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Formal Modelling Of The Fate Of Pyruvate Under Aerobic And Anaerobic Conditions Using Petri-Net

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Abstract
Human interest in studying biological systems is longstanding. Over the past three decades, advancements in cutting-edge genomics technologies have enabled scientists to generate vast amounts of complex biological data, including complete genomes. To better understand this complex data, including biological pathways, scientists use a range of modelling and simulation techniques. One such approach, Petri nets, has gained significant attention for modelling and simulating biological networks and pathways. Among the various Petri net models, hybrid functional Petri nets are particularly effective for modelling complex biological networks today.
In this study, we utilize Petri nets to model metabolic pathways, aiming to manage complex biological information and derive qualitative insights from the structural representation of pathways. Petri nets are introduced as a tool for computer-implementable pathway representation. They offer the potential to overcome current limitations and enable preliminary qualitative analysis through their various properties.
We apply Petri nets to model the different fates of pyruvate under aerobic and anaerobic conditions, which are crucial for cellular energy supply. During glycolysis, one molecule of glucose is converted into two molecules of pyruvate. Under aerobic conditions, pyruvate is converted into acetyl Co-A, while under anaerobic conditions, it is converted into lactate. Despite lower energy yield under anaerobic conditions compared to aerobic conditions, lactate can be a primary energy source for some cells and is useful in diagnosing disorders such as heart failure, shock, and cancer. When the body's oxygen demand exceeds supply, cells automatically switch from aerobic to anaerobic conditions. We systematically model, simulate, and validate qualitative models of these pathways using the well-established Petri net analysis technique.

Keywords*:* Perti nets; Biological networks; Pyruvate

1. Introduction

The human interest in studying biological systems is quite old. Biologists always try to understand the complex interactions of cells or organisms with great interest [9]. Several higher organisms contain billions of components with trillions of interconnections [7]. To understand the complete picture of cellular networksbiologists switch to computers where they complete the in-vivo or in-vitro and in-silico experiments. This led to the evolution of a new field called system biology. Systems biology is an increasingly popular new discipline encompassing molecular system computational modeling. The main goal of system biology is to analyze the behavior and interrelationships of biological systems. Systems Biology is aimed at the integration of different data analysis, visualization, modeling, and simulation to investigate biological systems [6], [18].
To perform computational analysis on a biological system, the information must be encoded in the simplest possible way. Existing knowledge representation techniques such as biological ontologies, structural neural models, and quantitative analysis models for simulation and analysis are used for analysis of the structural properties of biological networks [2]. These models are usually a graphic representation and are traditionally based on systems of ordinary differential equations [12]. More recent developments in mathematical models can play an important role in gaining insight into the systems' behavior and structure. They are widely used for the optimization of pathways to maximize understanding. Most models in systems biology can be classified into three different approaches of modeling, continuous and discrete, quantitative and qualitative, stochastic and deterministic [5]. Even though, the classification of these different modeling methods is neither exclusive nor independent. The variety of modeling approaches used in Systems Biology implies that the choice of method is carefully selected based on the specific biological system, the existing knowledge and data about that system, and the goals of the simulation study.
Some available models are either unsuitable for validating other models or are designed specifically for a particular type of system. Therefore an unambiguous representation of biological networks is needed which can manage these highly integrated networks computationally. Petri nets could play an integrating role by serving as a common intermediate. They can be used for both the mathematical representation of biochemical pathways and the validation of

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biochemical models. [5].

2. Petri Nets Basic

Carl Adam Petri introduced Petri Nets in 1962 during his Ph.D thesis, in which a mathematical model can be represented in graphical form to show the dynamic behavior of the system [17] [11]. Petri Net acts in both formalism which is mathematical and graphical. In a graphical formalism, it's easy to understand the system as it provides the visual treatment to the model both in analysis and designing phase [16] [13]. Since 1962, the theory of petri nets evolves at a very fast pace Figure 1.A Petri Net can be defined as a directed graph, where places are represented in graphical form as circles, which shows the various states, transitions are represented in graphical form as rectangular boxes which on certain conditions get fired (activated) and helps tokens to move to the next state, and arcs carry weight of tokens to be passed to next state, unit tokens doesn't show on arcs and is represented in graphical form as directed arcs [11]. Arcs can be from places to transitions or from transitions to places. A marking can be defined by '*M*', which shows the number of tokens in the places of a Petri Net model. During execution of a Petri Net model number and position may change. A Petri Net can be formally defined as a five-tuple ($N = (P, T, I, O, M_0)$), where:

- (P) is a finite set of places, denoted as ($\{p_1, p_2, \dots, p_m\}$).
- (T) is a finite set of transitions, denoted as ($\{t_1, t_2, \dots, t_n\}$), with the conditions that ($P \cap T \neq \emptyset$) and ($T \cap P \neq \emptyset$), ensuring that places and transitions are distinct.
- ($I: P \times T \rightarrow \mathbb{N}$) is the input function, which defines the directed arcs from places to transitions. Here, ($\mathbb{N}$) represents the set of non-negative integers.
- ($O: T \times P \rightarrow \mathbb{N}$) is the output function, which defines the directed arcs from transitions to places.
- ($M_0: P \rightarrow \mathbb{N}$) is the initial marking function, which assigns an initial number of tokens to each place in the Petri Net.

- Places (P): Represent conditions or resources in the system. They are depicted as circles in Petri Net diagrams.

- Transitions (T): Represent events or actions that change the state of the system. They are depicted as rectangles or bars in Petri Net diagrams.

- Input Function (I): Specifies the number of tokens that must be present in each place for a transition to fire. This is represented as directed arcs from places to transitions.

- Output Function (O): Specifies the number of tokens that are produced in each place when a transition fires. This is represented as directed arcs from transitions to places.

- Initial Marking (M_0): Defines the initial distribution of tokens across the places at the start of the Petri Net's operation.

This formalism allows for the modeling and analysis of systems with concurrency, synchronization, and resource sharing.

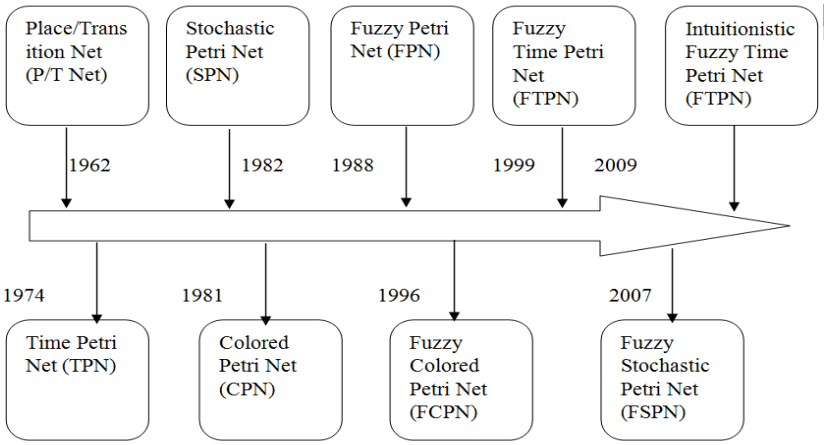

Fig. 1: Evolution of Petri Net Family ([14] [1]).

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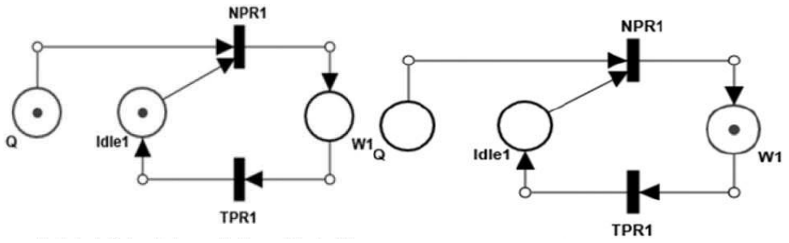

(a) Initial Marking, M_0 = (110) (before firing) (b) Initial Marking, M_0 = (001) (after firing)

Fig. 2: Change in marking after the firing of transition

2.1 Petri Nets Properties:

Petri Nets is a very useful tool for designing, describing and studying discrete event dymanic system. The properties and characteristics are defined as

Transition Firing: A Petri Net is executed by the event causing the firing of a transition, which occurs when the number of tokens inthe various places of a Petri Net model changes due to the occurrence of an event.

Enabling Rule: A transition can only be in enabled state when each input place has a minimum number of tokens equal to the weight of arc.

Firing Rule: Only the enabled transition can be fired which means it follows the enabling rule. From input places, number of tokens are moved to the transition and lastly to the output place equal to the weight of arc.
After firing of transition, each place always contains non- negative tokens. A source transition is defined where no input place is there, which is in enabled state and the sink transition is the one without the output place, which implies that no tokens can be generated after the firing of transition. Figure 2

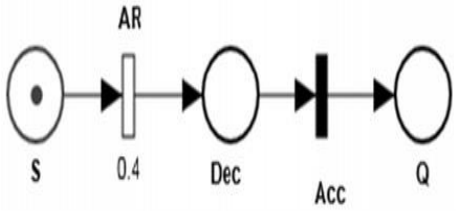

Fig. 3: Petri Net model for sequential execution

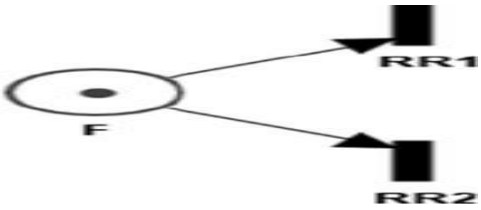

Fig. 4: Petri Net model for conflict

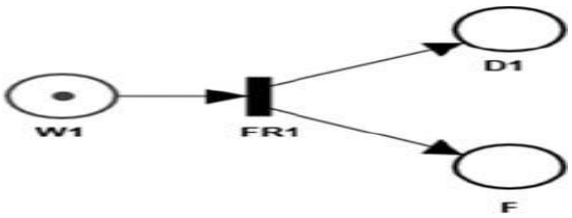

Fig. 5: Petri Net model for concurrency

2.2 Modeling Power of Petri Nets

Sequential Execution: In a sequential execution, transition Acc can only be fired after transition AR, which shows the precedence sequencing. Figure 3

Conflict: In this execution, transitions RR1 and RR2 are enabled, but the processing of one's request leads to the

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denial of the other transition. By assigning the probabilities to the transitions in conflict state, conflict can be resolved. Figure 4

Concurrency: In this execution, tokens can be distributed to one of the output places D1 or F which are in concurrency, after the firing of transition FR1. Figure 5

Synchronization: In this execution, firing of transition NPR1 depends on the places Q and Idle1 which leads to the synchronized behavior of a system. Figure 6

Mutually Exclusive: Whenever two concurrent processes are running, where a single resource has to be shared,then the processes are in mutually exclusive stage, i.e. a resource can be shared one by one by both the processes.

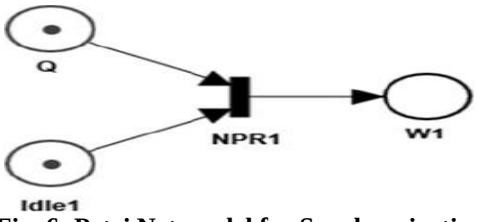

Fig. 6: Petri Net model for Synchronization

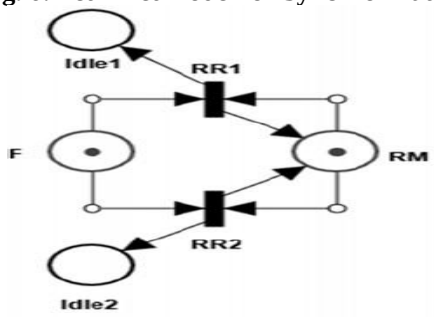

Fig. 7: Petri Net model for mutual exclusion

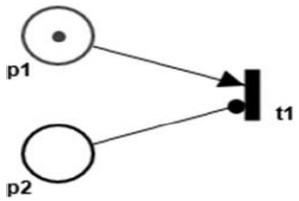

Fig. 8: Petri Net model for priorities

As shown in figure, RM is the resource which needs to be shared mutually by the transitions RR1 and RR2.Figure 7

Priorities: By introducing the inhibitor arc, priorities can be obtained, which is graphically represented by an arc with a black dot connected to it. A transition t_1 can only fire when places p_1 has minimum number of tokens equal to the weight of arc and no token in place p_2 where inhibitor arc is connected, which makes the change inenabling rule to the firing of transition. Figure 8

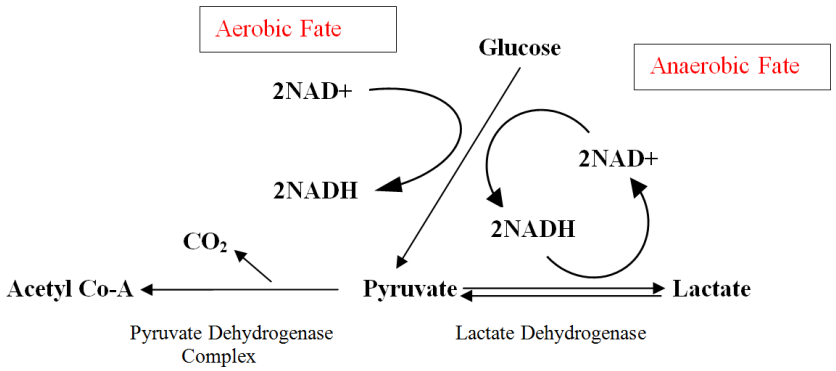

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Fig. 9: The metabolic fates of Pyruvate

3. Fates of Pyruvate Under Different Conditions

A metabolic pathway within a living organism is a series of different chemical reactions, which occurs in a cell that builds and breakdown metabolites for cellular processes. These metabolites generally participate in many different metabolic pathways, forming a complex network of reactions [8]. Out of many important pathways that occur in the living cell, Cellular respiration is of prime importance. In this pathway, the energy stored in the chemical bonds of metabolites is converted to ATP by the cellular metabolic machinery. Cellular respiration pathway could be aerobic or anaerobic. Glycolysis is the common step which occurs both in aerobic and anaerobic respiration. In humans, under aerobic conditions, the glucose is broken down in the cell cytoplasm form Pyruvate by a process called Glycolysis. During this process, one molecule of glucose is converted into two molecules of pyruvate. On the basis of specific cellular environment and specific conditions such as availability of oxygen, energy requirement, and the presenceor absence of mitochondria in the cell, the Pyruvate generated in the last step of glycolysis can be converted into different molecules. If the cell contains mitochondria, within the mitochondrial matrix, the pyruvate enters the citric acid cycle and undergoes oxidative phosphorylation and generates NADH and FADH2 [4]. These further enter into the electron transport chain, leading to the generation of 32 ATP per glucose molecule. However, as the enzymes for both the citric acid cycle and electron transport chain are present in mitochondrial matrix and within the inner mitochondrial membrane respectively, the cells which lack mitochondria (e.g., erythrocytes) cannot able to perform oxidative phosphorylation for energy production. Figure 9

In cells such as erythrocytes and oxygen deprived tissue such as contracted skeletal muscles, pyruvate remains within the cytoplasm and the lactate dehydrogenase enzyme converts pyruvate into lactate. The cell as an energy source cannot utilize lactate directly, but this reaction is necessary for the regeneration of NAD+ from NADH. In glycolysis, NAD+ is an essential oxidizing cofactor for maintaining the flow of glucose. NAD+ concentrations must remain high enough in earlier intracellular reactions of glycolysis to remain favorable. In comparison to oxidative phosphorylation, anaerobic glycolysis is significantly less efficient for energy production as it provides only a net production of only 2 ATP per glucose molecule (against oxidative phosphorylation which led to the production of 32 ATP per glucose molecule). However in organism where oxidative phosphorylation is absent glycolysis is the only for the production of ATP from glucose in the cells [4].

Whenever the oxygen demand exceeds from oxygen supply, cells shift from aerobic to anaerobic conditions. There- fore detecting the level of lactate can be useful in diagnosing and management of different conditions such as anemia, heart failure, shock, and cancer. Lactic acid measurements are useful for of such conditions [15] [3] and [10].

4. Pyruvate conversion under Aerobic and Anaerobic respiration

Pyruvate, the end product of glycolysis, undergoes different metabolic pathways depending on the availability of oxygen, leading to distinct outcomes in aerobic and anaerobic respiration. Here's a textual representation of these processes:

4.1 Aerobic Conditions (Cellular Respiration)

4.1.1 Conversion of Pyruvate to Acetyl-CoA:

Reaction: Pyruvate + CoA + NAD^+ → Acetyl-CoA + NADH + CO_2

Enzyme: Pyruvate dehydrogenase complex

Molecular Concentrations:

- Pyruvate: 3-carbon molecule
- CoA: Coenzyme A
- NAD^+: Nicotinamide adenine dinucleotide (oxidized form)
- Acetyl-CoA: 2-carbon molecule attached to CoA
- NADH: Reduced form of NAD^+
- CO_2: Carbon dioxide, a gas

Aerobic conditions involve the conversion of pyruvate to Acetyl-CoA, which then enters the citric acid cycle and electron transport chain to produce ATP, water, and CO_2.

4.2 Anaerobic Conditions (Fermentation)

4.2.1 Lactic Acid Fermentation:

Reaction: Pyruvate + NADH + H^+ → Lactate + NAD^+

Enzyme: Lactate dehydrogenase

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Molecular Concentrations:
- Pyruvate: 3-carbon molecule
- NADH: Reduced form of NAD^+
- Lactate: 3-carbon molecule
- NAD^+: Oxidized form of NADH

Anaerobic conditions lead to fermentation processes. In lactic acid fermentation, pyruvate is converted to lactate with the regeneration of NAD+. To visualize these processes, drawing diagrams typically involves illustrating each step of the pathways, the enzymes involved, and the flow of molecules between steps.

The human body switches from aerobic to anaerobic respiration primarily based on the availability of oxygen and the energy demands of the body. This switch occurs under conditions where oxygen supply is limited, such as during intense exercise. Here's an overview of how this switch happens, along with the chemical reactions and molecular concentrations involved.

Aerobic vs. Anaerobic Respiration
Aerobic Respiration process occurs when oxygen is available and provides a high yield of ATP (energy) through the complete oxidation of glucose. Anaerobic Respiration process occurs when oxygen is scarce and provides a lower yield of ATP. It relies on less efficient pathways such as lactic acid fermentation or alcoholic fermentation.

Aerobic Respiration involves the complete oxidation of glucose under aerobic conditions is described by the following chemical reaction:

$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O+ATP$

Molecular Concentrations (approximate values in cells):
- Glucose ($C_6H_{12}O_6$): Usually in the range of 1-10 μM in blood and lower in cells.
- Oxygen (O_2): 20-100 μM in tissues (depends on the tissue type and oxygenation level).
- Carbon Dioxide (CO_2): 10-50 μM in blood.
- Water (H_2O): Abundant.
- ATP: Typically 1-10 μM in cells.

Anaerobic Respiration (Lactic Acid Fermentation), is said when oxygen is limited, cells switch to lactic acid fermentation described by the following chemical reaction:
$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3+ATP$
$Pyruvate + NADH + H_+ \rightarrow Lactate + NAD^+$

Molecular Concentrations (approximate values in cells):
- Glucose ($C_6H_{12}O_6$): 1-10 μM (varies with availability and demand).
- Pyruvate ($C_3H_4O_3$): Intermediate concentration.
- NADH: Varies with metabolic activity (reduced form of NAD_+).
- Lactate ($C_3H_6O_3$): Increases during intense exercise, can be up to 20 μM.
- ATP: Typically 1-10 μM in cells.

Conditions to switch from aerobic to anaerobic respiration and vise versa
1. **Oxygen Availability:**
- Under normal conditions, cells use aerobic respiration for efficient ATP production.
- During intense exercise or when oxygen delivery cannot meet the demand, the body switches to anaerobic respiration due to insufficient oxygen.
2. **Regulation of Metabolism:**
- **Oxygen Debt:** When the oxygen demand exceeds the supply, cells shift to anaerobic pathways to produce ATP quickly, albeit less efficiently.
- **Lactate Production:** Pyruvate, the end product of glycolysis, is converted to lactate when oxygen is scarce, regenerating NAD^+ to allow glycolysis to continue.
3. **Recovery Phase:**
- After the exercise or when oxygen levels are restored, lactate is transported to the liver where it is converted back to glucose through gluconeogenesis. This process is known as the Cori cycle.

The switch between aerobic and anaerobic respiration is crucial for maintaining energy production under varying oxygen availability, ensuring that the body can respond to both low- and high-intensity activities. When cells shift from

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aerobic to anaerobic respiration, the concentration of oxygen (O_2) typically drops below a critical threshold. This transition occurs in response to insufficient oxygen supply relative to the metabolic demands. The exact concentration at which this shift occurs can vary based on the tissue type, metabolic rate, and specific conditions, but generally, it follows the following Oxygen Concentration Threshold patterns:

1. **Normal Aerobic Conditions:**
   - In well-oxygenated tissues, oxygen concentrations are usually between **20 to 100 µM** (micromolar). This range is sufficient to support aerobic respiration efficiently.
2. **Transition to Anaerobic Conditions:**
   - As oxygen consumption increases, such as during intense exercise or in conditions where oxygen delivery is compromised (e.g., hypoxia, ischemia), the oxygen concentration can drop significantly.
   - Typically, when oxygen levels fall below **10 µM**, cells start to rely more on anaerobic pathways. This threshold can vary depending on the type of tissue and its metabolic activity.
3. **Anaerobic Conditions:**
   - Under severe oxygen deprivation, oxygen levels can drop below **1 µM**, at which point anaerobic metabolism becomes predominant.

Following Factors Influencing the Shift between aerobic to anaerobic respiration and Vise versa

1. **Intensity of Activity:**
   - During high-intensity exercise, oxygen demand exceeds supply, causing a rapid decrease in local oxygen concentration.
   - For example, during sprinting, the oxygen levels in muscle tissues can drop significantly, leading to an increased reliance on anaerobic glycolysis.
2. **Tissue Type:**
   - Different tissues have varying thresholds. For instance, skeletal muscles might tolerate lower oxygen levels better than brain tissues, which are highly sensitive to oxygen deprivation.
3. **Blood Flow and Oxygen Delivery:**
   - Conditions like vascular occlusion or reduced blood flow can exacerbate the drop in oxygen levels, leading to an earlier switch to anaerobic metabolism.

5. Petri Net representation Pyruvate conversion under Aerobic and Anaerobic respiration

Petri Net representation Researchers now generate a vast amount of complex biological data using advanced techniques such as DNA sequencing and DNA microarrays. Several databases provide free access to this intricate data, including BRENDA, which offers enzyme-specific information and their interactions; OMIM, which details diseases and molecular data; and KEGG, which covers metabolic processes and complex biological pathways. Technological advances have made it easier to extract, interpret, and model this information effectively. Scientists commonly use Petri nets to reconstruct biological networks and biochemical reactions, allowing for direct translation of these networks into Petri net notation. Creating detailed diagrams for the conversion of pyruvate under aerobic and anaerobic conditions involves outlining the biochemical pathways involved.

Following model that shows how glucose converts into pyruvate (Figure 10) and how pyruvate transforms into various molecules under both aerobic and anaerobic conditions (Figures 11, 12, 13, 14, and 15).

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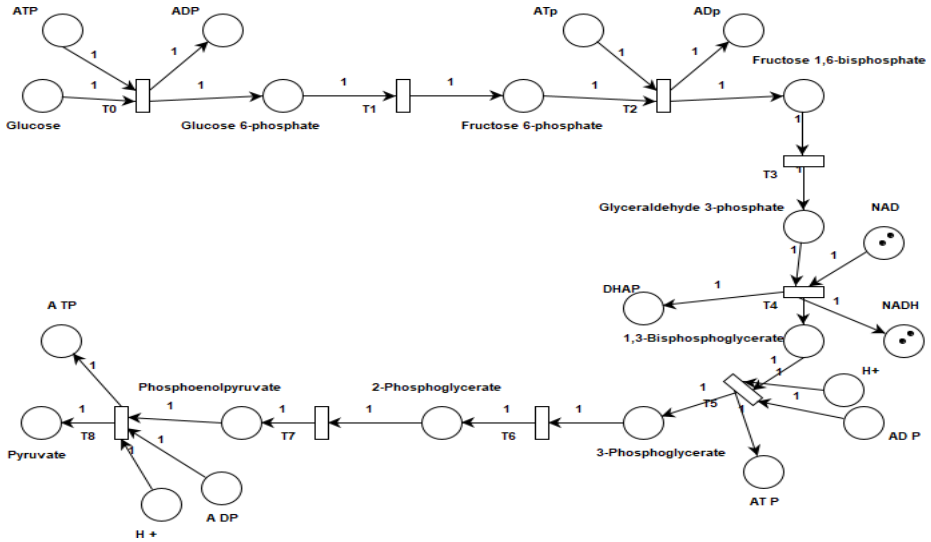

Fig. 10: Petri-Net representation for The Glycolytic Pathway

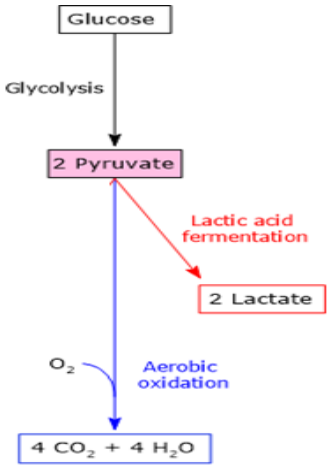

Fig. 11: Pyruvate conversion under aerobic and anaerobic conditions

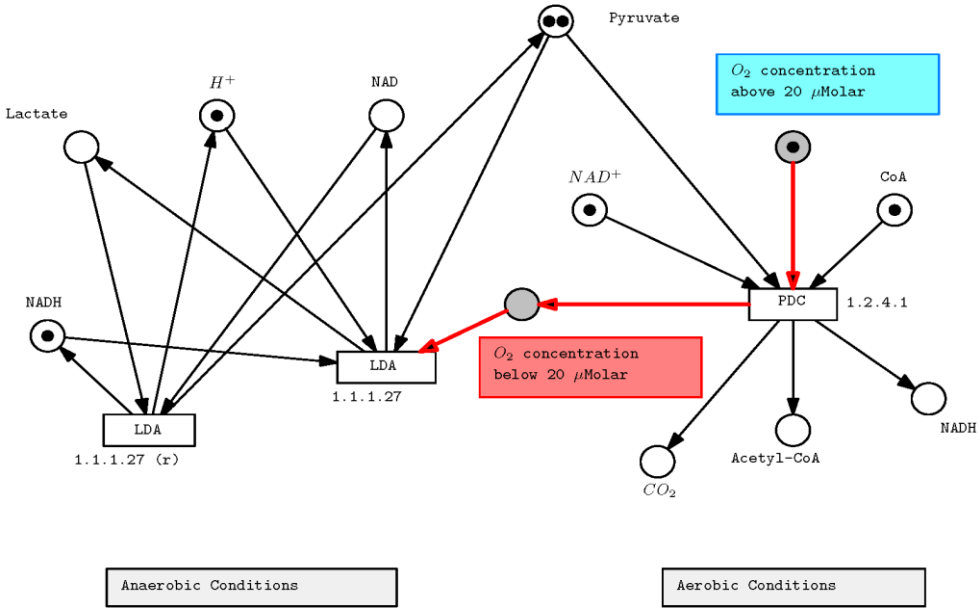

Fig. 12: Petri-Net representation for Pyruvate conversion under aerobic and anaerobic conditions (before firing)

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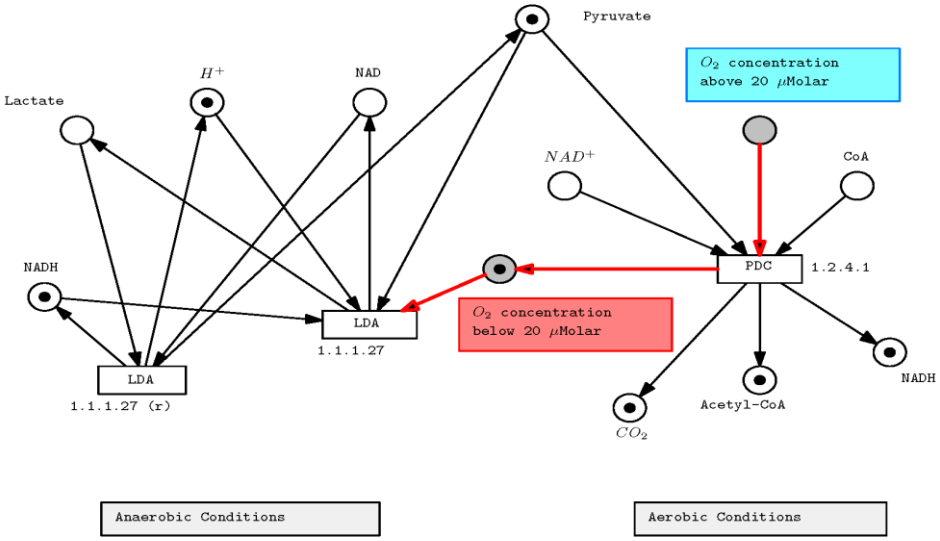

Fig. 13: Petri-Net representation for Pyruvate conversion under aerobic and anaerobic conditions (after aerobic firing)

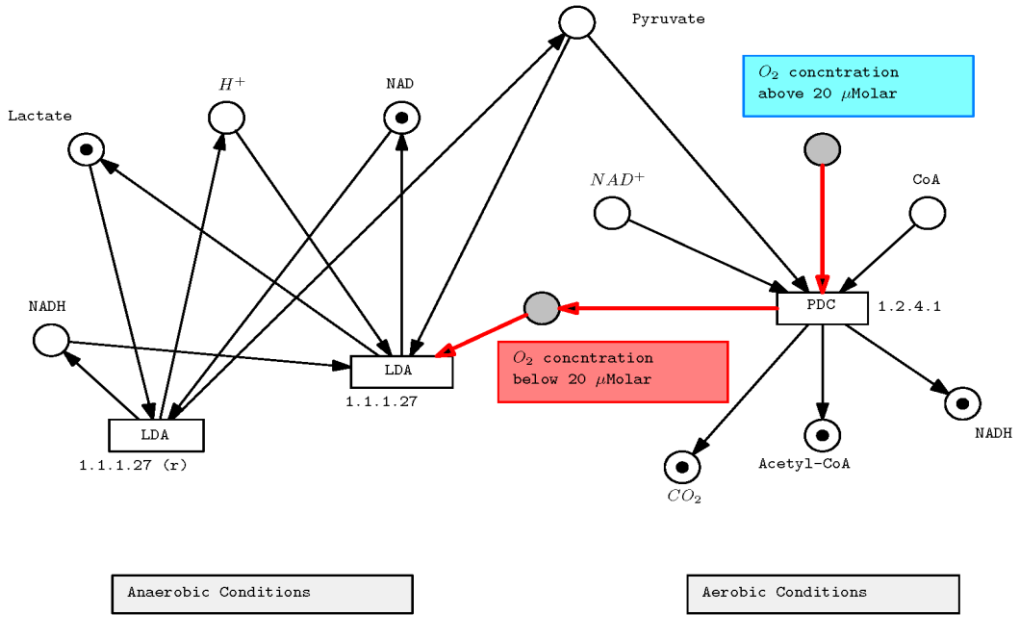

Fig. 14: Petri-Net representation for Pyruvate conversion under aerobic and anaerobic conditions (after anaerobic firing)

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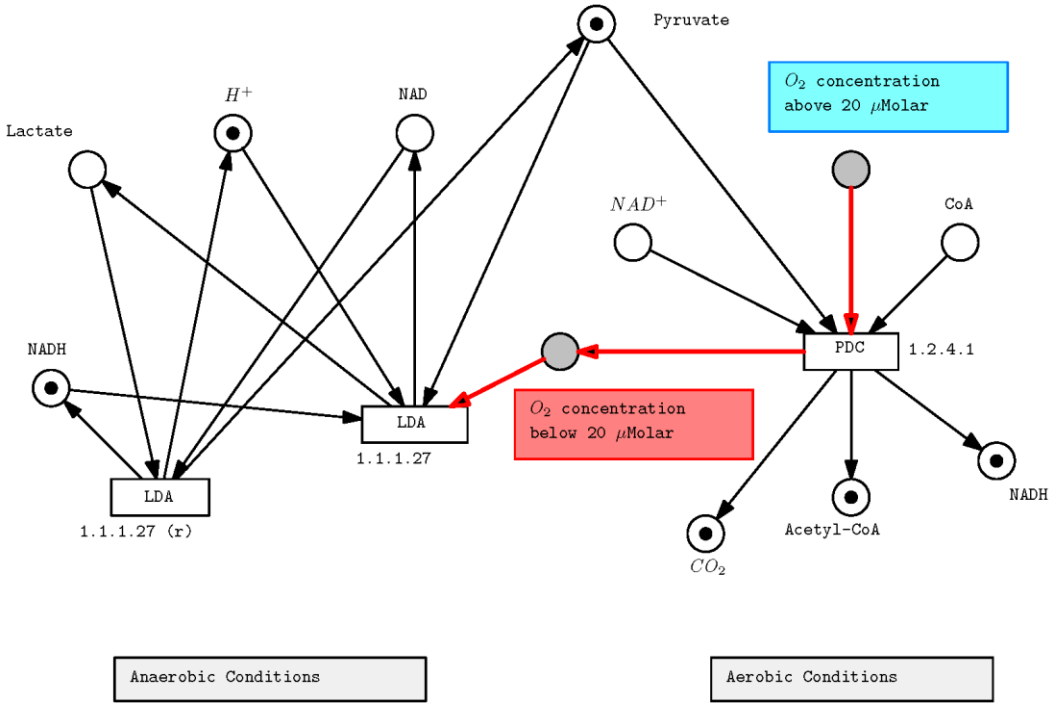

Fig. 15: Petri-Net representation for Pyruvate conversion under aerobic and anaerobic conditions (after anaerobic reverse firing)

6. Discussion on Petri net modeling and analysis

Figure 12 illustrates the human pyruvate conversion pathway represented as a Petri Net (PN) model under both aerobic and anaerobic conditions. This model includes three transitions and twelve places, reflecting various enzymes and pyruvate conversion molecules, with the transitions representing the numerous reactions between these molecules.

Description of the Model:

- **Places:** The twelve places in the Petri Net denote various compounds and enzymes involved in pyruvate conversion. Each place represents either a chemical compound's availability or the presence of an enzyme necessary for the conversion process.
- **Transitions:** The three transitions model the biochemical reactions that occur between the compounds. These transitions are categorized as source or sink transitions:
    - **Source Transitions:** Facilitate the production of chemicals by adding tokens to the relevant places, symbolizing the generation of products.
    - **Sink Transitions:** Represent the consumption of chemicals by removing tokens from the corresponding places, indicating the usage of substrates.
- **Enzyme Action:** Enzyme activity is modeled using test arcs in the Petri Net. Test arcs are used to check whether conditions are met for a transition to fire but do not consume tokens.

Verification and Examination:

- **Verification:** The Petri Net model should be verified to identify any discrepancies or metabolic blocks. This process ensures that the model accurately represents the biochemical pathway and functions without issues.
- **Animation Mode:** The animation mode in the Petri Net tool displays the firing of transitions, showing how the model progresses through the initiation and elongation phases. This visualization confirms that there is no impasse, meaning that all parts of the pathway are active and functioning as expected.
- **System Behavior:** By using the animation mode, one can gain insights into the real-life behavior of the metabolic pathway. This feature complements the advantages of using Petri Nets to model metabolic processes, as it allows for simulation and adjustment of the model before conducting quantitative analyses.

Pathway Dynamics:

- **Sink Transitions:** Prevent the accumulation of products, ensuring that chemicals do not build up excessively within the system.
- **Source Transitions:** Ensure a sufficient supply of substrates, which is crucial for the continuation of reactions and maintaining an active pathway.

Model Activity and Analysis:

- **Active Model:** The Petri Net model demonstrates no deadlocks (stalemates) during the fatty acid production process, indicating that the pathway operates effectively from its initial marking.

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- **State Space Analysis:** The state space analysis further supports the model's activity, confirming that the metabolic pathway is functioning correctly without any issues of deadlock or inefficiency.

In summary, the Petri Net model for human pyruvate conversion shows a well-functioning pathway with active transitions and proper flow of substances. The lack of impasse and the successful animation of transitions validate the accuracy of the model, ensuring that the pathway operates as intended and allowing for necessary adjustments before quantitative analysis.

The transition being fired from the matching state is shown by the arcs. The quantity of tokens that are present in each place in a given state is indicated by the system's state, which is a place vector. As long as the model remains unbroken, a concrete state model will not exist. Before examining a model's attributes, the produced PN model is examined and validated for the Pyruvate conversion under aerobic and anaerobic conditions in human, using the animation mode in the Petri net tool Snoopy 2.0 [19]. COPASI [23] is used to model the dynamics of biochemical processes including the conversion of pyruvate under aerobic and anaerobic respiration. Below, is the outline simple differential equations for each process.

6.1 Aerobic Respiration of Pyruvate

In aerobic respiration, pyruvate is converted into acetyl-CoA, which then enters the citric acid cycle. A basic differential equation can model the rate of change of pyruvate concentration as it is converted into acetyl-CoA and subsequently processed in the citric acid cycle.

Let:
- [P] represent the concentration of pyruvate.
- [A] represent the concentration of acetyl-CoA.
- k_1 be the rate constant for the conversion of pyruvate to acetyl-CoA.
- k_2 be the rate constant for the processing of acetyl-CoA in the citric acid cycle.

The differential equations can be expressed as:
Rate of change of pyruvate concentration:

$$\frac{d[P]}{dt} = -k_1[P]$$

Rate of change of acetyl-CoA concentration:

$$\frac{d[A]}{dt} = k_1[P] - k_2[A]$$

Here, $(k_1[P])$ represents the rate at which pyruvate is converted into acetyl-CoA, and $(k_2[A])$ represents the rate at which acetyl-CoA is utilized in the citric acid cycle.

6.2 Anaerobic Respiration of Pyruvate

In anaerobic respiration, pyruvate is converted into lactate or ethanol (depending on the organism) and regeneration of NAD^+ occurs. For simplicity, let's consider lactate fermentation.

Let:
- $[P]$ represent the concentration of pyruvate.
- $[L]$ represent the concentration of lactate.
- k_3 be the rate constant for the conversion of pyruvate to lactate.

The differential equations can be expressed as:
Rate of change of pyruvate concentration:

$$\frac{d[P]}{dt} = -k_3[P]$$

Rate of change of lactate concentration:

$$\frac{d[L]}{dt} = k_3[P]$$

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Here, $(k_3[P])$ represents the rate at which pyruvate is converted into lactate.

- Aerobic Respiration:

$$\frac{d[P]}{dt} = -k_1[P]$$

$$\frac{d[A]}{dt} = k_1[P] - k_2[A]$$

- Anaerobic Respiration (Lactic Acid Fermentation):

$$\frac{d[P]}{dt} = -k_3[P]$$

$$\frac{d[L]}{dt} = k_3[P]$$

These equations represent simplified models of the processes. In a more detailed model, additional factors such as enzyme kinetics, the impact of co-factors, and feedback mechanisms might be included.
The following behavioural results are also shown by the petrinet model:

Boundedness Status: Reasons

- Number of tokens in each place is finite at every reachable marking. Petri net is bounded
- There is at least one P-invariant that covers entire set of Petri net places. Assuming that initial marking is finite, petri net is bounded
- Petri net is not classified as State machine. Boundedness cannot be decided based on this classification

Conservativeness Status: Reasons

- There is at least one P-invariant that covers entire set of petri net places. Petri net is conservative
- Petri net is not classified as State machine. Conservativeness cannot be decided based on this classification

Deadlock free status: Reasons

- Each node of state space graph has at least one successor. Petri net is deadlock free (Lazy covering level used for this analysis: 1)
- Petri net is not live or the liveness test result status is undecidable. It is undecidable if a Petri net is deadlock free based on its liveness
- NMRT contains no deadlocks. Petri net is deadlock free

7. Conclusion

Systems Biology has the power which can be used to fulfill the scientists' dream about the virtual cell concept. Using different biological databases, now we can access the information available about the cell, biological processes,enzymes and metabolic pathways. Virtual cell representation can be done using these databases by integration and fusion of data. Once the data is retrieved, we need to build a useful model and simulation environment. During the last two decades, different variations of Petri-Nets have been used in system biology for various studies. Petri Nets are used for modeling of structural network for qualitative as well as for quantitative models analysis. The use of different kinds of Petri-Nets will be useful for the analysis of complex biological systems. In future it will be very useful to develop ever more integrated representations for complex biological networks.

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