Understanding the control of a vitamin B_{12} riboswitch

Mohammad Abbadi[1], Sarah Spurgeon[2], Naziyat Khan[3] and Martin Warren[4]

***Abstract*— Within the life sciences switching mechanisms are pervasive at all levels, from molecules to cells and tissues. Their operation can be a key determinant of health or disease. Whilst the existence and importance of switches is widely acknowledged within the biological literature, many life scientists do not deal explicitly with the switching behaviour. Frequently, steady-state behaviour before and after switching is the primary focus. Here methods for analysis of switched systems from control engineering are applied to the modelling and analysis of a riboswitch. The model has been developed by studying the dynamics of the vitamin B_{12} riboswitch. The simulation results have been validated using *in vivo* experiments by checking the bacterial growth when using *Escherichia coli* and *Salmonella enterica* where the action of the vitamin B_{12} riboswitch is known to be a determinant of system behaviour. The paper first describes a simple model for the B_{12}-riboswitch regulatory network in *E. coli* and applies the same analysis when changing the strain to *S. enterica*. Validation of the simulation results has been undertaken by linking the dynamics of the riboswitch to bacterial growth.**

I. INTRODUCTION

Vitamin B_{12}, the cyano-derivative of cobalamin, is a water-soluble vitamin whose biological forms play key roles in metabolism that affect the normal functioning of the brain and nervous system in humans. Herein, we use the term B_{12} generically to refer to cobalamin. B_{12} is unique among the vitamins in that it is made exclusively by only certain prokaryotes and there is significant interest in how B_{12} production is controlled within microbial communities and how the nutrient makes its way through the food chain into animals. For humans B_{12} deficiency is most often associated with an autoimmune disorder that prevents the body from absorbing the nutrient but people on a strictly vegetarian diet are also prone to deficiency as plants neither make nor require B_{12}. In fact, dietary B_{12} deficiency is a severe problem in many developing countries including the Indian subcontinent, Mexico, central and South America and selected areas of Africa [1]. Moreover, B_{12} deficiency is also a common problem for the 115000 sufferers of Crohn's disease in the UK and millions more worldwide as the inflammation caused by the condition can affect the end of the ileum, which is the main area where B_{12} is absorbed [2]. To understand the control of B_{12} availability it is important to study the gene regulatory network (GRN), which is a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression [3].

Riboswitches are naturally occurring RNA-based regulatory components that essentially function by first sensing specific metabolites such as cofactors, amino acids and nucleotides and then regulating the expression level of proteins in the corresponding metabolic pathways [4]. The switches undergo a conformational change in response to a small-molecule ligand whereby increasing ligand concentrations either increase or decrease gene expression. The steadily increasing number of examples of such natural RNA regulators that control gene expression through diverse mechanisms in different organisms has fostered growing interest in using RNA to build synthetic controllers. Both naturally occurring and synthetic riboswitches are seen to be highly tunable components capable of regulating gene expression.

Some work to model riboswitch function has taken place and useful guidelines have been developed for tuning, but this work has been performed under steady-state operating conditions. The literature on the tuning of riboswitches also focusses primarily on the riboswitch as an individual element rather than as a component within a wider biological pathway [5]. The mathematical model which is used in this paper is based on the riboswitch regulatory pathway [3]. This riboswitch is capable, in principle, of regulating BtuB, which is an outer membrane porin that mediates high affinity binding and TonB- dependent active transport of vitamin B_{12} across the outer membrane. Regulation at the transcriptional level occurs when a B_{12} molecule binds the riboswitch aptamer domain and causes the formation of a terminator structure in the riboswitch expression platform. After estimating the parameters of the model, the system has been validated by creating an equation for the bacterial growth and it is proved experimentally that the model reproduces the system behaviour and captures the system dynamics within suitable bounds. For validation purposes, the growth levels in vitamin B_{12}-dependent bacteria were determined when grown in environments containing different concentrations of vitamin B_{12}. *E. coli* and *S. enterica* strains were used in the experiments and the growth levels were measured using optical density data recorded on triplicates at 600 nm. The verification process was performed by comparing simulation and experimental results. In addition, the assessment of the saturation effect when the concentration is high or low has been studied. The organisation of the paper is as follows: first, a preliminary analysis for the model of vitamin B_{12} is conducted. Second, the model validation is undertaken and finally, a comparison between the analytical analysis and the experimental results is made.

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II. ANALYSIS OF A SIMPLE MODEL OF THE VITAMIN B_{12} RIBOSWITCH

The mathematical model which is used in this paper is based on the riboswitch regulatory pathway. Gene expression can be regulated at several levels in a cell. Differential gene transcription regulates which genes are allowed to be transcribed into RNA while selective nuclear RNA processing regulates which transcribed RNA can enter the cytoplasm and become messenger RNA. Genes can be regulated any time before, after or during the processes of translation and transcription. *E. coli* is incapable of synthesizing vitamin B_{12}. Instead, these bacteria actively transport the vitamin from the environment. BtuB, which is encoded by gene btuB, is an important component of the vitamin B_{12} transporter. Gene btuB is negatively regulated by vitamin B_{12} via a riboswitch. To develop a mathematical model for the B_{12}-riboswitch regulatory network in *E. coli*, Santillan et al. (2005) [3] identified three normalised model variables m, e and p which respectively represent the concentration of btuB mRNA , the concentration of BtuB and the concentration of vitamin B_{12}. The model itself is described by:

$$\dot{m} = \gamma\,[\phi(p) - m] \tag{1}$$

$$\dot{e} = \xi\,[m\,\theta(p) - e] \tag{2}$$

$$\dot{p} = \delta\,[\epsilon(P_{ext})\,e - p] \tag{3}$$

The biological meaning of the parameters in (1) - (3) are shown in Table I. The functions $\phi(p)$ and $\theta(p)$ denote the B_{12}-governed regulation at the transcriptional and translational levels respectively. These functions are shown to be given by the following Michaelis-Menten equations:

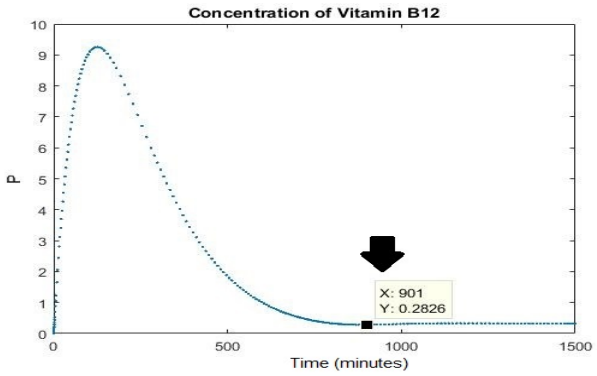
$$\phi(p) = \frac{K_\phi}{K_\phi + p}$$

$$\theta(p) = \frac{K_\theta}{K_\theta + p}$$

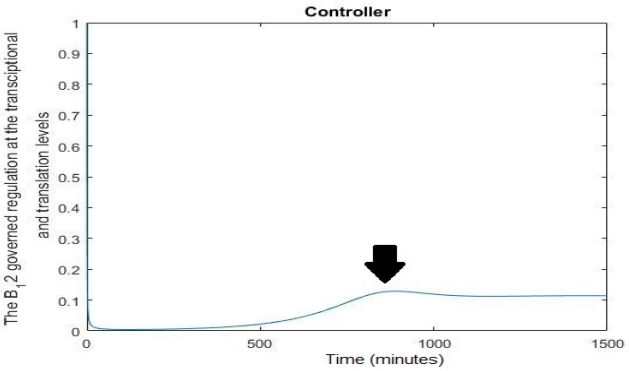
Santillan et al. [3] presents simulation results that have been undertaken for only 600 minutes, and the steady state value for the concentration of the vitamin B_{12} is 1. When the simulation is run for a longer time (1500 minutes), the concentration of vitamin B_{12} increases again after 900 minutes as shown in Fig. 1. This is an unexpected response and does not match the expected experimental results because the system should switch off when the concentration goes to zero. The controls $\Phi(P)$ and $\Theta(P)$ in the Michaelis-Menten equations depend on the value of the concentration of vitamin B_{12}, which is the third variable in the mathematical model (1) - (3) and thus the control system response is as shown in Fig. 1. As this does not match the expected experimental results, the parameters of the mathematical model (1) - (3) need to be modified. In the original paper [3], the dissociation constants at the transcription (K_ϕ) and translation (K_θ) levels are considered to be 1/4. Using this value, the steady-state value for the concentration will increase after 900 minutes. Based on the literature [8], [9] the dissociation constant for the reaction has a smaller magnitude. By changing the dissociation constant to 2.25 $\times$ 10^{-6}, the concentration output is correct and matches the results expected from experiments. The new parameter values are shown in Table I. The simulations using the modified parameters were carried out by solving (1) - (3) with MATLAB as shown in Fig. 2. Initial conditions of the normalised concentration of $btuB$ mRNA, the concentration of BtuB and the concentration of vitamin B_{12} were chosen as $m(0) = 1$, $e(0) = 1$ and $p(0) = 0$. All the simulations shown in this study used the same initial conditions. It is recognised from the simulation results of model (1) - (3) that the concentration of btuB mRNA has a rapid transient and thus it is assumed that

TABLE I: Pre-determined parameters in the mathematical model (1) - (3)

Symbol	Biological meaning	Original / New Experimental Value
γ	The mRNA degradation rate	7.1 $\times$ 10^{-2}
ξ	The btuB degradation rate	0.8 $\times$ 10^{-2}
δ	The B_{12} degradation rate	0.8 $\times$ 10^{-2}
$\epsilon(P_{ext})$	Represent the type of the strain	25
K_ϕ	The dissociation constant at the transcriptional level	1/4 / 2.25 $\times$ 10^{-6}
K_θ	The dissociation constant at the translation level	1/4 / 2.25 $\times$ 10^{-6}



(a) Concentration of vitamin B_{12}



(b) Controller

Fig. 1: The simulation results for the concentration of vitamin B_{12} and the controller in the original paper [3]

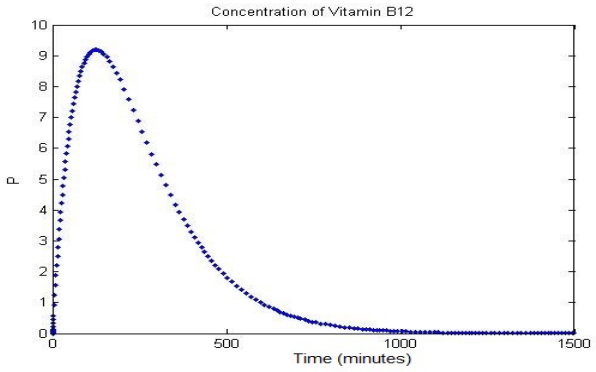


Fig. 2: Concentration of Vitamin B_{12} following simulation for 1500 minutes

this has reached steady-state in the subsequent analysis. The system's order is thus reduced to yield:

$$\dot{e} = \xi\left[\phi(p)\,\theta(p) - e\right] \tag{4}$$

$$\dot{p} = \delta\left[\epsilon(P_{ext})\,e - p\right] \tag{5}$$

using the steady-state value of m given by $\phi(p)$. There are several parameters that affect the reduced order mathematical model of the regulation equations for the B_{12} riboswitch as shown in (4) - (5). These parameters are the BtuB degradation rate, the B_{12} degradation rate and the value that is used to show which strain has been used (ϵ). The effect of varying these parameters on the concentration of vitamin B_{12} and the bacterial growth rate will be considered.

III. MODEL VALIDATION

To validate the model, it is necessary to model the growth curve from (4) - (5) and compare it with the growth curve obtained from the experiments. The comparison will include different levels of vitamin B_{12} concentration (50pM - 1nM). The effect of the parameters will be described in detail. For example, the relation between the concentration of vitamin B_{12} and the growth will be considered. Bacteria have three growth phases as shown in Fig 3.

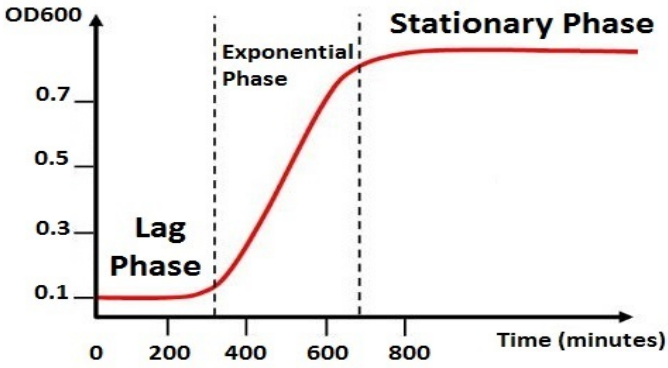


Fig. 3: Bacterial growth curve

1) **Lag phase**: when cells are transferred to fresh media, they require time to detect the environment, express specific genes, and synthesize components necessary for rapid growth. The cells are not dividing at this time.
2) **Exponential (log) phase**: binary fission occurs at a maximum rate, the cells are dividing as rapidly as possible.
3) **Stationary phase**: at this point, growth has stopped and there is no net increase or decrease in the number of cells. Bacteria use new forms of metabolism to survive in some cases producing secondary metabolites.

A model incorporating the effect of varying concentration of vitamin B_{12} with the OD600 bacterial growth output (which denotes the absorbance, or optical density, of a sample measured at a wavelength of 600 nm) is shown below:

$$OD_{600} = \begin{cases} OD_{Lag\,phase}, & \text{if } t \le t_l \\ [(\dfrac{0.057 \times P_{max}}{t_s - t_l}) \times (t - t_l)] + OD_{Lag\,phase} & \\ \text{if } t_l \le t \le t_s] & \\ OD_{t_s}, & \text{if } t \ge t_s \end{cases} \tag{6}$$

The parameters used in this equation with their biological meanings and experimental values are listed in Table II.

TABLE II: Pre-determined parameters in the growth equation (OD_{600})

Symbol	Biological meaning	Experimental Value
$OD_{Lag\,phase}$	The absorbance at OD_{600} when the growth is at the lag phase	0.1
t_l	Time to reach the log phase	400
t_s	Time to reach the saturation phase	Varies depending on vitamin B_{12} concentration
OD_{t_s}	The absorbance at OD_{600} when the growth is at the saturation phase	Varies depending on vitamin B_{12} concentration

The initial number of bacteria, bacterial type and the experimental environment depends on the particular experimental conditions. Table II presents a set of parameters consistent with the experiments performed. This choice may be justified by considering Fig. 4. P_{max} is defined to be the maximum value of the concentration of vitamin B_{12} and t_s is defined to be the time at which the maximum is reached. These values have been computed directly from the model (4) - (5) and can also be identified from the experimental results. It is clear that P_{max} and t_s vary with the change of Btub degradation rate ξ. Figure 5 shows P_{max} corresponding to different values of ξ. Changing the bacterial strain will also change P_{max} and this is demonstrated both from simulation and experimental results as shown in Fig. 6. Simulations were carried out by solving (4) - (5) and applying the simulation results in the growth equation. It is seen that by increasing the concentration of vitamin B_{12}, the absorbance at OD600

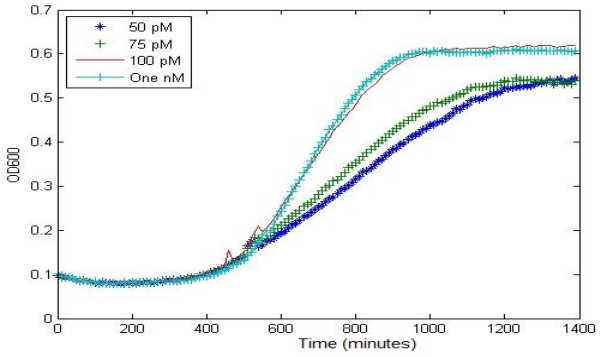
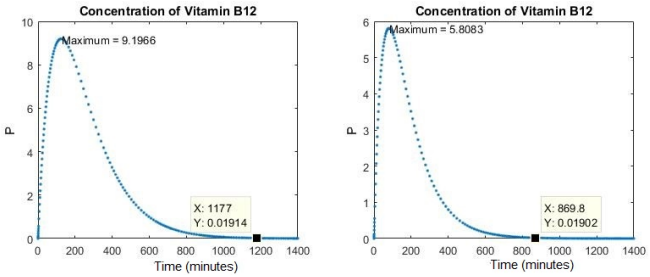
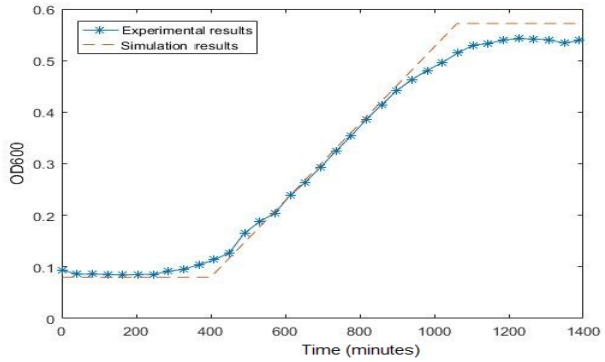


Fig. 4: Experimentally measured $E.coli$ growth curves with varying vitamin B_{12} concentration (50pM to 1nM)

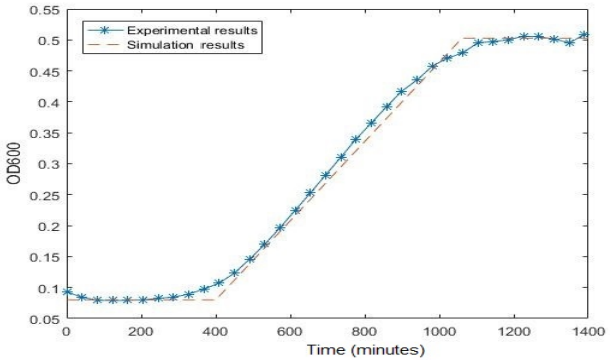


(a) 0.8×10^{-2} (b) 1.8×10^{-2}

Fig. 5: The concentration of Vitamin B_{12} when changing ξ from 0.8×10^{-2} to 1.8×10^{-2}



(a) Growth curve for 75pM when ϵ = 25



(b) Growth curve for 75pM when ϵ = 21.5

Fig. 6: Comparison between the experimental and the simulation results when changing the value of ϵ

will increase and the time required to reach the steady-state value will decrease. The time required to reach the stationary phase and the absorbance at OD600 for both simulation and experimental results for all concentrations is shown in Table III and Table IV. Fig. 4 shows the experimentally measured growth curve of *E. coli* with varying B_{12} concentration between 50pM and 1nM across three growth phases. In the lag phase, there is no change between the curves even when the concentration is changed. That is because the system requires time to detect the environment and synthesize components necessary for rapid growth while in the exponential phase. By changing the concentration of vitamin B_{12}, the growth curve changes. From Fig. 4, it is seen that the OD600 during the lag phase is 0.1 and t_s is 400 minutes. These two values vary from one plate reader to another as they are dependent on the number of bacteria present when the experiment is initialised and the atmospheric conditions in the laboratory, which may change. The exponential phase starts from 400 minutes to reach t_s. The value of t_s has a negative relation with the concentration of vitamin B_{12}, so when the concentration of vitamin B_{12} increases, the value of t_s decreases. In the stationary phase, the bacterial growth stops and there is no net increase or decrease in the number of cells. Varying the concentration of vitamin B_{12} will change the steady state value of absorbance. For example, when the concentration of vitamin B_{12} is 50pM, the value of absorbance at OD600 is 0.53 and when the concentration of vitamin B_{12} is 100pM, the value of absorbance at OD600 is 0.61 as shown in Fig. 7.

IV. Discussion

To validate the model, a comparison has been made between the experimental results and the simulation results as shown in Fig. 6. Table III shows a comparison between the time required to reach the stationary phase in simulation and experimentally while varying the concentration of vitamin B_{12}. It is clear that by increasing the concentration, growth is faster and it reaches the stationary phase more rapidly. In addition, by increasing the concentration the maximum value of the simulated concentration will increase and thus

TABLE III: Comparison between the time required to reach the stationary phase experimentally and the time required for the concentration to reach zero mathematically.

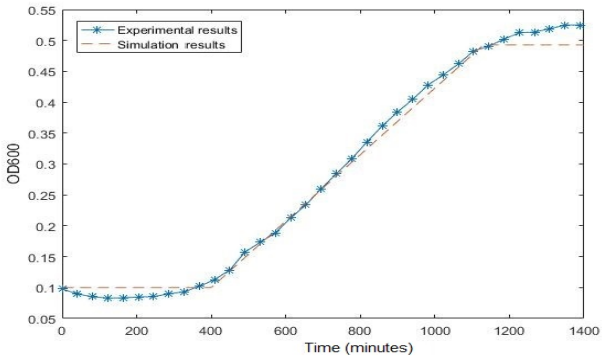
Concentration of Vitamin B_{12}	Time required to reach the stationary phase in Fig. 6, 7 (mins) Simulation Results	Time required to reach the stationary phase in Fig. 6, 7 (mins) Experimental Results
50 pM	1330	1497
75 pM	1170	1170
85 pM	1140	1135
100 pM	1090	1061

the absorbance at 600 nm will increase as shown in Table IV. Growth reaches the stationary phase when the concentration

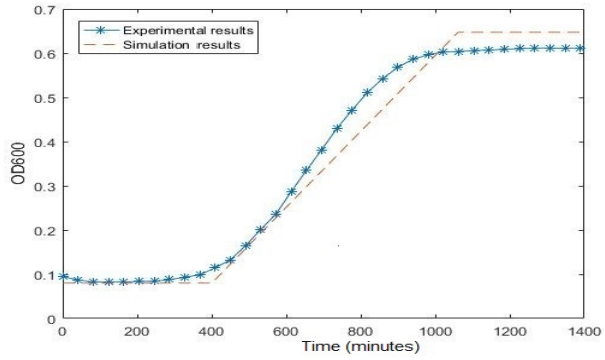
TABLE IV: Comparison between the absorbance at OD600 in the experiment and in the simulation.

Concentration of vitamin B_{12}	Simulation	Experiment
50 pM	0.503	0.532
75 pM	0.57	0.53
85 pM	0.61	0.57
100 pM	0.66	0.61

of vitamin B_{12} goes to zero. By increasing the concentration, the time to reach the stationary phase decreases. Fig. 7 shows a comparison between the experimental results and the simulation results for the growth curve when the concentration of vitamin B_{12} is 50pM and when it is 100pM. The simulation results have a similar trend to the experimental observations in terms of bacterial growth, peak values and steady-state values. The value of ϵ is related to the strain for each bacteria



(a) 50pM



(b) 100pM

Fig. 7: Comparison between the experimental and the mathematical growth curve with different concentration of Vitamin B_{12}

that has been used. Here, *E. coli* and *S. enterica* are used and they have different growth curves as shown in Fig. 6. The experiments correspond to the wild-type *E. coli* and 375 mutant strain *S. enterica* [10]. The values of ϵ employed in these simulations are as follows: ϵ = 25 (wild-type strain), ϵ = 21.5 (375 mutant strain). The value of absorbance at OD600

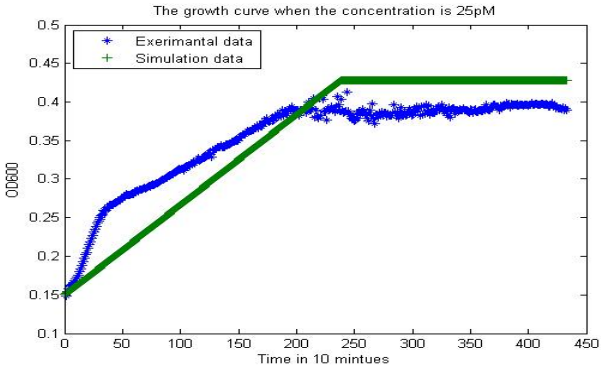
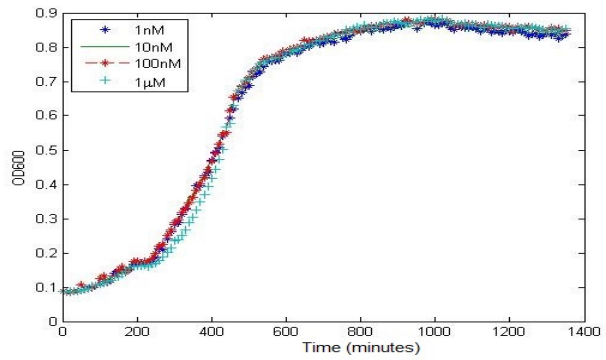


Fig. 8: Comparison between the simulation and experimental growth curve for E.coli with concentration of 25pM

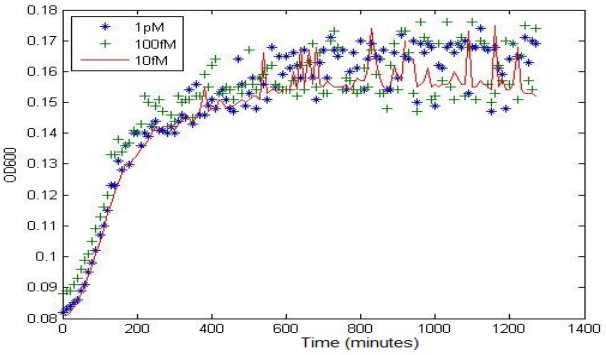
increases when the value of ϵ increases. From Fig. 6, the absorbance at OD600 is 0.63 when ϵ = 25 and it decreases to 0.52 when ϵ = 21.5.

After the model has been validated, an expected growth curve when the concentration of vitamin B_{12} is 25pM has been generated based only on the mathematical model. Experimental results were then obtained to verify the predictions. Fig. 8 shows the comparison between the simulated and experimental growth curve for *E. coli* with a concentration of 25pM. The model parameter t_l in (6) is zero and OD600 at the lag phase is 0.15. This setting is based on the plate that has been used for the experiments where it should be noted that results vary between plates.

It can be concluded that there is a positive relation between the concentration of vitamin B_{12} and bacterial growth, as when the concentration of vitamin B_{12} increases, the growth curve increases. Moreover, when the concentration of vitamin B_{12} increases to a certain level, it should be noted that bacterial growth will saturate. Comparing simulation and experimental results, it can be seen that the value of the concentration when the growth saturates is 1 nM; increasing the concentration of vitamin B_{12} by more than 1 nM, produces no change in the growth curve. Using the experimental results shown in Fig. 9a which are the experimental growth curve results for *E. coli* when the concentration of vitamin B_{12} is between 1nM and 100 μM, the experimental results agreed with the observed simulation results. In addition, this shows that for all the concentrations (1nM - 100 μM), the bacterial growth curve is approximately the same. Fig. 9b shows the growth curve for *E. coli* when varying the concentration of vitamin B_{12} between 1 pM and 10 fM. This also shows that when the growth is in the exponential phase, the growth is increasing slowly and the absorbance at OD600 is low. This also agrees with the simulation results, as when the concentration is low, the amplitude of the output is low. The growth curve remains in the exponential phase. When the concentration is decreased further, the absorbance at OD600 will also decrease to reach the same value as in the lag phase as shown in table V.



(a) high concentration



(b) low concentration

Fig. 9: Bacterial growth curve when the concentration of vitamin B_{12} are high and low

TABLE V: Simulation results for absorbance at OD600 with low vitamin B_{12} concentration.

Concentration of vitamin B_{12}	Simulation results
10 pM	0.2
5 pM	0.16
1 pM	0.11

V. CONCLUSIONS

In this paper, the effect of the vitamin B_{12} riboswitch has been tested at both the cellular and population level. For the cellular level, the concentration of $btub$ mRNA, the concentration of the BtuB and the concentration of vitamin B_{12} have been studied to determine how they affect the cells during transcription and translation. The dynamics of the vitamin B_{12} riboswitch have been incorporated in a model with *E. coli* bacterial growth. At the population level, the effect of varying the concentration of vitamin B_{12} has been tested on the bacterial growth curves of *E. coli*. The same analysis has been performed with different bacterial strains. The effect of changing the concentration of vitamin B_{12} in bacterium *S. enterica* has been tested. The results at both the cellular and population level have been linked and the simulation results obtained replicated the experimental results. The effect of enzyme inhibitors which are vitamin B_{12} analogues will be considered in future work. The long term objective of the study is to provide modelling and simulation tools to assist in the study of the impact of the vitamin B_{12} on health.

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