquid media flow rates. It should also be mentioned that the results of the process exergetic efficiency were more meaningful compared to those of rational exergy efficiency due to establishing a conceptual linkage between the process yield and the resources supplied to the photobioreactor in the process efficiency computations. On the other hand, the rational exergy efficiency dramatically dropped with increasing exergy destruction rate using both concepts since the rational exergy efficiency is inversely proportional to the rate of the exergy destruction (see Figs. 8 and 9). Accordingly, future works should include the optimization of the operational conditions of continuous photobioreactors via conceptual thermodynamic indicators such as process exergy efficiency through advanced optimization techniques like evolutionary algorithms for attaining eco-friendly and cost-effective biohydrogen production.

Finally, Fig. 10 expresses the effect of liquid media flow rate on exergetic improvement potential rate and sustainability index using both concepts during the 540 h of continuous fermentation. The sustainability index of the process varied from a minimum value of 1.97 to a maximum value of 6.47 using both concepts. Moreover, the exergetic improvement potential rate of the fermentation process was obtained in the range of 40.32–601.39 J/s via both approaches. Overall, it could be concluded that the exergy efficiency of the fermentation process could be maximized when the rate of exergy destruction was minimized. Moreover, the sustainability index of the process could be profoundly improved by increasing exergy efficiency or decreasing exergy destruction rate according to Equation (30). The comprehensive approach proposed herein will be very beneficial to those involved in design, simulation, and optimization of large-scale continuous photobioreactors for biohydrogen production to improve performance from the sustainability and productivity perspectives.

4. Conclusions

Exergetic performance parameters of a continuous photobioreactor for hydrogen production through the WGS reaction were examined using both conventional exergy and eco-exergy concepts at varying liquid media flow rate during 540 h of continuous carbon monoxide fermentation. The process exergetic efficiency varied in the range of 14.71–22.90% and 14.71–22.84% using the conventional exergy and eco-exergy concepts, respectively. Moreover, the normalized exergy destruction was found to be in the range of 0.97–3.06 using both concepts. Generally, optimization of bioreactor’s operation should be adjusted towards maximizing the process exergetic efficiency while minimizing the normalized exergy destruction to achieve the most sustainable mode of operation. Even though the eco-exergy approach did not lead to different results from those obtained using the conventional exergy concept, future works should be however directed towards the use of eco-exergy analysis for energy conversion systems involving living organisms. Furthermore, eco-exergy analysis showed to be a powerful tool for optimization of biological hydrogen production while simultaneous assessment of sustainability and productivity features are also considered. However, eco-exergy concept might not display significant differences in the exergetic performance parameters of bioreactors of low volume or when microorganisms of slow growth rate are involved. The results of the present survey also proved that the exergy analysis could enable engineers and researchers to identify the most environmentally- and financially-relevant pathway for renewable fuels production by providing inclusive information on process improvement potentials.
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