Electrical Communication in Bacterial Biofilms Based on Ion Channels

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Abstract

Recent discoveries indicate the use of potassium ion channels as a means of communication between distant bacterial biofilms. Results indicate that under metabolic stress, like lower glutamate levels, interior cells of the biofilm release intracellular potassium. This depolarises the neighbouring cells, and reduces their ability to intake glutamate, thereby amplifying and transmitting the signal further. This depolarised wave is followed by a hyperpolarized one, which may enable the cell to enhance glutamate concentrations. We studied these oscillations in bacterial biofilms with the help of a model similar to Hodgkin Huxley model of neuronal dynamics, seeing the similarity of our particular model with action potentials. We show that the modelling simulations match the experimental results to a very large extent .Then we move on towards plotting phase potraits for stability analysis and show that that there is an oscillation in membrane voltage and Extracellular Potassium null clines that explains the correlation between these two observed from experimental results shown in previous literature.

1 Introduction

Ion channels play an integral role in the generation of action potentials important for neuronal communication. These channels, including potassium, sodium, calcium,chlorine etc. have different behavior at different membrane potentials leading to the spiking behavior. Hodgkin and Huxley in 1952 modelled the biological cell as an electrical circuit and measured and quantified the spiking behavior[1]. Before it was thought that these ion channels were important for communication only in eukaryotes, but recent discoveries indicate that apart from playing a role in acid resistance and osmoregulation, they play an important role in electrical communication in the bacterial community.[5] In these microbial communities called biofilms, we can observe emergent phenomenon from the collective behavior of unicellular organisms. For this behavior, communication among different organisms in the community is key, and it was known this communication was through chemical signalling, a phenomenon known as quorum sensing[6]. But this could not possibly explain recent observations of metabolic oscillation between the interior and exterior members of the biofilm in glutamate depletion environment[4], as chemical messengers would diffuse very slowly throughout the biofilm. Upon investigating this thing in depth, recent discoveries point out that there could be electrical signalling going on in these biofilms of *B.subtilis*, analogous to action potential in neurons.

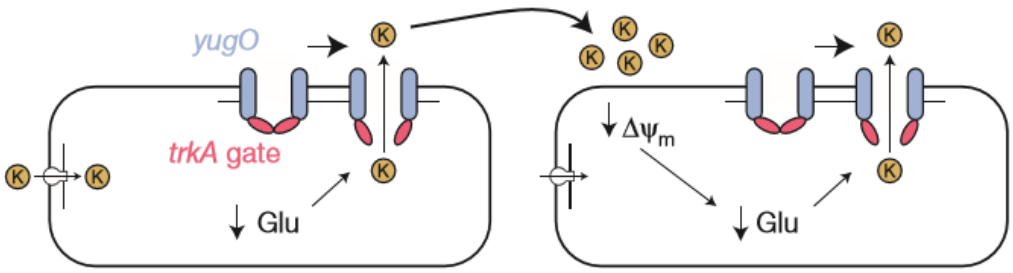


Figure 1: Potassium Signalling

Figure 1 depicts the basis of modelling bacterial communication.[5] The underlying principal is based on the fact that metabolically stressed cells release intracellular potassium, which imposes further stress on neighbouring cells.Depriving the cells of glutamate causes metabolic stress in the cell. This stress results in release of intracellular potassium as shown in the figure to neighbouring cells through YugO(potassium channel found in *B.subtilis*) gated potassium channels. Also, YugO gated channels play a major role in propagating potassium. This is proved by exposing the cells to bursts of extracellular potassium to simulate the dumping of potassium by the interior cells of the biofilm. We observe membrane depolarization, followed by a hyperpolarization phase that involves the increase in extracellular potassium by the aid of YugO channels. This model depicts the fact that stressed cells release intracellular potassium, which is aided by YugO channels that function as transporters of this potassium in extracellular space.

2 Material and Methods

2.1 Understanding the dynamics of a single bacterial cell

We investigate the mathematical model described by the study in depth.

Firstly we have the equation describing voltage dynamics:

$$\frac{dV}{dt} = -g_K n^4 (V - V_K) - g_L (V - V_L) \tag{1}$$

The equations are similar to the Hodgkin Huxley model of neuronal dynamics[1], with one notable difference being the absence of sodium term in the equations. This was done because experimental results from previous literature show that the sodium channel plays no role in these oscillations.

Next we have the gating variable equations:

$$\frac{dn}{dt} = \alpha(S)(1 - n) - \beta n \tag{2}$$

Here the opening rate of the potassium channel is dependent on the stress conditions. This is because the experimental results indicate that the oscillations happen only when the glutamate is limiting, and when supplied with a different nitrogen source like glutamine, the oscillations stops.

The opening rate increases when the stress is high, so we represent the dependence on stress with the Hill equation.

$$\alpha(s) = \frac{\alpha_0 S^m}{S_{th}^m + S^m} \tag{3}$$

High stress means that the glutamate levels are low in the cell, leading to the efflux of potassium ions which depolarises the nearby cells, and is actively transported to the outer layer to stop their intake of

glutamate, followed by a hyperpolarization wave which allows the interior cells to take up glutamate and stress levels falls.This oscillation is represented by the equation:

$$\frac{dS}{dt} = \frac{\alpha_S(V_{th} - V)}{exp(\frac{V_{th}-V}{\sigma}) - 1} - \gamma_S S \tag{4}$$

One more notable difference between this model and the Hodgkin Huxley model is the inclusion of a dynamic reversal potential term of potassium and the leak channel. This is because in neurons, we assume that the inflow and outflow of potassium is very fast, which is not the case with bacterial signalling. The efflux of potassium ions outside changes the reversal potential and hence is depicted by the following equations:

$$V_K = V_{KO} + \delta_K E \tag{5}$$

$$V_L = V_{LO} + \delta_L E \tag{6}$$

Extracellular potassium also varies with respect to time according to the voltage dependent behaviour of the potassium channel, with an additional parameter F that is related with the conductance of the cell and signifies the relation between membrane potential and extracellular potassium.

$$\frac{dE}{dt} = F g_K n^4 (V - V_K) - \gamma_e E \tag{7}$$

The dynamics of the Thioflavin T dye is represented by :

$$\frac{dT}{dt} = \alpha_t (V_{LO} - V) - \gamma_t T \tag{8}$$

This equation is important because the study[5] used a positively charged Thioflavin T(henceforth referred to as ThT) dye to quantify the membrane potential of the cell, the concept behind it being a decrease in ThT concentration indicates a decrease in the positive charge inside the cell, meaning that the membrane is becoming depolarised. Hence if we compare the ThT dynamics from the model with the experimental results, we can get a good idea about how accurate the model is.

2.2 Stability analysis

Often it is difficult to implicitly solve the differential equations, and in cases where it might be solved too, we get an added benefit by looking at it from a qualitative standpoint.This can be achieved by plotting null clines or analysing the phase space of the variables governing the differential equation. Null clines of a particular variable denote the region where that variable does not change with respect to time. If we have different variables, then the intersection of different null clines give us a particular point where there is no change with time, which is called an equilibrium point.By looking at the experimental data we hypothesised that we could reduce the dynamical parameters involved in the differential equations from five(given by equations (1) (2) (4) (7) (8) to two and then plot the null clines to get a better qualitative understanding.

2.3 Extending the model to space

We can determine whether cells can display long range communication or not by studying the propagation of the extracellular potassium through the biofilm. This helps to determine if the electrical signal obtained is caused by diffusion or is an active signal, simply from the fact that an active signal would not display decay over space. This can help understand that the oscillations in membrane potential across space can be mapped to the movement of extracellular potassium through the biofilm. To model this mathematically, we assume that the variables obtained so far represent local values for a particular square size

$$\Delta x = 30 \mu m \tag{9}$$

. We can extend it to N squares and describe potassium concentration

$$\frac{dE_i}{dt} = Fg_K n_i^4 (V - V_K) - \gamma_e E_i + \frac{D}{\Delta x^2}(E_(i+1) + E_(i-1) + 2E_i) \tag{10}$$

Here i labels the box along the chain and D is the diffusion coefficient.

Also we tried to emulate the series of research works done by the group(here in UCSD) like the discovery that nearby motile cells(not neccesarily of the same species) are attracted to the biofilm because of the dynamical potassium oscillation[2], and the time sharing of nutrients in nutrient poor conditions between different biofilms present nearby leading to enhanced communication strength as opposing to competition in nutrient rich conditions[3]. We thought that after replicating the results, we could move further by analysing the phase space using null clines(as described in Section 2.2) to distinguish the regions of competition and cooperation.

Table 1: Value of parameters used in this study

Parameter	Value
g_K	$30\ min^{-1}$
g_L	$0.2\ min^{-1}$
V_{KO}	$-380\ mV$
V_{LO}	$-156\ mV$
S_{th}	$40\ \mu M$
V_{th}	$-150\ mV$
α_O	$2\ min^{-1}$
β	$1.3\ min^{-1}$
m	1
F	$5.6\ mM/mV$
σ	$0.2\ mV$
δ_K	$1\ mV/mM$
γ_s	$0.1\ min^{-1}$
γ_e	$10\ min^{-1}$
γ_t	$4\ min^{-1}$
α_s	$1\ \mu M/(min\ mV)$
α_t	$1\ \mu M/(min\ mV)$
D	$13.8X10^{-6}\ cm^2/s$

3 Results

3.1 Dynamics of a single cell

The values of the various constant parameters were obtained from previous literature[5](again showed in Table 1). We then tried to first see what initial conditions should be selected, i.e what are the values of initial dynamic parameters at which we obtain constant behaviour with respect to time.

After some tweaking of the parameters we found out that at values shown in the figure 2, we see very little variation with time. Next, to simulate the conditions of rising extracellular potassium in a neighbouring cell, we provide an instantaneous flux of extracellular potassium of magnitude 200mM. Then we allow the system to adjust itself, the dynamics of which will be governed by the equations. We observe that there is an initial depolarization of the cell, denoted by a slight increase in V and a fall in ThT. This can occur probably due to the potassium ions entering the cell through the leak channels. Although in this particular part we are just simulating for a single bacterial cell, this depolarization wave will be transferred across the biofilm, leading to the exterior cells having a decrease in glutamate uptake. This wave will be followed by a hyperpolarization(shown below in figure 3), and since the glutamate uptake is driven by a proton motive force, the interior cells now uptake glutamate more readily than before.

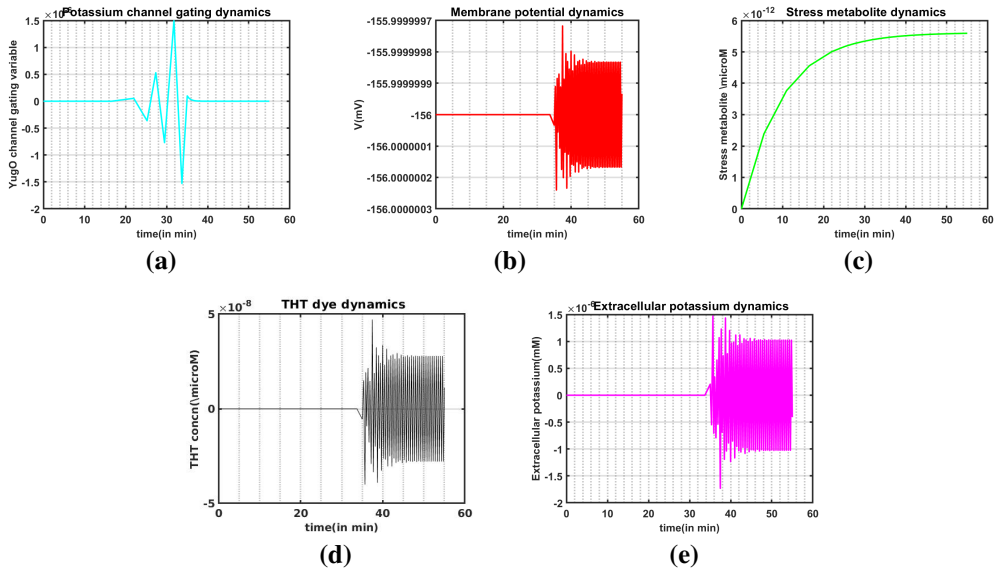


Figure 2: Resting Behavior of **(a)** Potassium ion channel gating variable **(b)** Membrane potential **(c)** Stress metabolite **(d)** ThT dye **(e)** Extracellular potassium

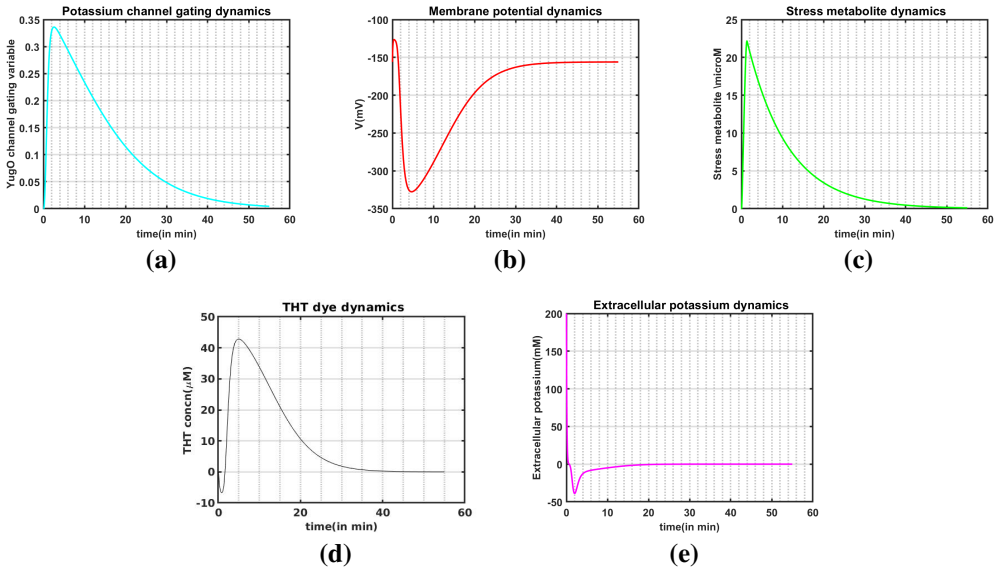


Figure 3: Dynamics of **(a)** Potassium channel gating variable. We see that there is a sharp exponential rise denoting the hyperpolarization phase where all the potassium leaves through the open gate,followed by a slow decay, showing the gates are no closing and the value approach the resting value **(b)** Membrane potential. Shows a short initial depolarization phase where potassium enters the cell followed by a hyperpolarization phase where potassium leaves the cell through the YugO channel, then it again returns to the resting potential when the gates start to close slowly. **(c)** Stress metabolite. A similar behavior is seen as the gating variable. When the potassium exits the cell it means that the cell is stressed(showing a sudden increase in stress levels) and when the hyperpolarization phase starts, i.e. when the cell begins to uptake glutamate, the stress decreases, although we notice that there is little delay between the beginning of the hyperpolarization phase and the stress levels falling. **(d)** ThT dye. The dye used to quantify membrane voltage shows a short drop followed by an exponential rise and then a slow decay(opposite to Membrane potential which is expected since it is positively charged) **(e)** Extracellular potassium. This graph has a little deviation from the expected results, and shows an exponential drop followed by a slow drop and then slow increase to the resting values.

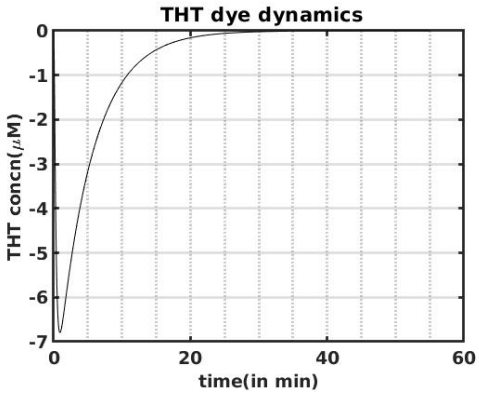
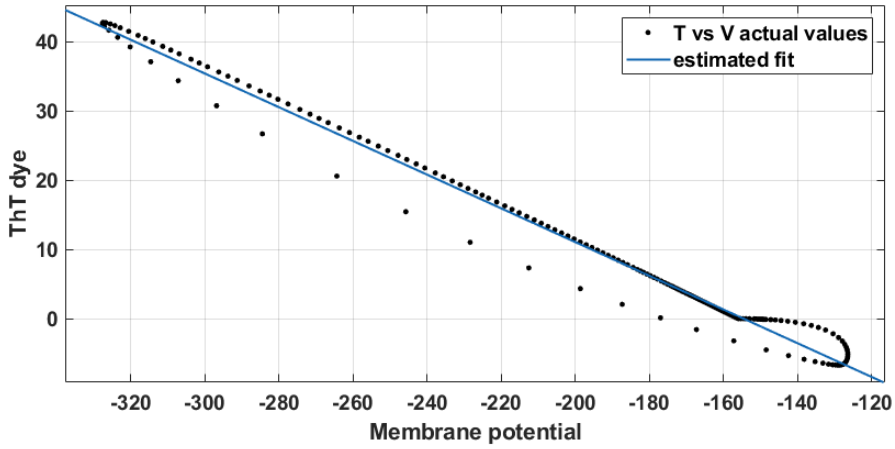


Figure 4: The dynamics of the ThT dye when the transport out of the cell is prevented by mutating the trkA gating domain of the YugO channel,and hence we observe that there is a depolarization seen by the sudden drop in ThT levels but there is no hyperpolarization phase (which is expected since this phase is because of the exit of potassium out of the cell through YugO channels) and instead it returns to the resting values

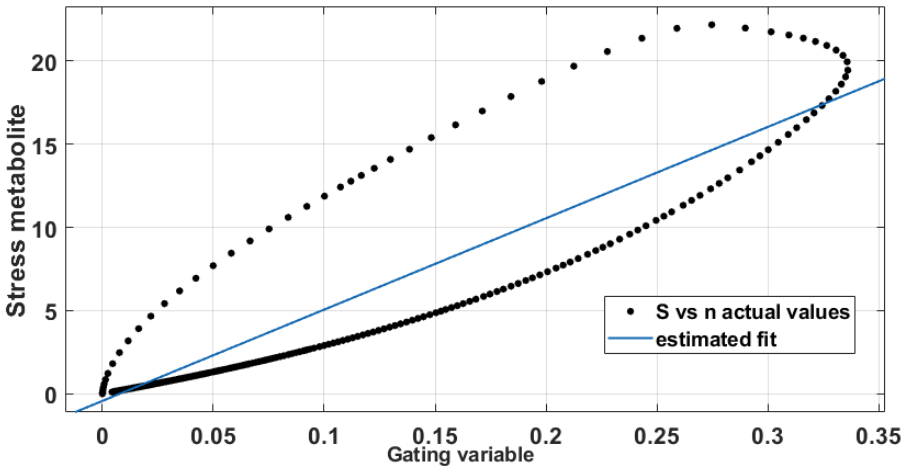
An interesting observation is that the extracellular potassium dynamics are different compared to the ThT dye, and the experimental results (in normal circumstances, no potassium clamp of 200mM) indicate similar behaviour of ThT and APG-4(a dye used to monitor the extracellular potassium). We think that this may be due to the artificial conditions caused by the clamping of potassium at 200mM. But since the model essentially considers that our single cell of study is a nearby cell surrounding the initial triggered cell,clamping is necessary so as to perturb the system to start oscillating.

3.2 Stability analysis

For the stability analysis, we try to reduce the number of parameters governing the set of ordinary differential equations. From Figure 3 we observe that the dynamics of the ThT dye is just the opposite of membrane voltage, which is expected since as discussed before, ThT dye helps in quantifying the membrane voltage and the characteristic of it being positively charged leads to opposite dynamics as membrane potential. We then estimate the exact dependence with a linear regression plot shown in figure 5. Another observation is a noticeable similarity between the gating variable n and the Stress parameter S, which also corroborates the experimental observation that the potassium channel opens only when the bacterial cell is stressed, like for example the depletion of glutamate. We linearly regress S(Stress parameter) on n(gating variable) also shown in figure 5.



(a)



(b)

Figure 5: **(a)** Fit of ThT dye vs membrane voltage. We can describe the fit based on the equation: $T = -0.2437 \cdot V + 37.67$ with a R^2value of 0.9877, where T is the ThT dye and V is the membrane voltage **(b)** Fit of Stress metabolite vs gating variable . Can be represented by the equation : $S = 54.88 \cdot n - 0.4328$ with a R^2 value of 0.8022, where S is the stress metabolite and n is the gating variable

And as noted before, we find that the behavior of extracellular potassium is slightly different from the experimental results, and when we tried regressing extracellular potassium on any of the parameters, we found no statistically significant fit. Hence instead of plotting phase portrait in 2 variables, we tried to do it in 3 variables. Here we tried to plot the null clines for a single fixed value of the third variable and then iterate it as many times as the length of the third variable. Thus we obtain a 3D null cline plot shown in figure 6. We observe that as we increase the extracellular potassium there is a kind of oscillation behavior on the V and E null cline. First at lower values of the extracellular potassium, the null clines lies in the intermediate regions of the phase potrait, shifting to lower regions at intermediate concentration of the extracellular potassium.If we increase the values of extracellular potassium further, we observe the V and E null clines shift to the upper side of the graph.

Also for majority of the part, there is no intersection between all the three null clines, meaning during this period there is no stable fixed point into which the system can fall into. There is an intersection between all three null clines at Extracellular Potassium concentration at about -38 mM(fig 6(a))(Also a point to note is the extracellular potassium or the ThT dye for that matter being negative signifies

that there is less extracellular potassium compared to intracellular, while for the ThT dye, it is the reverse i.e. more ThT is outside of the cell compared to the inside of the cell). The gating variable now changes with respect to time, but since the dependency on membrane voltage or extracellular potassium is lost(because of the way in which we have applied linear regression, see equation described in figure5), the N null cline doesn't change with respect to the changes in extracellular potassium or membrane voltage.

Lastly we conclude that the V and E null clines almost coincide at higher values of extracellular potassium, which agrees with the experimental observations perfectly as if the rate of change of two parameters is zero at particular values, then at those values there is a nearly 100 per cent correlation. This high correlation confirms the experimental data that say that change in membrane potential is because of the changes in extracellular potassium and in hindsight this qualitative analysis using phase space proved useful since we were not able to come at this conclusion just by looking at the dynamics obtained by solving the differential equations.

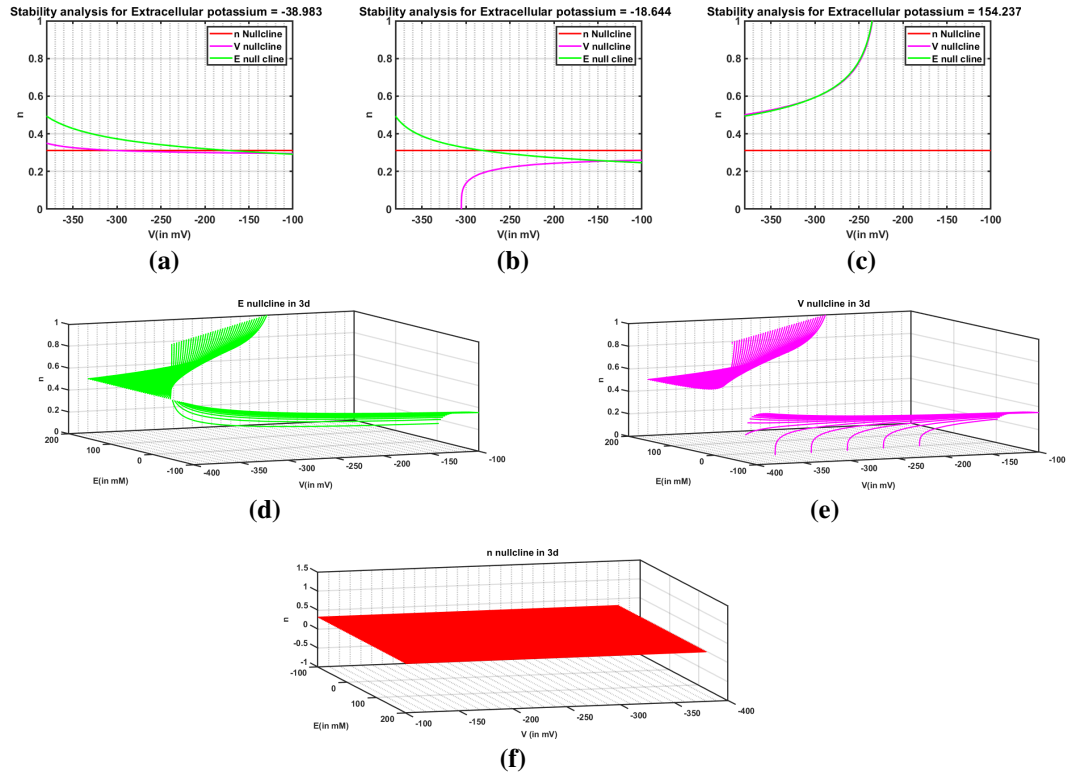


Figure 6: **(a)** Snapshot of the 3D plot at extracellular potassium(E)=-38.93mM, We observe an intersection of all the 3 null clines **(b)** Snapshot at E=-18.644, we observe that the E null cline shows a slow decrease(with respect to figure 6(a)) compared to the V null cline, which now lies on the lower region of the phase space **(c)** Snapshot at E=154.237, we observe that the E and V null clines now shift to the upper region and they both almost coincide **(d)** 3D null cline of Extracellular potassium **(e)** 3D null cline of membrane voltage **(f)** 3D null cline of potassium channel gating variable, we observe that it does not change with respect to the extracellular potassium values or the membrane voltage.

4 Conclusion and Future Scope

With our project we first emulated the experimental data and results of the study that discovered the phenomenon that bacteria use electrical signals to communicate among themselves in a biofilm. We corroborated the experimental data with our simulations showing that the dye concentration and membrane voltage are anti correlated and potassium channel gating variable and stress metabolite

show a positive correlation. Also we found that the extracellular potassium dynamics was a little different from expected, and this could be investigated further.

We then showed with our stability analysis that the V and E null clines behavior showed an oscillation from high to low and then again to high regions of the phase space, and although the reason why this behavior occurs could not be explained from the current simulations, we showed that it explains the correlation between extracellular potassium and membrane voltage observed in experimental results,and this surely presents an interesting prospect which has not been explored currently and could be investigated in the future.

We could not extend our dynamical mathematical model to space to prove that the signalling is actively amplified and is different from chemical signalling like quorum sensing as it proved difficult to simulate it in the time span of the project and since simulating this part involved using partial differential equations, it was also slightly outside the scope of the class.

On a concluding note, although the work may not seem important to the area of Neurodynamics at first, understanding how bacterial signalling works may turn out to be crucial for understanding the affect of bacterial infections in human neuronal diseases like Lyme's disease, Meningitis etc. Also the area of gut microbiome-neuronal interactions could utilise the effect of electrical bacterial signalling to enhance the knowledge of this already well studied field. The dynamics of potassium observed in bacterial electrical communication has a surprising similarity with cortical spreading depression (thought to be responsible for migraines) in neurons, where the neurons dump potassium in response to rising extracellular potassium levels. Studying the phenomenon of bacterial electrical signalling could provide more insights into this area.

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