Original Research Article

Using exergy to analyse the sustainability of fermentative ethanol and acetate production from syngas via anaerobic bacteria (Clostridium ljungdahlii)

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Abstract

In this study, exergetic performance assessment of ethanol and acetate fermentation in a batch bioreactor using Clostridium ljungdahlii under various syngas pressures in the range of 0.8–1.8 atm was carried out. The exergetic parameters of the bioreactor were determined using both conventional exergy and eco-exergy concepts in order to select the most sustainable and productive operational conditions. In general, a significant difference was found between the exergetic values of the process using the conventional exergy and eco-exergy approaches. Overall, the eco-exergy concept showed to be a more appropriate approach for analysing and optimizing energy conversion systems including living organisms due to the incorporation of the work of the genetic information embodied in the genomes of microorganisms. The lowest overall normalized exergy destruction was found to be 49.96 kJ/kJ Ac + EtOH and 40.54 kJ/kJ Ac + EtOH at initial pressure of 1.8 atm using the conventional exergy and eco-exergy concepts, respectively. Accordingly, this condition would be recommended to be applied in pilot- or industrial-scale bioreactors. Finally, the employed approach herein could be used to shed light on on-going attempts to improve the performance of the bioreactors for biofuel production considering the sustainability and productivity perspectives.

Introduction

Over the past century, non-renewable fossil-based fuels such as oil, gas, and coal have been the major resources to meet the global energy demands [1]. As a result of such enormous pressure on these global reserves, their rapid depletion would be inevitable in the near future. Furthermore, extensive combustion of fossil fuels and the consequent emission of greenhouse gases (GHG) has led to detrimental environment impacts [2]. To prevent this potentially-tragic trend and to achieve sustainable eco-friendly energy supplies, global attention has been paid to alternative energy resources like biofuels [3,4]. Biofuels are especially crucial in the transportation sector because the other alternative energy carriers such as wind and solar are too dilute to be used in internal combustion engines.

Among various biofuels, bioethanol is considered as the most promising candidate for future energy security [5]. However, bioethanol produced from food-grade feedstock (i.e., first generation bioethanol) has been widely criticized over the food vs. fuel competition [6]. On the contrary, bioethanol produced from lignocellulosic feedstock (i.e., second generation bioethanol) not only poses no threat to global food security but also could result in over 40% more reduction in GHG emissions compared with corn ethanol [7]. Among the different methods used for ligno-ethanol production, gasification of biomass to syngas followed by anaerobic fermentation has gained a growing interest due to its higher efficiency and cost-effectiveness [8]. Nevertheless, diagnostic renewability metrics like thermodynamic indicators should still be applied in order to monitor and recommend the best pathway...
for production of renewable fuels such as ligno-ethanol with respect to their sustainability and productivity issues.

Recently, thermodynamic-based approaches such as energy and exergy analyses have emerged as essential tools for design, analysis, and optimization of renewable fuel production systems. However, energy analysis which is based on the first law of the thermodynamics, is incapable of detecting the quality of various forms of energy [9–11]. This issue could be greatly overcome by introducing exergy concept based on both the first and second laws of the thermodynamics [12–14]. By employing exergy approach, not only magnitudes and locations of resource destructions through an energy conversion system can be transparently recognized but also useful insights into the energy efficiency improvements can be achieved [15–17]. It is worth quoting that exergy-based indicators are universally-accepted engineering metrics as decision-making tools for assessing and improving biofuel production systems.

During the last decade, numerous studies have been carried out on the use of exergy analysis for sustainability appraisal of various ethanol production routes [18–24]. For example, Ometto and Roma [19] used chemical exergy concept for atmospheric impact assessment of the life cycle emissions of a bioethanol-electricity cogeneration system from sugarcane in Brazil. In a different study, van der Heijden and Pratsinski [21] applied the exergy concept to analyze the ethanol production using syngas produced by a steam-blown indirect gasifier from woody feedstock. In a more recent investigation, Matteson et al. [24] compared the exergetic life cycle efficiency of the life cycle emissions of a bioethanol-electricity cogeneration system from sugarcane in Brazil. In a different study, van der Heijden and Pratsinski [21] applied the exergy concept to analyze the ethanol production using syngas produced by a steam-blown indirect gasifier from woody feedstock. In a more recent investigation, Matteson et al. [24] compared the exergetic life cycle efficiency of the life cycle emissions of a bioethanol-electricity cogeneration system from sugarcane in Brazil.

Materials and methods

Microbial cultivation and growth media

The detailed information on the ethanol and acetate fermentation via C. ljungdahlii ATCC 55383 (Culture Collection of University Boulevard, Virginia, USA) and its growth medium can be found in our previous report [8,27]. Briefly, an incubator shaker (Barnstead/Lab-Line, MaxQ 4000, USA) was used for growing the bacteria at 37 °C anaerobically. The reducing agent was prepared by adding 0.9 g NaOH, 4 g cysteine HCl, and 4 g Na2S·9H2O into 100 mL distilled water. Moreover, the Wheaton serum bottles (125 mL) were filled with 50 mL of the growth media for the fermentation experiments. Then, an oxygen-free gas mixture (containing N2 and CO2) was purged into each bottle to achieve anaerobic conditions. Subsequently, all the bottles were sealed completely using Gas impermeable butyl rubber septum-type stoppers and aluminum crimp seals and autoclaved.

The bottles were then cooled down to room temperature and the gas phase was replaced by syngas of different partial pressure ranging from 0.8 atm to 1.8 atm. Thereafter, 0.5 mL of the reducing agent and 5 mL of the seed culture were added into each serum bottle and the cultures were grown on an orbital shaker incubator (100 rpm) for 120 h. The syngas used consisted of 10% CO2, 15% Ar, 20% H2, and 55% CO (Air Products, Malaysia). Argon was used as an internal standard. The bottles were removed from the shaker for gas and liquid media sampling for about 2 min every 12 h. Liquid samples were directly withdrawn with a sterile syringe from the bioreactor through a septum located on the top of the bioreactor and were analyzed for optical density, as well as acetate and ethanol concentrations.
Microorganism, syngas, and liquid media analyses

A spectrophotometer (Cecil 1000 series, Cambridge, UK) was used for the determination of the cell dry weight concentration by measuring the optical density at 600 nm [8]. Then, the optical density was computed to cell dry weight according to the standard calibration curve. Furthermore, a gas chromatograph (Autosystem XL, Perkin Elmer, MA, USA) equipped with Carboxen 1000 column (Supelco, USA) and a thermal conductivity detector (TCD) was applied for analyzing the compositions of both inlet and outlet gases as described in our previous publication [8]. Another gas chromatograph (Perkin Elmer, Clarus 500) equipped with Carboxen 1000 column (Supelco, USA) and a flame ionization detector (FID) was used for measuring the acetate and ethanol concentration in the liquid media [8]. Notably, analytical reagents of ethanol and acetic acid were used for the quantitative analysis. Moreover, 10 calibration solutions in the range of 0.1–1 g/L for ethanol or acetic acid with 0.1 g/L intervals were used. It is worth mentioning that all experiments were replicated three times.

Theoretical consideration

The schematic view of the bioreactor used for sustainability assessment of ethanol and acetate fermentation, as a control mass with input and output terms, is depicted in Fig. 1.

According to Fig. 1, the exergy balance equation for the bioreactor consisting of liquid media, living organisms, and syngas can be written as follows:

\[
Ex_{CM} = Ex_{SG} + Ex_{MO} + Ex_{OS} = Ex_{CM,1,M} + Ex_{SG,1,M} + Ex_{MO,1,M} + Ex_{det.}
\]  

(1)

The following equation was used to determine the exergetic value of the culture media:

\[
Ex_{CM} = n \left( \sum x_i \epsilon_i + RT_0 \sum x_i \ln (x_i) \right).
\]  

(2)

It should be noted that the dead state temperature \(T_0\) was considered 37 °C, i.e., temperature of the shaker incubator.

The specific chemical exergy carried by organic materials used in liquid media preparation was calculated using the formula presented by Song et al. [28]:

\[
ex_{OM} = 363.439C + 1075.633H - 86.308O + 4.147N + 190.798S - 21.1A
\]  

(3)

Moreover, the following formula was employed in order to calculate the standard chemical exergy of the organic materials in the liquid culture media:

\[
ex_{OM} = M_{OM}ex_{OM}
\]  

(4)

Furthermore, the following basic equation was used to compute the standard chemical exergy of some inorganic substances, which were not available in the published literature.

\[
ex = -\Delta G + \sum n_j \epsilon_j - \sum n_k E_k
\]  

(5)

Additionally, the standard chemical exergy of the remained inorganic materials were adopted from the published literature [29] (Table 1).

The following equation was utilized in order to calculate the chemical exergy of the syngas in the bioreactor:

\[
ex_{SG} = n \left( \sum x_i \epsilon_i + RT_0 \sum x_i \ln (x_i) + RT_0 \ln \left( \frac{P}{P_0} \right) \right)
\]  

(6)

The dead state pressure was taken into account to be 101 kPa. Furthermore, the standard chemical exergy of the syngas components consisting of CO, H\(_2\), CO\(_2\), and Ar was found to be 275.10 kJ/mol, 236.1 kJ/mol, 19.87 kJ/mol, and 11.69 kJ/mol, respectively [29]. The exergy value of the microorganisms was found using the following equation:

\[
ex_{MO} = 18.7 m_{MO}
\]  

(7)

It is worth mentioning that 18.7 kJ/g represents the chemical exergy content of the detritus [30].

In addition to the conventional exergy approach, eco-exergy concept was taken into account in order to perform an accurate and acceptable analysis of the system. In fact, the application of the eco-exergy concept was essential owing to the fact that the living organisms such as C. ljungdahlii carry a noticeable amount of work energy in the form of genetic information compared with the non-living constituents which only carry chemical energy. Unlike the eco-exergy concept, such kind of genetic information is often ignored in the conventional exergy concept [30]. The following equations presented by Jørgensen et al. [31] were used for computing the eco-exergy content of the microorganisms:

Fig. 1. Schematic illustration of the bioreactor used for ethanol and acetate fermentation.
\[ \Phi = 7.34 \times 10^7 \text{Ex}_{\text{MO}} + \text{Ex}_{\text{MO}} \ln 20^{\text{(N(1 - NRG)/3)}} \]  

(8)

\[ \beta = 1 + \frac{\ln 20(\text{N(1 - NRG)})}{3 \times 7.34 \times 10^7} \]  

(9)

Eq. (9) can be simplified as follows by considering the \( \ln(20) \approx 3 \):

\[ \beta = 1 + \frac{(\text{N(1 - NRG)})}{7.34 \times 10^7} \]  

(10)

The weighting factor \( \beta \) was considered to be 8.5 for bacteria according to Jørgensen et al. [31]. Finally, the eco-exergy quantity \( \Phi \) of the living organisms was achieved by multiplying 8.5 by the exergy content of the microorganisms (Eq. (7)) as follows:

\[ \Phi = 18.7 \times 8.5 \text{m}_{\text{MO}} \]  

(11)

The amount of the exergy transferred to the culture media from the orbital shaker in the form of the mechanical work was obtained by applying the following equation:

\[ \text{Ex}_{\text{OS}} = m_{\text{CM}} \text{d}W \]  

(12)

It must be noted that the amount of the exergy transferred to the culture media in the form of mechanical work at each 12 h time interval was determined to be 6.71 kJ according to Eq. (12).
Finally, the last thermodynamic indicator namely the overall normalized exergy destruction as a decision making tool to determine the most suitable syngas pressure was obtained by dividing the amount exergy destroyed to the exergy of produced ethanol and acetate as follows:

$$N_{\text{Ex,\, des}} = \frac{E_{\text{Ex,\, des}}}{E_{\text{AC,\, EtOH}}}$$  \hspace{1cm} (14)

**Statistical analysis**

The exergetic calculations of ethanol and acetate fermentation process by C. ljungdahlii in a batch bioreactor were performed using Excel Ver.2007 (MS Office, 2007). Analysis of variance was carried out to find the effects ($p < 0.05$) of syngas pressure, fermentation time, and exergetic concept (i.e., conventional exergy and eco-exergy approaches) on the exergetic parameters of the ethanol and acetate fermentation from syngas. Multiple comparison tests were performed using the LSD’s test at the 95% confidence level. All the analyses were carried out by using the SPSS Statistics 15.0 (SPSS Inc., Chicago, IL, USA) software.

**Results and discussions**

Exergy-based sustainability assessment of ethanol and acetate synthesis from syngas in a batch bioreactor at various pressures was carried out using experimental data. Fig. 2 represents the variations in the exergy of the syngas at various pressures as a function of the fermentation time. According to the results achieved in our previous work shown in Fig. 3 [8], it was observed that the exergy of the syngas continually decreased ($p < 0.05$) for all the pressures towards the end of the process. This could be ascribed to the fact that the carbon monoxide available in the syngas was utilized by the microorganisms for microbial growth as well as for ethanol and acetate production. In fact, when CO is available in the gas phase, the following reactions are dominant:

$$6\text{CO} + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 4\text{CO}_2$$  \hspace{1cm} (15)

$$4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{CO}_2$$  \hspace{1cm} (16)

Furthermore, the CO with higher standard chemical exergy was utilized by the microorganisms through the Wood–Ljungdahl pathway for the production of CO$_2$ with lower standard chemical exergy. However, it should be mentioned that a portion of the produced or initially available CO$_2$ was utilized for ethanol and acetate production. In better words, when the partial pressure of CO$_2$ in the gas phase increases at the expense of a decrease in the partial pressure of CO, the following reactions become dominant:

$$2\text{CO}_2 + 6\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 3\text{H}_2\text{O}.$$  \hspace{1cm} (17)

$$2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}.$$  \hspace{1cm} (18)

These in turn lowered the exergy of the syngas towards the end of the fermentation process. Moreover, a sharp reduction in the exergy quantity of the syngas was observed at pressures of 1.6 atm and 1.8 atm. This could be attributed to the H$_2$ consumption by C. ljungdahlii at these elevated pressures for ethanol and acetate fermentation (see also Fig. 3). In fact, the following reaction becomes dominant because of the high partial pressure of H$_2$ and CO at these elevated syngas pressure values:

$$4\text{CO} + 6\text{H}_2 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2.$$  \hspace{1cm} (19)

In line with the findings of the present study, Abubackar et al. [32] also reported that increasing carbon monoxide pressure from 0.8 bar to 1.6 bar profoundly improved ethanol and acetic acid production. In better words, improvements in ethanol and acetic acid production were observed at pressures above 1.4 atm.
production was indicative of enhanced syngas consumption using *Clostridium autoethanogenum*. Similarly, Singla et al. [33] also found a linear increase in ethanol and acetic acid production by increasing the syngas pressure from 0.5 atm to 2 atm in an anaerobic bacterial mixed culture.

For lower pressure values (i.e., 0.8–1.4 atm), the reduction in the syngas exergy retarded after 60 h fermentation, possibly due to a decrease in ATP formation and subsequent CO uptake rate. Therefore, it seemed that pressurized bioreactors would be more suitable for ethanol and acetate synthesis by *C. ljungdahlii* possibly because of increased mass transfer rate and enhanced CO uptake rate. A comparison between the exergetic values of the syngas determined in the present study and the CO consumption rate reported in our previous publication as shown in Fig. 3 [8], revealed that the exergy of the syngas was profoundly affected by the CO uptake rate. It is also worth mentioning that the difference in the exergy of syngas at the end of fermentation process could be ascribed to the different compositions of the syngas after long time fermentation under different pressures.

The effect of syngas pressure on the exergy of the liquid culture media vs. the fermentation time is exhibited in Fig. 4. Obviously, the exergy content of the liquid culture media dropped ($p < 0.05$) during the early hours of the experiment due to the lower acetate and ethanol production as well as higher consumption rate of the produced acetate by microorganisms as shown in Fig. 5. Similarly, Hursta and Lewis [34] also reported that acetic acid and ethanol production by *Clostridium carboxidivorans* P7 were negligible during the early stages of fermentation process (up to 40 h). Nevertheless, in the present study, the exergy of the liquid culture media increased ($p < 0.05$) for all the pressures towards the end of the fermentation process. This phenomenon could be explained by, (1) the higher amount of ethanol and acetate formation by *C. ljungdahlii* toward the end of the process (Eqs. (15) and (16) and, (2) the lower acetate utilization by the microorganisms due to the cessation of the microbial growth after 48 h of the fermentation process. In agreement with the findings of the present study, Hursta and Lewis [34] in a study carried out for exploring the effect of carbon monoxide partial pressure on the metabolic process of syngas fermentation also argued that ethanol production initiated at least after 40 h of the fermentation process. Moreover, they found that the cell growth rate reached a plateau after 60 h of the fermentation process [34]. However, it should be mentioned that the exergy of the culture media in the present study did not show a clear trend ($p > 0.05$) with increase in syngas pressure.

![Fig. 5](image1.png)

*Fig. 5.* Effects of the syngas pressure on ethanol (A) and sodium acetate (532 B) production [8]. Copyright (2016), with permission from Elsevier.

![Fig. 6](image2.png)

*Fig. 6.* Effect of syngas pressure on the exergy of microorganisms using, (A) conventional exergy, and (B) eco-exergy concepts, respectively.

![Fig. 7](image3.png)

*Fig. 7.* Effects of the syngas pressure on cell dry weight [8]. Copyright (2016), with permission from Elsevier.
Fig. 6A and B present the effect of syngas pressure on both the conventional exergy and eco-exergy contents of the microorganisms as a function of the fermentation time. Obviously, the exergy quantity of the microorganisms increased ($p < 0.05$) towards the end of the fermentation process using both concepts due to an increase in the cell density of the culture. These findings were in a good agreement with the cell dry weight reported in our previous report as indicated in Fig. 7 [8] owing to the fact that both chemical exergy and eco-exergy of the microorganisms are straightforwardly related to the cell weight (see Eqs. (7) and (11)). A similar finding was reported by Hurst and Lewis [34] for the effect of carbon monoxide partial pressure on the average cell concentration of *C. carboxidivorans* P7 as a function of time. Unlike the exergy of the syngas, the exergy of the microorganisms in the present study did not show ($p > 0.05$) a clear trend by increasing the syngas pressures.

Furthermore, a sharp increase in the conventional exergy and eco-exergy contents of the microorganisms was observed between the 12 h and 60 h for the syngas pressures of 0.8 atm, 1.2 atm, and 1.4 atm because of the fast increase in cell population within this period. In case of more elevated pressure values of 1.6 atm and 1.8 atm, such rapid increments in the exergy and eco-exergy of the microorganisms were found to be in the range of 12–96 h. In addition, the exergy and eco-exergy contents of the microorganisms dropped at the end of the fermentation for the syngas pressures of 1.6 atm and 1.8 atm due to the death of the microorganisms under harsh conditions. Singla et al. [33] also argued that by increasing the syngas pressure beyond 2 atm cell growth in an anaerobic bacterial mixed culture was dramatically affected.

The exergy magnitude of the microorganisms using both concepts had a similar trend but with different values. More specifically, the eco-exergy value of the microorganisms was 8.5 times greater than their conventional exergy or chemical exergy content owing to the incorporation of the work of the information carried by living organisms in the eco-exergetic computations (see Eq. (11)).

The variations in the exergy destruction of the syngas fermentation process at various syngas pressures using both the conventional exergy and eco-exergy concepts are presented in Fig. 8A and B, respectively. Unlike the eco-exergy based destruction, the conventional exergy destruction exhibited a similar tendency ($p > 0.05$) for all the syngas pressures during the fermentation process. This occurred due to the inclusion of the work energy in the form of the genetic information embedded in the microbial genome by the eco-exergy approach. In better words, the work of the information embodied in the genomes of the living organisms was the most important factor affecting the exergy destruction based on the eco-exergy concept. Moreover, a comparison between the two graphs shown as Fig. 8 indicates that the exergy destruction based on the eco-exergy approach was lower ($p < 0.05$) than that of the conventional exergy concept at the inception of the fermentation process because of the rapid growth of the microorganisms at this period and the higher eco-exergy content, as previously explained. Interestingly, the exergy destruction based on the eco-exergy concept drastically increased ($p < 0.05$) towards the end of the process due to the trivial growth or even death of the microorganisms, particularly at higher syngas pressures of 1.6 atm, and 1.8 atm. 

Fig. 9A and B depict the exergy efficiencies of the ethanol and acetate fermentation process via *C. ljungdahlii* based on the conventional exergy and eco-exergy approaches at various syngas pressures, respectively. Clearly, the exergy efficiency of the fermentation process based on the eco-exergy approach was

![Fig. 8](image_url) Effect of syngas pressure on the exergy destruction using, (A) conventional exergy, and (B) eco-exergy concepts, respectively.

![Fig. 9](image_url) Effect of syngas pressure on the exergy efficiency using, (A) conventional exergy, and (B) eco-exergy concepts.
could be attributed to the lower exergy destruction using the eco-exergy concept, as previously illustrated. The lowest overall normalized exergy destruction was found to be 49.96 kJ/kg Ac + EtOH and 40.54 kJ/kg Ac + EtOH using the conventional exergy and eco-exergy concepts, respectively, at the syngas pressure of 1.8 atm. Consequently, the syngas with the initial pressure of 1.8 atm was found ($p < 0.05$) to be the most appropriate condition in order to synthesize acetate and ethanol by C. ljungdahli from the syngas investigated. This finding was in contrast those of our previous report showing that maximum acetate production could be obtained at the syngas pressure of 1.4 atm [8]. Moreover, the maximum ethanol formation could be achieved at the initial syngas pressures of 1.6 atm and 1.8 atm.

It is obvious that finding optimal operational conditions with an aim at sustainable biofuel production by solely relying upon process yield in such complex biochemical operations having multiple outputs would be impractical. Therefore, engineering tools like exergy concept should be taken into account as a decision making tool to comprehensively scrutinize the financial benefit and environmental impacts of various energy conversion routes. In general, the pressurized conditions should be employed to increase the sustainability and productivity of large-scale continuous bioreactors by taking into account the financial and environmental issues through the use of advanced exergy-based mythologies like exergoeconomic and exergoenvironmental analyses.

**Conclusion**

Sustainability assessment of acetate and ethanol synthesis in a batch bioreactor from syngas at different pressures was performed using both conventional exergy and eco-exergy concepts. Based on the results obtained, the initial syngas pressure of 1.8 atm was found to be the best condition for acetate and ethanol synthesis from syngas according to the overall normalized exergy destruction computed. At this condition, the exergy efficiency of the bioreactor was found to be in the range of 1.20–2.11% and 1.40–2.41% using the conventional and eco-exergy concepts, respectively, during 120 h of the batch fermentation process. Moreover, the outcomes of the current investigation demonstrated that exergy analysis could provide invaluable information about the irreversibilities occurring in the second generation ethanol production systems, e.g. from syngas. Besides, the eco-exergy concept was found to be a more useful engineering tool compared to the conventional exergy concept for scrutinizing energy conversion systems possessing living organisms because of considering their genetic information in its calculations. The future trends in this area point toward improved process understanding and optimization using advanced exergetic-based approaches like exergoeconomic and exergoenvironmental analyses together with advanced optimization techniques such as evolutionary algorithms for finding the most cost-effective and eco-friendly biofuel production systems.

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**References**


