Modelling Vibrio fischeri's behaviour Using P Systems

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Abstract. Quorum sensing is a cell density dependent gene regulation system that allows an entire population of bacterial cells to communicate in order to regulate the expression of certain or specific genes in a coordinated way depending on the size of the population. In this paper we present a model of the Quorum Sensing System in Vibrio fischeri using a variant of membrane systems called P systems. In this framework each bacterium and the environment are represented by membranes and the rules are applied according to probabilities computed using mass action law. This approach allows us to examine the individual behaviour of each bacterium as an agent as well as the behaviour of the colony as a whole and the processes of swarming and recruitment. Our simulations show that at low cell densities the bacteria remain dark while at high cell densities some bacteria start to produce light and a recruitment process takes place that makes the whole colony of bacteria to emit light. The above mentioned behaviour of our in silico bacteria maps well experiments and in vitro observations.

Keywords: quorum sensing, Vibrio fischeri, membrane computing

1 Introduction

Membrane Computing is an emergent branch of Natural Computing introduced by G. Păun in [12]. Since then it has received important attention from the scientific community. In fact, Membrane Computing has been selected by the Institute for Scientific Information, USA, as a fast *Emerging Research Front* in Computer Science, and [11] was mentioned in [19] as a highly cited paper in October 2003.

This new model of computation starts from the assumption that the processes taking place in the compartmental structure of a living cell can be interpreted as computations. The devices of this model are called P systems. Roughly speaking, a P system consists of a cell-like membrane structure, in the compartments of which one places multisets of objects which evolve according to given rules.

Most variants of membrane systems have been proved to be computationally complete, that is equivalent in power to Turing machines, and computationally efficient, that is able to solve computationally hard problems in polynomial time. Although most research in P systems concentrates on computational powers,

lately they have been used to model biological phenomena [1, 3, 14] and to specify artificial cell systems [16]. As P systems are inspired from the functioning of the living cell, it is natural to consider them as modelling tools for different biological systems.

In this paper we introduce a variant of P systems where instead of applying the rules in a maximal parallel manner we associate probabilities with each rule using mass action law, [2, 14]. According to these probabilities one rule is selected in each membrane and then all these rules are applied in parallel. This approach differs from the probabilistic approach taken in [1].

We have used this variant to model the Quorum Sensing System in the marine bacterium Vibrio fischeri. Bacteria are generally considered to be independent unicellular organisms. However it has been observed that certain bacteria have a gene regulation system that allows an entire population of bacterial cells to communicate in order to regulate the expression of certain or specific genes in a coordinated way depending on the size of the population. This cell density dependent gene regulation system is referred to as Quorum Sensing.

Up to now, most models of quorum sensing have used differential equations which focus on the description of the change of the average concentration of chemical compounds across the population. Here we use a new formalisation of this phenomenon in a computational framework which focuses on the description of the behaviour of each individual specifying the compartmental structure of the system (membrane structure) and the chemical reactions (rules) that take place in different regions of the system. This new approach allows us to examine the behaviour of each individual as an agent as well as the emergent behaviour of the whole population as a result of the processes of swarming and recruitment; processes which can also be easily studied in our model. Besides, differential equations treat reactions as continuous fluxes of matter which is correct if there is a very large number of molecules present in the system. Several authors argue that this approach is not correct in systems involving very small numbers of molecules. This is the case of quorum sensing systems where a small number of signal molecules inside a bacterium act as transcription regulators binding to a single 'molecule' of the DNA regulatory region. In this case stochastic approach are more accurate. In favour of our approach we also mention the easy understandability and programmability of this model, features which are not easily achieved in approaches which use differential equations.

The paper is organised as follows. P systems with Mass Action Dynamics are introduced in the next section. In section 3 a brief description of the Quorum Sensing System in Vibrio fischeri is given. The model of the Quorum Sensing System is presented in section 4. Results and discussions are exposed in the next section. Finally, conclusions are given in the last section.

2 P Systems with Mass Action Dynamics

In the structure and functioning of cells, membranes play an essential role. Cells are separated from the environment by means of a skin membrane, and they are

internally compartmentalised by means of internal membranes. It can also be thought that regions in the environment with different conditions are membranes where different processes take place. Inspired by these biological features Gh. Păun introduced P systems as an unconventional model of computation in [12]; for details and updated information on P systems we refer to [13, 20].

Next we give a formal definition of P systems with Mass Action Dynamics. A P system with Mass Action Dynamics is a construct:

$$\mathbf{\Pi} = (\Sigma, L, \mu, (w_1, l_1), \ldots, (w_n, l_n), \mathcal{R}) \quad \text{where:}$$

1. $n \geq 1$ is the degree of the system (number of membranes);
2. Σ is a finite alphabet of symbols representing chemical substances;
3. L is a finite alphabet of symbols representing labels for the membranes;
4. μ is a *membrane structure* consisting of n membranes identified with indexes 1, ..., n.
5. $w_1, \ldots, w_n$ are multisets over Σ and $l_1, \ldots, l_n$ are labels from L. Each (w_i, l_i) is associated with each membrane of the membrane structure μ.
6. $\mathcal{R}$ is a finite set of *rules* of the form:

$$u\,[\,v\,]_l \xrightarrow{k} u'[\,v'\,]_l$$

where $u, v \in \Sigma^*$ represent the reactants and $u', v' \in \Sigma^*$ represent the products of the reaction; $l \in L$ is the label of a membrane involved in the reaction and k is a number representing the kinetic constant, which depends on the physical properties of the molecules taking part in the reaction and the temperature of the system. In this sense the set of kinetic constants can be considered parameters of the system that may change depending on the current conditions of the system being modelled. A rule of the previous form should be interpreted as follows: the chemical substances u outside a membrane l and the chemical substances v inside a membrane l react together to produce u' outside l and v' inside l.

One of the main features that distinguishes one variant of P systems from another is the semantics for the application of rules. In most variants the rules are applied in a non-deterministic maximal parallel manner. Nonetheless, in our variant we will use mass action law to associate probabilities to each rule multiplying the kinetic constants and the current multiplicity of objects in the system. Then we will pick up only one rule in each membrane and we will apply them all in parallel.

Note that the larger the reaction rate and multiplicity of objects in the membrane are, the greater is the chance that a given rule will be applied in the next step of the simulation. Please note the similarities of this approach with mass action law applied in a continuous framework in [14].

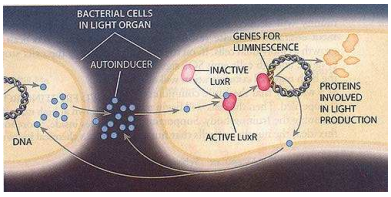
3 Quorum Sensing System in Vibrio Fischeri

Bacteria are generally considered to be independent unicellular organisms. However it has been observed that certain bacteria, like the marine bacterium *Vibrio*

Fischeri, exhibit coordinated behaviour which allows an entire population of bacteria to regulate the expression of certain or specific genes in a coordinated way depending on the size of the population. This cell density dependent gene regulation system is referred to as *Quorum Sensing*.

This phenomenon was first investigated in the marine bacterium *Vibrio fischeri*. This bacterium exists naturally either in a free-living planktonic state or as a symbiont of certain luminescent squid. The bacteria colonise specialised light organs in the squid, which cause it to luminesce. Luminescence in the squid is thought to be involved in the attraction of prey, camouflage and communication between different individuals. The source of the luminescence is the bacteria themselves. The bacteria only luminesce when colonising the light organs and do not emit light when in the free-living state.

The Quorum Sensing System in Vibrio Fischeri relies on the synthesis, accumulation and subsequent sensing of a signal molecule, 3-oxo-C6-HSL, an N-acyl homoserine lactone or AHL (we will call it OHHL). When only a small number of bacteria are present these proteins are produced by the bacteria at a low level. OHHL diffuses out of the bacterial cells and into the surrounding environment. At high cell density the signal accumulates in the area surrounding the bacteria and can also diffuse to the inside of the bacterial cells. The signal is able to interact with the LuxR protein to form the complex LuxR-OHHL. This complex binds to a region of DNA called the Lux Box causing the transcription of the luminescence genes, a small cluster of 5 genes, luxCDABE. Adjacent to this cluster are two regulatory genes for the transcription of LuxR and OHHL. In this sense OHHL and LuxR are said to be autoinducer because they activate their own synthesis.



The bacteria are effectively communicating, as a single bacterium is able to detect and respond to signals produced by the surrounding bacteria. Bacteria sense their cell density by measuring the amount of signal present; quorum sensing can therefore explain why the bacteria are dark when in the free living planktonic state at low cell density and light when colonising the light organ of squid at high cell density. A large number of Gram negative bacteria have been found to have AHL-based quorum sensing systems similar to Vibrio fischeri.

For a more detailed description of the Quorum Sensing System in Vibrio fischeri see the literature listed in the bibliography.

4 Modelling Quorum Sensing System in Vibrio fischeri

In this section we present a model of the Quorum Sensing System in Vibrio fischeri using P systems with Mass Action Dynamics. We will study the behaviour

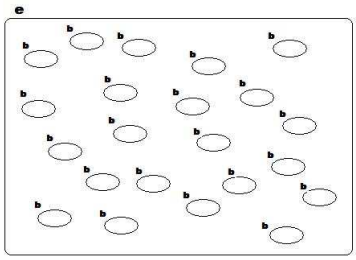
of a population of N bacteria placed inside a *single* environment by examining the evolution of the following P system:

$$\mathbf{\Pi}_{Vf} = (\Sigma, \{e, b\}, \mu, (w_1, e), (w_2, b) \ldots, (w_{N+1}, b), \mathcal{R}) \quad \text{where:}$$

(1) Alphabet: In the alphabet we represent the signal (3-oxo-C6-HSL), the protein LuxR, the complex protein-signal, the regulatory region LuxBox and the regulatory region occupied by the complex.

$$\Sigma = \{\text{OHHL}, \text{LuxR}, \text{LuxR-OHHL}, \text{LuxBox}, \text{LuxBox-LuxR-OHHL}\}$$

(2) Membrane Structure and Labels: In the Quorum Sensing System of Vibrio fischeri there are two relevant regions, namely the environment and the bacteria. The environment will be represented by the membrane labelled with e and each bacterium is represented by a membrane with label b. We can represent the membrane structure μ as a Venn diagram as follows:



(3) Initial Multisets: In the initial multisets we represent the initial conditions of the system. We are interested in examine how bacteria communicate to coordinate their behaviours and how the system moves from a downregulated state, where the protein and the signal are produced at basal rates, to an upregulated state. Therefore, in the initial multisets we will suppose there is nothing in the environment and in the bacteria we will only have the genome (LuxBox) to start the production of the signal and protein at basal rates.

$$w_1 = \emptyset,\ w_i = \{\text{LuxBox}\} \quad 2 \leq i \leq N+1$$

(4) Rules: In the rules we model the chemical reactions forming the Quorum Sensing System. Next we list the rules in $\mathcal{R}$ and we briefly describe the chemical reactions they represent:

• In an unstressed bacterium the transcription of the signal OHHL and the protein LuxR takes place at basal rates.

$r_1 :$ [LuxBox]$_b \overset{k_1}{\rightarrow}$ [LuxBox , OHHL]$_b$

$r_2 :$ [LuxBox]$_b \overset{k_2}{\rightarrow}$ [LuxBox , LuxR]$_b$

• The protein LuxR acts as a receptor and OHHL as its ligand. Both together form the complex LuxR-OHHL which in turn can dissociate into OHHL and LuxR again.

r_3 : [LuxR , OHHL]$_b$ $\stackrel{k_3}{\rightarrow}$ [LuxR-OHHL]$_b$

r_4 : [LuxR-OHHL]$_b$ $\stackrel{k_4}{\rightarrow}$ [LuxR , OHHL]$_b$

• The complex LuxR-OHHL acts as a transcription factor binding to the regulatory region of the bacterium DNA called LuxBox. The complex LuxR-OHHL can also dissociate from the LuxBox.

r_5 : [LuxBox , LuxR-OHHL]$_b$ $\stackrel{k_5}{\rightarrow}$ [LuxBox-LuxR-OHHL]$_b$

r_6 : [LuxBox-LuxR-OHHL]$_b$ $\stackrel{k_6}{\rightarrow}$ [LuxBox , LuxR-OHHL]$_b$

• The binding of the complex LuxR-OHHL to the LuxBox produce a massive increase in the transcription of the signal OHHL and of the protein LuxR.

r_7 : [LuxBox-LuxR-OHHL]$_b$ $\stackrel{k_7}{\rightarrow}$ [LuxBox-LuxR-OHHL , OHHL]$_b$

r_8 : [LuxBox-LuxR-OHHL]$_b$ $\stackrel{k_8}{\rightarrow}$ [LuxBox-LuxR-OHHL , LuxR]$_b$

• OHHL can diffuse outside the bacterium and accumulate in the environment. The signal OHHL in the environment can also diffuse inside the bacteria.

r_9 : [OHHL]$_b$ $\stackrel{k_9}{\rightarrow}$ OHHL [ ]$_b$

r_{10} : OHHL [ ]$_b$ $\stackrel{k_{13}}{\rightarrow}$ [OHHL]$_b$

• OHHL, LuxR and the complex LuxR-OHHL undergo a process of degradation in the bacterium and in the environment.

r_{11} : [OHHL]$_b$ $\stackrel{k_{10}}{\rightarrow}$ [ ]$_b$

r_{12} : [OHHL]$_e$ $\stackrel{k_{10}}{\rightarrow}$ [ ]$_e$

r_{13} : [LuxR]$_b$ $\stackrel{k_{11}}{\rightarrow}$ [ ]$_b$

r_{14} : [LuxR-OHHL]$_b$ $\stackrel{k_{12}}{\rightarrow}$ [ ]$_b$

(5) Parameters: As said before, the kinetic constants depend on the physical properties of the molecules taking part in the reaction and the temperature of the system, see also [2]. Therefore they can be considered as parameters of the system.

5 Results and Discussions

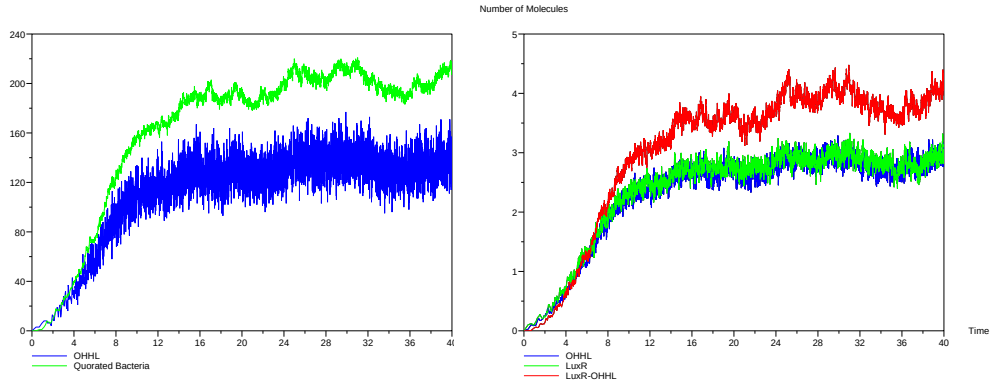
The model presented in the previous section has been represented in SBML, Systems Biology Markup Language, a computer-readable format for representing models of biochemical reaction networks [21]. And it has been implemented using Scilab, a scientific software package for numerical computations providing a powerful open computing environment for engineering and scientific applications [22]. The Scilab code was generated automatically from the SBML code using a translator written in Java.

In order to implement our model we have chosen the following set of parameters, $k_1 = 2, k_2 = 2, k_3 = 9, k_4 = 1, k_5 = 10, k_6 = 2, k_7 = 250, k_8 = 200, k_9 = 1, k_{10} = 50, k_{11} = 30, k_{12} = 15, k_{13} = 20, k_{14} = 20$. These values have been set such that the degradation rates $(k_{11}, k_{12}, k_{13}, k_{14})$ compensate the basal production of the signal and the protein (k_1, k_2) and such that the production rates

when the regulatory region is occupied (k_7, k_8) produce a massive increase in the transcription of the signal and the protein.

We have studied the behaviour of the system for two populations of different size to examine how bacteria can sense the number of bacteria in the population and produce light only when the number of individuals is big enough.

First we have considered a population of 300 bacteria. Next we show the evolution over time of the number of quorated bacteria [1], the number of signal (OHHL) in the environment and the evolution over time in the average bacterium across the population of the number of signal (OHHL), protein (LuxR) and the complex (LuxR-OHHL).



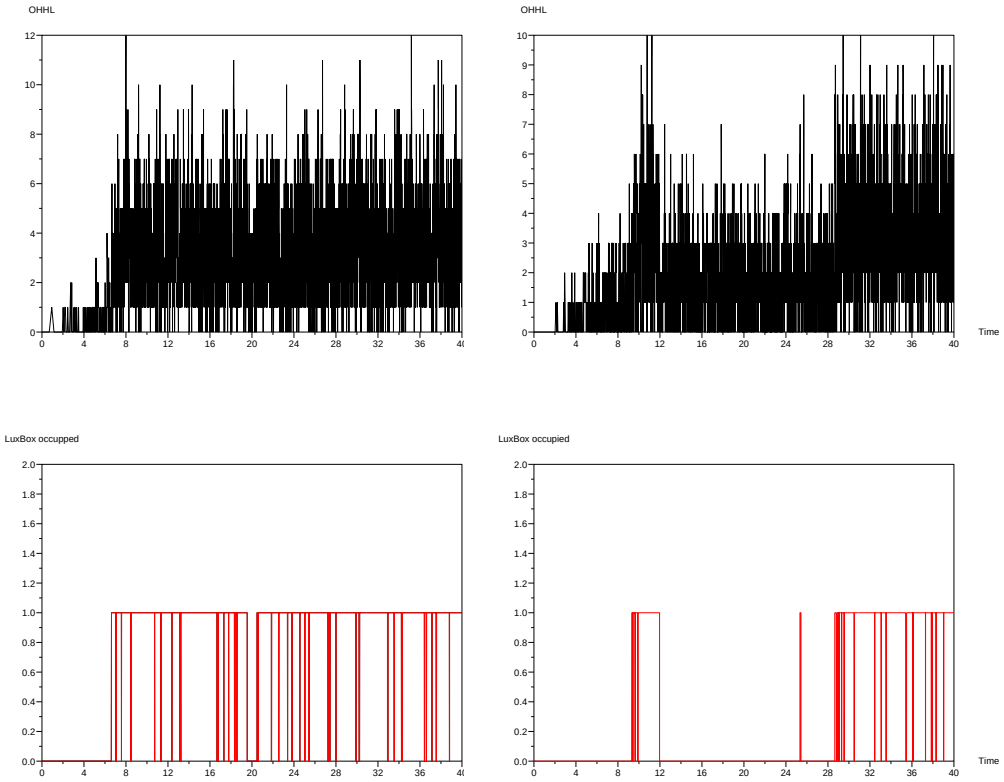
Observe that the signal, OHHL, accumulates in the environment until saturation and then, when this threshold is reached, bacteria are able to detect that the size of the population is big enough. At the beginning, a few bacteria get quorated and then they accelerate a process of recruitment that makes the whole population behave in a coordinated way.

There exists a correlation between the number of signal in the environment and the number of quorated bacteria such that, when the number of signal in the environment drops, so does the number of quorated bacteria and when the signal goes up it produces a recruitment of more bacteria.

Also note that in the average bacterium there is a correlation between the signal OHHL, the protein LuxR and the complex LuxR-OHHL. Besides, the pattern in the evolution of the average number of complexes across the population and the number of quorated bacteria are similar.

In our approach the behaviour of each individual in the colony can be tracked. We have taken a sample of five bacteria and have studied the correlation between the number of signal inside each bacterium (first row) and the occupation of the LuxBox by the complex (second row) which represents that the bacterium has been quorated.

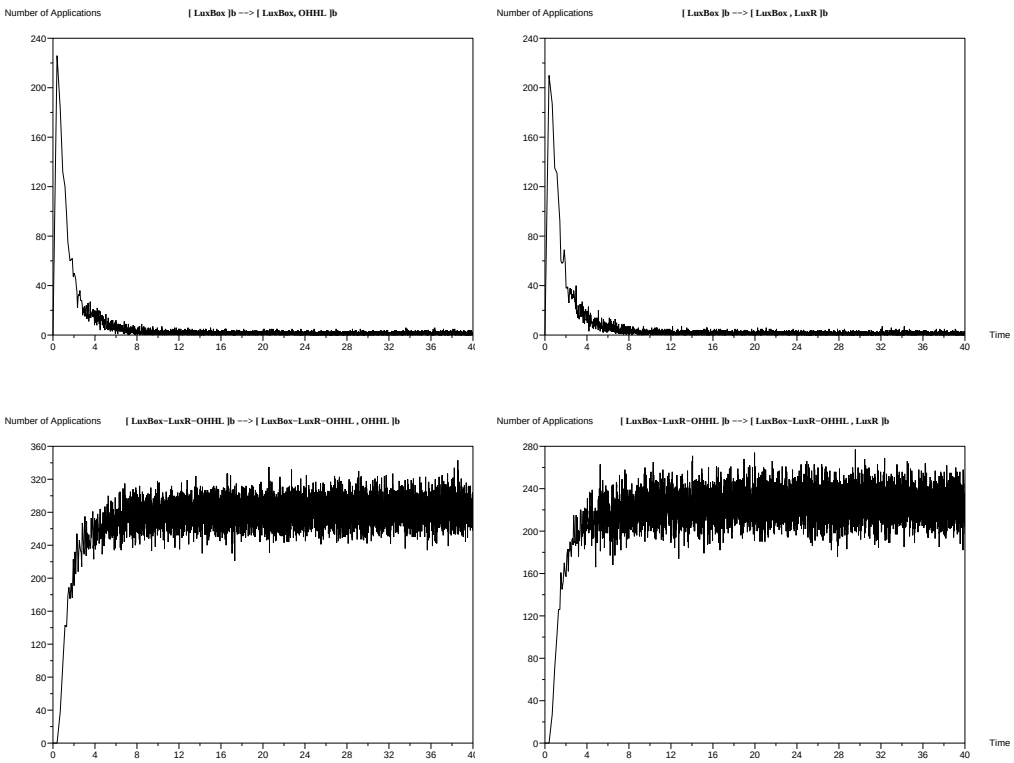
[1] We will say that a bacterium is quorated if the LuxBox in this bacterium is occupied by the complex producing the transcription of the enzymes involved in the production of light.



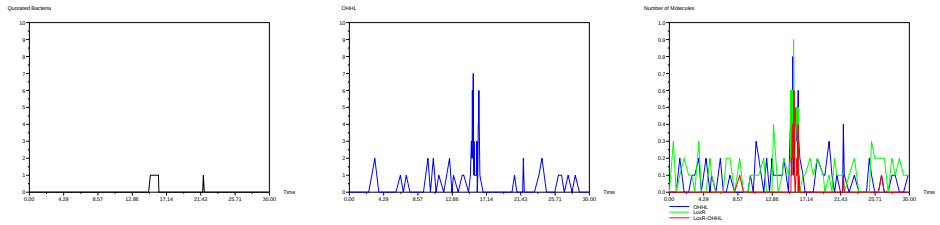
Above it is shown that the number of signal molecules inside the bacterium has to exceed a threshold of approximately 7 molecules in order to recruit the bacterium. Observe that when the number of molecules is greater than 7 the LuxBox is occupied, that is, the bacterium is quorated or upregulated but when there is less than seven signals the bacterium switches off the system and it goes downregulated.

We can also study how rules are applied across the evolution of the system. In the next page we show the evolution of the number of applications of the rules representing the basal production (first two graphs) and the rules representing the production of the signal and protein induced by the binding of the complex to the LuxBox.

Next it is depicted how at the beginning the basal production rules are the most applied rules while they other two are seldomly applied. But then, as a result of the recruitment process the bacteria sense the size of the population and they behave in a coordinate way applying massively the third and fourth rules. So the system moves from a downregulated state to an upregulated state where the bacteria are luminescence.

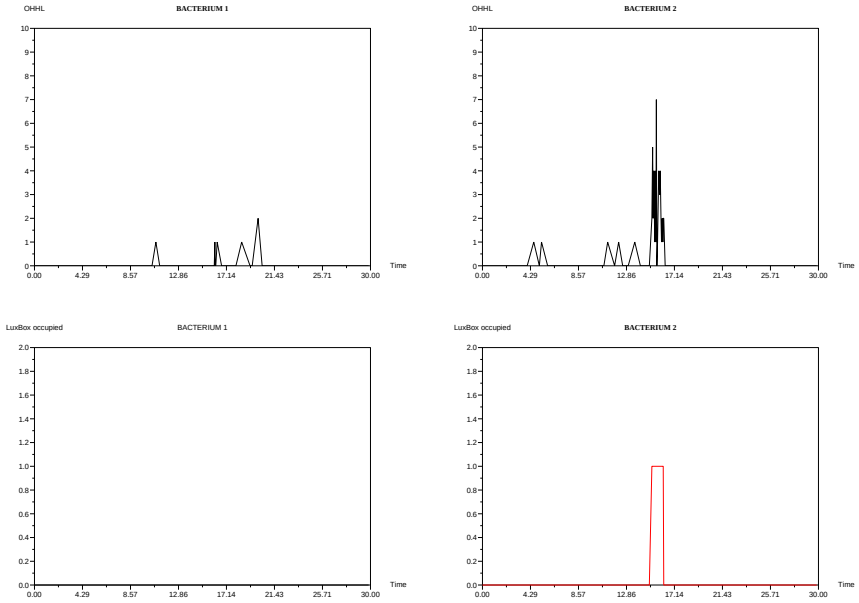


Finally, in order to study how bacteria can sense the number of individuals in the colony and get quorated only when the size of the colony is big enough we have examine the behaviour of a population of only 10 bacteria.

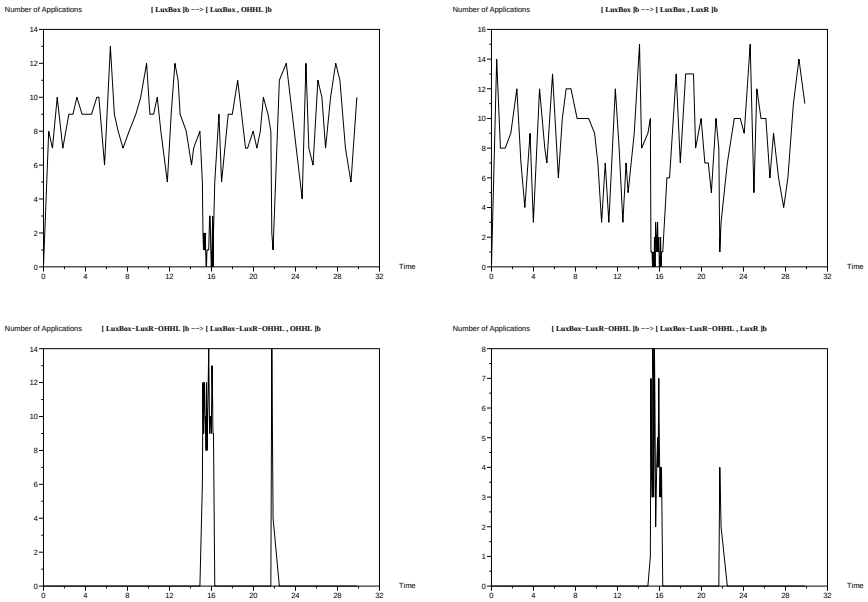


In this case no recruitment process takes place and only one of the bacteria guessed wrong the size of the population and got upregulated. But then, after sensing that the signal does not accumulate in the environment, it switched off its systems. In the average bacterium the number of molecules shows no pattern which means that the colony is not coordinating its behaviour.

In the next page it is depicted the behaviour of two bacteria in the population; one that never got quorated and the one that got quorated. Observe that this bacterium got quorated because the number of signal inside it exceeded the threshold of 7 signals.



Finally, observe that for only 10 bacteria the system remains in an downregulated state only applying the rules representing the basal productions while the rules associated with the production of light are seldomly applied.



Summing up, our simulations show that Vibrio fischeri has a Quorum Sensing System where a single bacterium can guess that the size of the population is big enough and start to produce light. Then this bacterium starts to massively produce signals, if the signal does not accumulate in the environment meaning that the guess was wrong it switches off the system. On the other hand if the signal does accumulate in the environment meaning that the number of bacteria in the colony is big a recruitment process takes place that makes the whole population of bacteria to luminescence. These results agree well with in vitro experiments and with results obtained using differential equations [4].

6 Conclusions

In this paper we have introduced a variant of P systems where the rules are applied following the mass action law. We have used this variant to develop a model of the Quorum Sensing System in Vibrio fischeri. The results of our model show that on the one hand bacteria remain dark at low cell densities and on the other hand in big size populations bacteria are able to sense the number of individuals and the whole colony starts to emit light in a coordinated way. This results agree well with in vitro observations.

A similar approach to ours has been used in an artificial life framework in [16, 17]. In their case the system is supposed to be a single volume or cell; while in our study we have examined the behaviour of a population of bacteria and their colective and coordinated activity as result of the communication system. In our approach we consider bacteria populations as ecological systems and simulate the chemical oscillations found in the quorum sensing, instead of considering bacteria as independent individuals.

Acknowledgement

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21. SBML Web Site `http://sbml.org/index.psp`
22. SciLab Web Site `http://scilabsoft.inria.fr/`