Modeling of Metabolic Pathways using Petri Net

Jelili Oyelade[1], Itunuoluwa Isewon[1], Olanrewaju Ogunbona[1], Olufemi Aromolaran[1]
[1]Department of Computer and information Sciences
Covenant University
Ota, Nigeria
ola.oyelade@covenantuniversity.edu.ng

Jumoke Soyemi[2]
Department of Computer Science
The Federal Polythecnic
Ilaro, Nigeria

***Abstract*— Plasmodium falciparum which is one of the four species that causes malaria has been found to be the deadliest in Africa. Over the years, attempts have been made to find a way of eradicating this disease thereby reducing the casualties as a result of the disease. Computational models such as Boolean networks, Graph theory have been used to simulate and model real life systems as well as biological systems as a method of identifying the way the systems behave in different situations. But, the petri net model which is also a computational model developed by Carl Adams Petri would be used in this project, this is due to its ability to model inconsistency that has been found to be in existence in most real life systems. In this study, the Petri net model constructed with the PNML (Petri Net Markup Language) based on the stoichiometric matrix is used to represent the TCA cycle (TCA cycle data obtained from Biocyc database) a system that is involved in producing energy for the Plasmodium falciparum parasite, it is also one that has been found to be at the heart of all the energy developing processes. The constructed model is then used to identify choke points (essential reactions) that could be altered and used as a way to cause the death of the parasite due to the lack of energy production.**

Keywords*— *plasmodium falciparum, Petri net, Petrinet Markup Language, TCA, Parasite

I. INTRODUCTION

Metabolic networks are a complete set of metabolic and physical processes that determine the physiology and biochemical properties of a cell. It consists of reactions which connect the metabolites, they are very useful in studying and modeling metabolism. This method of modeling and analyzing concurrent, asynchronous systems was invented by Carl Adam petri [1]. As a mathematical tool petri net models can be described by a set of linear algebraic equations, or other mathematical models that reflect the behavior of systems. It allows for the formal analysis of such systems. Petri nets are suited to represent logical interactions among parts of activities in a system. A Petri net is a simple bipartite directed graph with two types of nodes (places and transition). Places represent the conditions or resources represented with circles in the petri net model and they contain the tokens. Transitions represent the activities that change the state of the resources. They consume and produce resources from the output to the input places respectively. They are represented with a rectangle. It has directed arcs that connect the places to the transitions and transitions to places. The arcs do not connect arcs to arcs and transitions to which it can carry. When it is not weighted then it has the default weight of one. Another element that exists in a petri net model is the token. It represents one unit of the resources; tokens could mean anything based on the system being modeled.

II. RELATED STUDIES

The petri net model has been used for the following biological processes; Application of petrin net modelling to yeast pheromone pathway to identify how cell can dynamically adapt the pathway to continue reproduction under severe environmental changes [2]. The research answered the two questions: (1) the conditions in which the pheromone pathway responds positively and (2) the kind of changes in the cell that would cause a negative response to change to positive. Their study showed that a cell can overcome effects of conditions by using more concentration of additional proteins. Simulation of a Petri net-based Model of the Terpenoid Biosynthesis Pathway was carried out by [3]. Their model describes the biosynthesis of terpenoids via two independent pathways, one involving mevalonate (MEV) and one involving methylerythritol phosphate (MEP). HFPNE which was found that the simulation and validation processes performed using the petri net model is consistent with known biological information and data. The model provides a better understanding of the reactions involved in both pathways and how they affect each other. Model validation of biological pathways using petri nets was demonstrated by [4] for Apoptosis to describe a regulated intrinsic cell. Study by [5] allows the cell to control its cell numbers and tissue size as well as protect itself from deadly cells. Cells that undergo apoptosis could exhibit plasma membrane blebbing, cell shrinkage and so on due to the important role that is played by apoptosis during neural development, it is necessary to make use of a computational model that can represent the pathway in a quantitative manner. Petri Net Modeling and Analysis of Biological Processes Implicated in Amyotrophic Lateral Sclerosis (ALS) [6]. ALS is a progressive neurodegenerative disease that damages the nerve cells of the brain and the spinal cord. It was concluded that the petri net model is able to represent accurately the processes that take place in a cell, the ability to also manipulate initial tokens and firing rates makes it possible to identify different effects on the system. Study by [7] applied Petri Net modelling for Granulomatous Inflammation which is an implication for IL-10 Mediated Control of Leishmania donovani Infection.

978-1-5386-2652-8/17 $31.00 © 2017 IEEE
DOI 10.1109/CSCI.2017.224

The research identifies distinct patterns of effectors function which suggests a means for maintenance of parasites.

TABLE 1. RELATIONSHIP BETWEEN ELEMENTS IN THE METABOLIC NETWORK AND PETRI NET MODEL[8])

PETRI NET	***Metabolic Network***
Input places	Substrates, reagents
Output places	Reaction products
Transitions	Reactions, interactions
Arc weights	Stoichiometric coefficients
Number of tokens in places	Enzymes

The TCA cycle of the metabolic pathway of *Plasmodium falciparum* is the pathway that would be modeled. We shall identify the choke points (essential genes).

III. MATERIALS AND METHODS

In this research the data used in the construction of the net was obtained from the Biocyc database [9]. Petri Net Markup Language (PNML) was used for the construction of the network and the Snoopy tool to display the petri net model and also identify the essential reactions in the TCA cycle pathway.

The TCA cycle also known as Citric acid cycle is cyclic in structure with its final products; CO_2, water and energy. It is the central hub of carbon metabolism connecting glycolysis, gluconeogenesis, respiration, amino acid synthesis and other biosynthetic pathways. Citric acid cycle is aerobic due to the fact that it requires oxygen to produce Nictonamide adenine dinucleotide (NAD^+) in the mitochondrion. Substrate channeling which is as a result of enzymes being associated with each other and the causing products to pass directly from one another could occur in the TCA cycle. Its overall reaction of the citric acid cycle is:

$$3NAD^+ + FAD + GDP + P_i + \text{acetyl-CoA} \rightarrow 3NADH + FADH_2 + GTP + CoA + 2CO_2$$

The products from the intermediates in the TCA cycle were used in the biosynthesis of vital cellular constituents. Anaplerotic reactions that replenish the citric acid intermediates must be performed as a result of the use of supplied components during synthesis.

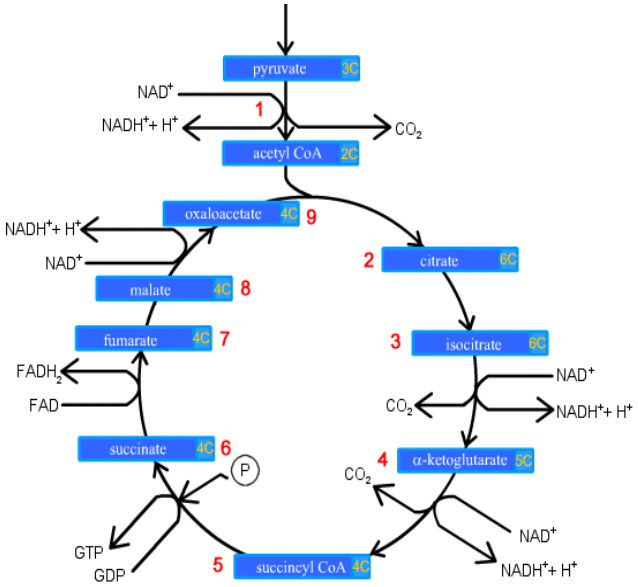

Fig 1: The structure of the TCA cycle in human

A. *Formal definition of Petri Nets*

1) *Definition 1:*

Petri was defined by [10] as an ordinary petri net as well as a marked petri net as follows;

***Ordinary Petri Net*:** This is a petri net whose arcs weigh one. This kind of petri net is said to be a safe petri net. Mathematically, this petri net is a four-tuple $N= (P, T, I, O)$ where $\forall\, p \in P$, $\forall\, t \in T$, $I(p, t) \leq 1$ and $O(p, t) \leq 1$

***Marked Petri Net*:** A marked petri net is a five-tuple with $N = (P, T, I, O, M)$ where

- P is the finite set of places $\{p_0, p_1, \ldots, p_n\}$
- T is the finite set of transitions $\{t_0, t_1, \ldots, t_n\}$
- I is the set of input places
- O is the set of output places
- M is the set of markings with M_0 being the initial set of markings

2) *Definition 2:*

Chaouiya et al, [10] defined petri net as a 5-tuple: $(N, M_0) = < P, T, Pre, Post, M_0 >$ where;

- P is a finite set of places (output and input places)
- T is a finite set of transitions, with $P \cap T = \emptyset$ *and* $P \cup T = \emptyset$
- Pre : $P \times T \rightarrow N$ defines weighted arcs between places and transitions
- Post : $T \times P \rightarrow N$ defines weighted arcs between transitions and places

M_0: $P \rightarrow N$ is the initial marking (an integer number of tokens associated with each place).

In some petri net graphs, there are test arcs that are represented by two directed arrows to and fro the transition and the place. Matrices can be generated for *Pre, Post* and M_0. The matrices are then used to obtain the incidence matrix *C* and also the next marking M′. the incidence matrix C is synonymous with the stoichiometric matrix of a metabolic pathway.

$$Pre = \begin{bmatrix} 2\,0\,1\,0\,0 \\ 0\,1\,0\,0\,0 \\ 0\,0\,0\,1\,1 \end{bmatrix} \qquad Post = \begin{bmatrix} 0\,1\,0 \\ 2\,0\,0 \\ 0\,0\,1 \\ 1\,0\,0 \\ 0\,0\,1 \end{bmatrix} \qquad M_0 = \begin{bmatrix} 2 \\ 0 \\ 0 \end{bmatrix}$$

$C = Post^T - Pre$
$M' = M + C.u$

$$M_1 = \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix} = \begin{bmatrix} 2 \\ 0 \\ 0 \end{bmatrix} + \begin{bmatrix} -2 & 2 & -1 & 1 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

Where u_i is the firing vector with $|T|$ components, all of its components except the i^{th} component are zero. This i^{th} component has a value of one to show that t_i fires (t_i is the i^{th} transition).

A transition is said to be enabled when the token(s) in the input place(s) are less or equal to the marking on the arc, i.e. $\forall p \in P$, *Pre (p, t)* $\leq M(p)$ [11].

For all the places in set *P* whose weights from input place *p* to transition *t* are lesser than or equal to the marking *M* of that input place *p*, the transition is enabled and can fire, firing allows a particular amount of token(s) to be consumed by the transition from the input place and produced by that transition to the output place. The number of tokens consumed could differ from the number of tokens produced by the transition. This process is crucial in a petri net model because this is how resources are transferred to places. Deadlock occurs when the firing rule cannot hold; this is the point where the resources (tokens) cannot be fired.

The stoichiometric matrix generated from the pathway information obtained from the Biocyc database showing the reactants with (negative) and products (positive) is provided in table 2 below. The matrix also contains the reversible reactions with "a" to show the first reaction and "b" to show the second reaction.

IV. RESULTS AND DISCUSSIONS

Using the data obtained from the Biocyc database, the stoichiometric matrix of the cycle was generated (This was explained in the third chapter). Using the stoichiometric matrix, the petri net model was constructed with the Systems Biology Markup Language; the snoopy tool is then used to view the constructed net.

A metabolic Petri net was constructed for the TCA cycle in Plasmodium Falciparum. This validates [12] definition of a metabolic petri net because it contains places (P) that represents biological compounds and transitions (T) that represents the biochemical reactions between metabolites that are being catalyzed by enzymes.

The tokens placed in the petri net model before firing are placed based on the fact that the place is an output place but not an input place. Places that are output places and also input places are not initialized with a token. The number of token is determined by the stoichiometric coefficient related to the compound in the chemical reaction.

The petri net model for the TCA cycle as the place oxoaloacetate with two token because it is enabling two transitions.

The Petri net is a hierarchical Petri net due to the fact that it is arranged in the order of the reactions in the cycle. The TCA cycle contains 10 reactions with a total of 21 reactants and 24 products. There are a total of 5 reversible reactions and 5 irreversible reactions.

TABLE II. STOICHIOMETRIC MATRIX GENERATED FROM THE PATHWAY INFORMATION OBTAINED FROM BIOCYC DATABASE SHOWING REACTION WITH NEGATIVE AND PRODUCTS POSITIVE

	1	**2a**	**2b**	**3a**	**3b**	**4**	**5**	**6a**	**6b**	**7**	**8a**	**8b**	**9**	**10a**	**10b**
Oxa	-1	0	0	0	0	0	0	0	0	0	0	0	1	1	-1
aCoA	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H_2O	-1	1	-1	-1	1	0	0	0	0	0	1	-1	0	0	0
Citrate	1	-1	1	0	0	0	0	0	0	0	0	0	0	0	0
CoA	1	0	0	0	0	0	-1	-1	1	0	0	0	0	0	0
Hp	1	0	0	0	0	0	0	0	0	0	0	0	0	1	-1
Cis-a	0	1	-1	-1	1	0	0	0	0	0	0	0	0	0	0
Dti	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0
NADPp	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0
Oxo-2	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0
Co_2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
NADPH	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
NADp	0	0	0	0	0	0	-1	1	-1	0	0	0	0	-1	1
S-CoA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
NADH	0	0	0	0	0	0	1	0	0	0	0	0	0	1	-1
Suc	0	0	0	0	0	0	0	-1	1	-1	0	0	0	0	0
ATP	0	0	0	0	0	0	0	-1	1	0	0	0	0	0	0
ADP	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
Phosphate	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
an-ubiquinone	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0
Fumarate	0	0	0	0	0	0	0	0	0	1	1	-1	0	0	0
An-ubiquinol	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
(S)-malate	0	0	0	0	0	0	0	0	0	0	-1	1	-1	-1	1
a quinone	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0
a quinol	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

TABLE III. REACTIONS IN THE TCA CYCLE

Reaction Number	Reaction Name / Gene Name	Reaction layout
Rx1	Oxoaloacetate transacetase / PF10_0218	Oxaloacetate + acetyl-CoA + H_2O → citrate + coenzyme A + H^+
Rx2	Aconitase / PF13_0229	Citrate ↔ cis-aconitate + H_2O
Rx3	PF13_0229	Cis-aconitate + H_2O ↔D-threo-isocitrate
Rx4	Oxalosuccinate decarboxylase / PF13_0242	D-threo-isocitrate + $NADP^+$ → 2-oxoglutarate + Co_2 + NADPH
Rx5	a-ketoglutarate oxidative / PF08_0045	2-oxoglutarate + coenzyme A + NAD^+ → succinyl-CoA + Co_2 + NADH
Rx6	Succinate thiokinose / PF14_0295	Succinate + ATP + Coenzyme A ↔ succinyl-CoA + ADP + phosphate
Rx7	Succinic dehydrogenase / PF10_0334	Succinate + an ubiquinone$_{[inner\ membrane]}$ → fumarate + an ubiquinol$_{[inner\ membrane]}$
Rx8	Fumarase / PFI1340W	(S)-malate ↔ fumarate + H_2O
Rx9	PFF0815W	(S)-malate + a quinone → oxaloacetate + a quinol
Rx10	Malic dehydrogenase / PFF0895W	(S)-malate + NAD^+ ↔ oxaloacetate + NADH + H^+

TABLE IV. REACTIONS LAYOUT OF THE TCA CYCLE

	Abbreviations	Full Meanings
1	$NADP^+$	Nicotinamide Adenine Dinucleotide phosphate
2	NADPH	Reduced Nicotinamide Adenine Dinucleotide phosphate
3	NAD^+	Nicotinamide Adenine Dinucleotide
4	NADH	Reduced Nicotinamide Adenine Dinucleotide
5	ATP	Adenosine Triphosphate
6	ADP	Adenosine Diphosphate

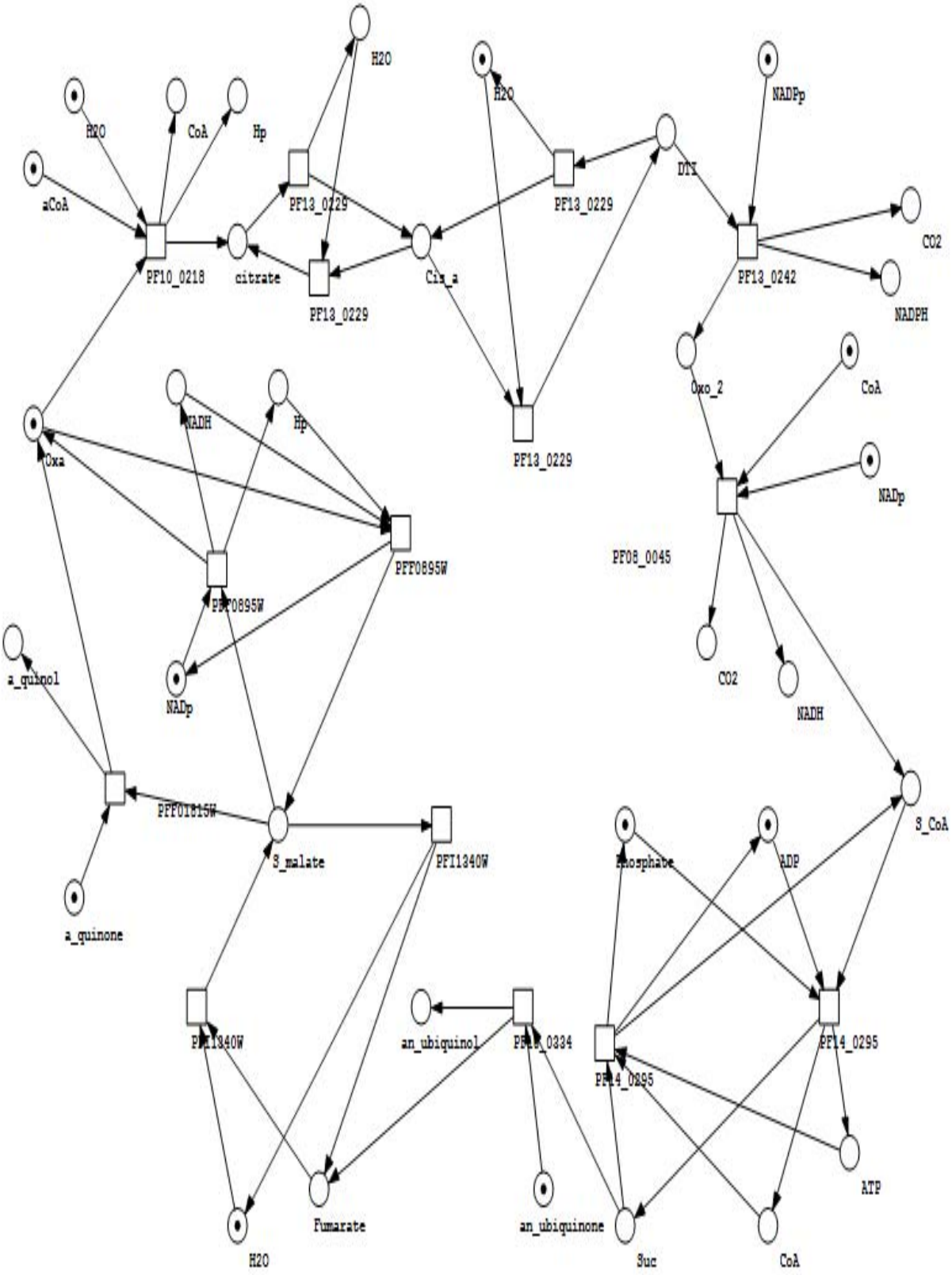

Fig 2: The petri net model before firing

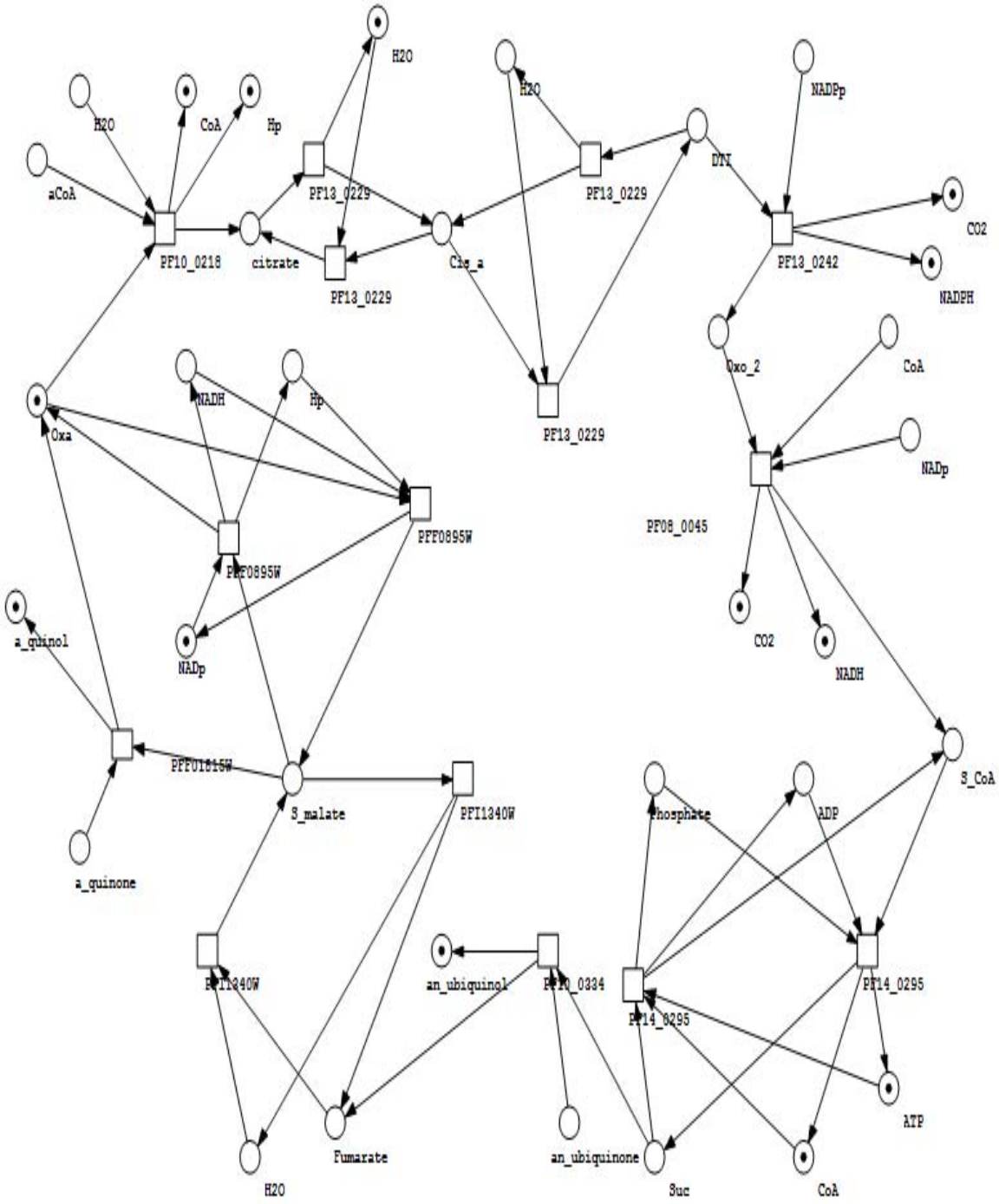

Fig 3: After firing the net

V. CONCLUSION

The Plasmodium falciparum is a major causative parasite of the malaria disease. Several attempts have been made to provide a means of reducing the impact of this disease in the country. This project takes the first step towards the reduction of the malaria impact.

The TCA cycle like the metabolic pathway is an energy producing pathway. It produces energy for the plasmodium falciparum and has also been found to be at the center of all the energy producing process. This makes the pathway an interesting one because if it is altered it can affect the process of energy production in plasmodium falciparum consequentially, the death of the protozoa.

The constructed petri net model and the identified choke points (essential reactions) that cause a deadlock in the model can be used to cause deadlock in the real pathway that could lead to the death of the parasite due to lack of energy.

ACKNOWLEDGEMENT

We acknowledge the support of Covenant University Center for Research, Innovation and Discovery, Covenant University, Ota, Nigeria.

REFERENCES

[1] C.A. Petri and R. Wolfgang, "Petri net," Scholarpedia, vol. 3, pp. 6477, 2008.

[2] A. Majumdar, S.D. Scott, J.S. Deogun and S. Harris, "Yeast phremone pathway modelling using petri nets," BMC Bioinformatics, vol.15, pp. 1-13, 2014.

[3] A.H. Hawari and Z.M. Hussein, "Simulation of a petri net-based model of the Terpenoid biosynthesis pathway," BMC Bioinformatics, vol. 11, pp. 1-11, 2010.

[4] M. Heiner, I. Koch and J. Will, "Model validation of biological pathway using petri nets – demonstrated for apoptosis," Computational Methods in Systems Biology. CMSB 2003. Lecture Notes in Computer Science, vol. 2602. Springer, Berlin, Heidelberg.

[5] J.F. Kerl, A.H. Wyllie and A.R. Currie, "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics," PubMed, vol. 26, pp. 239-257, 1972.

[6] S. Knowlton, "Petri Net Modeling and Analysis of Biological Processes Implicated in Amyotrophic Lateral Sclerosis," Bio-Grid REU, 2012.

[7] L. Albergante, J. Timmis, L. Beattie, and P.M. Kaye "A Petri Net Model of Granulomatous Inflammation: Implications for IL-10 Mediated Control of Leishmania donovani Infection," Plos Computational Biology, vol. 9, pp. 1-13, 2013

[8] P. Baldan, N. Cocco, A. Marin and M. Simeoni, " Petri nets for modeling metaboloic pathways : A survey," Natural Computing, vol. 9, pp. 955-989, 2010.

[9] Biocyc Database: : https://thebiogrid.org/

[10] A. Halder, "A study of Petri nets modeling Analysis and simulation," Indian Institute of Technology, India, 2006.

[11] C. Chaoiya, E. Remmy and D. Thieffry, " Qualitative petri net modeling of genetic networks," Transactions on Computational Biology, vol. 4, pp. 95-112, 2006.

[12] I. Koch, B.H. Junker and M. Heiner, "Application of Petrin net theory for modelling and validation of sucrose breakdown pathway in the potato tuber," Bioinformatics, vol. 21, 1219-1226, 2005.