Exergy and exergy cost analysis of biochemical energy conversion process: Application to the metabolic model of living cells

Assal SELMA[a], Mauro REINI[b], Michele GIANI[c] and Francesca MALFATTI[d]

[a] Dept. of Engineering and Architecture, University of Trieste, Polo di Pordenone-Italy,
assal.selma@phd.units.it,
[b] Dept. of Engineering and Architecture, University of Trieste, Polo di Pordenone-Italy,
reini@units.it,
[c] Instituto nazionale di oceanografia e di geofisica sperimentale - (TS) - Italy, mgiani@inogs.it,
[d] Instituto nazionale di oceanografia e di geofisica sperimentale - (TS) - Italy, fmalfatti@ogs.it,

Abstract:

The idea of a Thermodynamic cost associated with flows in a network of irreversible thermodynamic processes is widely developed in the context of energy engineering, but the general formulation of the Exergy Cost Theory-ECT allows in principle its application also to the biochemical networks.

This paper describes the application of the exergy analysis to a generic metabolic network and an approach for calculating the exergy costs associated with all the flows present in the network, according to the ECT. The main perspective is to use the exergy cost information for defining additional constraints in the Flux Balance Analysis- FBA of the bacterial metabolic network. Which could help identifying directions for the optimization of the biomass production process, and the enhancement of the biofuel use in industry. In fact, this approach mainly relays on the maximization of the produced biomass, for identifying all metabolites fluxes in a biochemical network, with the constraints expressed by the stoichiometric relations of all reactions within the network. Therefore, some additional constraints have to be introduced, in order for guiding the optimization algorithm towards thermodynamically feasible solutions.

The expectation is that, by introducing the actual exergy cost, with their clear physical meaning, the results would be more consistent with the experimental finding, reported in literature, for a wide range of possible environmental conditions.

By applying the unit exergy cost concept, a deeper understanding of the reason why the reaction paths of the same metabolic network changes, in different environmental conditions, is also expected to be achieved.

Keywords:

Exergy analysis; Exergy Cost Theory-ETC; Energy Conversion Process; Thermodynamic Cost, Biomass Production Optimization; Biofuel; Flux Balance Analysis-FBA; Biochemical Networks Of Living Systems Far From Equilibrium.

1. Introduction

Living cells are known as complex and dynamic systems that play a vital role in maintaining the balance of life on Earth. They are composed of various subsystems that interact with each other in a coordinated manner, with each subsystem contributing to the overall functioning of the cell.

Understanding the behavior and performance of these systems is critical for comprehending the underlying mechanisms of cellular processes and for improving the efficiency of biotechnological processes. One approach to analyzing the behavior and performance of living cells is exergy analysis. Exergy analysis is a thermodynamic method that quantifies the amount of energy available for conversion from one form to another and has been widely used in the analysis of energy systems, such as power plants and industrial processes.

In recent years, exergy analysis has been applied to the study of living cells, it has been widely used in the study of living cells, providing a comprehensive understanding of the thermodynamics of these complex and dynamic systems. The application of exergy analysis to living cells has resulted in new insights into cellular behavior and performance, and has led to improvements in the design and operation of bioreactors and bioprocesses. One of the key areas where exergy analysis has been applied to the study of living cells is in the analysis of metabolic pathways.

By quantifying the energy inputs, outputs, and losses in cellular processes, exergy analysis can provide a detailed understanding of the efficiency of metabolic pathways, and identify areas for improvement. This

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information can be used to optimize cellular processes, leading to improved performance and greater sustainability. Another area where exergy analysis has been applied to the study of living cells is in the analysis of ion transport processes, such as ion pumps and channels. By quantifying the energy inputs and outputs of these processes, exergy analysis provides a valuable framework for assessing their efficiency and optimizing their performance.

In this context, exergy analysis has also been used in the study of bioreactors and bioprocesses, providing a thermodynamic framework for the analysis of energy and matter exchange in these systems.

In this paper, we describe the application of exergy analysis to a generic metabolic network, with a focus on calculating the exergy costs associated with all the flows present in the network.

The Exergy Cost Theory (ECT) provides a framework for this analysis, allowing for the formulation of a thermodynamic cost associated with flows in a network of irreversible thermodynamic processes. While the ECT has been widely used in energy engineering, this paper explores its application to biochemical networks. Our main perspective is to use the exergy cost information to define additional constraints in the Flux Balance Analysis (FBA) of the bacterial metabolic network, which is a powerful technique in bioinformatics, used for identifying directions for the optimization of biomass production processes and the enhancement of biofuel use in industry. Nevertheless, the approach mainly relies on the maximization of the produced biomass, with the constraints expressed by the stoichiometric relations of all reactions within the network. This approach requires the introduction of additional constraints for guiding the optimization algorithm towards thermodynamically feasible solutions.

We expect that by introducing the actual exergy cost, with their clear physical meaning, the results would be more consistent with experimental findings reported in literature for a wide range of possible environmental conditions. We also expect to achieve a deeper understanding of why the reaction paths of the same metabolic network change in different environmental conditions by applying the concept of unit exergy cost. Overall, our paper demonstrates the potential of exergy analysis, and the ECT in particular, for optimizing metabolic networks in bioengineering.

2. Exergy analysis of cellular process

Exergy analysis of cellular processes starts with a thermodynamic analysis of the energy exchanges that take place in the cell, including the inputs and outputs of energy, matter, and entropy[1].

The exergy of a process is defined as the maximum useful work that can be obtained from a system, and it is calculated as the difference between the actual enthalpy of the system and the reference enthalpy[2]. The reference enthalpy is the enthalpy of the system in a state of equilibrium at a reference temperature and pressure, usually taken to be the temperature and pressure of the environment.

Exergy analysis can be used to assess the efficiency of various cellular processes, such as metabolic pathways, ion transport, and biochemical reactions[3]. For instance, it is possible to use it to determine the energy conversion efficiency of metabolic pathways, as well as the exergy losses due to internal irreversibilities, [4] [13] such as entropy production.

Additionally, exergy analysis can be used to assess the efficiency of ion transport processes, such as ion pumps and channels, by quantifying the inputs and outputs of these processes from an energetic point of view [5]. By quantifying the energy inputs, outputs, and losses in cellular processes, exergy analysis could be utilized for assessing theefficiency of various metabolic pathways [6].

The late mentioned method provides a valuable framework for the optimization of cellular processes, by identifying areas for improvement in energy and matter exchange. This information can be used to improve the efficiency of cellular processes [7].

3. Exergy Analysis of Biotechnological Processes

Evaluating the potential for energy utilization and efficiency in a given system or process is a key point to fulfill optimization. In the context of biotechnology, exergy analysis is a suitable tool to evaluate the performanceof bioprocesses such as fermentation, biorefining, and biogas production, etc..

The basic principle of exergy analysis is to calculate the maximum useful work that can be obtained from a system or process, based on the availability of energy in the system and the environment.
In bioprocesses,[7] the exergy of the process inputs (such as substrates and nutrients) and the exergy losses (such as waste heat and other by-products) can be quantified. These information can then be employed to identify areas for improvement in terms of energy efficiency and sustainability.

There are several benefits to using exergy analysis in biotechnology. One of the main benefits is that it provides a comprehensive and quantitative assessment of the energy utilization and efficiency of a bioprocess.

Such information is quite useful for enhancement the process conditions, reduce energy costs, and minimize environmental impact.

These kind of analysis is indeed, a valuable tool for evaluating the performance of bioprocesses in terms of energy utilization and efficiency. As the information obtained from an exergy analysis could provide meaningful insights to optimize the process conditions, reduce energy costs, and minimize environmental impact, making bioprocesses more sustainable and environmentally friendly.

4. Glycolysis pathway of Escherichia coli

Escherichia coli is a type of bacterium that uses glycolysis as its primary source of energy, which is a metabolic pathway that breaks down glucose (a simple sugar) into pyruvate, producing energy in the form of ATP [9]. The following is a summary of the glycolysis pathway in *E. coli*:

- Conversion of glucose to fructose-1,6-bisphosphate: Glucose is converted to fructose-1,6-bisphosphate through the action of hexokinase, which adds a phosphate group to the molecule.
- Cleavage of fructose-1,6-bisphosphate: The next step is the cleavage of fructose-1,6- bisphosphate into two three-carbon molecules, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, by the action of aldolase;
- Phosphorylation and isomerization: Both glyceraldehyde-3-phosphate and dihydroxyacetone phosphate are converted to their isomer, 1,3-bisphosphoglycerate, by the action of triose phosphate isomerase. This reaction is also accompanied by the addition of a phosphate group, producing energy in the form of ATP.
- Conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate: The next step is the conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate through the action of phosphoglycerate kinase, which adds another phosphate group, producing more ATP.
- Conversion of 3-phosphoglycerate to 2-phosphoglycerate: The next step is the conversion of 3-phosphoglycerate to 2-phosphoglycerate through the action of phosphoglycerate mutase.
- Conversion of 2-phosphoglycerate to phosphoenolpyruvate: The final step of glycolysis is the conversion of 2-phosphoglycerate to phosphoenolpyruvate (PEP) through the action of enolase. This reaction releases energy in the form of ATP.
- Conversion of phosphoenolpyruvate to pyruvate: The conversion of phosphoenolpyruvate to pyruvate is catalyzed by the enzyme pyruvate kinase. This final step of glycolysis completes the degradation of glucose to pyruvate, producing a net gain of two ATP molecules.

In addition to producing ATP, the degradation of glucose in glycolysis also generates the building blocks for other metabolic processes, including the citric acid cycle, which generates more energy.

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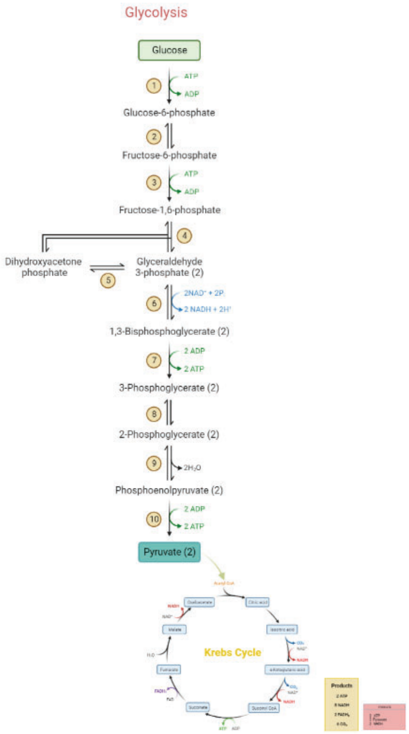

Figure. 1. Glycolysis pathway of *Escherichia. Coli*.

4.1. Exergy analysis and exergy cost of the glycolysis metabolic pathway of *Escherichia coli*

Exergy analysis is a thermodynamic method that assesses the maximum useful work that can be obtained from a system. In the context of the glycolysis metabolic pathway of *E. coli*, the exergy analysis would involve calculating the change in exergy (useful work potential) for e each step of the reaction sequence.

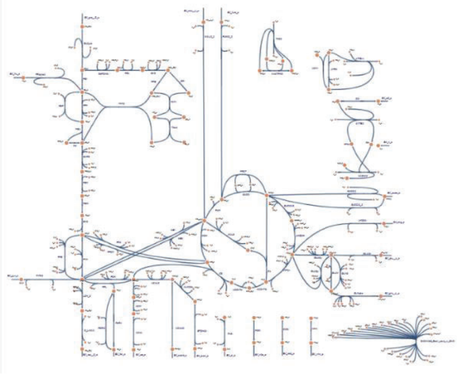

Figure. 2. ESCHER map of the metabolic pathway of *E.coli*. core (**a simplified model**).

(https://escher.github.io/#/app?map=e_coli_core.Core%20metabolism&tool=Builder&model=e_coli_core)

Performing the exergy analysis of the glycolysis pathway involves estimating the change in exergy (by representing the useful work potential) at each step of the whole reaction sequence.

In order to perform exergy analysis, it is necessary to determine the chemical exergy values of the involved compounds in the metabolic pathway. These values represent the maximum amount of work that can be obtained from each compound through chemical reactions under standard conditions.

By taking into account the exergy inputs and outputs of each reaction, the exergetic cost associated with the targeted pathway could be calculated.

Identifying possible relevant compounds in the pathway is also possible through the exergy of the metabolic compounds. (Figure3)

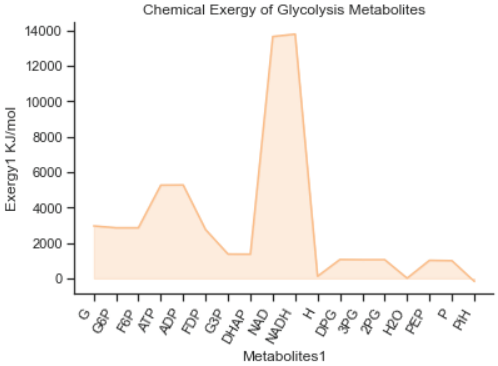

Figure. 3. The chemical exergy of the glycolysis metabolites in standard conditions.

The chemical exergy values in the plot represent the maximum amount of work that can be obtained from the metabolites through chemical reactions, and under standard conditions (298 K, 1 atm, pH 7). The data used toproduce the plot was originally extracted from literature [11].

It demonstrate that the higher the chemical exergy value of a metabolite, the greater its potential for doing useful work. Based on the plot, we can see that glucose-6-phosphate (G6P) has the highest chemical exergy value among all the metabolites in the glycolysis pathway, followed by fructose-6-phosphate (F6P) and 3-phosphoglycerate (3PG).

Which could suggests that these metabolites have the greatest potential for performing useful work in the pathway. In contrast, the lowest chemical exergy values are observed for pyruvate and ATP, indicating that these metabolites have less potential for performing useful work in the pathway.

However, it is important to note that ATP is a crucial energy carrier in the cell and plays a key role in energy metabolism, as showed, the plot gives insight into the chemical potential of the metabolites in the glycolysis pathway and can be used to guide the optimization of the pathway for increased efficiency and energy utilization. (View Figure.3)

The exergy cost represents the irreversibilities or inefficiencies within a giving system and it is a good tool to quantifies the amount of available work that is lost or degraded during the process. Exergy cost calculation involves the comparison between the exergy values of the reactants and the exergy of the products for each reaction in the metabolic pathway.

For accurate K* calculations, reliable chemical exergy values for the compounds involved in glycolysis are crucial as a primary step.

By applying exergy analysis approach and calculating the exergetic cost for each compound in the glycolysis pathway, it is possible to determine the steps with the highest exergy losses.

This sort of information is a suitable way to guide efforts for the optimization of certain pathways and improvement of the overall efficiency and energy utilization of the whole process.

Using the exergy analysis and exergy cost calculation of compounds in the glycolysis metabolic network may be a way to provide valuable insights into the thermodynamic efficiency and sustainability of this essential metabolic process. Furthermore, It enables researchers to evaluate the performance of the pathway, determine areas for potential improvement, and to develop more efficient strategies, especially for metabolic engineering and optimization.

Through the optimizing the glycolysis pathway based on exergy analysis, it is possible to enhance energy efficiency, reduce waste, and improve the utilization of resources in *Escherichia coli* metabolism.

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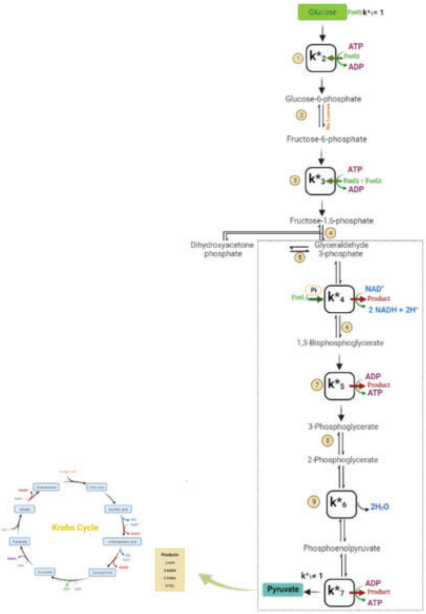

Figure. 4. Reproductive structure of the glycolysis pathway.

K* represents the exergy cost

In this context, glycolysis pathway can be seen as a reproductive structure within cellular metabolism, where the participated metabolic compounds act as fuels and products.

This pathway is one of the important and relevant pathways in most metabolic networks, as it serves as a central hub for energy generation and the production of essential molecules that sustain cellular functions.

In glycolysis, glucose, is regarded as a primary fuel source, it is initially converted into two molecules of pyruvate through a series of enzymatic reactions. This breakdown of glucose generates energy in the form of ATP and NADH. These energy-rich compounds are considered as vital fuels for various cellular processes in biology.

Moreover, the selected pathway is responsible for the production of other important metabolic compounds (that act as products). For instance, during the conversion of glucose to pyruvate, intermediates such as glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), and 3-phosphoglycerate (3PG) are formed and regarded as “internal products” in this process. These compounds play crucial roles in biosynthetic pathways, providing building blocks for the synthesis of essential molecules such as nucleotides, amino acids, and lipids, etc..

The reproductive nature of the glycolysis metabolic network is evident in the recycling of metabolic compounds, where NADH, generated during glycolysis, is further oxidized to NAD+ in a subsequent cellular processes, such as oxidative phosphorylation. This recycling mechanism ensures the continuous availability of key cofactors and maintains the energy flow within the metabolic network.

The identification of metabolites as fuels and products highlights the dynamic and interconnected nature of cellular metabolism.

Glycolysis not only generates ATP, the primary energy currency of the cell, but also provides essential precursors for the synthesis of biomolecules involved in the cellular growth.

Consequently, understanding the reproductive structure of the glycolysis pathway and the roles of its compounds (By its identification as fuels and products in the process) is vital for comprehending cells from an energetic point of view, optimizing certain metabolic pathways, and also designing strategies for metabolic engineering and biotechnological applications.

Therefore, by manipulating the glycolysis pathway and its associated metabolic compounds, it would make possible to ameliorate energy production, redirect metabolic fluxes, and achieve desired cellular outcomes.

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4.2. Exergy and exergy cost analysis of biochemical networks in livingsystems far from equilibrium

These kind of analyses represents a potential approach to understand the thermodynamic behavior of biological systems and to identify the most energetically efficient pathways for energy conversion and utilization. In living systems, biological processes often occur far from thermodynamic equilibrium, which means that traditional exergy analysis approaches may not be applicable.

For this matter, it is important to develop new methods and theoretical frameworks for exergy analysis in far from- equilibrium systems. One example of a theoretical framework is the concept of exergy efficiency, which is utilized to quantify the efficiency of energy conversion and utilization in living systems. This framework takes into account the entropy production of the system, as well as the exergy changes that occur in the system. Another important area of research is the application of exergy and exergy cost analysis to specific biological systems, such as metabolic pathways, cellular respiration, and photosynthesis. As these sort of studies can provide wider insights into the energetics of these processes and furthermore, help in the identification of potential targets for improvement in terms of energy and energy efficiency.

Despite that the approach of exergy analysis of biochemical networks in living systems far from equilibrium is still developing, it has already produced valuable insights into the energetics of these systems and has the potential to provide important guidance for energy-efficient design and operation in a wide rangeof biological and biotechnological application.

5. Analogical analysis: Industrial system (Engine) vs Biological system (Bacterial environment)

One of the fascinated things about nature is that it is the origin of all human kind works, same as industrial systems that has been inspired from the biological system present in our environment, as understanding the nature of the work process in a biological environment could be the key to make a development and a step forward in Human work, and vice- versa. In an engine, the exergy analysis would involve quantifying the energy losses and inefficiencies that occur as fuel is converted into useful work. This analysis can help identify areas where energy is being wasted, such as through heat losses or frictional losses, and guide efforts to optimize the engine for improved efficiency.

Similarly, in a bacterial environment, the exergy analysis would involve quantifying the energy losses and inefficiencies that occur as nutrients are converted into biomass or other bioproducts. This analysis can help identify areas where energy is being wasted, such as through the production of useless bi-products, and guide efforts to optimize the metabolic pathways of the bacteria for improved efficiency.

A key difference between the two mentioned systems is that an engine is a closed system, whereas a bacterial environment is an open system that interacts with its surroundings.

This means that the exergy analysis of a bacterial environment must take into account the energy inputs and outputs from the surrounding environment, such as through the exchange of nutrients, gases, and heat. To say better, the analogy between the exergy analysis of an industrial system and a biological system highlights the importance of understanding the thermodynamic efficiency of systems, whether they are designed by humans or evolved through natural selection.

By optimizing the efficiency of these systems, we can minimize energy losses and waste, and improve our ability to produce useful work or bioproducts.

6. Microbial production of fuels

Microbial production of fuels refers to the process of using microorganisms, such as bacteria and yeast, to convert biomass into biofuels. The most common type of biofuel produced using this method is bioethanol, which is made from sugars or starches in crops such as corn, sugarcane, and wheat.

The process of microbial production of bioethanol involves the fermentation of sugars by yeast or bacteria to produce ethanol and carbon dioxide. In addition to bioethanol, other biofuels such as butanol and biomethane can also be produced using microbial processes. Butanol, for example, can be produced by the fermentation of sugars and starches by bacteria such as Clostridium. Biomethane can be produced by the anaerobic digestion of organic matter by microorganisms, producing methane that can be used as a fuel source. Microbial production of biofuels has gained increasing attention as a sustainable and renewable alternative to traditional fossil fuels.[13]

The use of biomass as a feedstock reduces the carbon footprint of biofuels and helps to reduce dependence on finite fossil fuel resources. However, the efficiency and scalability of microbial production of biofuels still pose challenges, and further research and development is needed to make this technology more economically viable.

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7. The challenges

The efficiency and scalability of microbial production of biofuels are two important factors that determine the commercial viability of this technology. Efficiency refers to the amount of biofuel produced per unit of biomass used as a feedstock. Improving the efficiency of the process is crucial in order to make it economically viable, as it directly affects the cost of production.

The efficiency of microbial production of biofuels is influenced by a number of factors, such as the type of microorganisms used, the composition of the feedstock, and the conditions of the fermentation process. The efficiency and scalability of microbial production of biofuels are two important factors that determine the commercial viability of this technology.

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Scalability refers to the ability to increase the production of biofuels as demand increases. Scalability is a challenge for microbial production of biofuels, as the process is often limited by factors such as the availability of suitable feedstocks, the cost and availability of the microorganisms used, and the cost of the necessary equipment and infrastructure. Additionally, scaling up the production process can also affect the efficiency of the process, as the conditions required for optimal growth and fermentation of microorganisms may change at larger scales.

Despite these challenges, research and development in the field of microbial production of biofuels continues, with the goal of improving both the efficiency and scalability of the process. Advances in genetic engineering and synthetic biology have led to the development of more efficient microorganisms, and the development of new technologies such as consolidated bioprocessing and metabolic engineering have the potential to significantly improve the efficiency and scalability of the process in the future.

Conclusion

Exergy analysis is a powerful tool for the analysis of living cells and biotechnological processes. It provides a thermodynamic framework for quantifying the efficiency of cellular processes and biotechnological systems, and it has been widely applied to the study of energy systems, such as power plants and industrial processes. lately, exergy analysis has been applied to the study of living cells, providing new insights of the metabolic behavior, and offering new avenues for optimization and improvement.

It has been used to assess the efficiency of metabolic pathways, ion transport, and biochemical reactions, and to identify areas for improvement in bioreactor design and operation. The application of exergy analysis to living cells and biotechnological processes continues to grow, as researchers seek to gain deeper insights into the complex and dynamic nature of these systems. With its ability to provide a thermodynamic framework for the analysis of energy and matter exchange, exergy analysis is poised to play an increasingly important role in the study of living cells and biotechnology in the years to come.

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