Modelling and Simulation of Biological Regulatory Networks by Stochastic Petri Nets

Iftikhar A. Sheikh, Jamil Ahmad, and Muhammad T. Saeed

***Abstract*–Biological Regulatory Networks (BRNs) depicts the basic interactions in between various nodes in all biological systems. Inherently, all chemical and biological actions are governed by continuous functions, however, they have been modeled in discrete domain through Rene Thomas' Formalism. While the knowledge of steady states is sufficiently captured by this formalism, the dynamical properties of the changes taking place are not described. Hybrid Modeling was introduced to cover this aspect as it associated a range of delays with the changes in expression levels of various genes and hence insight in the dynamical behaviour of BRNs was achieved. However, the chemical reactions as well as the biological interactions are all stochastic in nature and could therefore assume any rate of change within the range of delays determined through Hybrid Modeling. Therefore, we have extended the Hybrid Modeling framework to Stochastic Modeling using an improved and simplified approach for conversion of BRN to Stochastic Petri Nets (SPNs) in which the random delay in firing of the transitions aptly captures the stochastic behaviour of changes in expression levels of genes. The proposed framework has been applied to the mucus production in Pseudomonas Aeruginosa BRN and results given by the Stochastic Petri Nets are in agreement with the Hybrid Modeling results from which establishes the accuracy of this approach as well as provide more insight in the dynamical behaviour of BRNs through simulation.**

***Index Terms*–Biological Regulatory Networks (BRNs), René Thomas Framework, Hybrid Modelling, Stochastic Petri Nets (SPNs)**

I. Introduction

BIOLOGICAL Regulatory Networks (BRNs) are used to describe almost all biological functions to cover the interactions taking place in various chemical reactions. The nature of these interactions is continuous and stochastic in nature and the rate of change in the underlying dynamics of these changes is not always fixed. Large number of formal approaches devised for modeling the structure of BRNs exist which are used for analysis of dynamical properties [1]. René Thomas Discrete Modelling technique [2] called for representing the change in expression level in a gene or biological entity with a logical function having discrete values. However, it ignores the time taken for the activation or degradation levels. Hybrid Modelling Technique [3] addressed this short coming by associating delays in BRNs by considering the change in levels as piece-wise linear functions thus making it possible to perform model checking and obtain various useful properties. This approach, however, only considers a linear rate of change in the levels and as a result it associates a range of delays within which a certain dynamical behaviour is possible.

Our approach is to extend the Hybrid Modelling Framework to Stochastic Modelling keeping in view the stochastic nature of the changes taking place in biological interactions. Stochastic Petri Nets (SPNs) is a useful tool for analysis of dynamics in Concurrent Systems and it has been applied to different biological networks [4, 5]. The random behaviour in the changes can be represented by using stochastic transitions in the SPN model. Chaouiya et al. have proposed the qualitative modelling of BRNs by Petri Nets in [6] and have proposed an approach for Petri Nets mapping of discrete multi-level biological regulatory models [7]. Based on these works, we have simplified the conversion of Biological Regulatory Networks to Stochastic Petri Nets. Petri Nets also offers powerful analysis tools for the modelled systems through simulation and checking of their various properties is also possible. We take the interesting case of mucus production in Pseudomonas Aeruginosa as a running example in this paper as it involves Activation, Inhibition and Self-regulation actions and also has a multi-valued Interaction Graph. The simulation results obtained from the modelling through our proposed simplified approach are in agreement with the previous results thus validating its applicability to BRNs.

The rest of paper is organised as follows: Section 2 discusses basics of René Thomas Discrete Modelling Framework and Hybrid Modelling Framework, the principles of Stochastic Modelling are described in Section 3 along with the Stochastic Petri Net model followed by its different properties and simulation results. The paper is concluded in Section 4.

II. Hybrid Modelling

A. Definitions

The René Thomas Discrete Modelling Framework for representation of Biological Regulatory Networks (BRNs)

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has been enriched with Parametric Time Delays in Hybrid Modelling presented in [3]. The main features of Discrete Modelling is based on Graph Theory as defined below:

Definition 1. (Graph)

Graph R is the ordered pair *R = (V, E)* in which, V, having typical element v, is the vertices set representing entities or dimensions, and E, having a typical element $e = (v_m, v_n)\ \forall\ v_m, v_n \in V$ is the edges set which represent the connections between the vertices.

A special class of graph is the directed graph in which the edges are directed from one vertex to another which implies that the pair constituting the edges is ordered and therefore, $e_1 = (v_m, v_n) \neq e_2 = (v_n, v_m)$.

The degree of each vertex in the directed graph is assigned on the basis of its connections with the other vertices, defined as:

Definition 2. (Degree).

Degree of vertex $v \in V$ is defined as the edges that a selected vertex has with other vertices in the graph. The degree is of two types; In degree: those edges that terminate in the selected vertex, and Out degree: those edges that originate from the selected vertex.

In Biological Regulatory Networks the genes are always interacting through each other's proteins thus limiting the edges to connect a gene with a protein and vice versa at a time. The graph formed by such network is known as bipartite graph and defined as below:

Definition 3. (Bi-partite Graph).

Graph *R = (V, E)* is bi-partite if and only if: $V = A \cup B$ such that $A \cap B = \emptyset$, and $E \subseteq (A \times B) \cup (B \times A)$. In BRNs, the edges are of two distinct types, Activation and Inhibition. The activation edge exerts a positive influence of the originating gene to the target element whereas the inhibition edge represents the negative influence. These distinct types of interactions lead to different behavioural dynamics which could not be represented by the static representation of the simple directed graphs. Therefore, the graph is converted to a dynamic network through the application of Kinetic Logic proposed in René Thomas' formalism.

A. Logic Formalism by René Thomas

Initially, René Thomas proposed a qualitative framework based on Boolean logic applicable on BRNs [2, 8] which closely approximated the ODE models. Later, it was realized that the Boolean model is insufficient to represent various interactions taking place in BRNs at varying gene expression concentrations. This led to the presentation of kinetic logic formalism which allows the modelling of discretely abstracted concentration levels other than Boolean as well [9]. Qualitative modeling approach have been used to model behavior of several biological networks including MAL-associated BRN [10], dengue virus pathogenesis and clearance mechanism [11], tail-resorption in tadpole's metamorphosis [12] and immunity control mechanism in bacteriophage lambda [13].

Thomas' Formalism is based on a modified graph called Biological Regulatory Network (BRN) which is adapted from [2, 9, 14] and formally defined below:

Definition 4. (Biological Regulatory Network).

Graph *R = (V, E)* is a Biological Regulatory Network when, each edge (v_m, v_n) is labelled by the pair $(j_{v_m,v_n}, \eta_{v_m,v_n})$ such that j_{v_m,v_n} is a positive integer and represents the concentration threshold level required for interaction, and $\eta_{v_m,v_n} \in \{+, -\}$ describes the type of interaction, i.e., '+' for activation and '−'for inhibition.

Definition 5. (Qualitative States).

These are the BRN configurations based upon the discrete threshold levels of its nodes. The qualitative state depicts a single system configuration which is different from other configurations in threshold level of one node at least. The System State Space is constituted by gathering all the system configurations. When all these configurations are represented in the form of directed graph then it is called the BRN State Graph.

Definition 6. (Resources).

When a single or a set of activators of a particular node is present (resp. inhibitors is absent) then it is described as the resource(s) of that node. The various sets of resources for the node are generated by different combinations of inhibitors and activators.

Definition 7. (Logical Parameters).

The BRN evolution is governed by the logical K parameters which contain the set of resources for a particular node.

The BRN now contains the required information to represent its dynamic behaviour, however it still does not represent the resulting trajectories. Such dynamics is represented by the State Graph (SG) which is generated by the BRN against a particular set of logical parameters governing the behaviours of each entity as a function of resources available for that entity in a given state.

B. Hybrid Modelling Framework

Hybrid modelling combines the discrete modelling domain of René Thomas with the continuous time domain. In effect, the levels of the entities remain the same, however each entity is assigned a clock which is responsible for measuring time for that entity [3]. Likewise, each entity is also assigned respective activation and inhibition delays which measure the time required by an entity to activate or deactivate from one discrete level to another. Fig 1 (a) shows a hypothetical graph showing the piecewise linear dynamics of the hybrid model.

III. Stochastic Modelling

The nature of most of the molecular processes is stochastic, therefore, the application of stochastic simulations is very suitable to these processes [15]. The Stochastic Petri Nets were first proposed for the modelling of various processes in Biological Regulatory Networks in [4] and several case studies have been discussed in [16, 17].

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A. Principles

The explanation of Stochastic Petri Nets as given in [18] is based on the assumption that the structure of the network given by the qualitative Petri Net which is independent of time is the same as its quantitative Stochastic Petri Net that is dependent on time. This implies that the discrete marking along with the structure is maintained in Stochastic Petri Net. The main difference in SPN is in the firing of the transitions which are enabled if pre places of that transition are marked appropriately, but, a waiting time *t* elapses before the enabled transition is fired with the actual firing not consuming any time. This waiting time is governed by the exponential distributed random variable $W_t \in [0, \infty)$ having a probability distribution function (pdf) as:

$$f_{W_t}(\tau) = \lambda(m) \cdot e^{-\lambda(m)\tau}, \tau \geq 0 \qquad (1)$$

Our approach.

In hybrid modelling, the piece-wise linear curve used for modelling the change in gene X expression assumes a constant rate of change w.r.t time. i.e., ΔX / Δt as shown in Fig 1 (a). Whereas, we know that the chemical reactions being stochastic in nature can have different rates which result in the change in the gene's expression level when its concentration reaches a particular threshold level. This is represented in Fig 1 (b) which shows that with different values of Δt, the slope of curve changes. In our approach of stochastic modelling, we have represented this varying time Δt with a random variable W_t. We have then used Stochastic Petri Nets to model this behaviour where the transitions are fired after a waiting time controlled by the random variable W_t with the actual firing not consuming any time. In this way, the stochastic nature of change in expression levels of genes is appropriately taken into account during simulations.

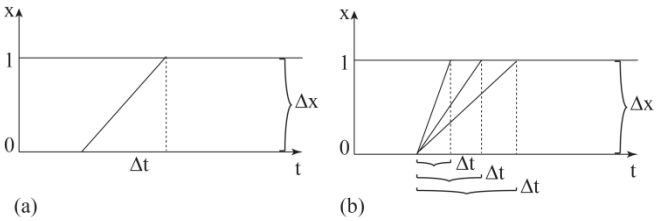

Fig. 1. (a) Piece-wise linear dynamics of change in expression level of a gene in hybrid modelling with constant rate of change. (b) Change in expression level of a gene in stochastic modelling can take place with different rates.

Modelling of stochastic variable.

Continuous Time Markov Chain (CTMC) describe the SPN semantics as it is similar to the graph of reachability in which the edges in between states is governed by the rates of the transitions. This implies that all reactions defined in the network structure in stochastic Petri nets still occurs, but its likelihood is dependent on the distribution of their probability. Therefore, the same techniques of analysis which are applicable to qualitative Petri nets can also be successfully applied to stochastic Petri nets. A timer local to each transition is assigned during simulation and starts with an initial value once enabled (i.e., its predecessor places are appropriately loaded). Then, depending on the probability distribution assigned to the particular transition, a waiting time is computed. During each simulation, the value of this waiting time comes out to be different. The transition will fire once the time elapses after the decrementation of timer is carried out with constant speed. In case of conflict between two transitions, the one with the smaller waiting time will fire before the other. After the firing of one transition, all waiting times are set to zero and the computation of waiting times is carried out again for the transitions which are enabled in the next state. It is highlighted that the firing of transition does not consume any time.

B. Definition

Based on the works of Chaouiya et al. [19, 7], in terms of converting BRNs to discrete PNs, as well as the descriptions of PNs given in [15, 20], the standard PNs, SPNs, and their properties are defined below:

Definition 8. (Stochastic Petri Net).

Stochastic Petri Net in the context of BRN is a tuple $SPN_{BRN} = (N, E, g, w, n_0)$, where:

- N, E are the number of places and transitions respectively.
- $g: ((\mathrm{N} \times E) \cup (\mathrm{E} \times \mathrm{N})) \longrightarrow \mathbb{N}_0$ assigns a positive integer to each arc.
- w is the array of firing rates λ(m) associated with the transitions in E where each λ is the parameter associated with random variable W_t.
- $n_0: N \longrightarrow \mathbb{N}_0$ defines the initial value of the places.

C. SPN Model of Pseudomonas Aeroginosa

We take the gene regulation of Pseudomonas Aeruginosa as our running example to show our stochastic modelling approach and compare it with the results of hybrid modelling. The Regulatory State Graph of production of mucus in Pseudomonas Aeruginosa is shown in Fig 2 (a) in which U represents Gene AlgU and V its inhibitor gene whereas its State Graph is shown in Fig 2 (b) which corresponds to the set of Thomas' K parameters given in Table 1.

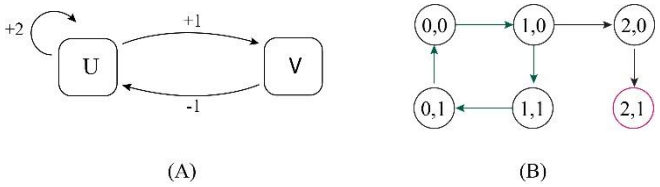

Fig. 2. (a) Regulatory Graph and (b) State Graph of mucus production in Pseudomonas Aeruginosa.

Table I.
K-parameters for Pseudomonas Aeruginosa

Parameter	Resources	Value
K_u	{}	0
	{U}	2
	{V}	2
	{U,V}	2
K_v	{}	0
	{U}	1

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D. Proposed Algorithm

A detailed procedure for converting a Biological Regulatory Network to a Petri Net has been outlined by Chaouiya et al. [6] in which each biological entity P is represented by two places (P primary and cP complementary) and each K parameter by two transitions (T+ source and T- sink) respectively. This approach results in a very complex Petri net. Here we have proposed a simplification in this algorithm by introducing only one transition $\mathcal{K}_i$ for each K parameter thus achieving a simplified Petri net representation with only half the number of transitions as compared to the method proposed in [6]. The pseudo-code for our proposed algorithm for converting a BRN to its compatible Petri net is shown in Fig 3. The application of this algorithm to our running example of Pseudomonas Aeruginosa is outlined in following steps:

3.4.1 Determining Places.

Each of the genes of Pseudomonas Aeruginosa, U and V, are represented by two primary places $\mathcal{G}$, (U and V), and two complementary places $c\mathcal{G}i$, (cU and cV) respectively.

3.4.2 Determining Transitions.

Instead of representing each resource of genes with two transitions as outlined in Chaouiya [7], we assign a single stochastic transition $\mathcal{K}_i$ to each resource which in this case are Tu_0, Tu_U, Tu_V and Tu_UV for gene U and Tv_0 and Tv_U for gene V respectively. This step greatly reduces the complexity of the resulting Stochastic Petri Net model and is easier to construct.

3.4.3 Determining Tokens.

The number of tokens are set according to the highest level of expression of each gene. For running example, the maximum expression level for gene U and gene V is 2 and 1 respectively, therefore, 2 and 1 tokens are placed in places cU and cV respectively. Moreover, the tokens are conserved in the complementary places for each gene. It implies that in all the markings of this SPN, the total number of tokens will not exceed 2 for places U and cU together for gene U. Similarly, the total number of tokens for gene V will be 1 for places V and cV together. Hence, this Petri Net is in the class of Bounded Petri Net.

3.4.4 Determining Edges.

The places $\mathcal{G}_i$ are connected with the transitions and transitions with the places as per the resources of each gene. For all resources which increase the level of gene U, the edges are directed from cU to U through the transitions Tu_V and Tu_UV. For K parameters which decrease the level of gene U, the edges are directed from U to cU through the transition Tu_0. Similarly, for gene V, the edges are directed from cV to V for transition Tv_U and from V to cV for transition Tv_0.

3.4.5 Determining Test Arcs.

Test arcs are also required to be used in SPN model as these are used for testing a certain number of tokens at the places to which it is connected and gets enabled only when the number of tokens equals the weight of the test arc. For those genes which act as activator of other genes, test arcs are connected from complementary place of that gene to those transitions of other genes which acts as resource for decrease in expression level of other gene. Similarly, the primary place of each gene will be connected to those transitions of other gene which act to increase the expression level of that gene. In this example, gene U being the activator of gene V, the test arcs are connected from complementary place cU to Tv_0 and from primary place U to Tv_U. This behaviour is completely reversed for the genes which acts as inhibitor for other genes. This can be seen in case of gene V where its primary place V is connected to transition Tu_0, while its complementary place cU is connected with transitions Tu_V and Tu_UV with test arcs. The weight of the arcs is set as per the corresponding value $\mathcal{V}_i \ of$ K-parameter.

Data: $\mathcal{G}_i$ [ ]; $\mathcal{K}_i$ [ ]; v_i [ ]; // No of Genes, K-parameters, and its Value
Result: Model $\mathcal{M}$ // Output Model containing Places, Transitions and Edges
initialization; // For Simple Edges
$for\ (i = 1\ to\ \mathcal{K}_i)\ do$
  $if\ (T.Source = T.Target)\ then$ // Self-regulation
    $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{G}_i, \mathcal{K}_i)$;
    $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{K}_i, \mathcal{G}_i)$;
  $else$
    $if\ (v_i = 0)\ then$
      $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{G}_i, \mathcal{K}_i)$;
      $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{K}_i, c\mathcal{G}_i)$;
    $else$
      $\mathcal{M} \leftarrow draw\ edge\ (c\mathcal{G}_i, \mathcal{K}_i)$;
      $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{K}_i, \mathcal{G}_i)$;
    end
  end
end
// For Test Edges
$for\ (i = 1\ to\ \mathcal{G}_i)\ do$
  $for\ (i = 1\ to\ \mathcal{K}_i)\ do$ // $\mathcal{K}_i$ for other genes than $\mathcal{G}_i$
    $if\ (\mathcal{G}_i\ is\ activator\ of\ \mathcal{K}_i)\ then$
      $if\ (\mathcal{G}_i\ is\ resource\ of\ \mathcal{K}_i)\ then$
        $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{G}_i, \mathcal{K}_i)$;
      $else$
        $\mathcal{M} \leftarrow draw\ edge\ (c\mathcal{G}_i, \mathcal{K}_i)$;
      end
    $else$ // $\mathcal{G}_i$ is inhibitor of $\mathcal{K}_i$
      $if\ (\mathcal{G}_i\ is\ resource\ of\ \mathcal{K}_i)\ then$
        $\mathcal{M} \leftarrow draw\ edge\ (c\mathcal{G}_i, \mathcal{K}_i)$;
      $else$
        $\mathcal{M} \leftarrow draw\ edge\ (\mathcal{G}_i, \mathcal{K}_i)$;
      end
    end
  end
end
$return\ \mathcal{M}$

Fig. 3. Pseudo code for our proposed algorithm for converting a BRN to its corresponding Petri net.

3.4.6 Self-Regulation.

For the case of self-regulation of a gene, the arcs to the transition which contains the gene itself as the resource, are

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drawn slightly differently than those described above. In this case, edges are drawn from the primary place to transition representing the K parameter for self-regulation with a weight equal to the threshold at which self-regulation is activated and back to primary place. Moreover, there are test arcs from all other genes to this transition as per the previous defined rules. i.e., for activator genes, test arcs from complementary places and for inhibitor genes, test arcs from primary places. For the case of Pseudomonas Aeruginosa we see that Gene U is a self-regulator at an expression threshold of 2, therefore, an edge is drawn from primary place U to Tu_U transition and back to U with a weight of 2. Moreover, a test edge is extended from primary place V to Tu_U transition as it is inhibitor of Gene U.

The resulting model of Stochastic Petri Net which represents the production in Pseudomonas Aeruginosa is constructed using the 'SNOOPY' tool [21] and is shown in Fig 4.

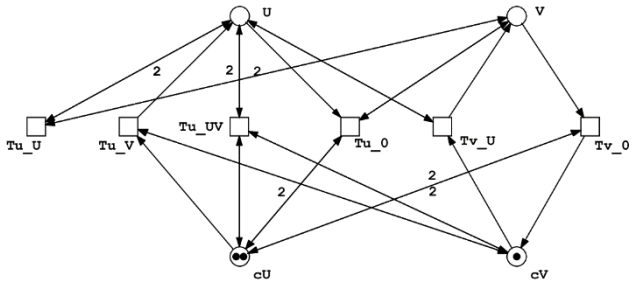

Fig. 4. Stochastic Petri Net model of mucus production in Pseudomonas Aeroginosa using the 'Snoopy' tool.

E. Comparison between Proposed and Existing Methods

The proposed method for conversion of Biological Regulatory Network to its corresponding Petri Net model considerably reduces the transitions and the interactions between transitions and places as compared to the existing method given by Chaouiya et al. [7] which results in a more efficient, simplified and improved Petri Net model. A comparison between the existing and our proposed method is given in Table II below. It can be seen that the number of transitions, directed arcs and test arcs are less in our proposed method as compared to the existing method. This implies that the proposed method leads to a much simplified model which is also evident from the time taken for 1000 simulation runs. The Petri net constructed by the proposed method takes 220 msec to complete the simulation whereas the Petri net made with the existing method completes the simulation in 385 msec.

Table II.
Comparison between Chaouiya et al. [7] and proposed methods for constructing Petri net for BRN of Pseudomonas Aeroginosa

Method	No of Places	No of Transitions	No of Directed Arcs	No of Test Arcs	Time taken for 1000 Simulation Runs
Chaouiya et al. [7]	4	12	21	12	385 msec
Proposed	4	6	8	10	220 msec

The advantage of proposed method in terms of less complexity and reduced simulation time would be more significant for BRNs having more number of genes. Moreover, there would be less number of conflicting transitions in the proposed method which means that there would be less variations in the observed behaviour of the Petri Net model during simulation.

F. Reachability Graph and Structural Properties.

The reachability graph of this SPN model is obtained from the 'CHARLIE' tool [22] and is depicted in Fig. 5 which is the same as given in the literature against the set of K parameters defined earlier. This confirms that the SPN model correctly represents the behaviour of mucus production in Pseudomonas Aeruginosa. Various structural properties of the SPN model can be quickly deduced which gives us further insight in the behaviour of the model. It is found that the model is Bounded, Conservative, Repetitive and Consistent but it is not Live.

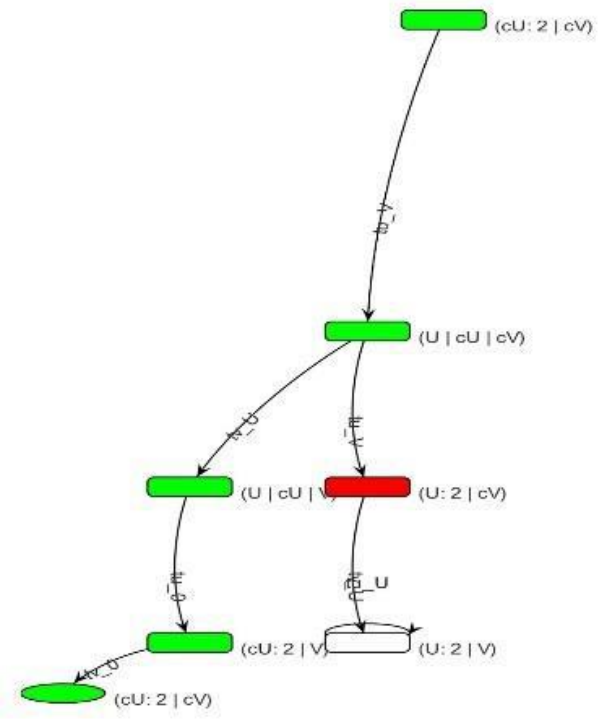

Fig. 5. Reachability Graph obtained from SPN using the 'Charlie' tool.

G. Simulation Results

A main feature of modelling in Petri Nets is the simulation of its behaviour w.r.t. time. So the SPN model of Pseudomonas Aeruginosa was simulated by assigning different probabilities to the conflicting transitions Tu_V and Tv_U. It can be seen that when the model is in state (1,0), firing of transition Tu_V will lead to the steady state (2,1) whereas firing of transition Tv_U will result in a cycle.

As can be seen in Fig 6 that for cases (a), (c) and (d) the expression levels for Genes U and V reach the values of 2 and 1 respectively which indicates that the mucus production has entered the steady state (2,1). Whereas, in case (b) it can be seen that the values of 0.5 for both genes U and V indicate that the mucus production is in a cycle between states (0,0), (0,1), (1,1) and (1,0).

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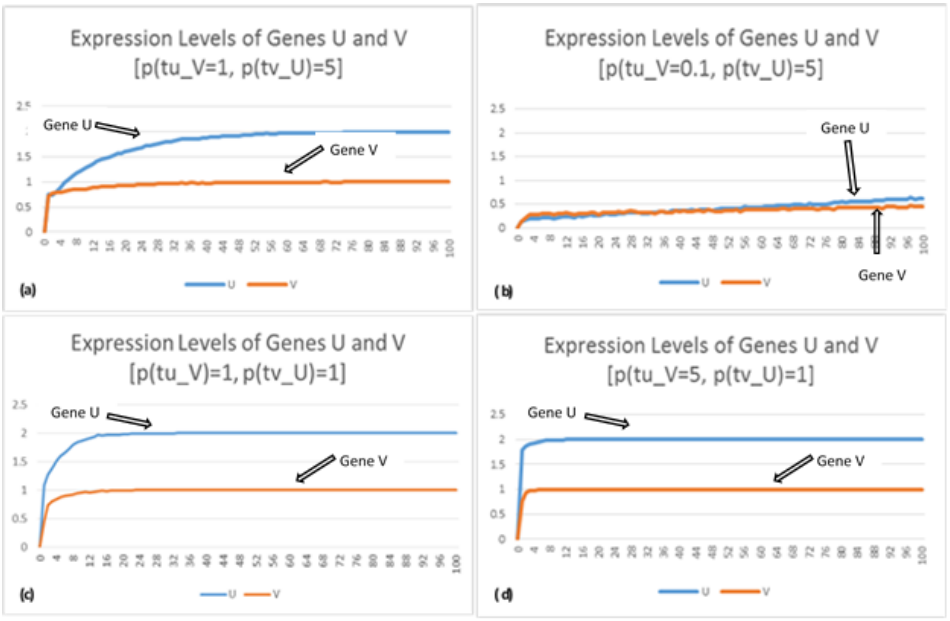

Fig 6. Simulation results for SPN Model with different probabilities for conflicting transitions

IV. Conclusion

Petri Nets offers a powerful framework for simulation and modelling of Biological Networks. Nature of biological interactions and chemical reactions demands that we consider their stochastic nature while modelling. It can be done through stochastic PNs. We have proposed a simplified approach for stochastic modelling of Biological Regulatory Networks with Stochastic Petri Nets which will make it easier to handle larger networks. The proposed scheme has been applied to develop a Stochastic Petri Net model of mucus production in Pseudomonas Aeruginosa. Simulation of this model with different probabilities of interactions show interesting behaviours. Moreover, various structural properties of the PN model have also been evaluated.

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