Model of Bacterial Conjugation using a Cellular Automaton and Evolutionary Computation

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Dedication

I want to dedicate this thesis to my loved son; to my wife, who supports me unconditionally in every step; to my mother and brother because they always believed in me and they did not let me give up; to my Grand-parents, who always have given me good advices and last but not least, to my father.

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First, I want to thank to my advisor Jonatan Gómez Perdomo for so many hours of corrections and discussions, for supporting me to continue when I wanted to stop trying. Second, I would like to thank to Engineer Arles Rodriguez, not just for all the help with my thesis; besides, for the commitment with the Alife Research Group and the unconditional help to each person who needs it. Finally, I want to thank to the Alife research Group and all friends of mine who were involved in this thesis, with advices, corrections or just for supporting me with words that I needed to listen, Thanks.

Resumen

Esta tesis presenta un modelo basado en autómatas celulares y computación evolutiva que permite abstraer el comportamiento de una población bacteriana en un ambiente hóstil. Se define un ecosistema donde convive una población de bacterias y a cada bacteria se le definen un conjunto de propiedades; las bacterias interactúan con el ambiente y con las bacterias que la rodean permitiendo que la colonia bacteriana evolucione. En las bacterias a través de varias generaciones pueden surgir mutaciones, procesos de conjugación y muerte de manera natural o a causa de la acción de antibióticos. Los resultados experimentales muestran que el uso de un antibiótico apropiado, con la dosis apropiada, puede causar la desaparición de la colonia bacteriana; al contrario, si el proceso de suministro de antibióticos no es el apropiado, se genera la resistencia a antibióticos como una propiedad emergente en la colonia bacteriana.

Palabras clave: (Conjugación Bacteriana, Autómatas Celulares, Mecanismo de Acción de Antibióticos, Computación Evolutiva, Mutación, Plásmidos, Resistencia a Antibióticos.

Abstract

This thesis presents a model based on cellular automata and evolutionary computation, the purpose of the model is to abstract the behaviour and properties of a bacterial colony. In the bacterial colony, each bacterium has a set of properties and interacts with the environment and other bacteria. Depending on environment characteristics, a bacterium can carry out a set of actions (to reproduce itself, transfer plasmids, die). The obtained results show that the application of the correct use of antibiotics (the correct type of antibiotics, at the correct generation, with the correct dosis) may cause the extinction of the bacterial colony; on the opposite, the antibiotic resistance is obtained as emergent property in the bacterial colony if the mechanism of action of antibiotics is not appropriate.

Keywords: Bacterial Conjugation, Cellular Automata, Mechanism of Action of Antibiotics, Evolutionary Computation, Mutation, Plasmids, Antibiotics Resistance.

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1 Introduction

Nowadays, antibiotic resistance has become a wide world problem, the world is asking for alternatives because the traditional solutions are not working anymore [?]. In the last forty years the production of new classes of antibiotics has decreased and bacteria are acquiring resistance to conventional antibiotics. Some studies claim that the bacterial resistance problem may be due to the way that antibiotics are used [?]; in fact, the incorrect use of antibiotics can neutralize the effect of other ones.

This problem can be due to the fact that there are not treatments neither antibiotics to treat the new strains of bacteria; statistics continually show that antibiotic resistance is dynamic and is spreading worldwide [?], which increases untreatable bacterial strains. In this way, biologists have described efficient mechanisms of genes exchange between bacteria which allows the propagation of antibiotic resistance; in fact, horizontal gene transfer has played an important role in evolution of antibiotic resistance. In particular, three processes are considered the key of horizontal gene transfer in bacteria [?]:

The first one is transformation, which allows a bacterium to take exogenous DNA up and recombine DNA into its chromosomes [?]; the second process is titled transduction where DNA from a bacterium is transferred to another bacterium by a virus; the last process is called bacterial conjugation where a horizontal transference of genetic material [?] is performed between two bacteria by using a structure called pilus located on the bacteria surface, this structure allows to hook with other bacteria and transfer the pieces of DNA called plasmids, even though they are not essential for bacteria, plasmids can provide new characteristics depending on their codification. Due to the lack of time, in this work the Bacterial Conjugation process will be studied and the other two processes will not be considered.

Some studies claim [?] that conjugation is a mechanism that provides new behaviours and characteristics to the recipient bacterium; one of the most important characteristics provided is the resistance to antibiotics. Authors such as Sengelov and zur Wiesch [?] have described the bacterial antibiotic resistance as an answer to the pressure caused by antibiotics on the environment.

Many characteristics of the bacterial populations have been modelled with tools of Artificial Life; one of the most interesting models developed is a simulator called “Bacsim” [?], which

allows to observe the growing of biofilms and emergent behaviours of bacteria; anyway, "Bacsim" is not focused on a specific design of bacterial conjugation or antibiotic resistance.

Other authors such as Simoes and Costa [**?**] propose a genetic operator called "VMEA" which is inspired by the conjugation process; this operator allows to replace the crossover operator considering the best individuals to be donors and the remaining individuals to be recipient; then, part of the genetic material of donors is transfered to recipient bacteria and the population of the next generation is the combination of resultant bacteria with the donor bacteria.

However, the work of Simoes and Costa does not capture in a natural way the process of bacterial conjugation due to the lack of biological processes involved in "VMEA" and the unnatural process of selecting the donor and recipient bacteria. In this thesis, a simulator that models the basic biological process involved in bacterial conjugation is developed, this model includes the populations of donor and recipient bacteria in a hexagonal cellular automaton, the plasmid concept which represents the emergence of new characteristics in a recipient bacteria, the diffusion of a mechanism of action of antibiotics based on a sand-pile model and the transference of plasmids between donor and recipient bacteria.

General Objective

The general objective of this work is to provide a simulator where can be modelled the process called Bacterial Conjugation, this simulator contains a bacterial colony where simulated bacteria can interact, live, die, transfer plasmids and the simulator also allows to display mechanisms of action of antibiotics. In this work, antibiotic resistance is an emergent property and can emerge depending on the environment conditions such as antibiotic pressure, quantity of nutrients and number of bacteria.

Specific Objectives

- **To design and implement a virtual environment of bacteria populations by using cellular automata.** In this work, a hexagonal automaton is used to represent the environment where bacteria interact and is composed by two layers. The "Physical Layer" shows the interactions between bacteria and the "Concentration Layer" shows the chemical properties in the environment. A first approach to the model of plasmids transference is proposed, which consists in dividing the bacterial population into two groups, donor bacteria and recipient bacteria (Donor bacteria have a pilus that allows to transfer plasmids to recipient bacteria that are in direct contact). Besides, initial experiments are conducted to analyse a mechanism of action of antibiotics; the diffusion of antibiotics is based on a sand pile model; donor bacteria are resistant to antibiotics

while recipient bacteria are susceptible and can die in the presence of such antibiotics.

- **To design a model of plasmid transference by using techniques of evolutionary computation.** Once the virtual environment has been developed, an evolutionary computation model is used to add diversity and evolution to the system. This work includes the development of new evolutionary operators, genetic code of plasmids and the rules to determine the action to be initiated by bacteria in each generation.

- **To develop a mechanism of action of antibiotics.** Once the model based on evolutionary computation is developed, the mechanism of action of antibiotics must change to be adequate for interaction with the new rules and with the environment. In addition,the genetic code for antibiotics and the set of rules to determine susceptibility or resistance of bacteria to antibiotics are included and experiments are run to determine the applicability of the model as an optimization technique.

- **Evaluate the performance of the model.** On one hand, this work analyses experimental results and evaluates the emergence of Antibiotic Resistance. On the other hand, some experiments are designed to evaluate the performance of the model in four well-known test problems in the Genetic Search Strategies research area.

Main Contributions

A simulated environment of bacteria

A simulated environment is developed where initial populations of bacteria can interact according to the characteristics of the environment; the interactions of a bacterium is regulated by its metabolism, the quantity of nutrients in its specific cell, the number and state of its neighbours and the action that the bacterium made in the previous generation. An article called "Bacterial conjugation simulation using a hexagonal cellular automaton" [**?**] was published in the 7th Colombian Computing Congress (7CCC), which presents a first approach of simulated environment of bacteria, in this article the environment is composed of two layers in order to represent the physical and chemical interactions of bacteria; in the "Physical Layer" the interactions of bacteria are simulated and the conjugation process is represented. Donor bacteria have a red line in one of their edge to represent the pilus, when a donor bacteria is in direct contact with a recipient bacterium and the conditions of the environment are adequate for conjugation, the donor bacterium transfers one plasmid to the recipient bacterium and turns into a donor bacterium in the next generation. The "Chemical Layer" displays the chemical properties that determine the environmental stress for bacteria, which generates an appropriate response in the Physical Layer.

A first approach to a Bacterial Conjugation Model

A mechanism of Bacterial Conjugation is developed to transfer genetic material between bacteria; new characteristics and techniques of evolutionary computation are added to the simulator in order to improve the abstraction of the conjugation process. Plasmids are represented by arrays of bits and have two important elements, an origin of replication (just for reducing the amount of similar plasmids) and resistance genes (genes that provide resistance to some types of antibiotics); bacteria can contain more than one plasmid affecting the metabolism of bacteria (higher the number of plasmids, higher the metabolism); when bacteria are not in a environmental stress some plasmids disappear due to the cost that represents to keep them.

A model of mechanisms of action of antibiotics

Studies [**?**] show that conjugation is one of the mechanisms that provides antibiotic resistance and transferring plasmids is one of the ways such antibiotic resistance is acquired by bacteria; hence, if plasmids start to disappear due to the lack of pressure on the environment, the rate of conjugation process would decrease. In this work, a mechanism of action of antibiotics is developed in order to exert pressure on selective places on the environment, the diffusion of the mechanism is based on a sand pile model which allows to spread the doses of antibiotics. Susceptibility rules, used to determine the efficacy of the antibiotics against a bacterium, are designed. These rules evaluate when the characteristics provided by plasmids to a bacterium are efficient against a respective type of antibiotic.

A Bio-inspired Optimization Technique

The model can be used as an optimization technique because the purpose of the plasmids is to transfer solutions between bacteria(individuals) that try to resolve the problem of surviving to a mechanism of action of antibiotics; therefore, the plasmids contain the solutions to some problems and the antibiotics indicate which problem will be optimized. In this way, four functions are initially used to evaluate the performance of the model for optimization purposes, these functions are chosen due to the complexity that they exhibit for genetic algorithms(Deceptive-3, Deceptive-4, MaxOnes and Royal Road); the obtained results are shown in chapter 3.

Thesis Outline

The structure of the thesis is as follows:

- **Chapter 2.** Provides a description about bacteria, antibiotics, cellular automata, evolutionary computation, bacteria simulations and bio-inspired algorithms.

- **Chapter 3.** First, this chapter introduces the development of the bacterial environment and interactions between resistant bacteria, sensitive bacteria and antibiotics. Then, this chapter introduces new characteristics to the model and improvements to the simulation such as evolution and new mechanisms of evaluation to determine resistance to antibiotics. Finally, the proposed model is used to optimize four functions: MaxOnes, Deceptive-3, Deceptive-4 and Royal Road.
- **Chapter 4.** Conclusions and future work.

2 Background

2.1 Bacteria

Bacteria are prokaryotic organisms that are involved in many processes in humans and other organisms, for example on host metabolism [**?**], immune systems [**?**] and many other processes, some bacteria also produce infections and diseases. Besides, bacteria are grouped in biofilms [**?**] where interesting social behaviours occur.

2.1.1 Gram-Positive and Gram-Negative Bacteria

Species of bacteria can be Gram-Positive o Gram-Negative depending on the chemical and physical properties of their cell walls and the quantity of a polymer called peptidoglycan. In order to classify the bacteria into Gram-Positive or Gram Negative, a method, called "Gram Staining", is used [**?**]. Gram-Positive bacteria are stained purple thanks to their thicker concentration of peptidoglycan, while Gram-Negative bacteria do not keep the purple stain.

2.1.2 Bacteria Shapes

Bacteria can be classified into Gram-Positive and Gram-Negative, but there is also a classification based on the shape of bacteria as follows:

Coccus

Coccus bacteria are sphere shaped or oval and are, depending on the arrangement or patterns formed, classified as follows: Diplococci are arranged in pairs, Streptococci are arranged in rows or chains, Staphylococci [**?**] are grapelike clusters of cells, Sarcinae are groups of eight or more cells and tetrads are four cells that are grouped in a square arrangement.

Bacillus

Bacillus species have a rod shaped [**?**], these species are Gram-positive bacteria and can grow in presence of oxygen. A bacterium of the *Bacillus* specie is usually found alone by itself and sometimes forming groups of two-cells pairs (Diplobacilli); in chains (Streptobacilli); or arranged in different angles (Palisades).

Spirilla

These species of bacterium have a spiral shaped and twisted body, according to some differences they can be classified as: *Vibrio* (curved rod shape like comma shape), *Spirilla* (twisted body like a spiral); and *Spirochaete* bacteria (long, helical and flexible body).

2.1.3 Bacteria Motility

Bacteria can also be classified based on their motility as flagellar and gliding.

Flagellar motility

The bacterial flagella [**?**] are filaments that allow a bacterium to move towards nutrients and other attractants. At the base of each flagellum, there is a rotate motor that generates the force that is required for moving the bacterium.

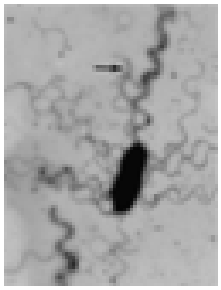


Figure **2-1**: Flagella.

Gliding motility

Some studies have shown that there is not just a mechanism of gliding motility in bacteria [**?**, **?**]. The mechanism depends on the kind and strain of bacteria being under study, in some cases, the gliding mechanism is based on the movement of a bacterium in different surfaces and in the absence of flagella.

2.1.4 Conjugation Process as a mechanism of antibiotic resistance

In some works [**?**, **?**], antibiotic resistance has been presented as the emergence property of bacteria dealing with the high pressure exerted by antibiotics. In 1947, Lederberg and Tatum introduced the first set of evidences of a genes recombination process in a population of bacteria *Escherichia Coli* [**?**], that can only be explained by the existence of a sexual process in bacteria. Then, there are basically three processes of genetic transference that have been titled for some scientists as "sexual processes of bacteria" regarding to the similarity to

eukaryotic sex. One of these processes is called bacterial conjugation, where a horizontal transference of genetic material [**?**] takes place between two bacteria.
Bacterial conjugation (see Figure **??**) is a process of fast transference of few pieces of DNA called plasmids; even though they are not essential for bacteria, plasmids can provide new characteristics depending on their codification. Plasmids (see Figure **??**) are transferred from one bacterium to another by using a structure called pilus that is located on the bacterium surface. This structure allows to hook the donor bacterium with other bacterium and transfer the plasmids.

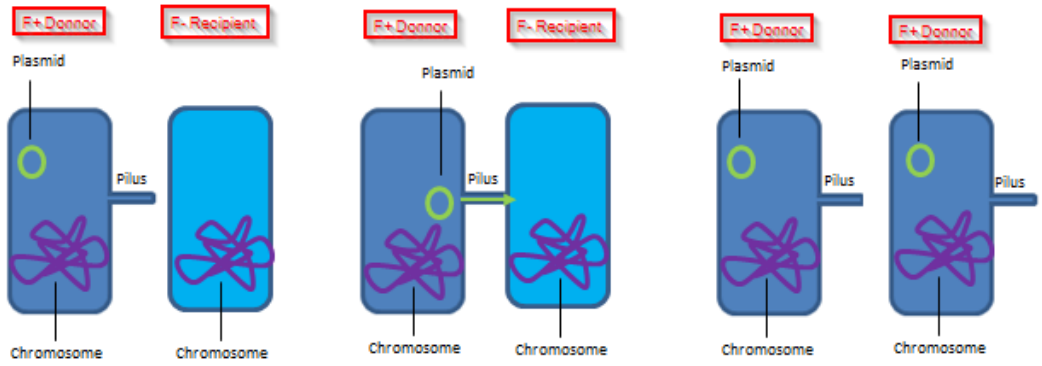


Figure **2-2**: Bacterial Conjugation.

An antibiotic resistance evidence can be found in the well-studied bacterium *Escherichia coli*, this bacterium causes several diseases such as urinary tract infections (UTI), respiratory problems and pneumonia. Depending on the health conditions of the patient and the type of bacteria involved, cephalosporins, semisynthetic penicillins and quinolones are the most used types of antibiotics to treat UTI. Unfortunately, some *E. Colli* strains are becoming resistant at least to one or several groups of these type of antibiotics [**?**, **?**].
The Antibiotic Resistance is not a new issue; resistant bacteria have been detected even before the penicillin was introduced [**?**], in fact, some studies display evidence of bacteria containing resistance genes four millions years back [**?**].

Antibiotics

Antibiotics are chemicals derived from micro-organisms that are used to treat infections caused by micro-organisms such as bacteria; these antibiotics can kill bacteria (bactericidals) or slow the growth of bacteria (bacteriostatics). Depending on the type of infections an antibiotic can treat, antibiotics can be classified as follows:

Penicillins: Penicillins [**?**] are the oldest group of antibiotics and are derived from the fungi *Penicillium*. The Penicillin's action mechanism is to interfere with the bacterial cell wall synthesis; some antibiotics that belong to this group are: amoxicillin, ampicillin, carbeni-

cillin, dicloxacillin and oxacillin.

Cephalosporins: Are a group of β-lactams antibiotics derived from the fungus *Cephalosporium* [**?**]. The Cephalosporin's action mechanism is the same as penicillins, they disrupt the synthesis of the peptidoglycan layer of bacterial cell walls; some antibiotics that belong to this group are: cefaclor, cefadroxil, cefalexin.

Tetracyclines: Are used to treat bacterial infections such as pneumonia and other respiratory tract infections; infections skins, and genital and urinary systems. The Tetracycline's action mechanism is based on the inhibition of bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome [**?**]; some antibiotics that belong to this group are: tetracycline, doxycycline and minocycline.

Aminoglycosides Are one of the most potent broad-spectrum classes of antimicrobials [**?**], it is used for treatment to Gram-negative infections, This group of antibiotics can act simultaneously with antibiotics β-lactams; some antibiotics that belong to this group are: gentamicin, amikacin, and tobramycin.

Macrolides This group of antibiotics is effective against a broad spectrum of Gram-positive bacteria, Macrolides are used to treat infections such as respiratory tract and soft-tissue infections; some antibiotics that belong to this group are: erythromycin, clarithromycin, azithromycin and telithromycin.

Fluoroquinolones This group of antibiotics are used to treat respiratory and urinary tract infections, these antibiotics are effective against a broad spectrum of Gram-positive and Gram-negative bacteria; some antibiotics that are catalogued into this group are: ciprofloxacin, levofloxacin, and norfloxacin.

Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) defines the problem that is facing the world where microorganisms are resistant to antimicrobials that they were sensitive before. Antimicrobial resistance is becoming a global problem, the mobility of patients around the world generates the mobility of the superbugs (resistant organisms to many type of antibiotics).

AMR affects patients, healthcare providers, pharmaceutical industry and society, becoming more than a health problem; treatments in patients with resistance bacteria are more expensive than treatments to patients with "sensitive" bacteria. One of the causes for the global spread of AMR is due to the wrong use of antibiotics, in some countries, antibiotics are prescribed in irrational amounts; incorrect indications, erroneous frequency of application and

imprecise duration in the treatment; furthermore, some patients are treated with antibiotics when the cause is viral. [**?**].

Scientists are asking if we are entering a post antibiotic era, some studies claim that no successful discoveries of new classes of antibiotics have been made since 1987, while the crisis due to antibiotic resistance, increases; the industry is not focused on facing this issue, in fact, the number of new systemic antibiotics approved by the FDA (U.S. Food and Drug Administration) have fallen from sixteen (16) between 1983-1987 to just two (2) in the last five years [**?**].

Plasmids

Plasmids are circular genetic code embedded into the bacteria (see Figure **??**); plasmids can be classified as F plasmids, R plasmids or others. Fertility plasmids (F plasmid) allow to transfer genetic material from a bacterium (Donor) to another bacterium (Recipient). Donor bacteria that carry the F plasmid are defined as F+ and recipient bacteria are known as F-; besides, bacteria with the F+ plasmid, promote the synthesis of pili on the bacterium surface.

R plasmids provide capabilities of antibiotic resistance, depending on the resistance genes that plasmids contain. Some plasmids can be F Factor and R factor [**?**], therefore they are responsible for transferring genetic material and for drug resistance.

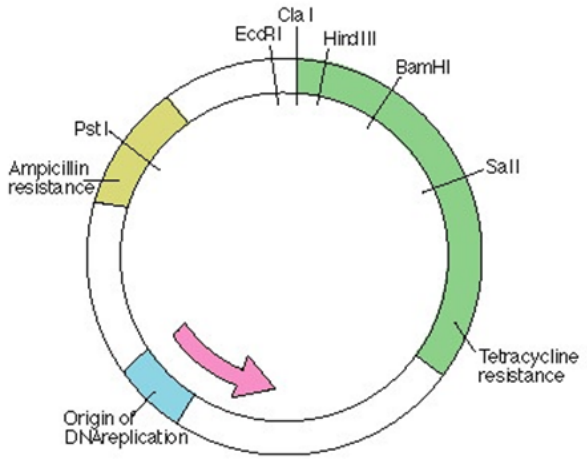


Figure **2-3**: Plasmid PBR322, *Escherichia coli*. Taken from: `http://lnx.futuremedicos.com/Revista_future/Articulos&Trabajos/Basicas/bq/clonacion_DNA_archivos/pbr322.jpg`

Some studies claim that plasmids can affect bacteria metabolism [**?**]; actually, a bacterium with many plasmids can consume more nutrients or affect the growth rate than a bacterium

that has fewer plasmids. Eventually, plasmids can be more a burden than a benefit depending on the pressure over the environment; in hostile environments, plasmids can be efficient to resist the mechanism of action of antibiotics, but in neutral environments, the metabolic fitness of a bacterium can be reduced due to the plasmids carriage [**?**].

In nature, plasmids contains two parts that are important to abstract to the model. The first part is the origin of replication which works such as a control for different characteristics, most important functionalities that Origin of Replications control are the number of copies that a plasmid can maintain and the incompatibility of plasmids with the same origin of replication. The second one is the resistance antibiotic regions, these regions encode some characteristics that provide resistance, to a bacterium, to different types of antibiotics; resistance plasmids often have many resistance-encoding genes, therefore a strain of bacteria can be resistance to many antibiotics by acquiring just one resistance plasmid.

Superbugs: Pseudomonas aeruginosa, Staphylococcus aureus

Superbugs are bacteria that carry several resistance genes; therefore, they can be resistant to several types of antibiotics. In this section, two bacteria that have strains defined as superbugs, are introduced; these types of bacterium are known as *Pseudomonas aeruginosa* and *Staphylococcus aureus*:

Pseudomonas aeruginosa This type of Gram-Negative bacterium has demonstrated resistance to several antibiotics; *Pseudomonas aeruginosa* is cause of hospital-acquired infections (HAIs) and chronic lung infections in patients with Cystic fibrosis (CF).

A type of *P. aeruginosa* bacterium, shows resistance to aminoglycosides, fluoroquinolones and β-lactams; a factor for this behaviour is the low permeability of its outer membrane which limits the rate of penetration of antibiotics due to the functionality such as a selective barrier; this and others mechanisms can be reached or enhanced by mutations in the genetic code of this type of bacterium [**?**].

Staphylococcus aureus *S. aureus* is the most common cause of hospital-acquired infections (HAIs) [**?**] and some strains are resistant to many classes of antibiotics:

- MRSA: methicillin-resistant *Staphylococcus aureus*. This strain was first described in 1960s, this is a strain of *S. aureus* that is resistant to β-lactams antibiotics; hence, these bacteria are resistant to penicillins(methicillin, dicloxacillin, nafcillin, oxacillin, others) and Cephalosporins; MRSA strains are treated with vancomycin.
- VISA/GISA: Vancomycin intermediate resistant *Staphylococcus aureus*/Glycopeptide-intermediate *Staphylococcus aureus*, is a strain of *S. aureus* that is not completely

susceptible to vancomycin; however, large doses of vancomycin can kill this strain of *S. Aerus* [**?**].

- VRSA: Vancomycin-Resistant *Staphylococcus aureus* is a strain of *S. aureus* which includes a high-level of vancomycin resistance, some VRSA isolates have been reported in the last ten years in United States, Iran, India, Europe and Latin America.

2.2 Cellular Automata

Cellular Automata are mathematical models built in a simple way but can represent complex behaviours [**?**], cellular automata can be considered discrete systems which evolve depending on the cell's states and a set of rules locally defined. Interesting patterns are formed in a cellular automaton due to local interactions, these patterns have been used to simulate and study complex systems in several areas; in artificial life are mainly combined with multi-agent systems (MAS) [**?**] and evolutionary algorithms [**?**] to design different types of simulations like biological systems, social interactions and economical behaviours.

John von Neumman

John von Neumman proposed the creation of a model to represent an autonomous self-replication machine. The self-replication problem was boarded by John von Neumann through a cellular automaton [**?**] by designing a model called "the Universal Constructor". The self-replication problem was approached by using a grid where each space represents a cell, these cells can have a finite number of states (In this case, 29 states). Each cell can go from state A to state B depending on its current state and the states of the cells in its neighbourhood. The "von Neumann Neighbourhood" consists of 4-cell that surround a central cell, also known as a neighbourhood of range one (1).

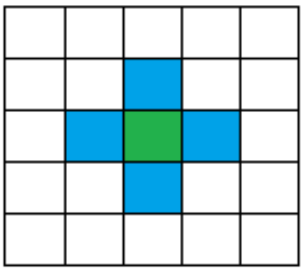


Figure **2-4**: von Neumann's neighborhood of range one (1).

John Conway: Game of Life

Decades later, John Conway was interested in the work made by John von Neumann. So, J. Conway designed a game titled "Game of Life" [**?**] published in the Journal "Scientific Amer-

ican", the game consists in creating an initial configuration (in a two-dimensional cellular automaton) and observing how the cellular automaton evolves depending on the following rules:

Survivors: Each cell with two or three cells in its neighborhood, survives for next generation.

Death: A bacterium can die due to two factors:

- Underpopulation: Each cell with less than two cells in its neigborhood, dies.
- Overcrowding: Each cell with more than three cells within its neighborhood, dies.

Reproduction: Each empty cell surrounded by exactly three live cells, in the next generation will become a live cell.

Stephan Wolfram

Stephan Wolfram carried on several studies about celullar automata and different characteristics that they can exhibit; he established that, depending on the rules and the initial configuration (state), a cellular automaton can generate different patterns and behaviours [**?**] like the following ones:

Behaviours

- Disappears with time.
- Evolves to a fixed finite size.
- Grows indefinitely at a fixed speed.
- Grows and contracts irregularly.

Patterns

- Spatially homogeneous state.
- Sequence of simple stable or periodic structures.
- Chaotic aperiodic behaviour.
- Complicated localized structures, some propagating.

2.3 Evolutionary Computation

Evolutionary computation is based on Evolution Theory; evolutionary computation is mainly used to solve problems, especially for solving optimization problems; given a population of individuals in an environment where individuals must compete and the environmental pressure causes natural selection, the fitness (a measure of optimality) of the population increases in each generation [**?**]. There are many techniques of evolutionary computation in order to solve different problems, but all the techniques are based on some notions:

2.3.1 Representation

Mechanism to represent the possible solutions of "the real world" (phenotype) in "the virtual world" (genotype). The representation is the mapping from the phenotypes onto a set of genotypes; the genotype and the phenotype can be very different; the search is always done in the genotypic space; therefore, the genotypic space must be able to represent, in a correct way, the phenotypic space.

2.3.2 Population

The population is a multi-set of genotypes where processes will take place; the population can be as simple as a set of individuals or can be composed of different structures such as distance measures and neighbourhoods, depending on the problem to be resolved. The diversity of the population is important in order to apply the selection mechanisms, for example, in most cases, the best individual of a generation is chosen as seed of the next generation and the worst individual is replaced. There are three widely-used populations models:

Global Model: This is the classical method, where the population is the one hundred percent of individuals and depending on the selection mechanisms and fitness function, some individuals are replaced in the population.

Island models (migration): This model is based on the parallel computers architecture, the process consists of dividing the whole population into sub-populations. Each sub-population has many generations of isolated evolution; then, some individuals are distributed to other sub-populations, this process generates diversity and exchange of information between sub-populations [**?**, **?**].

Diffusion Model: In this model, all individuals disperse and reproduce. These models are widely used to represent social and economical behaviours, and biological models [**?**, **?**].

Parent Selection: This mechanism is about taking the best individuals as parents of the next generation. Parents are selected by probabilistic methods. In one hand, an individual

which high qualities, has higher probability to be chosen as a parent; on the opposite hand, an individual with low qualities, has lower probability.

2.3.3 Variation Operators

Variation operators allow the evolutionary algorithm to generate new individuals for next generations according to current generation of individuals. The variation operators can be classified according to their arity (number of objects as input), the most used operators are unary (one object) and binary (two objects).

Mutation

Mutation is an unary operator that makes random changes in an individual, this variation operator alters one or more gene values in the representation (genotype); the main purpose of this operator is to generate diversity in the population. There are several branches for mechanisms of mutation, some of them are:

Flip Bit: A bit is taken and is flipped.

Gaussian Mutation: this mutation adds a unit Gaussian distributed random value to the gene.

Cauchy Mutation: Adds a Cauchy distributed random value to the gene.

Adaptive Lévy Mutation: This mechanisms is used to produce four mutated offsprings for each parent by using four different Lévy's probability distributions [**?**].

Combined mutation Operators Combine one or more operators of mutation.

Crossover

Crossover or recombination is a variation operator which generally involves two individuals; therefore, crossover is an operator of arity two (binary). The main objective of crossover is to mate two individuals with good features, resulting in a offspring that will keep features of both. There are kinds of crossover which involve more than two parents, but these recombinations with higher arity, do not have biological equivalence. Some types [**?**] of binary crossover are:

Single point crossover: A crossover point is chosen randomly, the offspring is the combination of the chromosome of one parent before the crossover point, and the chromosome of

the second parent since the crossover point.

Multiple point crossover: There are several crossover points. Uniform crossover: The parent's chromosomes are compared and changed according to a fixed probability.

2.3.4 Fitness Function

The fitness function is the basis for selection, the purpose of the fitness function is to assign a quality measure to the phenotype of each individual, so the fitness function decides if an individual is good or bad depending on the problem to be solved.

Selection

Selection is also called replacement, the purpose is to select the individuals with the best fitness to be part of the next generation; this process is conducted after creating the offspring. There are several methods:

Elitist Selection: The best individuals are kept for the next generation [**?**].

Tournament Selection: A tournament is held among a certain number of competitors, the winner of each tournament is taken to the mating pool [**?**].

Roulette Wheel Selection: Each individual has a probability of selection depending on its fitness; therefore, individuals with better fitness have high probability of being selected, while individuals with low fitness have low probability of being selected. It is similar to a roulette wheel where each segment has a different size, based on each individual's relative fitness; then, a random selection is performed.

Rank Selection: The mechanism consists in sorting the population of individuals in an ascending order according to the individual fitness [**?**].

2.4 Algorithms and Simulations

In this section, the most representative literature of studies and simulations about bacterial colonies and genetic exchange is presented; several processes are studied, such as communication, theories about how bacteria live and emergent behaviours. The first part of the section is based on bio-inspired algorithms and the second one is about different applications in the field of artificial life.

2.4.1 Algorithms bio-inspired in Bacteria

One of the most studied algorithms, the "Bacterial Foraging Optimization Algorithm (BFOA)" or "Bacterial Foraging Algorithm (BFA)" [**?**], is specifically inspired in bacteria *E. colli*, and *M. xanthus*. The algorithm is based on the chemotaxis bacterial behaviour, where bacteria can perceive changes or chemical substances in the environment such as nutrients or antibiotics; bacteria swim towards concentration or flee depending on the perceived signals. The strategy of the algorithm consists in simulating the chemotaxis by designing the attractants as optimums. The rules of the algorithm are the following:

- Chemotaxis, depending on the concentration of surrounded cells, the cell behaviour changes.
- Reproduction, cells or bacteria with good fitness are selected to reproduce.
- Elimination, old cells are discarded and new cells are inserted.

This optimization algorithm has been studied and modified with the purpose of improving its performance and convergence time. In first instance, some authors take the "BFA algorithm" and improve the behaviour of bacteria by using intelligent techniques of swarming [**?**]; resulting in better results in most cases. Cho, Park and Jeong [**?**], claim that algorithm BFA does not take into account chemical communications such as quorum-sensing, which allows bacteria to coordinate social behaviour; these authors develop a new algorithm and obtain better results in a Multimodal Gaussian-Like Function.

Gao, Gao, Qi and Yin [**?**], claimed that the algorithm BFA has problems in cost and time to converge; therefore, they proposed a way to speed BFA up by using differential operators of evolution in order to improve the swarming motility of bacteria. This approach always performs better than BFA, improving the time of convergence; but in some problems the algorithm converges to a local optimum.

Some authors focused on hybrid methods to improve the performance of the algorithm; for example, some authors use differential equations to study bacteria reproduction in a dynamical environment; simulations are inspired on natural processes such as chemotaxis, swarming groups, reproduction, death and division in bacteria [**?**]. The main problem of the model, is to deal with differential equations because it is not possible to find explicit solutions considering that coefficients differ in a complex way through time.

Nicolau, Burrage and Nico [**?**] propose an algorithm considering the movement of *Escherichia coli*, these bacteria use a simple biochemical mechanism of memory to perform simple comparisons. The objective of the algorithm is to evaluate the best route, if comparison indicates

an improvement, bacteria have a high probability to follow in a straight line direction; otherwise, bacteria turn around. In fact, there are many algorithms based on the concept of chemostaxis [**?**, **?**]; however, these algorithms seem to have problems with quadratic and multimodal functions.

Finally, there is a field that focused on creating new genetic operators inspired on genetic exchange between bacteria. A first algorithm called "Genetic Algorithm pseudo-bacterial" [**?**], consists on replacing the crossover operator by an operator called "gen of transference"; the population of individuals is divided into two sub-populations, the first subpopulation consists of individuals with the best fitness and in the second subpopulation consists of the other individuals. Then, at random is selected one individual in each subpopulation and a part of the best individual's chromosome is randomly transferred to the chromosome of the individual of the second subpopulation, this is repeated for all the individuals.

Other algorithm is called "Bacterial Memetic Algorithm (BMA)" [**?**], this algorithm is based on three steps: The first step is the creation of clones (copies of a selected chromosome); next, the clones are mutated several times and the best clone is maintained and the other ones are deleted. Then, the Levenberg-Marquardt method is iterated during seven (7) to ten (10) times; finally, in the third step a gene transfer operation is applied to a partial population.

The previous algorithms have been useful as a first approach to bio-inspired algorithms in bacteria; however, these algorithms are focused on solving optimization algorithms instead of representing adequately the bacterial processes. In the next subsection, computational models focused on representing bacterial processes, are presented.

2.4.2 Simulations of bacterial colony and bacterial processes

In the field of simulations, one of the main objectives is to represent behaviours that occur in bacterial colonies. Paton and Gregory developed two models titled [**?**, **?**]: "Computing Systems of Microbial of Communications (COSMIC I)" and "Rule-Based Bacterial Model (RUBAM)", these models are focused on simulating the emergent behaviours and shows that it is important to design adequately three main components: The artificial environment, the population and the set of genetic operators.

Other studied model [**?**] was developed in 2002. This is a stochastic model where the growing bacteria process is studied taking into account different emergent patterns. First, a deterministic model is introduced where there are bacteria that exhibit motility and others ones that are motionless. Next, fluctuations of concentrations and the changes that concentrations cause in the environment, are added in order to design a stochastic model. Finally,

simulation experiments were conducted in one and two dimensions for both models; the best results were obtained with the stochastic model.

Others authors simulated the process of chemostaxis (movement of an organism in response to a chemical stimulus) and the emergent behaviours produced by this process. Eyiyurekli, Manley, Lelk and Breen [**?**] studied individual cells that emit an answer to chemical impulses; each cell has its own program that allows a different answer to a similar impulse. A cell can carry out several actions (to emit and perceive impulses, movement, join to other cells, division, get old and die).

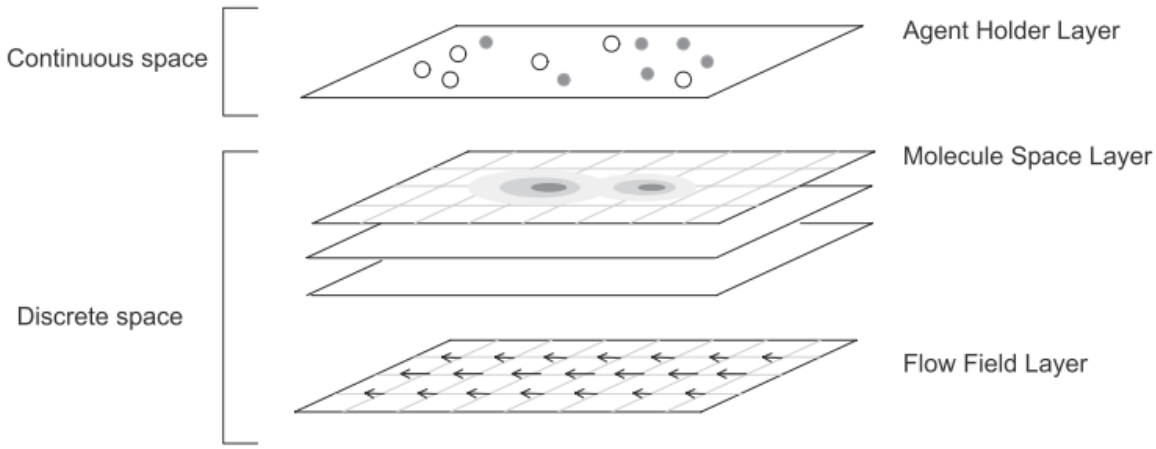


Figure **2-5**: Hybrid model built in three layers.

Some simulations are based on hybrid models. Guo, Sloot and Tay [**?**] desgined a hybrid model that uses differential equations and agents, the model consists of three layers (see Figure **??**): A container layer where agents reside; a layer where the molecular distributions are saved and a flow layer where different concentrations are modeled. Each cell is the composition of the three layers and the interactions between them are carried out by using differential equations.

Finally, there are two tools to facilitate the development of simulations of bacterial colonies. The first one is called "The Swarm Simulation System" [**?**], this tool is based on simulations of swarming, it supports hierarchical models and provides libraries with components that can be reused for new implementations. The second model called "BACSIM" [**?**], studies the growing and behaviour of bacterial colonies; each bacterium is represented as an instance of an agent class, these agents are represented by circles in an environment 2D and each agent can carry out a set of actions.

Summary

This chapter introduced a full background of biological processes and computational models based on bacterial processes. On one hand, the studied simulations presented models based on general processes of bacterial colonies and are not focused specifically in the conjugation process. On the other hand, the works that are focused on the process of transference of plasmids, do not take into consideration several mechanisms and biological processes (different types of plasmids, diffusion of antibiotics) or the authors were interested in optimization problems; therefore, they partially studied the biological process. In the next chapter, an approach to the design and development of a bacterial conjugation model in a cellular automaton with evolutionary computation techniques, is introduced.

3 Evolutionary Cellular Automaton Bacterial Conjugation Model

In the previous chapter, a full background of biological processes and computational models based on bacterial processes, was presented. As shown in chapter 1, a model combining the adequate tools to simulate the process of bacterial conjugation, has not been proposed. In order to design an appropriate model to represent the process of bacterial conjugation; the first step is the design and development of an adequate environment where bacteria can interact. Hence, in this chapter, an approach to the design and development of the bacterial conjugation model, is presented.

The first section of this chapter introduces the construction of an environment to represent a bacterial colony; the environment is designed with a Hexagonal Cellular Automaton, where each cell can contain or not a bacterium. The number of bacteria in the environment depends on the initial values set up of the simulation. There are two types of bacteria, recipient bacteria (sensitive) and donor bacteria(resistant to antibiotics); according to its type, each bacterium contains a set of characteristics:

Recipient bacteria (sensitive bacteria) are vulnerable to antibiotics, do not contain plasmids but can receive them from a donor bacterium. A recipient bacterium that receives a plasmid is transformed into a new donor bacterium (resistant bacterium); therefore, the bacterium can transfer the plasmid obtained to recipient bacteria.

Donor bacteria (resistant bacteria) contain at least one plasmid which provides resistance to antibiotics and can be transferred by direct contact to recipient bacteria. A donor bacterium has one pilus represented by a red line in one of its edges; a donor bacterium can only transfer a plasmid, if the pilus is pointing out to a recipient bacterium.

The first section also introduces a mechanism to evaluate the actions to be performed by bacteria, this mechanism is based on a fuzzy function that takes as input the concentration of each cell and returns as output an array with a membership degree for each possible action. Then, a set of simulations to study the simulated bacterial colony under different initial conditions, are presented.

The second section introduces new mechanisms and characteristics for improving simulations behaviour. These mechanisms and characteristics are: First, new processes are carried out in the simulations such as Life-cycle of a bacterium, its metabolism, reproduction and death; these processes are simulated in a layer titled "Nutrient's Layer". Next, new mechanisms for improving the representation of a bacteria, are introduced; some of these mechanisms are: the inclusion of multiple pili in a donor bacterium and the transference of plasmids between donor bacteria; therefore, a donor bacterium can have more than one plasmid. In the third section, a mechanism of action of antibiotics is introduced; this mechanism includes. The design of the genetic code of antibiotics and the implementation of an antibiotic resistance function that evaluates the susceptibility of a bacterium against a type of antibiotic.
This chapter is organized as follows. First, the design of the model is described; next, improvements and new included mechanisms, are explained. Finally, a mechanism of actions of antibiotics, is introduced.

3.1 Representation of Bacterial Colonies

In this work, bacterial colonies are simulated with a hexagonal cellular automaton with toroid borders and hexagonal neighbourhood (see Figure **??**). The environment is conformed by two layers similar to the architecture mentioned by Guo [**?**]. The first layer is the "Physical Layer", it is located at the top of the model and is used to represent physical properties of bacteria. The second layer is the "Concentration's Layer", this layer contains chemical representations based on the concentration values of each cell, these values are determined by the type of bacterium in the cell and the type of bacteria in the cell's neighborhood.

3.1.1 Physical Layer

The Physical layer represents the physical environment simulated in a hexagonal cellular automaton where bacteria interact, each hexagonal cell can contain or not a bacterium. A donor bacterium is represented with a dark blue color and a red line in one edge, represents the pilus; a recipient bacterium is represented with a light blue color and does not have a pilus; and the absence of a bacterium in a cell is represented with a white color.

Figure **3-1**: Hexagonal Neighbourhood

In Figure **??**, the bacterial layer is shown, where bacteria interact with the environment. On one hand, recipient bacteria do not perform any action; on the other hand, donor bacteria can perform three actions:

Turn left (counter clockwise): A bacterium turns 60°counter clockwise, when environment conditions are appropriate to transfer plasmids, but the pilus is not pointing out a recipient bacterium.

Turn right (clockwise): A bacterium turns 60°clockwise. This action has the same objective as "Turn left", to find a recipient bacterium to transfer plasmids.

Conjugate: When conditions in the environment are appropriate and the pilus of a donor bacterium, is pointing out a recipient bacterium; the donor bacterium transfers a plasmid to the recipient bacterium.

3.1.2 Representation of Concentration's Layer

The second layer is called the "Concentration Layer" that represents the chemical properties in the environment (see Figure **??**). In this work, these properties are represented by a variable called "Concentration". The concentration values determine the condition of the environment for bacteria, these values must be within some limits or the environment becomes hostile for bacteria. On one hand, antibiotics change the concentration to values out of the limits. On the other hand, plasmids maintain the concentration values within the limits.

The concentration of each cell, depends on the type of bacterium (donor or recipient) that each cell contains, the properties of the environment and the cell neighbourhood; instead, the concentration of empty cells (no bacteria) is determined just by its neighbourhood.

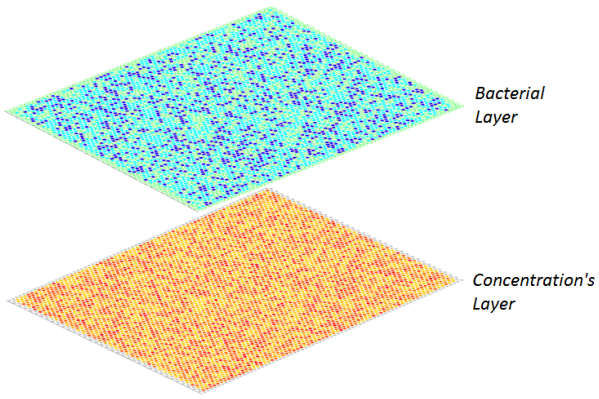
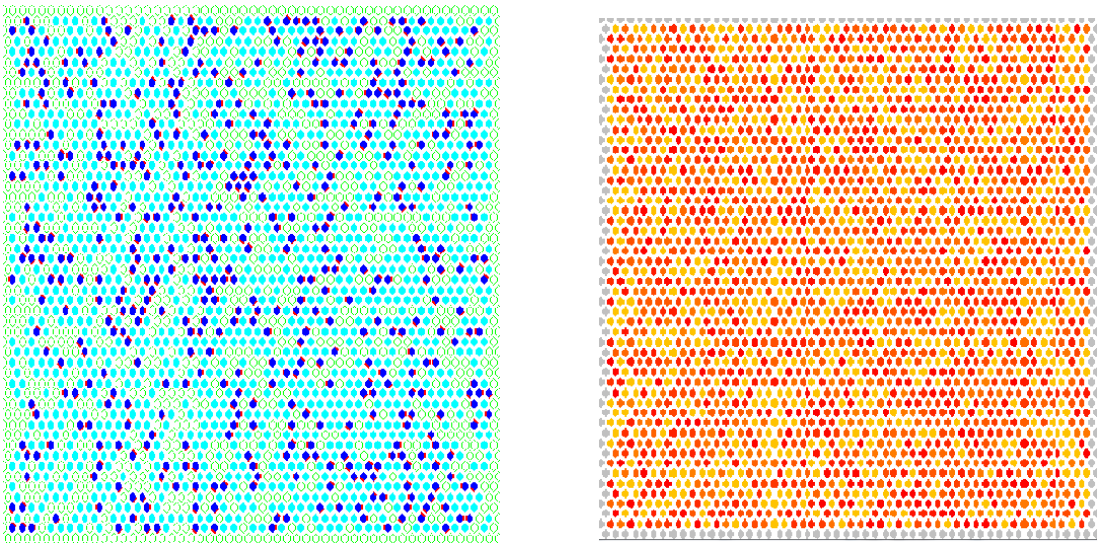


Figure **3-2**: Bacterial and Concentration Layer

The simulator allows users to change the displayable layer in any generation. Therefore, it is possible to display the simulation in "Physical Layer" to observe interactions between bacteria, or display the "Concentration's Layer" to evaluate how antibiotics and plasmids are affecting the concentration values.

Figure **??** shows the Physical Layer and Concentration's Layer in generation 0. Groups of bacteria are represented using the following colors code: yellow regions represent lack of bacteria, orange regions represent groups of recipient bacteria and red regions represent groups of donor bacteria.



(a) Physical Layer (b) Concentration's Layer

Figure **3-3**: Environment at generation 0.

3.1.3 Plasmids

Plasmids provide to bacterial colonies new characteristics, such as antibiotic resistance [**?**] and transference of plasmids [**?**, **?**]. In this model, plasmids act directly on the values of the concentration to keep them into a established range, actually, the local interactions and the global behaviour of bacterial, change in the simulation depending on the impact of the plasmids.

On one side, a bacterium that contains plasmids, is a donor bacterium and is resistant to some antibiotics, in this model. On the opposite side, a bacterium, in the absence of plasmids, is considered a recipient bacterium and is vulnerable to the antibiotics.

Four functions are used to define the impact of plasmids in the environment. In this first approach, the functions were defined with a threshold of fifty (50), which is used as a control value to define the limits of the concentration and the design of a membership function; anyway, this value is arbitrary and can be changed in any way, (see equations (**??**), (**??**) and (**??**)).

- t is time, where t + 1 is the next generation.
- nc is concentration in t + 1.
- c is concentration in t.
- k is a constant.

Constant plasmid: When a bacterium contains a plasmid, a constant value is added or subtracted from the cell's concentration that contains the bacterium depending on the current concentration value (see equation (**??**)).

$$nc = \begin{cases} c + k & c < 50 \\ c - k & c > 50 \end{cases} \tag{3-1}$$

Linear Plasmid: The impact of the plasmid in the concentration value follows a linear function (see equation (**??**)).

$$nc = c - k\,(c - 50) \tag{3-2}$$

Cubic Plasmid: The impact of the plasmid in the concentration value follows a cubic function (see equation (**??**)).

$$nc = c + \sqrt[3]{-c + 50} \tag{3-3}$$

Plasmid 0: It does not change the current concentration of the cell, but it is used to represent the conjugation process and antibiotic resistance.

3.1.4 Evaluation of Actions

In each generation, actions that a bacterium can perform, are evaluated according to the next steps: first, a cell that contains a bacterium is chosen. Next, the cell's concentration and the neighborhood concentrations, define the input for a membership function. After that, the function defines the grade of membership for each action, therefore, the output of the function is an array with a grade of membership for each action. Then, a process similar

to the Roulette Wheel Selection is conducted where any action has a probability of being chosen; however, the actions with higher membership degree, have higher probability to be selected; finally, the selected action is performed by the bacterium. In particular, when the selected action is "conjugation" but the donor bacterium's pilus is not pointing a recipient bacterium, the action changes to "turn left" or "turn right" in order to find a recipient bacterium.

3.1.5 Mechanism of antibiotics diffusion

The antibiotics diffusion is based on a hexagonal sand pile model (see Figure **??**); the model is composed of the following four steps:

1. A cell is selected, where the units of antibiotic will be applied.
2. In each generation, three units of antibiotic are applied on the selected cell.
3. If the number of units of antibiotic in the cell is bigger than six, one unit of antibiotic, is spread to the cell's neighbors, and six units of antibiotic are subtracted from the cell.
4. The step 3 is performed for each cell that at least contains one units of antibiotic.

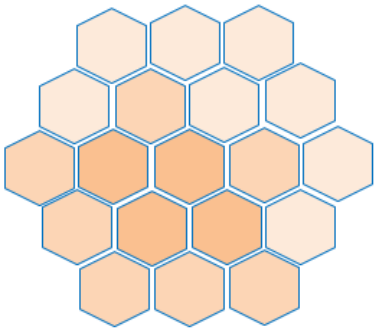


Figure **3-4**: Mechanism of Antibiotics Diffusion.

3.1.6 Simulations

Two types of bacteria are simulated; the first type of bacteria are the donor bacteria, which objective is to transfer plasmids to recipient bacteria. The second type are the recipient bacteria, which objective is to survive in the environment.

This section introduces both, the interaction between a donor bacterium with a recipient bacterium and the initial model of transference of plasmids. In this transference model, a donor bacterium can only transfer plasmids to a recipient bacterium; moreover, a donor

bacterium can have just one plasmid. The whole population of donor bacteria are factor F+ and R; hence, a donor bacterium transfers plasmids and provides antibiotic resistance to a receipt bacterium that becomes a donor bacterium. Besides, a mechanism for simulating the action of antibiotics is also introduced, this mechanism is based on a sand-pile model.

Simulations are focused on studying the simulated bacterial colony under different initial conditions (defined as parameters of the simulator and rules). The first simulation is based on changing the dimensions of the lattice and observe how many generations takes to transfer the antibiotic resistance to the whole population. In the second simulation, the four functions of plasmids are used in different executions, after fifty generations, the number of donor bacteria are counted and compared with the initial number of donor bacteria. In the third simulation, a mechanism of action of antibiotics is included, the antibiotic is spread in the bacterial colony during one hundred generations and only donor bacteria survive to the contact with such antibiotic. Then, some graphical patterns, obtained after running the third simulation, are studied. This is done because these patterns resemble some patterns displayed in bacterial colonies.

Transference of plasmids

The purpose of this set of simulation experiments is to determine how many generations takes to transfer the antibiotic resistance to the whole population. In these experiments, the initial conditions are set to: Fifty (50) percent of donor bacteria, fifty (50) percent of recipient bacteria and zero (0) percent of empty cells; constant function of plasmids and no death or reproduction in the bacterial colony. Thirty (30) different runs are executed under these conditions with different cellular automaton dimensions: 10x10; 20x20; 40x40; 60x60, 100x100 (see Figure **??**). These conditions create an ideal environment for bacteria; therefore, concentration values always are within the limits and donor bacteria are always trying to transfer plasmids.

Figure **??** shows the process of transference of plasmids in different cellular automaton dimensions. As can be seen, the initial population of bacteria has a random distribution with half of donor bacteria (dark blue) at generation 0. The transference of plasmids shows a logarithmic behaviour where the rate of bacteria in process of conjugation is very high during the first ten (10) generations (see second and third columns in Figure **??**). After that, the conjugation rate decreases until generation twenty five (25); where the number of donor bacteria is close to one hundred (100) percent for cellular automaton dimensions 10x10 and 20x20; instead, the cellular automaton dimensions 60x60 and 100x100, still count with some recipient bacteria. However, around the fifty (50) generation, the total number of resistant bacteria is close to ninety nine percent (99%) (just for the evaluated dimensions).

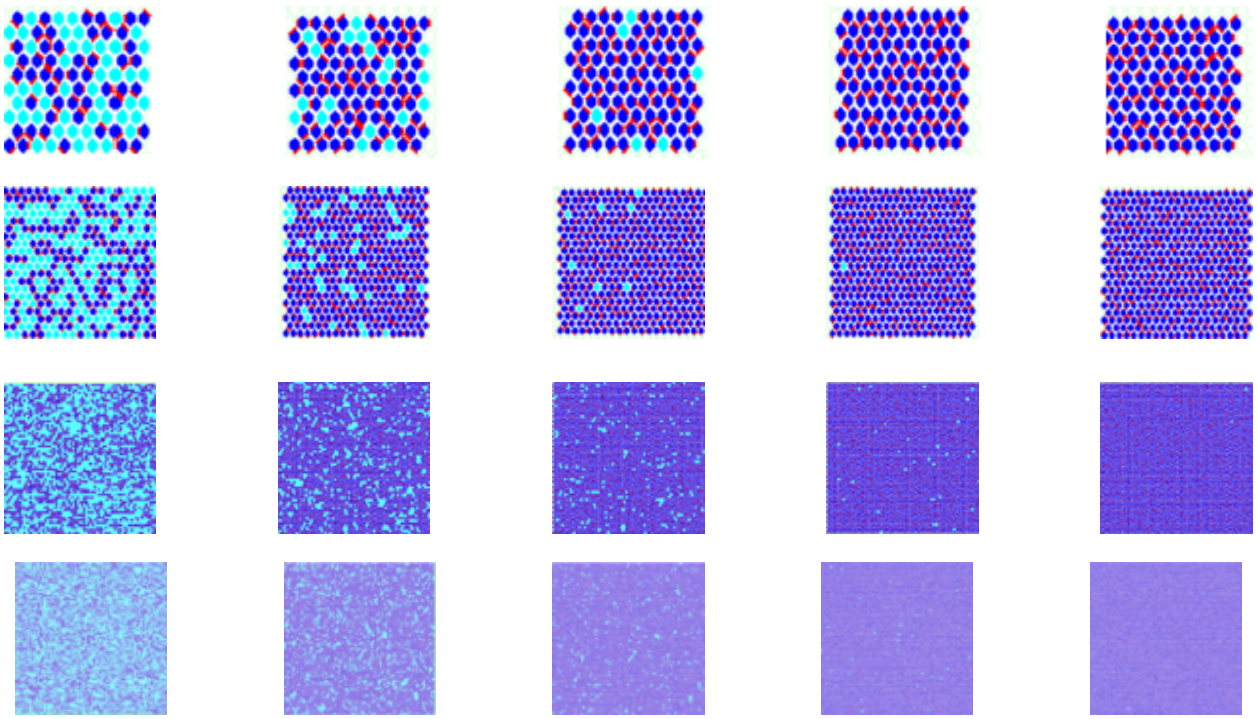


Figure **3-5**: Transferring plasmids during fifty (50) generations with different automaton dimensions. Dimensions per row: 10 x 10, 20 x 20, 60 x 60 and 100 x 100; Generations per column: 0, 5, 10, 25, 50. (Constant Function of plasmids)

Impact of plasmids in the bacterial colony

The purpose of this set of simulation experiments is to evaluate the impact of each function of plasmids in the bacterial colony. In these experiments, the initial conditions are set to: Twelve (12) percent of donor bacteria, twelve (12) percent of recipient bacteria and seventy six (26) percent of empty cells, no death or reproduction in the bacterial colony. Thirty different runs are executed under these conditions during fifty (50) generations, with four different functions of plasmids: Constant plasmid; linear plasmid; cubic plasmid and plasmid 0.

Figure **??** shows the rate of conjugation for each plasmid function during the first fifty (50) generations and the Table **??** shows the number of Donor Bacteria and Recipient Bacteria after fifty (50) generations. As expected, the behaviour of the bacterial colony changes according to the used plasmids function. On one hand, the function "plasmid 0", shows a slow performance of bacterial conjugation due to the absence of pressure that this function performs over the environment. On the other hand, The constant and linear functions, present a similar performance in the simulation, resulting in populations of donor bacteria close to eighty (80) percent after fifty (50) generations. However, The cubic function presents the higher rate of transference, with a population of donor bacteria close to eighty eight (88)

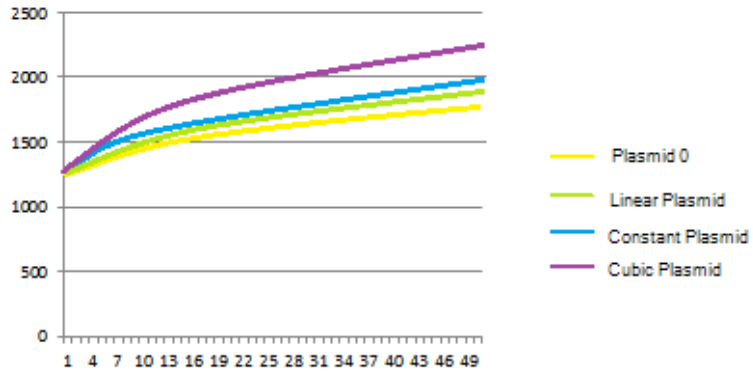


Figure **3-6**: Performance of Plasmids Functions during fifty generations.

percent after fifty (50) generations.

Plasmid Function	Donor Bacteria	Recipient Bacteria
Constant	1750	750
Linear	1900	600
Constant	2000	500
Constant	2200	300

Table **3-1**: Results of Plasmid Functions (After fifty (50) generations).

Diffusion of antibiotics

The purpose of the experiments is to observe the behaviour of applying a constant dose of antibiotic, the antibiotic diffusion when it is applied to a cell that is located in the middle of the cellular automaton, and the impact over the bacterial colony (if a recipient bacterium is reached by the antibiotic, it dies). In these experiments, the initial conditions are set to: Twenty five (25) percent of donor bacteria, twenty five (25) percent of recipient bacteria and fifty (50) percent of empty cells; bacteria can not reproduce and the cellular automaton dimension is 100X100. Thirty (30)different runs are executed under these conditions during one hundred (100) generations.

Figure **??** shows the antibiotic diffusion and how the antibiotic affects the simulated bacterial colony during one hundred (100) generations. An initial dose of antibiotic is applied at generation zero (0) (see the first column of Figure **??**). Next, recipient bacteria die due to the direct contact with the antibiotic, however, the number of affected bacteria is small due to the poor concentration of antibiotic in the environment during the first fifteen (15) generations (see second and third columns in Figure **??**).After that, around generation fifty

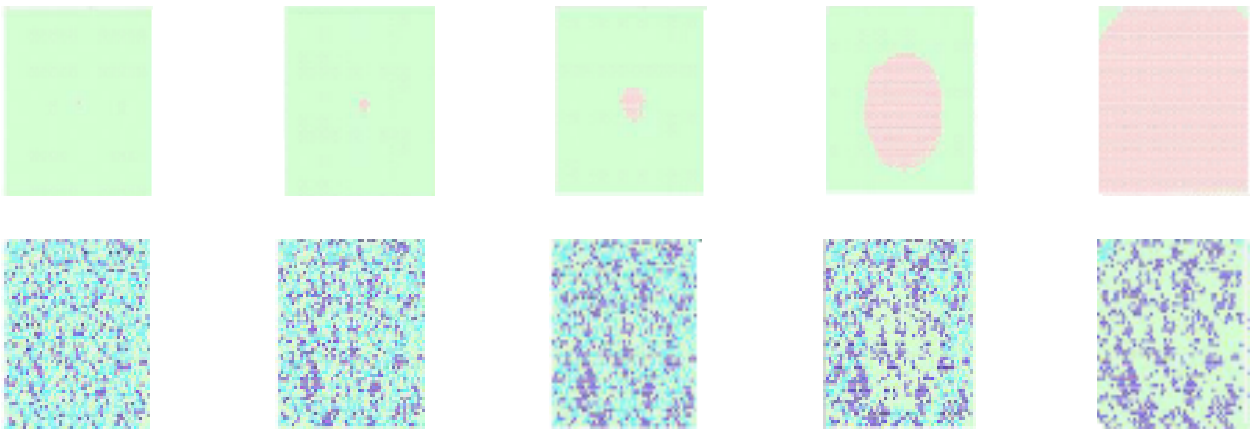


Figure **3-7**: Antibiotic Diffusion Experiment during one hundred (100) generations. Row 1. Antibiotic Diffusion. Row 2. Physical layer affected by antibiotics)

(50), the quantity of antibiotic is high and a large number of recipient bacteria have died; therefore, only donor bacteria remain in the center of the cellular automaton (see fourth column in Figure **??**. Finally, the antibiotic has spread through the bacterial colony and just donor bacteria have survived at generation one hundred (100).

The mechanism of antibiotics diffusion is efficient to spread the antibiotic through the bacterial colony (see Figure **??**). Therefore, a large number of bacteria (recipient) die due to the direct contact with the antibiotic, while donor bacteria survive to the antibiotic diffusion, resulting in interesting patterns produced by the donor bacteria that remain in the environment (see Figure **??**). These patterns are graphically similar to some patterns that are displayed in bacterial colonies.

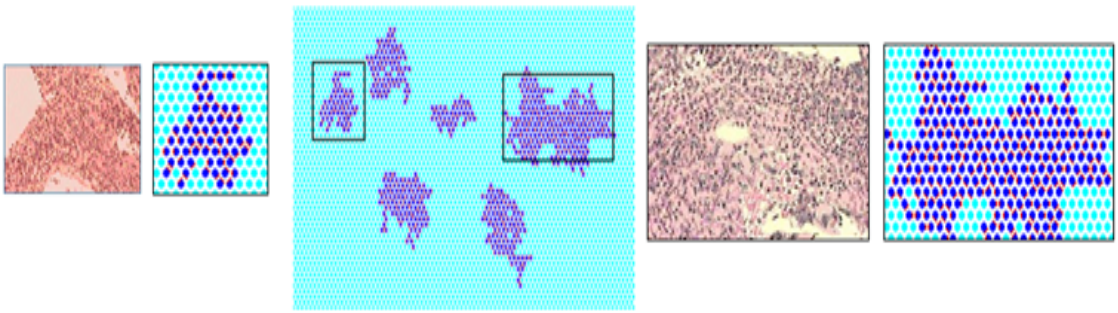


Figure **3-8**: Bacteria Colonies vs Our Model

3.1.7 Summary

This section presented the design and development of the bacterial environment, where interactions between donor and recipient bacteria are carried out. In first instance, the physical and concentration layers are presented; in these layers, the physical and chemical interactions are conducted. Besides, the plasmids are represented as a mechanism of transferring fertility and antibiotic resistance characteristics, from a donor bacterium to a recipient bacterium.

The mechanism of actions selection and the actions that can perform the donor bacteria, were introduced. The mechanism is defined by two steps; in the first step, a membership value is given to each action; then, in the second step, a random process is conducted to select the action to be performed; in this process, any action with a membership value higher than zero, can be chosen; however, higher the membership degree; higher probability of being selected.

A mechanism of antibiotics diffusion, based on a hexagonal sand pile model, was presented. This mechanism allows antibiotics to diffuse across the bacterial colony; donor bacteria are antibiotic resistant, but recipient bacteria, die, if they are in direct contact with the antibiotic.

Although the structure of the environment allows simulations to be conducted, there is a lack of biological characteristics that should be considered in order to improve the simulations. In the next section, new mechanisms are included in the model, such as evolutionary computation, resistance genes, different types of antibiotics and a mechanism of action of antibiotics.

3.2 Evolution and New Characteristics

In this section, the model (introduced in the previous section) includes new mechanisms and characteristics such as evolutionary computation, resistance genes, different types of antibiotics and a mechanism of action of antibiotics. First, a new layer titled “Nutrient’s Layer”, is introduced. Next, a summary of the new add-ins and changes performed to bacteria, are presented.

After that, new characteristics such as multiple pili, new actions, genetic code of plasmids and variation operators, are introduced. Finally, a mechanism of action of antibiotics and a resistance function that evaluates the antibiotic resistance, are designed.

3.2.1 The Nutrient's layer

Bacteria need adequate enviromental conditions to survive and grow; for instance, nutrients are one of the most important growing factors. In this model, nutrients are a necessary resource and an important factor for the evolution of the bacterial colony. On one hand, If there is lack of nutrients, bacteria limit their actions, on the other hand, over-quantity of nutrients can generate an increasing on the growing rate and can lead to overpopulation. Therefore, the first step is to design a mechanism based on nutrients that replaces the mechanism of concentration, shown in the previous section.

In first instance, the nutrients layer is based on a regeneration model that follows these steps. First, each cell starts with a random quantity of nutrients between a range of values at generation 0 (see Figure **??**); then, the bacterial colony consumes the cells' nutrients during the first ten (10) generations (see Figure **??** and Figure **??**). Finally, a new quantity of nutrients is generated and added to each cell at generation eleven (11). The amount of nutrients consumed by each bacterium depends on the bacterium's metabolism (according to the number of plasmids that each bacterium contain); nutrients that are not consumed by bacteria remain in the environment.

(a) Generation 0. (b) Generation 5. (c) Generation 10.

Figure **3-9**: Decreasing of nutrients in the bacterial colony for the first ten generations.

3.2.2 New Bacteria Characteristics

The model includes new mechanisms and characteristics for improving the representation of bacteria; besides, some characteristics shown in the previous section, are replaced. These mechanisms and characteristics are the following:

A Donor Bacterium has multiple pili, therefore, if a donor bacterium is in contact with another bacterium within its neighbourhood, the donor bacterium can transfer a plasmid to the recipient bacterium. In Figure **??**, a donor bacterium (in yellow) can transfer a plasmid to four recipient bacteria (in purple) because they are in direct contact with the

donor bacterium.

In nature, bacterial strains with multiple plasmids are common; however, this is determined by several factors such as incompatibility groups, plasmids with the same replication mechanism or some properties in the origin of replication [**?**]. The incompatibility of plasmids in the model, is simplified in the next way. Plasmids with the same first four bits are incompatible and can not be maintained in the same bacterium.

Bacterial metabolism is affected by the number of plasmids; while a bacterium contains more plasmids, the bacterium needs more nutrients to survive.

Figure **3-10**: A donor bacterium (in yellow) has a pilus in every one of its six edges and recipient bacteria (in purple) are in direct contact with it; therefore, the donor bacterium can transfer plasmids to any of the four purple bacteria.

Plasmids

Simulated plasmids contain a genetic code of one hundred (100) bits; first four (4) bits represent the origin of replication, which goal is to control the incompatibility of plasmids in a bacterium. Therefore, a bacterium can not have two plasmids with the same origin of replication. The last ninety six (96) bits, contain four (4) resistance genes of plasmids (see Figure **??**). The purpose of each resistance gene is to provide resistance to a determined group of antibiotics (see Figure **??**). The resistance genes are:

1. Resistance Gene 1: This gene is located from bit sixteen to twenty six, this gene provides resistance to antibiotics of Type I.

2. Resistance Gene 2: This gene is located from bit thirty eight to forty eight, this gene provides resistance to antibiotics of Type II.

3. Resistance Gene 3: This gene is located from bit sixty four to seventy four, this gene provides resistance to antibiotics of Type III.

4. Resistance Gene 4: This gene is located from bit seventy nine to eighty nine, this gene provides resistance to antibiotics of Type IV.

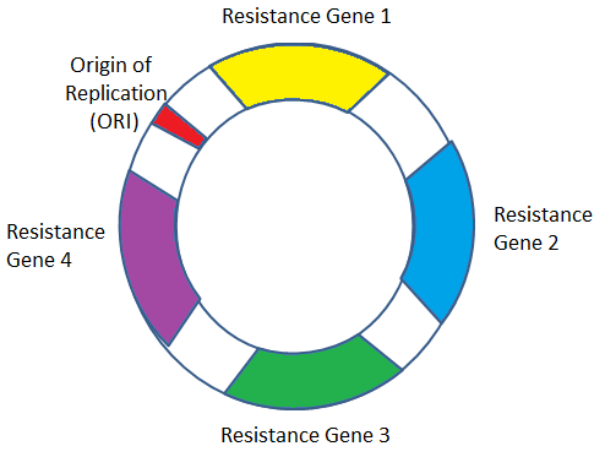


Figure **3-11**: Plasmid

3.2.3 Actions of Bacteria

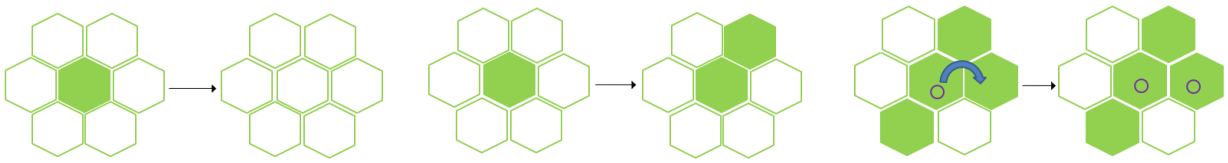
In the previous section, three actions are presented and explained: turn left, turn right and conjugate. However, actions such as reproduction and death were not taken into consideration. In this section, death and reproduction are included to the model, and new features are included to the existent actions. Therefore, the model includes the following actions:

Motility: Bacteria do not exhibit motility, movements turn right and turn left are removed because the objective of these movements is to find a bacterium for transferring a plasmid; however, this is not required anymore because a donor bacterium has multiple pili.

Death: A bacterium may die due to old-age or starvation; in this model, when a bacterium dies, it is removed from the Physical Layer (see Figure **??**).

Reproduction: A bacterium can reproduce if the environment has the appropriate conditions; in fact, a bacterium reproduces when the next requirements are fulfilled: the quantity of nutrients in its cell is adequate, the rate of reproduction is in zero and there is a empty neighbour cell (see Figure **??**).

Transference of Plasmids: A donor bacterium has multiple pili; therefore, when a donor bacterium is in direct contact with a recipient bacterium and enviromental conditions are adequate; the donor bacterium can transfer a plasmid to a recipient bacterium (see Figure **??**).



(a) Death. (b) Reproduction. (c) Conjugation.

Figure **3-12**: Actions of Bacteria.

Actions in a Neutral Environment

The environment can be neutral (no antibiotics) or stressed (antibiotics in the environment); therefore, behaviours of a bacterium changes according to the environmental pressure or absence of this. When there is not pressure over the environment, two linguistic variables are used to determine the action that a bacterium must carry out. The first linguistic variable is titled "Nutrients", this linguistic variable can take four linguistic values: low, medium, high and very high (see Figure **??**). The second linguistic variable is titled "Number of Plasmids", this linguistic variable can take three linguistic values: low, medium and high (see Figure **??**).

Each generation, the action to be performed by a bacterium is defined according to the number of contained nutrients in the cell where the bacterium resides and the number of plasmids that a bacterium contains. On one hand, a bacterium does not carry out any action when the quantity of nutrients is slow or medium; on the other hand, a bacterium reproduces just when the quantity of nutrients are high or very high. The Fuzzy Association Matrix is shown in Table **??**.

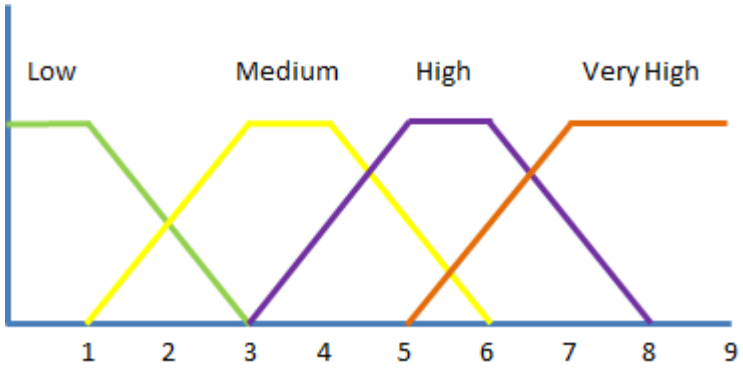


Figure **3-13**: Linguistic Variable - "Nutrients"

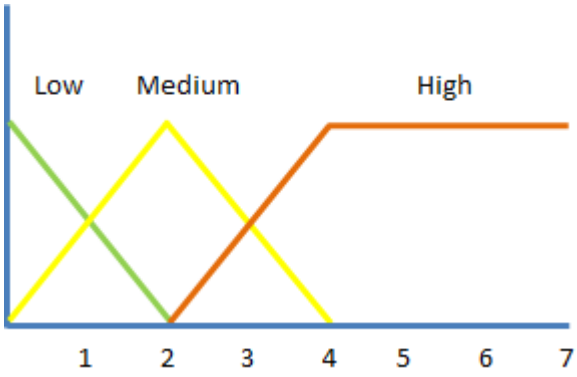


Figure **3-14**: Linguistic Variable - "Number of Plasmids"

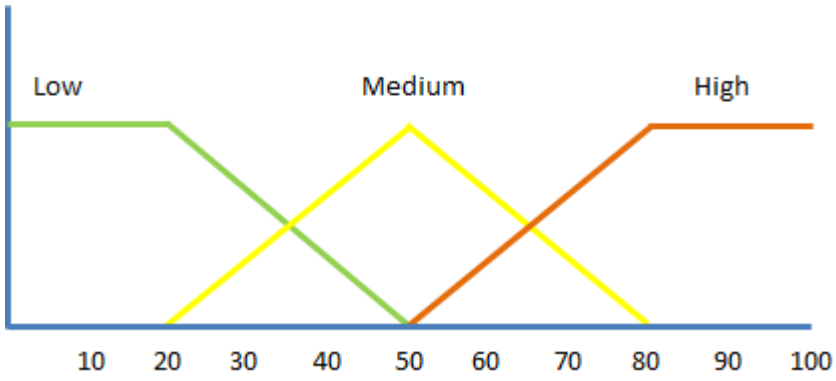


Figure **3-15**: Linguistic Variable - "Antibiotic Resistance".

Nutrients					
		Low	Medium	High	Very High
Number of Plasmids	Low	Still	Still	Reproduce	Reproduce
	Medium	Still	Still	Reproduce	Reproduce
	High	Still	Still	Reproduce	Reproduce

Table **3-2**: Fuzzy Association Matrix. Nutrients vs Number of Plasmids.

Actions in a stressed environment

The presence of a type of antibiotics in the environment, changes the behaviour of the bacterial colony. Two linguistic variables are used for determining the action to be carried out by a bacterium in a stressed environment. The linguistic variable called "Nutrients", is used again, keeping its linguistic values: low, medium, high and very high (see Figure **??**). The second used linguistic variable, is called "Antibiotic Resistance"; this linguistic variable

returns a value depending on the antibiotic resistance of a bacterium to a type of antibiotic. The linguistic values for this variable, are the following: low, medium and high (see Figure **??**).
A bacterium remains still or carries out two actions (reproduce or conjugate) under stressed conditions. The action is defined by a Fuzzy Association Matrix (see Table **??**) that is created depending on the number of nutrients in a determined cell, and the antibiotic resistance of a specific bacterium.

	Nutrients				
		Low	Medium	High	Very High
Bacterium Resistance	Low	Still	Still	Reproduce	Reproduce
	Medium	Still	Reproduce	Conjugate	Conjugate
	High	Still	Conjugate	Conjugate	Reproduce

Table **3-3**: Fuzzy Association Matrix of Nutrients vs Antibiotic Resistance.

3.2.4 Quantity of required nutrients for performing each action

In each generation, the quantity of nutrients consumed by a bacterium depends on the action performed by the bacterium in the current generation and the number of plasmids that the bacterium contains. The second column in Table **??**, shows the quantity of nutrients that are consumed by a bacterium according to a performed action and the third column shows the quantity of added nutrients to each performed action depending on the number of plasmids that a bacterium contains (Plasmids Factor).

Action	**Nutrients**	**Plasmids Factor**
Still - Survive	0.5	0.1
Reproduction	1	0.1
Conjugation	1	0
Death	0	0

Table **3-4**: Metabolism

Rules

Reproduction: The environment must achieve several factors in order to provide adequate conditions for bacterium's reproduction; the main factors are temperature, levels of acidity(pH), levels of humidity and quantity of nutrients. In this model, the first three variables are not taken into consideration; instead, a minimal quantity of nutrients is required to

carry out a bacterium's reproduction. Conditions that must be accomplished for bacterial reproduction are the following:

- Reproduction must be selected in the mechanism of actions selection.
- A neighbour cell must be empty.
- The cell which contains the bacterium, must contain at least one (1) unit of nutrients plus the plasmids factor (see column three in Table **??**).

Mutation: Mutation is carried out when a bacterium is in process of reproduction. The mutation (Flip Bit: bit is taken and is flipped) is performed in one selected plasmid; the mutation probability in a reproduction depends on the bacterium's resistance probability; while higher the resistance probability, lower the mutation probability.

Death A bacterium dies due to three different factors:

1. Age: Bacterium dies due to old-age (when a bacterium is born, the bacterium has a variable with the number of life generations).
2. Starvation: A bacterium does not have nutrients to consume for three generations; therefore, the bacterium dies for starvation.
3. Mechanism of Action of Antibiotics: When a Bacterium is in a stressed environment due to a mechanism of action of antibiotics and the bacterium is not resistant to the type of antibiotics being used, or the antibiotic resistance is not enough to resist the quantity of antibiotics, the bacterium dies.

Conjugation A Bacterium can transfer a plasmid when the next conditions are accomplished:

- Conjugation is selected as the action to be carried out by the mechanism of actions selection.
- The donor bacterium must be in direct contact with other bacterium.
- The recipient bacterium must not contain a plasmid with the same origin of replication (First four bits).
- The cell that contains the donor bacterium, must have one (1) unit of nutrients.

3.3 Antibiotics

The purpose of the mechanism of action of antibiotics, is to eliminate the bacterial colony or affect the growth rate. A first approach to a diffusion antibiotic mechanism based on a sand-pile model was presented in the first section; however, the mechanism of action of antibiotics was very simple (recipient bacteria die and donor bacteria survive) and it is not adequate for acting against bacterium's characteristics provided by Resistance genes (see Figure **??**). Therefore, the mechanism of action of antibiotics changes according to the characteristics that each bacterium may exhibit.
There are four types of antibiotics that can be included in a mechanism of action, the purpose of each type of antibiotics is to act against specific characteristics of a bacterium. The types of antibiotics are as follows:

- **Type i:** Antibiotic of type i, acts against provided characteristics by Resistance Gene i.
- $1 \leq i \leq 4$

3.3.1 Codification of Antibiotics

Antibiotics are designed as an array of twelve (12) bits (see Figure **??**), two (2) initial bits indicate the mechanism of action (it specifies the bacterium's characteristic where the mechanism acts). The last ten (10) bits are the body of the antibiotic and are used to evaluate the susceptibility of each bacterium to a type of antibiotics (see Algorithm **??**).

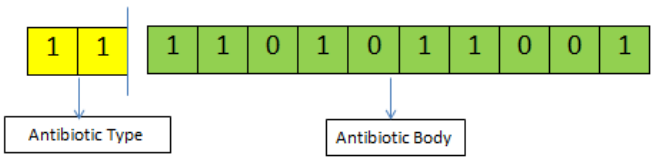


Figure **3-16**: Antibiotic Gene.

Antibiotic Resistance Function

Bacteria have developed mechanisms of antibiotic resistant, studies [**?**] show that some mechanisms are derived from different kind of plasmids that provide a bacterium with new characteristics. A mechanism of action of antibiotics, is used to act against specific characteristics in a bacterium; for instance, the mechanism of action of penicillin is to interfere with the bacterial cell wall synthesis [**?**]. In this subsection, a mechanism of action of antibiotics is designed to evaluate the performance of a type of antibiotics against a bacterium.

The mechanism is composed of a set of rules that evaluates the susceptibility of a bacterium to a type of antibiotics. The genetic code of the antibiotic (see Figure **??**), is evaluated against each plasmid that a bacterium contains according to the algorithm "Evaluation of Antibiotic Resistance" (See Algorithm **??**).

The process for evaluating the bacterium's antibiotic resistance, follows two steps: First, the two initial bits of the antibiotic, are examined to define bacterium's characteristics that will be affected by a type of antibiotics (when a bacterium does not contain plasmids, the bacterium dies). Second, The algorithm "Evaluation of Antibiotic Resistance" (See Algorithm **??**), is used for evaluating the probability of antibiotic resistance depending on the quantity of antibiotics on the bacterium's cell (this algorithm compares the last ten (10) bits of the antibiotic with the ten (10) bits of the selected resistance gene).

Algorithm 1 Evaluation of Antibiotic Resistance

```
1: IsBacteriaResistant(codePlasm,codeAnt)
2: if codePlasm is complement of codeAnt then
3:    probRes ← 0
4:    return false
5: else if difCode ≤ limSup and difCode ≥ limInf then
6:    if concAnt ≥ limAntibiotic then
7:       probRes ← ResistenciaBact
8:       return false
9:    else
10:      probRes ← ResistenciaBact
11:      return true
12:   end if
13: end if
```

3.4 Bacterial Growth Curve and Optimization

In the previous section, the proposed model is explained and simulations of it can be conducted; however, this model is not compared against biological mechanisms, neither computational mechanisms; therefore, in this section, the proposed model is compared with a biological model called "Bacterial Growth Curve" and for evaluating the model performance by solving four optimization functions. First, some experiments for observing bacterial growth in the model, are conducted; these experiments are compared with a model titled "Bacterial Growth Curve". Next, a set of experiments to find the correct parameters for the regeneration of nutrients in order to stabilize the population in a medium value, are conducted. Finally, the last experiments show the emergency and evolution of resistance to

antibiotics (antibiotic resistance remains in the bacterial colony under stressed conditions).

3.4.1 Bacterial Growth Curve

A bacterial colony exhibits a pattern of growth that can be represented by a curve of four phases, this curve is titled "Bacteria Growth Curve" [**?**][**?**]. The bacterial growth curve has four different phases (See Figure **??**).

Lag Phase: In this phase, a bacterium is not able to reproduce because the bacterium is adjusting to the environment and the bacterium's metabolism starts to accelerate. the time that a bacterial colony is in a lag phase, depends on the quantity of nutrients and conditions of the environment (neutral or stressed environment).

Exponential/Logarithmic Phase: This phase is characterized by a high rate of bacteria reproduction; therefore, the bacteria's metabolism increases exponentially/logarithmically.

Stationary Phase: In this phase, death and reproduction rates are similar; therefore, population remains in a stationary period where the the number of bacteria is stable during several generations.

Death Phase: In this phase, the rate of bacterium's death is high; therefore, the number of bacteria decreases at each generation, because the death rate is higher than the reproduction rate. This behaviour may be produced by the fast consumption of nutrients, the quantity of waste materials and the production or existence of inhibitory substances such as antibiotics.

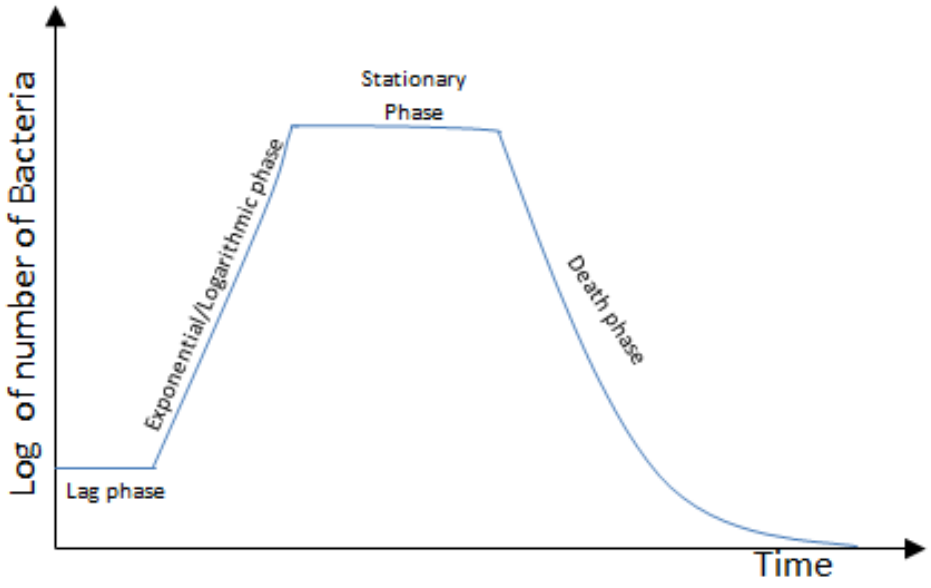
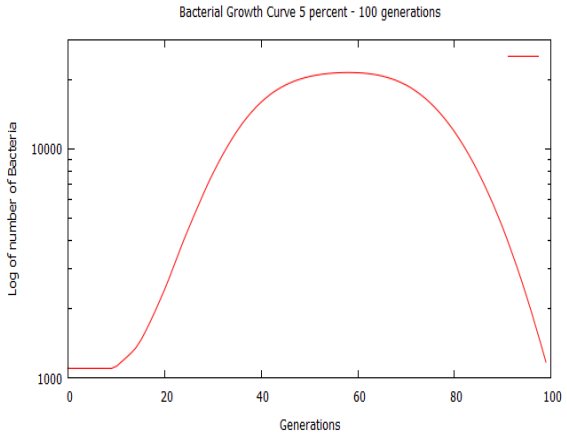


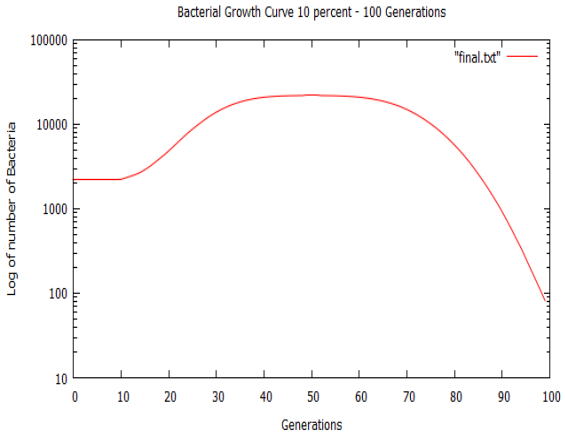
Figure **3-17**: Bacterial Growth Curve

Representation of Bacterial Growth Curve

The purpose of this set of simulation experiments is to evaluate the produced Bacterial Growth Curve by the model. In these experiments, the initial conditions are set to: Constant quantity of nutrients per cell (no regeneration); perfect reproduction (binary fission generates bacteria exactly equal, no mutation), neutral environment (no mechanism of action of antibiotics) and the cellular automaton dimension is 220X100. Thirty (30) different runs are executed under these conditions during one hundred (100) generations, with four different initial percent of bacteria: five (5), ten (10), twenty (20) and thirty (30) percent of bacteria. Figures **??** and **??** correspond to the average of the thirty (30) runs.



(a) Five (5) percent

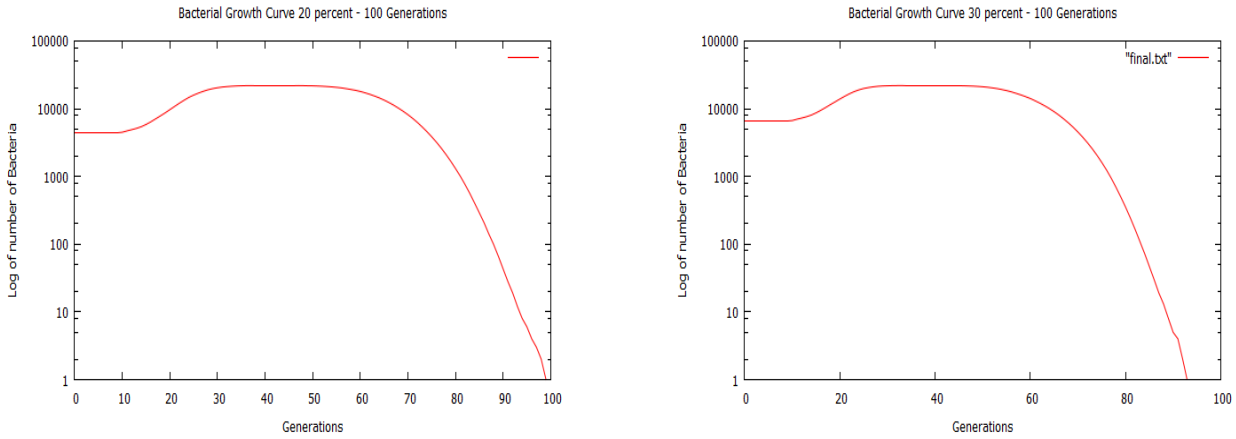


(b) Ten (10) percent

Figure **3-18**: Bacterial Growth Curve for initial population of five (5) and ten (10) percent of bacteria.

Curves generated by the model (see Figures **??** and **??**), are similar to the studied Bacterial Growth Curve (see Figure **??**). However, the initial bacterial population changes the graphic of the curve and the time of generations that takes to finish and start a new phase. Curves generated with an initial bacterial population of twenty (20) and thirty (30) percent (see Figure **??**), present a similar behaviour, where the exponential phase is too short. The curve produced by an initial bacterial population of ten (10) percent (see Figure **??**), presents a short exponential phase; however, the stationary and death phase are the longest ones of the four simulations. Lastly, the generated curve with an initial bacterial population of five (5) percent (see Figure **??**), presents adequately the four phases in one hundred (100) generations; therefore, this curve shows the most similar behaviour to the Bacterial Growth Curve.

In first instance, the generated simulation with the designed model, may represent similar behaviours to the Bacterial Growth Curve; however, the purpose of the model is to observe



(a) Twenty (20) percent

(b) Thirty (30) percent

Figure **3-19**: Bacterial Growth Curve for initial population of twenty (20) and thirty (30) percent of bacteria.

the emergency of antibiotic resistance; therefore, a number of live bacteria must remain in the bacterial colony during several generations. In the next subsection, some simulation experiments are conducted for finding the adequate parameters to keep the bacterial colony in the stationary phase in a large number of generations.

Stationary Phase

The previous experiments show that the designed model may generate similar behaviours to the Bacterial Growth Curve; however, this behaviour in the bacterial colony is not useful for the simulations, because the population has been not enough time in a stationary phase in order to apply a mechanism of action of antibiotics and observe, interactions and effects of these actions over the bacterial colony. Therefore, the purpose of this set of simulation experiments is to find the adequate parameters to maintain the bacterial colony in a stationary phase.

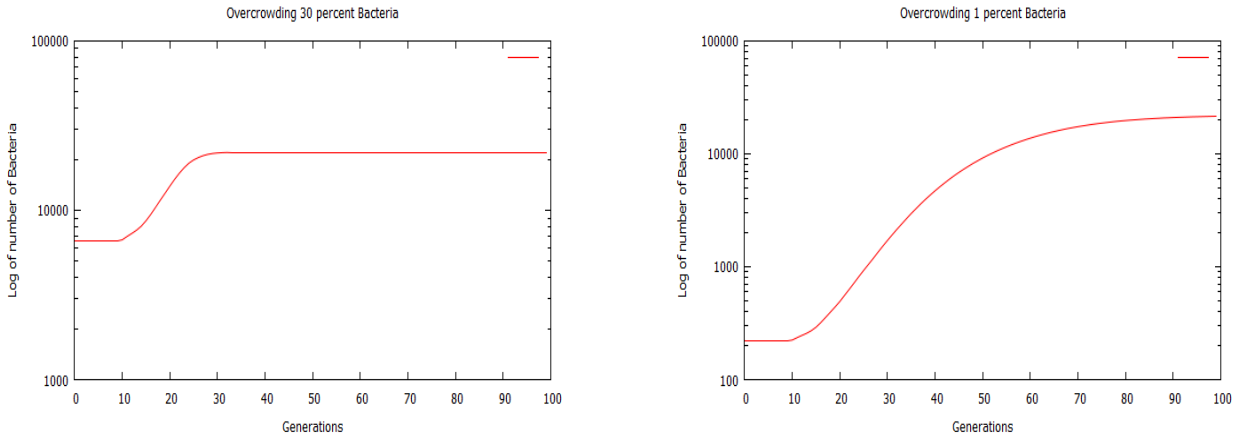
The strategy to keep the colony in a stationary phase is based on nutrients regeneration; therefore, a quantity of nutrients is randomly added to each cell every ten generations. Several simulations are conducted for finding the correct superior and inferior limits of nutrients to keep the colony in a stationary phase without losing diversity (bacterial population must change and evolve).

Overpopulation

The purpose of this set of simulation experiments is to observe the behaviour of the bacterial colony with a large number of available nutrients. In these experiments, the initial conditions

are set to: A quantity of one hundred (100) nutrients per cell, regeneration of nutrients every ten generations, perfect reproduction (binary fission generates bacteria exactly equal, no mutation or conjugation), neutral environment (no mechanism of action of antibiotics) and the cellular automaton dimension is 220X100. Thirty (30) different runs are executed under these conditions during one hundred (100) generations, with two different initial percent of bacteria: Thirty (30) percent and one (1) percent of bacteria.

The obtained results show that the population with an initial population of thirty (30) percent of bacteria, is effectively stabilized close to thirty (30) generations (see Figure **??**); instead, the curve for a initial population of one (1) percent (see Figure **??**), is stabilized in a stationary phase close to (60) generations. However, the exhibited behaviour is not adequate due to generation of overpopulation; therefore, the diversity of the bacterial colony is not adequate. In conclusion, a bacterial colony with a large quantity of nutrients generates overpopulation independently to the initial population; therefore, the range of values of nutrients must change for stabilizing the model in a stationary phase with adequate diversity conditions.



(a) Thirty (30) percent

(b) One (1) percent

Figure **3-20**: A) Bacterial Growth Curve for initial population of twenty (20) and thirty (30) percent of bacteria.

Stationary phase with diversity

The purpose of this set of simulation experiments is to evaluate different parameters of regeneration of nutrients for keeping the bacterial population between thirty (30) to sixty (60) percent. In these experiments, the initial conditions are set to: Thirty (30) percent of initial bacteria, reproduction with a probability of mutation (no conjugation), neutral environment (no mechanism of action of antibiotics) and the cellular automaton dimension is

220X100. Thirty (30) different runs are executed under these conditions during ten thousand (10000) generations, with different regeneration parameters: between zero (0) units and ten (10) units (see Figure **??**), between two (2) units and ten (10) units (see Figure **??**), and between four (4) and ten (10) units of nutrients (see Figure **??**). The Figures correspond to the average of the thirty (30) runs.

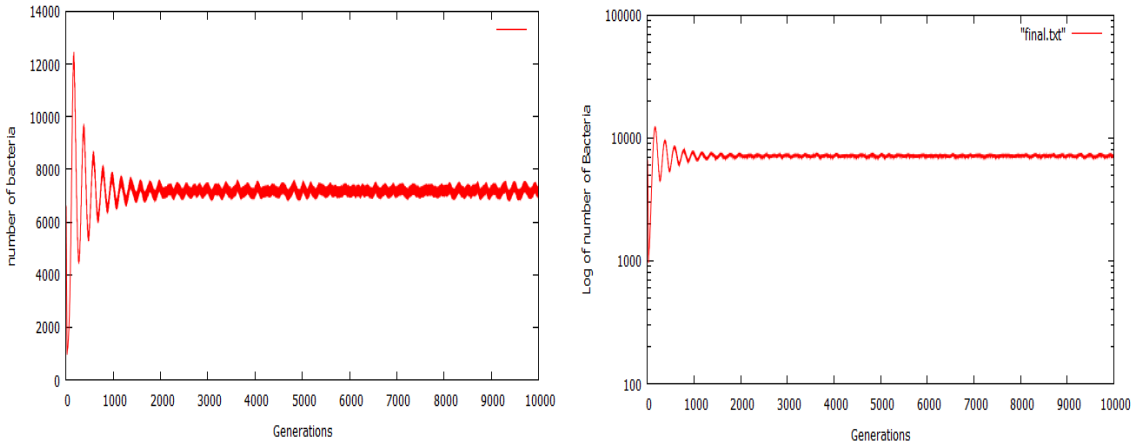


Figure **3-21**: Nutrients regeneration parameters - random values between 0-10 each ten generations.

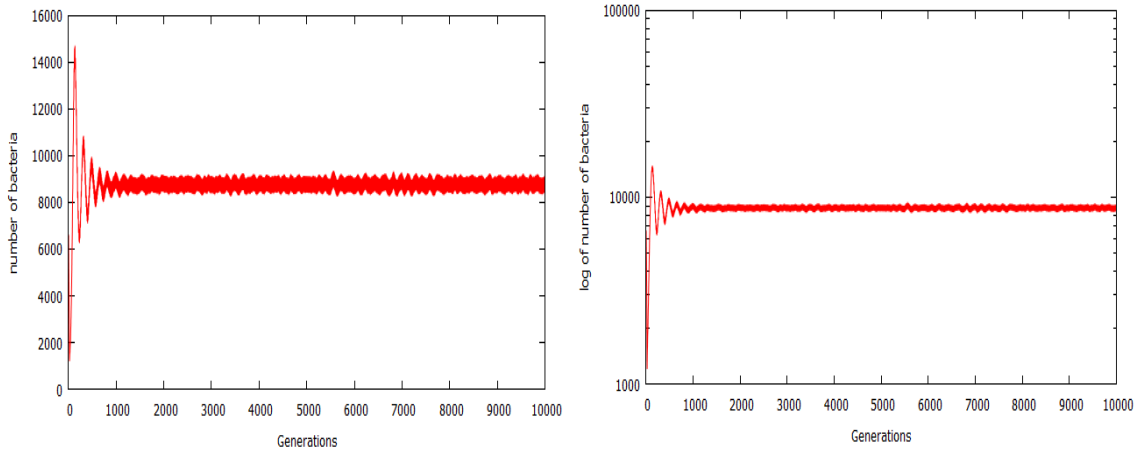


Figure **3-22**: Nutrients regeneration parameters - random values between 2-10 each ten generations.

The obtained results show that simulation experiments conducted with ranges between zero (0) to ten (10) and two (2) to ten (units), may effectively maintain the bacterial population with adequate diversity conditions (see Figures **??** and **??**); however, simulations show high oscillations in the first one thousand (1000) generations; instead, experiments conducted with parameters of regeneration between four (4) to ten (10) units of nutrients, show a faster

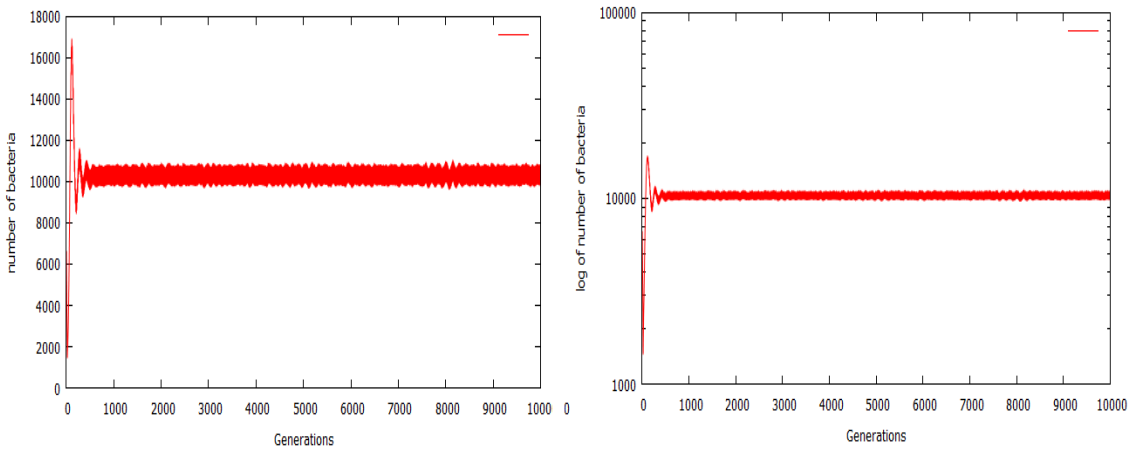


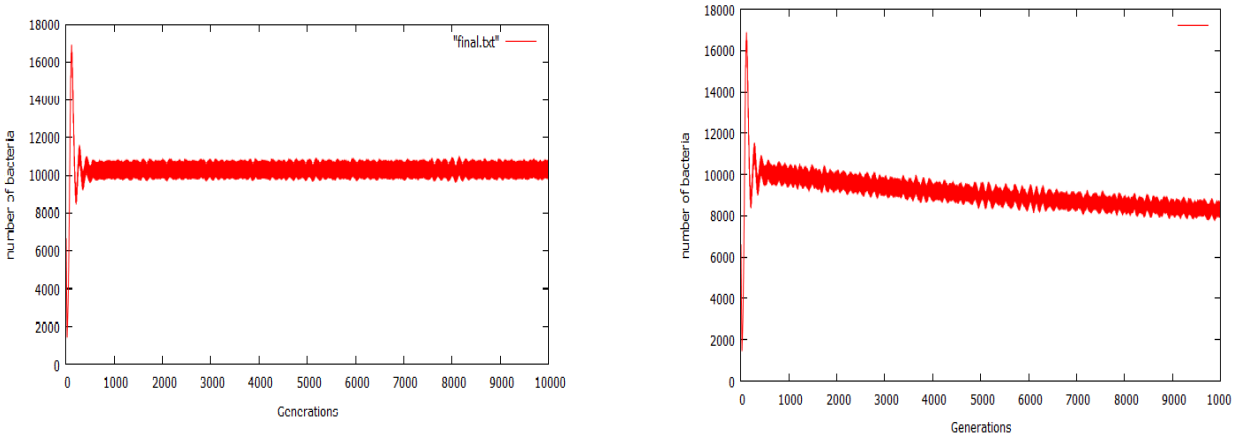
Figure **3-23**: Nutrients regeneration parameters - random values between 4-10 each ten generations.

stabilization of the size of the population. Therefore, the selected range for regeneration, is between four (4) to ten (10) units of nutrients every ten generations.

Mechanism of action of antibiotics

The purpose of this set of simulation experiments is to evaluate the impact of antibiotics over the bacterial population. In these experiments, the initial conditions are set to: Thirty (30) percent of initial bacteria (no antibiotic resistance), reproduction with a probability of mutation (no plasmids), stressed environment (a constant mechanism of action of antibiotics) and the cellular automaton dimension is 220X100. Thirty (30) different runs are executed under these conditions during ten thousand (10000) generations. The average of these thirty (30) runs is shown in Figure **??**.
In Figure **??**, the obtained graphical in a stressed environment, is compared to the graphical in a neutral environment (additional conditions are kept the same). The stressed environment shows a decreasing behaviour (see Figure **??**) compared to the stable population shown in Figure **??**; however, the cellular automaton dimension is too high in order to observe a significant decreasing of the size of the population in ten thousand (10000) generations. Therefore, new simulation experiments are conducted to clearly observe the impact of the mechanism of action of antibiotics in a cellular automaton with low dimensions. In these new simulation experiments, the initial conditions are set to: Thirty (30) percent of initial bacteria (no antibiotic resistance), reproduction with a probability of mutation (no plasmids), stressed environment (a constant mechanism of action of antibiotics) and the cellular automaton dimension is 100X60. Thirty (30) different runs are executed under these conditions during ten thousand (10000) generations.

The obtained results (see Figure **??**) show a significant decreasing of the size of the popula-



(a) Neutral Environment (b) Stressed Environment

Figure **3-24**: Bacterial Population in a neutral environment vs stressed environment.

tion, this behaviour is produced by the mechanism of action of antibiotics. As expected, the mechanism of action of antibiotics acts appropriately against the proliferation of bacteria and may cause the extinction of the bacterial colony if there is not a mechanism in opposition to the antibiotics. In the next subsection, plasmids are included in the proposed model and some simulation experiments are conducted in order to evaluate the impact of plasmids in the bacterial colony.

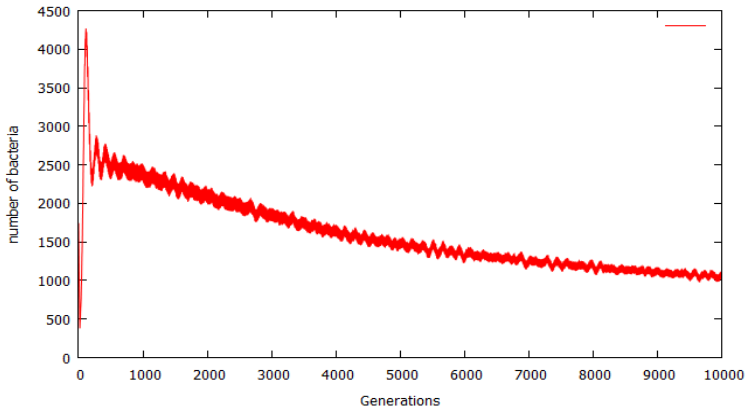


Figure **3-25**: Bacterial population in a stressed environment - (Cellular Automaton Dimension 100x60)

Mechanism of action of antibiotics in a stressed environment that exhibits conjugation

The purpose of this set of simulation experiments is to compare the last results of the population decreasing with a population that contains plasmids. The initial conditions are set to: Thirty (30) percent of initial bacteria (fifteen (15) percent of initial bacteria, contain a plasmid), reproduction with a probability of mutation, transference of plasmids (conjugation), stressed environment (a constant mechanism of action of antibiotics) and the cellular automaton dimension is 100X60. Thirty (30) different runs are executed under these conditions during ten thousand (10000) generations.

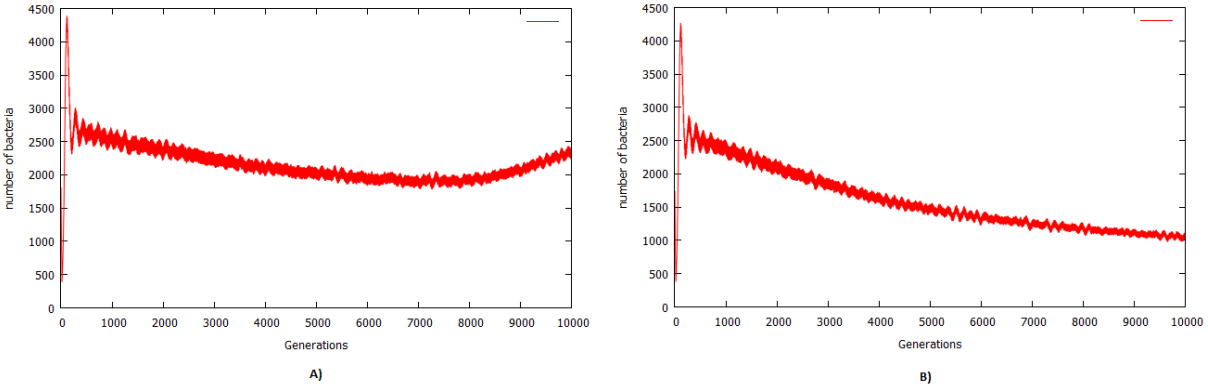
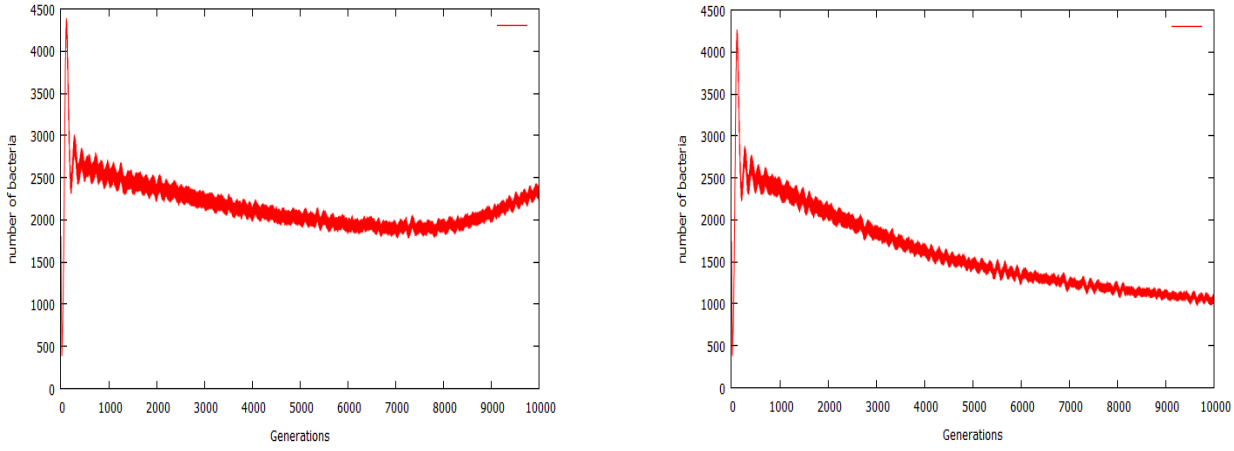


Figure **3-26**: A)Stressed environment in a bacterial colony with plasmids, B)Stressed environment (no plasmids).



(a) Stressed environment (plasmids)

(b) Stressed environment (no plasmids)

Figure **3-27**: A)Stressed environment in a bacterial colony with plasmids. B) Stressed environment (no plasmids).

Figure **??**, shows a bacterial colony in a stressed environment after ten thousand (10000) generations. Figure **??** shows the obtained graphical in a stressed environment where bacteria can transfer plasmids, and Figure **??** presents a stressed environment where bacteria do not contain plasmids. On one hand, both plots are very similar (see Figure **??**) during the first one thousand (1000) generations; on the other hand, in the stressed environment with plasmids, the population decreases approximately until generation seven thousand (7000); then, the size of the bacterial population starts to increase close to generation eight thousand (8000); clearly, plasmids provide characteristics which allow bacteria to resist a mechanism of action of antibiotics. Furthermore, the number of bacteria increases after several generations even though the mechanism of action of antibiotics is active; therefore, the antibiotic resistance emerges in the bacterial colony and it is provided by plasmids.

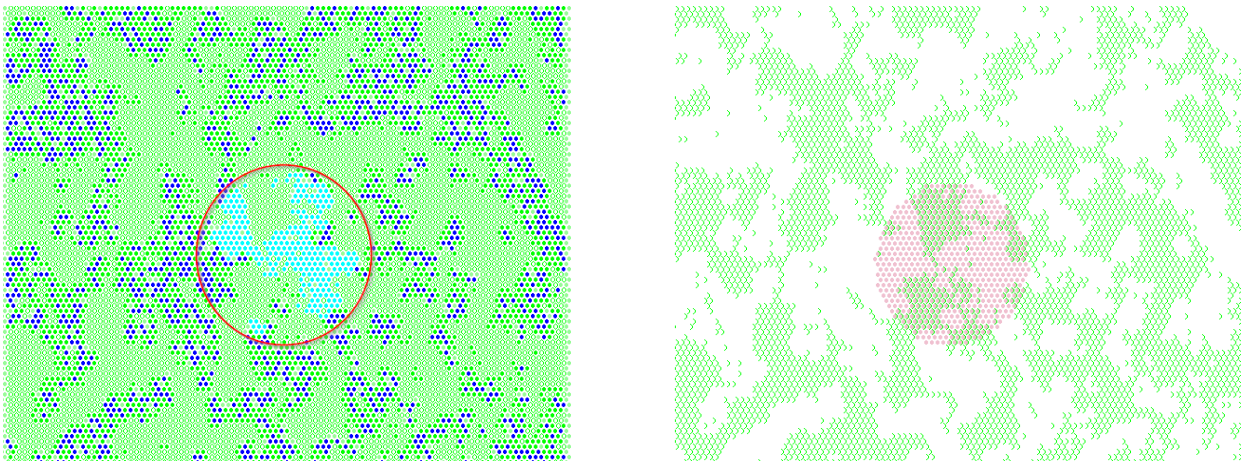
Emergence of Antibiotic Resistance

The purpose of this set of experiments is to evaluate the emergence and evolution of antibiotic resistance in the bacterial colony. The initial conditions are set to: Thirty (30) percent of initial bacteria (fifteen (15) percent of initial bacteria, contain a plasmid), reproduction with a probability of mutation, transference of plasmids (conjugation), stressed environment (a constant mechanism of action of antibiotics) and the cellular automaton dimension is 100X100. Ten (10) different runs are executed under these conditions during one thousand (1000) generations.

Antibiotic	Generations			
	200 Res.	400 Res.	800 Res.	1000 Res.
010111111011	0011101011 0.6	0011101011 0.6	0111111011 1.0	0111111011 1.0
110110001100	1111100101 0.45	0110001100 1.0	0110001100 1.0	0110001100 1.0
011101110000	1001101000 0.6	1001101000 0.6	1101110000 1.0	1101110000 1.0
001010100000	1010000000 0.8	1010100000 1.0	1010100000 1.0	1010100000 1.0
001001011010	1101101010 0.6	1101101010 0.6	1001010010 0.8	1001011010 1.0
010000000000	0011000001 0.6	0011000001 0.6	0000000000 1.0	0000000000 1.0
001001000011	1011000101 0.6	1001000011 1.0	1001000011 1.0	1001000011 1.0
111110010111	1110000111 0.8	1110010111 1.0	1110010111 1.0	1110010111 1.0
010000000010	0000000000 0.8	0000000000 0.8	0000000010 1.0	0000000010 1.0
001000010110	1000000101 0.6	1000000101 0.6	1000010110 1.0	1000010110 1.0

Table **3-5**: Emergency of Antibiotic Resistance.

In table **??**, the first column shows the genetic code of the mechanism of action of antibiotics used. The next columns show the best plasmid (plasmid that provides the best characteristic/mechanism to resist the mechanism of action of antibiotics) and the antibiotic



(a) Resistant Bacteria (in cyan))

(b) Antibiotic Diffusion (in pink)

Figure **3-28**: Emergence of antibiotic resistance at generation one thousand (1000); bacteria in cyan color are completely resistant to the mechanism of action of antibiotics being used.

resistance that the plasmid provides to a bacterium at generation two hundred (200), four hundred (400), eight hundred (800) and one thousand (1000). The Obtained results show that the efficiency of the best plasmid, increases through time; resulting in resistant bacteria approximately at generation eight hundred (800). In Figure **??**, the emergency of antibiotic resistance is shown after one thousand (1000) generations. Strains of resistant bacteria (in cyan) appear (see Figure **??**) where the antibiotic (in pink) has spread.

3.5 Optimization

In the previous section, the obtained results showed the emergency of antibiotic resistance by transferring plasmids from a donor bacterium to a recipient bacterium, and by performing mutations in the genetic code. Resulting in bacteria that exhibit resistance to a type of antibiotics after approximately eight hundred (800) generations. In this section, the proposed model is used as an algorithm to solve indirectly optimization problems; in this case, the genetic code of plasmids, contains solutions to different problems, and the genetic code of the antibiotic, contains the problem to be resolved. Actually, a bacterium is not trying to solve the optimization problems, instead, the bacterium tries to survive against a mechanism of action of antibiotics. The antibiotic resistance is the solution provided to the optimization problem normalized between zero (0) to one (1) (a bacterium with a value of resistance in (1) is completely resistant to the mechanism of action of antibiotics and has the solution to the optimization problem).

3.5.1 Plasmids

The purpose of plasmids is to transfer solutions from a donor bacterium to a recipient bacterium. Plasmid's Genetic code changes in order to contain solutions (resistance genes) (see Figure **??**) for four different problems (MaxOnes, Deceptive-Three, Deceptive-Four and Royal Road). In fact, each optimization function is a mechanism of action of antibiotics and each resistance gene provides characteristics/mechanisms to a bacterium for solving a determined problem. Resistance genes are defined in the following way:

- MaxOnes Resistance: This gene has a size of one hundred (100) bits and provides characteristics for solving the optimization problem "MaxOnes".
- Deceptive-3 Resistance: This gene has a size of thirty (30) bits and provides characteristics for solving the optimization problem "Deceptive-3".
- Deceptive-4 Resistance: This gene has a size of forty (40) bits and provides characteristics for solving the optimization problem "Deceptive-4".
- Royal Road Resistance: This gene has a size of eighty (80) bits and provides characteristics for solving the optimization problem "Royal Road".

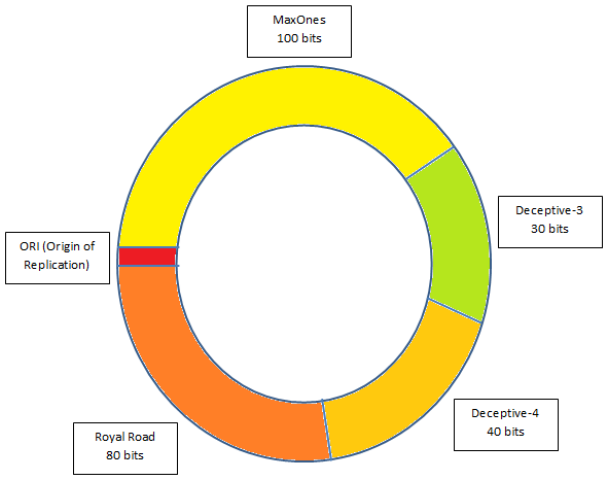


Figure **3-29**: New Plasmid

3.5.2 Antibiotic

The purpose of the antibiotic is to define the problem to be optimized instead of containing the problem itself; the plasmid's genetic code just contains two bits (see Table **??**)that indicate the mechanism of action of antibiotics (optimization problem).

Bits	Optimization Problem
00	MaxOnes
01	Deceptive-Three
10	Deceptive-Four
11	Royal Road

Table **3-6**: Bits indicate the problem to be optimized.

Antibiotic Resistance Function

The antibiotic resistance function is a mechanism that is composed by a set of rules that evaluates the susceptibility of a bacterium to a type of antibiotics. The genetic code of the antibiotic is evaluated against each plasmid that a bacterium contains. In fact, the resistance function keeps the same functionality; however, the mechanism of action of antibiotics (optimization function) is not contained by the genetic code of antibiotics; instead, it is included in the resistance function. Therefore,there is an antibiotic resistance function for each optimization problem.

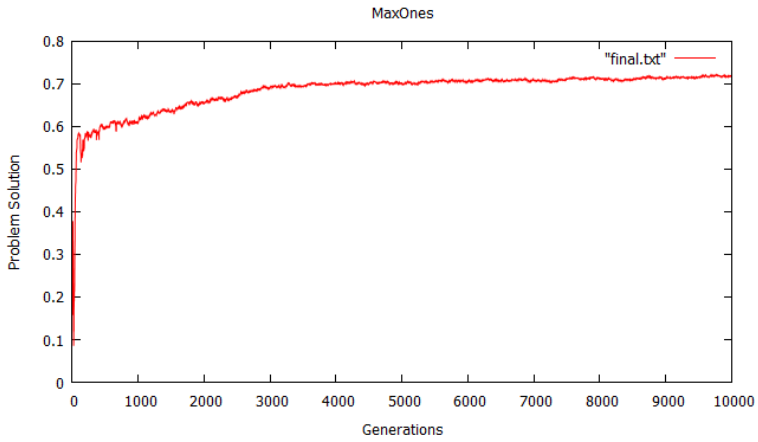


Figure **3-30**: MaxOnes optimization problem

3.5.3 Simulation Experiments

The purpose of this set of simulation experiments is to evaluate the performance of the proposed model for solving four optimization problems: MaxOnes of one hundred (100) bits, Deceptive-Three of thirty (30) bits, Deceptive-Four of forty (40) bits and RoyalRoad of eighty (80) bits. In these experiments, the initial conditions are set to: Thirty (30) percent of initial bacteria (each bacterium contains one plasmid), reproduction with a probability of mutation, transference of plasmids (conjugation), stressed environment (optimization problems) and

the cellular automaton dimension is 100X100. Thirty (30) different runs are executed under these conditions during ten thousand (10000) generations for each optimization problem. The obtained results are normalized so that complete resistance to antibiotics (problem solution) has a value of one (1).

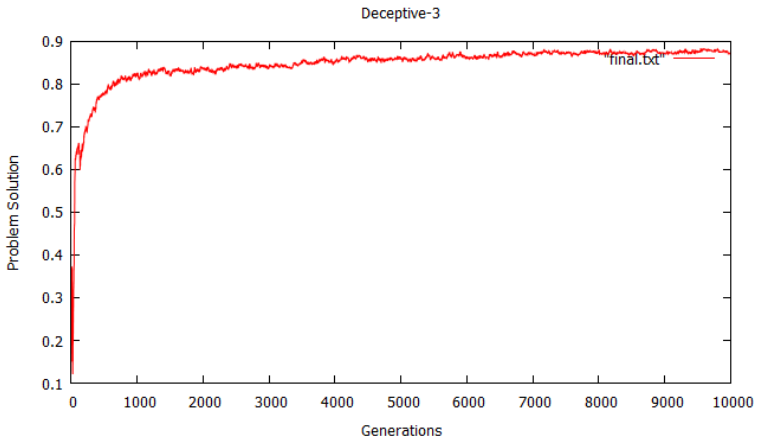


Figure **3-31**: Deceptive-Three optimization problem

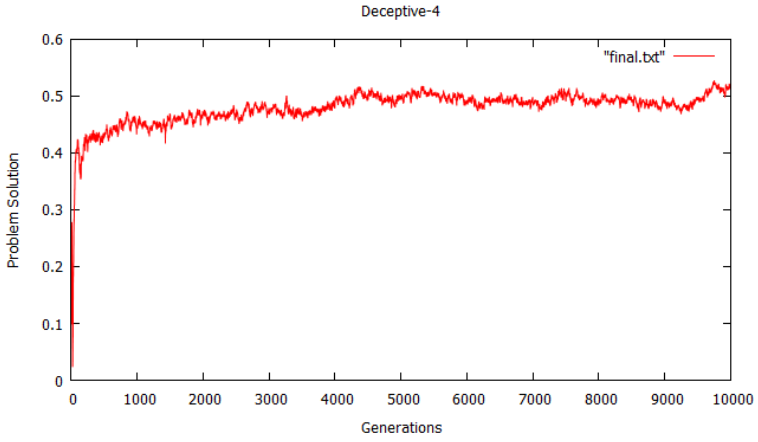


Figure **3-32**: Deceptive-Four optimization problem

The proposed model gets the best results with Deceptive-Three optimization problem; the provided solution is close to eighty seven (87) percent (see Figure **??**). Instead, the worst solution is found for RoyalRoad; this function shows an interesting behaviour as a mechanism of action of antibiotics because it is very effective against the bacterial colony; therefore, the final solution for RoyalRoad is close to zero (0) percent with the proposed model. The solutions obtained for MaxOnes (see Figure **??**) and Deceptive-Four (see Figure **??**) are close to seventy two (72) percent and fifty three (53) percent respectively. The obtained results show that the proposed model obtains acceptable results by considering that the

model is not designed for optimization; however, in order to evaluate the performance of the proposed model for solving optimization problems, this is compared against the algorithm "Hill Climbing".

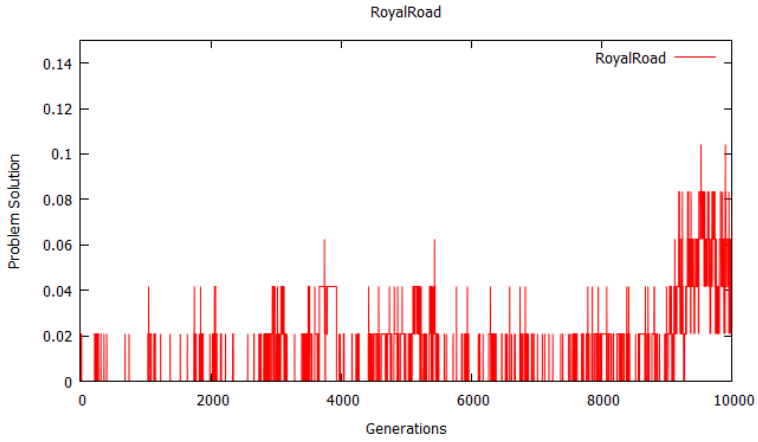


Figure **3-33**: Royal Road optimization problem

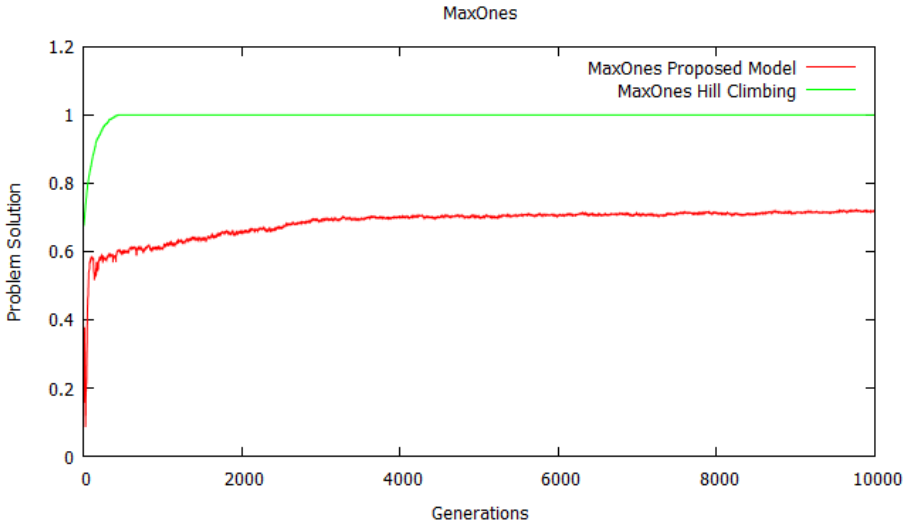


Figure **3-34**: MaxOnes solved by proposed model and Hill Climbing.

Comparisons with the algorithm Hill Climbing

The purpose of this subsection is to compare the obtained results with the proposed model, with the obtained results with the algorithm "Hill Climbing", the initial conditions for the algorithm "Hill climbing" are set to: Three thousand (3000) individuals and ten thousand (1000) evolution generations. Thirty (30) different runs are executed under these conditions with four different optimization problems (MaxOnes, Deceptive-Three, Deceptive-Four and

RoyalRoad). The Figures correspond to the average of the thirty (30) runs and the values are normalized between zero (0) and one (1).

The algorithm "Hill Climbing" solves the MaxOnes optimization problem in short generations due to the shape of the problem. Instead, the proposed model obtained a solution close to seventy three (73) percent (See Figure **??**). However, the proposed model starts with a solution close to (0) at generation zero(0) against a solution close to seventy (70) percent with the algorithm "Hill Climbing". Besides, Individuals in algorithm Hill Climbing are focused on optimizing and finding a solution; on the opposite, a bacterium is focused on surviving and only on finding a solution (resistance to antibiotic) when a mechanism of action of antibiotics is in direct contact with it.

The obtained results for the Deceptive-Three Optimization problem (see Figure **??**), are very similar for both algorithms. The difference between the proposed model and the algorithm "Hill Climbing" is only six (6) percent. These are excellent results for the proposed model by considering that the optimization is indirectly conducted.

For the Deceptive-Four optimization Function, the "Hill Climbing Function" shows a better performance that the proposed model (see Figure **??**). On one hand, Hill Climbing obtains results close to seventy five (75) percent; on the other hand, the proposed model obtains results close to fifty (50) percent.

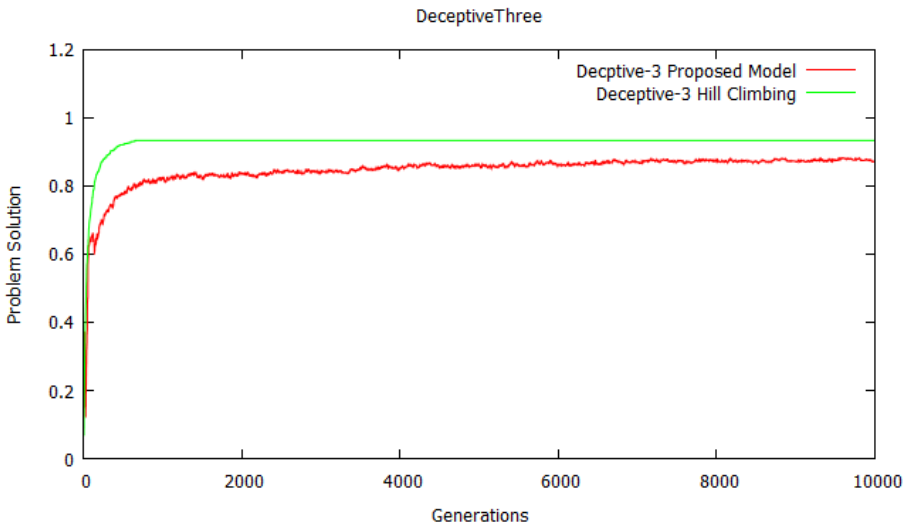


Figure **3-35**: Deceptive-Three solved by proposed model and Hill Climbing.

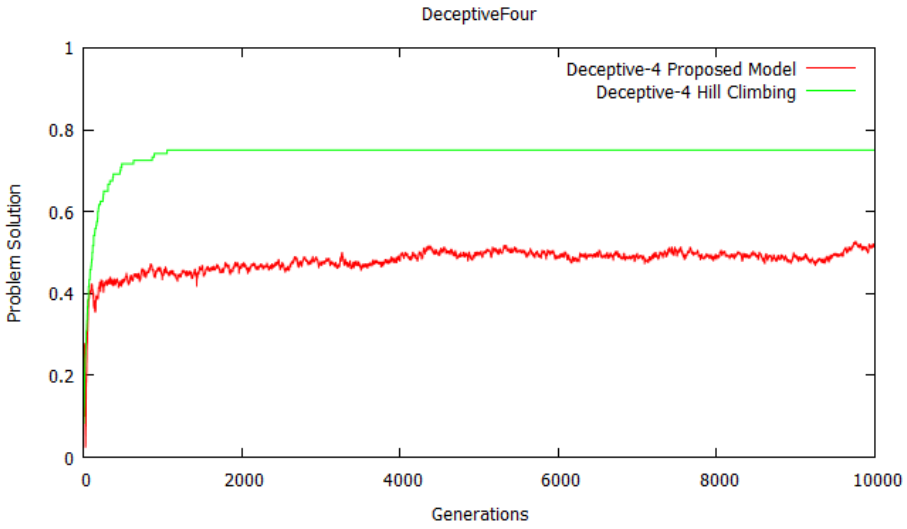


Figure **3-36**: Deceptive-Four solved by proposed model and Hill Climbing.

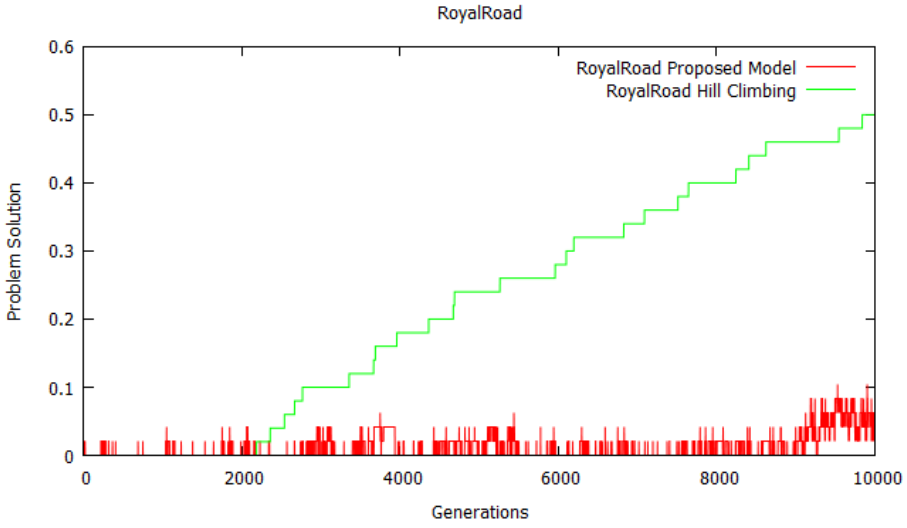


Figure **3-37**: RoyalRoad solved by proposed model and Hill Climbing.

This optimization problem was very difficult for both algorithms; however, Hill Climbing obtains widely better results that the proposed model (Hill Climbing close to fifty (50) percent, the proposed model is close to zero (0) percent)(see Figure **??**). However, this is similar to behaviours in bacterial colonies when an effective mechanisms of action of antibiotics is eliminating the bacterial colony.

Summary

In this chapter was presented the design of the model for representing bacterial conjugation. The proposed model was designed in two phases. In the first phase, a simplified model was designed in order to design the virtual environment based on a cellular automaton where two groups of bacteria interact (donor bacteria and recipient bacteria). A donor bacterium contains a plasmid that provides characteristics of antibiotic resistance, the plasmid may be transferred to a recipient bacterium by direct contact when the environment conditions are appropriate.

In the simplified model, the environment is composed of two layers: The Bacterial Layer displays the two groups of bacteria; in this layer, a donor bacterium can carry out three actions (turn right, turn left and conjugate); the purpose of these actions is to find a recipient bacterium to transfer the plasmid. A second layer (Concentration Layer)is designed in order to evaluate the conditions of the environment according to concentration values, these vales are determined by the elements interacting in the bacterial layer in each cell of the automaton.

Besides, a first approach to a model to of diffusion of antibiotics was proposed. The antibiotics are spread by a sand-pile model through the environment; on one hand, when the antibiotic is in contact with a recipient bacterium, the bacterium dies. On the other hand, donor bacteria are resistant to antibiotics. These simple rules formed patterns similar to the ones that can be observed in real bacterial colonies.

In the second phase, some changes to the model were introduced to abstract some characteristics that were not considered in the first phase. First, the concentration layer was divided into two layers (nutrient layer and antibiotic layer); the purpose of the nutrient's layer is to generate enough nutrients to allow the proliferation of the bacterial colony but not overpopulation. The purpose of the antibiotic layer is to display the mechanism of action of antibiotics and determine the impact of the antibiotics in the bacterial colony according to some rules (see Algorithm **??**). Other characteristic added to the model was the codification of plasmids and antibiotics in order to connect them for evaluating the impact of an antibiotic depending on the plasmids that a bacterium contains; besides, a bacterium may have more than one plasmid and can transfer a plasmid to bacteria in its neighbourhood.

Reproduction, mutation and death by several factors (old-age, starvation, action of antibiotics) are introduced; therefore, the bacterial colony has diversity and evolves through time. The most interesting behaviour is the emergency of antibiotic resistance; this occurs when the bacterial colony is in constant stress by a mechanism of action of antibiotics and the antibiotic is not so efficient to eliminate the bacterial colony; in consequence, a strain of bacteria emerges with a plasmid that provides characteristics to resist the mechanism of

action of antibiotics; then, the plasmid is transferred to other bacteria.

Finally, although the proposed model is not designed for solving optimization problems; the model was used for solving four classical optimization problems (Maxones, DeceptiveThree, DeceptiveFour and RoyalRoad). The obtained results were compared against the algorithm "Hill Climbing" that obtained better results. However, the obtained results are acceptable by considering that bacteria are not directly optimizing and the optimization problem is only being solved by bacteria in direct contact; therefore, it is required that several generations have passed in order to increase the number of bacteria in contact with the antibiotic; besides, some bacteria with good solutions (plasmids) may die due to old-age or starvation.

4 Conclusions and future work

4.1 Conclusions

The process of modelling a biological process is really difficult for a systems engineer because of two factors: The lack of knowledge about the subject and the complex process of abstraction of the biological process in order to fit in a computational model. First, it was necessary to study many biological processes where most of the definitions were unknown, therefore, it was a slow and not a simple process. After that, it was a challenge to break the barrier between the specific detail that biology requires and the adequate abstraction of characteristics and mechanisms to build a consistent model, in order to represent some bacterial processes with computational tools. In spite of these difficulties, the author thinks that the proposed model has obtained very interesting results for representing the bacterial conjugation process; this process is affected for so many factors and conditions that it was impossible to abstract every detail and to set it in the model. However, step by step the model was designed and incrementally new characteristics were included. The obtained results show that the proposed model can exhibit several behaviours similar to real bacterial colonies such as formation of bacterial patterns, growth of the bacterial colony and antibiotic resistance.

The adequate distribution of nutrients through the bacterial colony was not a trivial task. Therefore, it was necessary to find an appropriate mechanism to represent the nutrients in the bacterial colony in order to maintain the population in appropriate values to generate diversity and evolution. Furthermore, the process of reproduction, death and regeneration of nutrients, must be in equilibrium in order to maintain the population within an interval (if a process is faster than the other ones, overpopulation or extinction of the bacterial colony are generated). After several simulation experiments, the obtained mechanism maintains the population within an interval (stationary phase) in a neutral environment (no mechanism of action of antibiotics).

Another challenge was to define an adequate mechanism of action of antibiotics that could connect with all the characteristics and mechanisms that the proposed model already contained. At this point, many questions appeared such as “Is the antibiotic lethal for a bacterium?, if not, how to determine the resistance of a bacterium? or how to determine if a plasmid provides resistance against a type of antibiotic?. Therefore, it was necessary to

study types of antibiotics, mechanisms of action of antibiotics and how plasmids provide characteristics to a bacterium for resisting the action of antibiotics. As a result, it was possible to abstract the general properties that were involved in this process to design an efficient mechanism of action of antibiotics. After several simulations involving all the characteristics and mechanisms in the bacterial colony (reproduction, mutation, conjugation, mechanisms of action of antibiotics); the antibiotic resistance emerged when the mechanisms of action of antibiotics was constant during several generations.

Even though the proposed model is not designed for solving optimization problems. The author thinks that the antibiotic resistance could be considered as a solution for a given problem. So, the model was used to solve four classical optimization problems (MaxOnes, Deceptive-Three, Deceptive-Four and RoyalRoad) and then the results were compared against the algorithm "Hill Climbing". Clearly, the results of "Hill Climbing" are better for solving optimization problems; mainly the proposed model is not good for resolving the problem RoyalRoad due to the efficiency of this function as a mechanism of action of antibiotics. therefore, the process of conjugation takes place in a low rate and the antibiotics resistance does not emerge (this behaviour is similar to real bacterial colonies when a mechanism of antibiotics is appropriately applied). Besides, the author claims that the purpose of a bacterium just is to survive and the solution of the problem emerges; hence, a bacterium is not solving an optimization problem in a direct way; for this reason, the author considers that the obtained results for solving optimization problems (MaxOnes, DeceptiveThree and DeceptiveFour) are acceptable and better results could be achieved with small changes in the model.

4.2 Future work

Although the antibiotic resistance emerges in the proposed model, there are some aspects that can be addressed in future works:

A further study could take into consideration other biological aspects for improving the simulations. For example, aspects such as temperature, different environments for simulations (dishes of petri, tissues) and processes such as transduction and transformation.

It would be interesting to observe the behaviour of the bacterial colony under several mechanisms of actions of antibiotics through time. First, an initial dose of antibiotic can be applied over the bacterial colony; then, a new type antibiotic can be applied after some generations that the previous antibiotic has ran out. These experiments could be conducted several times with different interval of generations among antibiotics. The purpose would be to evaluate the performance of the antibiotic resistance.

Although the proposed model is not designed for solving optimization problems, it was used indirectly to solve four classical optimization problems. This is possible because a bacterium transfers plasmids (solutions) when it is in contact with a type of antibiotic (optimization problem). It would be interesting to solve several optimization problems at the same time, by using several mechanisms of action of antibiotics in different regions of the cellular automaton.

For optimization purposes, the bacterial colony could start with a minimal quantity of antibiotics (optimization problem) in the bacterial colony. The purpose would be to force bacteria to optimize since generation 0; resulting in better results for optimization problems.

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