Simulation and modeling of microorganisms in Biofilm

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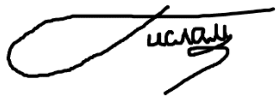
Appendix V –Declaration Form

DECLARATION

I hereby, declare that this manuscript, entitled “Simulation and modeling of microorganisms in Biofilm”, is the result of my own work except for quotations and citations which have been duly acknowledged.
I also declare that, to the best of my knowledge and belief, it has not been previously or concurrently submitted, in whole or in part, for any other degree or diploma at Nazarbayev University or any other national or international institution.



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Date: 18.03.2021

Abstract

Biofilms are microstructured microbial communities that form at interfacies. In biofilms, microbial cells are encased in a matrix comprising polysaccharides, proteins, and extracelluar DNA. In the environment, biofilms contribute to biogeochemical cycles and pollutant degradation. However, biofilms are also responsible for more than 60% of infections and 80% of chronic infections [1, 2]. The phenotype of microorganisms in the biofilm is different compared to those in the planktonic state. Bacteria in biofilms have increased resistance to antibiotics, faster mutation accumulation rate, and higher virulence [3-10]. Biofilm are heterogenous and dynamic systems, in which biomass self-organize in response to nutrient availability, flow conditions, etc. While numerous techniques exist for biofilm growth and analysis, biofilm modeling allows simulating a broad range of growth conditions and provide preliminary information that reduce the number of experiments. Among others, agent-based models (ABM) have been used to simulate biofilm formation and growth. In these models, cells are described as individual automata that follow a simple set of rules (rules of life) to interact between each other and with the surrounding environment. While ABMs can be coded in any language, they are easy to implement in NetLogo, a simplified multi-agent programmable environment for modelling designed specifically for ABMs. In this thesis, the bacterial attachment, growth and biofilm propagation were modeled using the NetLogo. The model was run using parameters extracted from previous literature. This work provides a simple framework to test biofilm formation, propagation and dispersal in capillaries and other simple biomedical devices.

Acknowledgements

First and foremost, I would like to express my gratitude to the Biomedical Engineering Department of Nazarbayev University, for opening their doors to their first students in 2019, believing the students and providing them an opportunity to explore the field that integrates life sciences into engineering disciplines. I would also like to thank my supervisors, Dr. Enrico Marsili and Dr. Carlo Molardi, for their mentorship, continuous support, valuable advice and time. My appreciation also goes to my family and friends for their persistent support throughout the entire course of my studies.

Table of Contents

List of Abbreviations and Symbols

PA	Pseudomonas Aeruginosa
ABM	Agent-based model

List of Figures

List of Tables

Chapter 1- Introduction

1.1 General

A biofilm is a microstructured community of microorganisms enclosed in a microbially-produced extracellular polymeric matrix (EPS) [11]. While some aspects of biofilm resemble a multicellular organism, it is rather a community of distinct independent microorganisms [2]. In the environment, biofilms are often composed of multiple species of microorganisms, including bacteria, protozoa, fungi, algae and archaea [2]. Biofilms are genetically and structurally heterogeneous with sophisticated interspecies and intraspecies syntrophic interactions, including mutualism, commensalism and parasitism [2].

Biofilms are prevailing systems that exist on practically any solid substrate exposed to or submerged in water [12]. They can be found on the surfaces of the living tissues, drinking water pipes, indwelling medical devices and natural aquatic systems [13]. It was estimated that approximately 65% of the entire bacterial infections are related to bacterial biofilms, including device and non-device related infections. Biofilms are responsible for the formation of 2% of bacterial infections on breast implants, 4% on mechanical heart valves and 40% on ventricular-assisted devices [2].

The biofilm was firstly observed on the tooth surfaces with a primitive microscope by Anton van Levenhuk in the XVII century [14]. Since then, various research projects were conducted to observe bacteria on different surfaces. For instance, in 1973, Characklis discovered high viscosity and resistance of microbial slimes to chlorine disinfectants in industrial water systems. Since then, studies were focused on identifying the impacts of biofilm on the environment and the public health by discovering the underlying mechanism of biofilm formation. While early biofilm studies were conducted with scanning electron microscopy (SEM) or standard microbiological culture-based methods, a significant improvement in biofilm analysis was achieved by the application of confocal laser scanning microscope (CLSM), recombinant DNA technology, and optical coherence tomography [13, 15]. It is likely that future biofilm research will involve complex computational modeling to simulate biofilm development and growth in fluids [16].

1.2 Aims and Objectives

The goal of this thesis project is to develop a simplified computational model for a single species bacterial biofilm attachment and dispersal in a capillary. While no specific microorganism is considered, the model is designed for biofilm-forming bacteria commonly occurring in catheters and other indwelling devices, like *Pseudomonas aeruginosa*. A 3D discrete agent-based modelling (ABM) approach was chosen, due to its simplicity. This thesis provides the reader with the general concepts of biofilms, their growth mechanism, a brief explanation of the coding tools adopted, and the limitations of the study.

1.3 Thesis statement

Computational simulations based on agent-based modelling (ABM) can help predicting the time for cells attachment and biofilm formation on biomedical devices. ABMs can reduce the need for preliminary laboratory experiments, thus saving time and resources.

1.4 Methodologies and techniques

The research project consists of two main parts: an extensive literature review and a simulator based on ABMs. The ABM model was created in NetLogo software. The biological parameters for the model were extracted from relevant literature on *P. aeruginosa* and other biofilm-forming microorganisms. The initial plan includes the experimental validation of the model results in laboratory biofilm experiments. However, this work was not carried out due to the access limitations imposed by the COVID-19 pandemic.

1.5 Scope and constraints

P. aeruginosa bacteria species was selected as model microorganism for the simulation These microorganisms have been studied in details, and the mechanism of biofilm formation is mostly known. Further, *P. aeruginosa* can be grown rapidly under laboratory conditions, thus experimental validation of the model could be carried out in the future [17].

The model adopted was simplified, and only a few parameters involved in biofilm formation were included. A more comprehensive model will require computational power beyond that of a mid-range laptop. In the future, it is expected to run a complete version of the simulation on a faster machine, which will be available at high performance computing (HPC)-NU.

Chapter 2- Literature review

2.1.1 Biofilms

Biofilms are prevailing systems that exist on practically any solid substrate exposed to or submerged in water [12]. They can be found on the surfaces of the living tissues, water system piping, indwelling medical devices and natural aquatic systems [13]. It was identified that approximately 65% of the entire bacterial infections are related to bacterial biofilms, including device and non-device infections. Data demonstrated in these studies show that biofilm was responsible for the formation of 2% of bacterial infections on breast implants, 4% on mechanical heart valves and 40% on ventricular-assisted devices [2]. The formation of diverse biofilms on the surface of a medical device and an industrial water system are demonstrated in figure 1 shown in the Appendix section of the paper. It can be seen that the biofilm in the water system is highly sophisticated, containing bacteria, clay material, corrosion products and fresh water diatoms, whereas the biofilm on the medical device composed of single coccoid-shaped bacteria and the corresponding EPS matrix [13]. Biofilm is formed in three stages: adherence, maturation and dispersal. The representation of this biofilm growth cycle is depicted in figure 2 shown in the Appendix section of the paper [18]. In comparison with the planktonic bacteria, those growing in the biofilms have unique transcriptional responses. The latter ones can modify their physiology from aerobic to anaerobic metabolism and downregulate cell wall, protein and synthesis of the DNA.

2.1.2 Composition of Biofilms

In the biofilm, microorganisms account for 5-35% of the volume whereas the remaining volume comprised of the extracellular matrix [2]. There are different component types present in the EPS: protein (>2%), polysaccharides (1-2%), RNA molecules (<1%), DNA (<1%), ions and water (97%) [2]. The favorable growth environment and sufficient nutrient content created by bacterial aggregation, serve as a reservoir for bacteria [18]. Interestingly, the bacteria within this reservoir are hidden from the host immune system and express a unique phenotype compared to the planktonic states of the same species [18].

2.1.3 The Life cycle of bacteria in biofilms

Depending on the conditions of the environment, some of the microorganisms in the biofilm can have at least four metabolic states: anaerobic growth, aerobic growth, dormant cells and dead cells. It is assumed that these exceptional physiological states are responsible for the

formation of persisters or dormant cells and resistance to antibiotics, thus imposing an evolving and unique challenges to clinicians and patients [18, 19]. It was reported that they have a substantial impact on the prognosis and progression of a variety of diseases such as urinary tract infections, infective endocarditis, keratitis and chronic otitis media. Moreover, they are commonly associated with long-term chronic disease states of patients [19]. In some cases, the complete eradication of bacterial biofilms may be impossible [18].

2.1 Biofilm modeling

As it was mentioned previously, biofilms are responsible for the formation of medical and industrial problems ranging from antibiotic resistance to tooth decay and ship fouling. However, they can also benefit the environment by cleaning hazardous waste and thus contributing to the environmental purification. These ambiguous roles of the biofilms sparkle the interest in understanding their processes of growth, metabolism, acquisition of antibiotic resistance, death, communication and persistence [2]. Due to the difficulty in controlling and examining the real biofilms, the particular attention of these processes was brought by computer modelers determined to clarify the correlation between various physical-biological mechanisms and biofilm properties. Biofilm models present excellent means to explore the principles of biofilm composition, formation, function and structure [20]. They are created to provide a useful instrument predicting and controlling biofilm behavior, thus enabling the effective utilization and management of biofilms in medical and industrial fields [20, 21]. The biofilm models are becoming increasingly complex due to the elevating number of research studies in this field. In spite of this, there is still a discrepancy between experimental and computational results [21]. In general, biofilm models are classified into four groups: cell-centered models with discrete cells (cellular automata), individual-based models (IbM), continuum models (continuum automata) (CA), hybrid models and Agent-Based models [2, 22]. Continuum models express the biofilm as a continuum material, usually made of the nutrients and a polymer, gel or viscous fluid. These models do not consider the dynamics of an individual bacteria as they evaluate biomass as a homogenous substance [20]. For the representation of the biofilms, these CA models use nonlinear bacterial reactions and mass transport equations. Interfacial and boundary conditions are critical in providing the outcomes of the solutions that are mostly in the numerical form. It was identified that more effective approximation to the real model can be obtained with the thin film approximations. These models have one major shortcoming: biofilm morphology and material properties change responding to the environmental conditions are neglected in them. For instance, the biofilm can become less viscous or harden, disintegrate bacteria or other substances in response to the changes in the

nutrient concentration, flow, presence of chemical hazards in the environment, etc. Microorganisms in the IbM models are considered as solid particles evolving according to reaction-diffusion equations. Due to the fact that various processes such as nutrient diffusion and reproduction occur in different dimensional scales, the models are extremely dependent on the grid size. Using these models is always accompanied by a dilemma: coarse grids result in considerable deviations, whereas the fine grids demand high computational effort. In addition, as a drawback, IbM models have a large number of parameters with unknown values. CA models rely on probability principles and set of simple rules, including reproduction, feeding, death. In these IbM models, the biomass is distributed over a cell grid and performs the modifications according to the probability rules. Reliance on the biological principles, simplicity in computer simulations and affordable cost make these models favorable compared to other models because of the reliance on biological principles. CA models available in the market already include several simple bacterial mechanisms in their default setups. However, these CA models are not well-studied for simulations of more sophisticated mechanisms, including microorganism attachment to surfaces, EPS matrix production, quorum sensing to create biofilms and interaction with the environment. The limitations of the two models are circumvented in some of the hybrid models that combine discrete and continuous cell descriptions. For instance, the EPS matrix can be modeled as an incompressible viscous flow that contains the discrete microorganism cells. These three models have their own limitations and advantages and none of them is ideal for every case study. In most cases, the user will have to determine which is more suitable for the particular case [2]. Agent-Based models are computational and mathematical models in which agents are autonomous and unique entities locally interacting with other agents and their environment [22, 23]. ABMs are flexible and intuitive in representing macro-systems with complex behaviors from relatively simple rules and micro-behavior of individual agents [24]. It should be noted that ABMs and IbMs have similarities in their purposes. They both demonstrate the behavior of individual entities in disciplines requiring the assessment of intricate systems [25]. In some cases, these two terms are used interchangeably [24]. Nevertheless, ABM is a more generic term for IBM. The autonomous individuals in ABM may not necessarily be individual agents, they can be an entity operating individually among other individually operating entities within the complex system [25]. Such intricate systems can also be modeled using approaches such as Knowledge-Based models, (KBMs), Systems Dynamics (SDs), Couple Component Models (CCMs) and Bayesian Networks (BNs) [26].

No matter what type of computational model is chosen for the study, it should include three main elements of biofilm growth: transport mechanism, cell growth and biofilm loss [20]. The transport mechanism is usually achieved by diffusion and it serves to deliver growth substrates, nutrients or other vital elements to the biofilm cells. The consumption of these substrates should result in cell proliferation and growth in the model. Afterward, the cells must be damaged or died to eradicate the biofilm. It should be noted that several other factors can also contribute to biofilm growth such as cell-ECM or cell-cell adhesion and biofilm reactions to the shear stress caused by fluid flow [2].

2.2 Programs used to examine biofilms

There are different programs that can be used to analyze the bacterial development in biofilms. In this paragraph, there will be listed several types of biofilm simulation software. CompuCell3D is a 3D C++ simulation software for solving biocomplexity problems and integrating a variety of mathematical models [27]. It is able to simulate reactions of the cells to exterior chemical stimuli including secretion, resorption, haptotaxis and chemotaxis. In general, C++ general-purpose language is suitable for creating different algorithms that predict the behavior of biofilms. Most of the researches use this programing language in their biofilm studies [28]. NetLogo is a software allowing to conduct multi-agent programmable modeling including system dynamics and participatory simulations [29]. More detailed information about NetLogo software will be provided in the next sections of the paper. MatLab programming tools can also be used for designing biofilm models. Despite the fact that numerical computing is the main purpose of this software, an additional Simulink package adds graphical simulation with multiple domains and dynamic model designs [30]. MASON is a fast multi-agent simulation environment created in Java. MASON is able to provide lightweight simulations and 2D/3D model visualizations [31]. Vcell is an open-source comprehensive platform for the simulation and modeling of cell systems [32]. BSim is an agent-based computational instrument for evaluating the behavior of microorganisms as a community. Unlike other simulation tools, BSim is able to model the bacterial dynamics in more realistic 3D, sophisticated environments [33]. Biofilm modeling can also be achieved by using the Visual Studio code editor. The main advantage of this editor is that it has built-in support for Node.js, JavaScript and TypeScript along with the broad spectrum of extensions for other languages such as C++,Java, Python [34].

2.3 Biofilm cultivation

There is a number of methodologies available for analyzing bacterial biofilm formation. The typical biofilm culturing method involves the separation of adherent bacteria from those growing planktonically. The culturing technique can be modified to mimic certain system features in which the research is specialized [35]. Static Microtiter Plate Assays is one of the first methods used for quantifying the formation of the biofilm. This method is designed to evaluate the extent of microbial attachment to the abiotic surface. Short incubation times of up to 2 hours provide the information of initial attachment to a surface, whereas longer incubation times of up to 20 hours data regarding biofilm formation. Static Microtiter Plate Assay is relatively high-throughput and suitable for the evaluation of bacterial attachment to the surface and identifying the response of the biofilm growth to the change in the environmental conditions. The flow cell system is comprised of a chamber in which the biofilm bacteria are cultivated and a coverslip. In this system sterile, fresh medium is constantly flown through the chamber stimulating surface-attached bacteria growth and eliminating planktonic cells. Individual cells can be monitored in real-time by adding the fluorescent dyes and proteins to the system. This system can be further modified to achieve a better resemblance to the biologically relevant surfaces. Flow cells can be coated with the biological molecules to assess the bacterial attachment on the surface of the tissues. However, it should be noted that in vitro and in vivo conditions drastically differ from each other and the current state system is only a distant representation of host dynamic environment. In addition, despite the ideal suitability of the flow cells to visualize the biofilms in real-time, it is hard to collect the biomass from the system and they do not provide high-throughput assays. Similar to the flow cells, tube biofilms can be used to study the biofilm development under flow. However, in tube biofilms, the cells are grown on the internal surface of the silicone tubing. Compared to the flow cell system, the biomass can be gathered easily by scrapping from the tubing. This method can be used to quantitatively analyze the impact of antibiotics on biofilms by measuring colony forming units before and after the treatment. Shortcomings of this method are the amenability to microscopy and difficulty in achieving high-throughput evaluation. A colony biofilm method investigates the development of biofilm at the air-surface interface, without exposing the biofilm to liquid. In this technique, the biofilm is grown on the semi-permeable filter made of polycarbonate and they can populate extensively in a short time period. This method is especially useful for investigating the susceptibility of microorganisms to antibiotics. However, it is difficult to handle the flimsy membrane after the total bacterial coverage of the membrane. As compared to all the described methods, peg lids provide the opportunity to cultivate several biofilm

samples at one time. In addition, it also possible to detach the pegs for thorough microscope analyzes. The rotating disk reactors (RDR) and concentric cylinder reactors (CCR) have been developed to expose the biofilms to shear stress during their development. These methods express high reproducibility and can simulate real antibiotic therapy by delivering the chemicals through continuous flow in the medium [35]. The main drawback of these systems is their inability to concurrently test several biofilms.

2.4 Parameters of Pseudomonas aeruginosa

In order to create a successful agent-based program, it is crucial to identify the details of Pseudomonas aeruginosa bacterial growth, propagation and biofilm formation. For this reason, during this academic semester, a thorough literature review has been conducted.

2.4.1 Mobility of bacteria

The Shigematsu et al. conducted the research to identify the swimming velocities of Pseudomonas aeruginosa and Vibrio cholera bacteria using a computer-assisted video tracking method [17]. This method was declared as an accurate and simple analysis for tracking bacterial movement. The experimental results revealed the tendency of bacteria to frequently alter swimming velocity and direction. The patterns of their motion were described as “small spirals or circles”. In the experiments of Shigematsu et al, bacteria did not swim in a continuous and straight manner. The average swimming velocity of five strains of Pseudomonas aeruginosa was 51.3 +/- 8.4 μm/s. For future experiments, researchers suggested studying the behavior of bacteria in viscous media. Particular interest to this issue was attributed due to the features of the slime layer in the respiratory tract or intestinal in which the bacteria may act in a different manner [17]. A more recent Pseudomonas Aeruginosa movement investigation was conducted by the research team led by Hook et al [36]. They used the quantitative method of label-free tracking and imaging of individual bacteria concurrently within the solid-liquid interfaces and bulk liquid. This method involved the utilization of two-dimensional total internal reflectance microscopy (TIRM), differential interference contrast (DIC) and three-dimensional digital holographic microscopy (DHM). Hook et al. stated that the average speed of P.aeroginosa cells in planktonic conditions is equal to 59±4 μm/s. Despite the considerable contribution of Shigematsu et al., the latter research team used the novel method of microorganism analysis. Therefore, it was decided to extract the data provided by Hook et al [36].

2.4.2 Bacterial growth rate

Bacterial growth is a sophisticated and intricate process. This complexity stems from wide range of factors, such as the unique microenvironment, composition and intracellular interaction of microorganisms [37]. Therefore, the current aim of biomedical engineers is to understand the bacterial growth in natural environments before creating a code in the programming software. In order for the program to maximally imitate the natural behavior of bacteria, it is required to import precise bacterial growth data. The protocol provided by LaBauve and Wargo declares the usage of Lysogeny broth (LB) [38]. This type of broth was utilized in the experiments of Wijesinghe et al. 2018 and Yang et al [39, 40]. The latter research team applied FISH technique for targeting bacterial rRna for counting the number of microorganisms. According to these research findings, PA01 strain Pseudomonas Aeruginosa has a specific growth rate of 1.3/h and Intensity/Cell Volume of 440 [41].

2.4.3 Other parameters

The following table represents the data describing the other specific parameters of Pseudomonas aeruginosa.

Table 1 Parameters of Pseudomonas aeruginosa (PA)

Parameters	Value	Reference
Dimensions	0.5 to 0.8 μm by 1.5 to 3.0 μm	[42]
Width of peptidoglycan layer	2.41 (±0.54) nm	[43]
Concentration of overnight culture	2.2×10^9±0.2×10^9 CFU/mL	[43]
Half maximal induction of quorum sensing by C4-HSL (C4-homo-serine lactone)	2.5 μM	[43]
Doubling time of PA in CF patient	1.9 - 2.4 hours	[43]
PA velocity	41 (±2) nm/s	[43]
Concentration of c-di-GMP in planktonic cells	< 30 pmol per mg of total cell extract,	[44]
Concentration of c-di-GMP in biofilms	75–110 pmol per mg of total cell extract	[44]
Concentration of c-AMP in planktonic cells	within the range of 0 to 10 mM	[45]
Concentration of c-AMP in biofilms	0,4 $nmol^{-1}$	[46]
Average speed of PA cells in planktonic conditions	59±4 μm/s	[46]

Chapter 3- Modeling

3.1 NetLogo multi-agent programmable environment for modelling

Thorough analysis and literature review of existing software led to the decision of choosing the NetLogo software for accomplishing this project. It is a multi-agent programming language capable of modeling sophisticated natural and social phenomena [47]. In spite of relative simplicity, it has been extensively used in the field of education and research [48]. NetLogo was created by Uri Wilensky and first released in 1999. Since then, it experiences constant improvements and updates. It should be mentioned that NetLogo has an extensive library of ABM models included in the software. The software is free for downloading and currently is on version 6.2 [49]. The dictionary of the software can be accessed at official NetLogo website [48]. In addition, the simplified web-version can be run at http://NetLogoweb.org/. The most prominent feature of this modeling environment is the suitability for modeling substantial collections of agents evolving over time. Modelers can provide concurrent instructions to thousands of independent agents with any design and investigate the micro-level interactions between agents leading to macro-level behavior patterns. By altering various conditions and parameters of the simulation, the users are able to explore the influence of external or internal conditions on the behavior of ABM. The user interface consists of 3 sections: a tab where code in NetLogo is written, a visualization tab where the user can view the dynamics of agents in the model and a tab where the user can write or read the detailed information about a certain model [49].

3.2 Simulation of biofilm formation in NetLogo

The purpose of this NetLogo project is to simulate bacterial attachment to the surface. The model covers the multi-step process of biofilm formation, including initial attachment, irreversible attachment, microcolony formation, maturation and dispersion. The simulation starts from creating the user-defined number of planktonic cells in the system and the distribution of energy to the patches and bacteria. The energy is needed for the microorganisms for exhibiting regular physiological behavior attributed to bacteria. The level of energy decreases each time bacteria moves and increases each time it consumes the nutrients. The most crucial role of the energy parameter is performed in the dispersion step, where low energy level in the neighboring sessile cells is accompanied by the production of new planktonic cells. Referring back to the first steps of biofilm formation, the initial and irreversible attachment processes are controlled by c-di-GMP and AHL-level parameters. The first parameter known

as c-di-GMP is the secondary chemical messenger, responsible for the transformation of planktonic cells to sessile cells. In nature, planktonic cells contain a lower concentration of c-di-GMP compared to sessile cells. The same principle is exploited in this simulation model. The concentration in planktonic cells increases by hitting the walls and neighboring sessile cells by a certain quantity, selected by the user. In the meantime, the second parameter is known as AHL-level assigned to the patches also increases each time they experience contact with bacteria. The AHL parameter was introduced to patches in order to mimic the quorum-sensing signaling interference between cells. The c-di-GMP level of planktonic cells and AHL concentrations elevate until they reach the user-defined threshold level. After planktonic cells gain the threshold level of c-di-GMP, they stick to the high AHL-level zones of the surface wall and become sessile cells. The sessile cells attached to the surface of the walls then move randomly and accumulate in certain regions of the simulation box, creating a microcolony. If the number of sessile cells in a radius of 2 patches increases to 4, then they disperse and produce new planktonic cells to the system that in turn repeat the cycle again. The schematic of the process is shown in Figure 3.

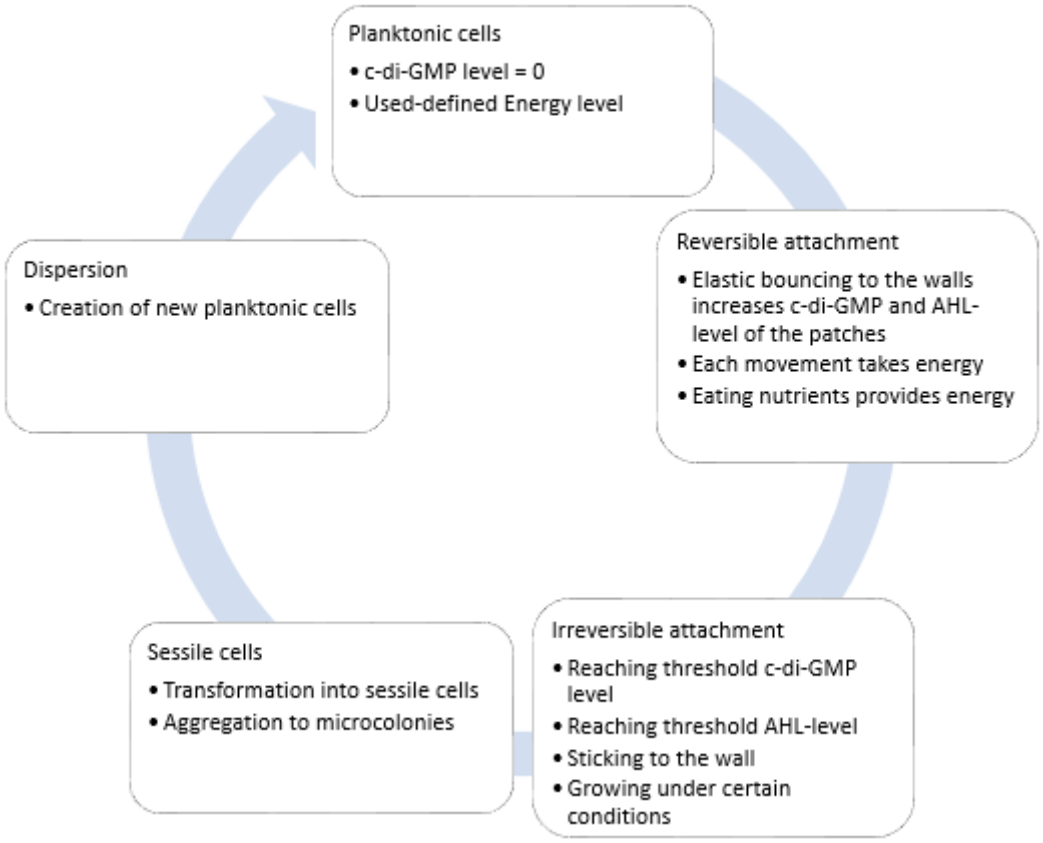

Figure 3. The growth cycle of the model

3.3 Interface and output of the model

This section is intended to introduce the reader to the interface and the visualization outputs of the model. Figure 4 is the interface with the elements described in Table 2. Figures 5-7 represent the results of the simulation, where blue indicates planktonic cells, red- sessile cells and green- newly-created planktonic cells.

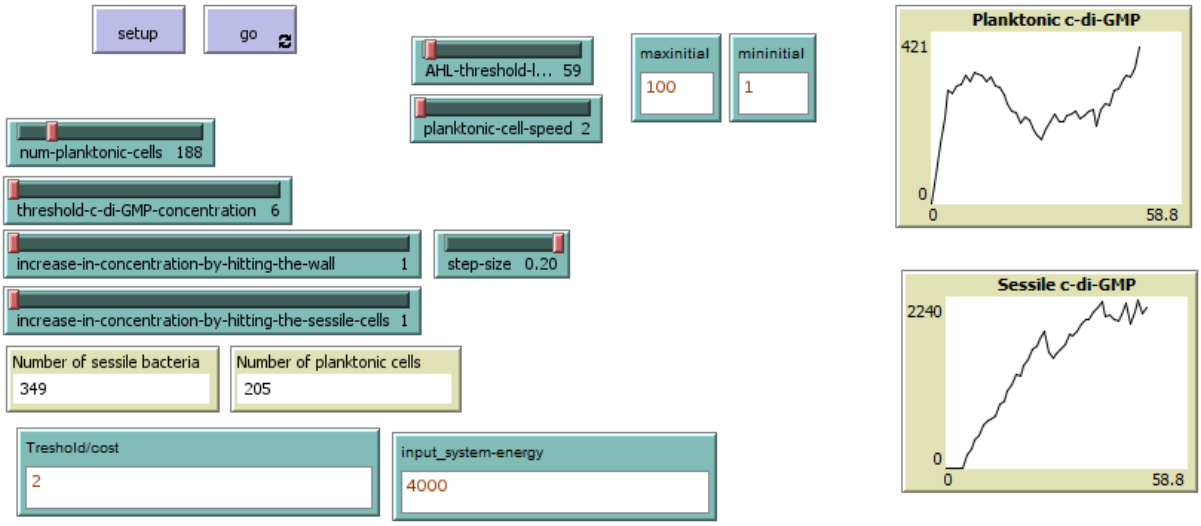

Figure 4. Planktonic cells in the world

Table 2. Elements of the interface and their function

Interference elements	Names	Function
Slider	num-planktonic-cells	Initial number of planktonic cells in the model
Slider	threshold-c-di-GMP-concentration	The threshold c-di-GMP concentration, responsible for transforming planktonic cells to sessile cells
Slider	increase-in-concentration-by-hitting-the-wall	The level of c-di-GMP increase in planktonic cells resulted from each contact with the walls
Slider	increase-in-concentration-by-hitting-the-sessile-cells	The level of c-di-GMP increase in planktonic cells resulted from each contact with the sessile cells
Slider	AHL-threshold-level	The threshold AHL concentration in patches, responsible for sticking

		planktonic cells to certain zones of the world
Slider	planktonic-cell-speed	Speed of planktonic cells
Slider	step-size	Speed of sessile cells
Input	maxinitial	Maximum initial energy provided to bacteria
Input	mininitial	Minimum initial energy provided to bacteria
Input	Treshold/cost	The threshold energy required for reproduction of sessile cells
Input	input_system-energy	Total energy provided to the patches
Output	Number of sessile bacteria	The display showing the number of sessile cells in the world
Output	Number of planktonic cells	The display showing the number of planktonic cells in the world

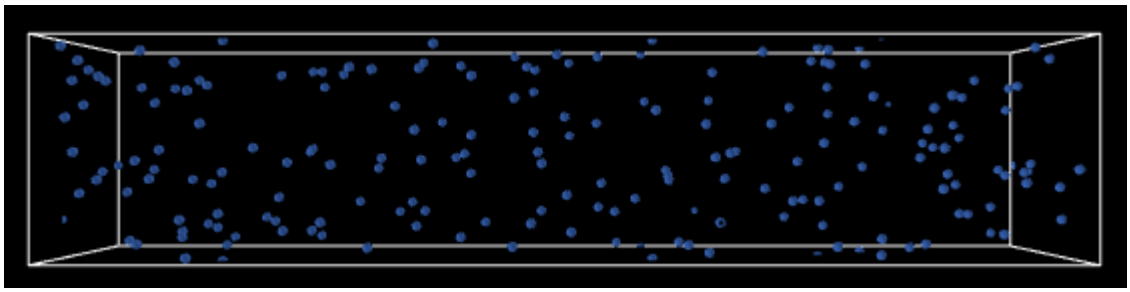

Figure 5. The agents in the model: blue- planktonic cells

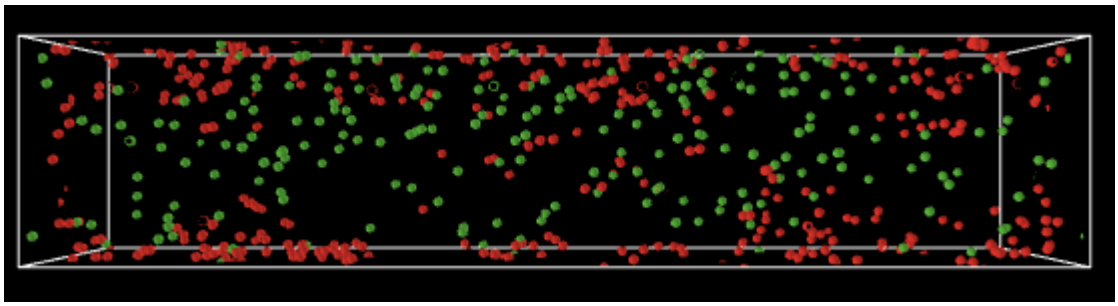

Figure 6. The agents in the model: red- sessile cells, green – newly-created planktonic cells

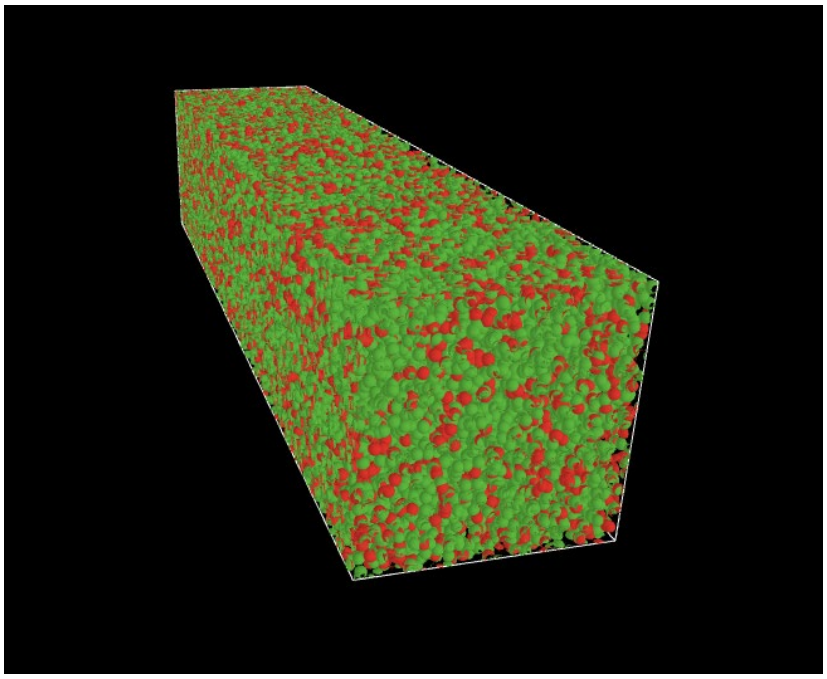

Figure 7. The representation of created biofilm in NetLogo

Chapter 4-Results and Discussion

The results of the simulation were retrieved from the NetLogo-integrated BehaviorSpace tool (Figure 8). This instrument is considered a powerful assistant to the modelers. It allows running the simulation many times, systematically altering the parameters of the model and recording the data of each run. Such features of the BehaviorSpace tool provide the opportunity to explore multiple possible behaviors of the agents and determine the specific parameters causing the behavior of interest. The simulation is accomplished in parallel, one per core in the computers with multiple processor cores [50].

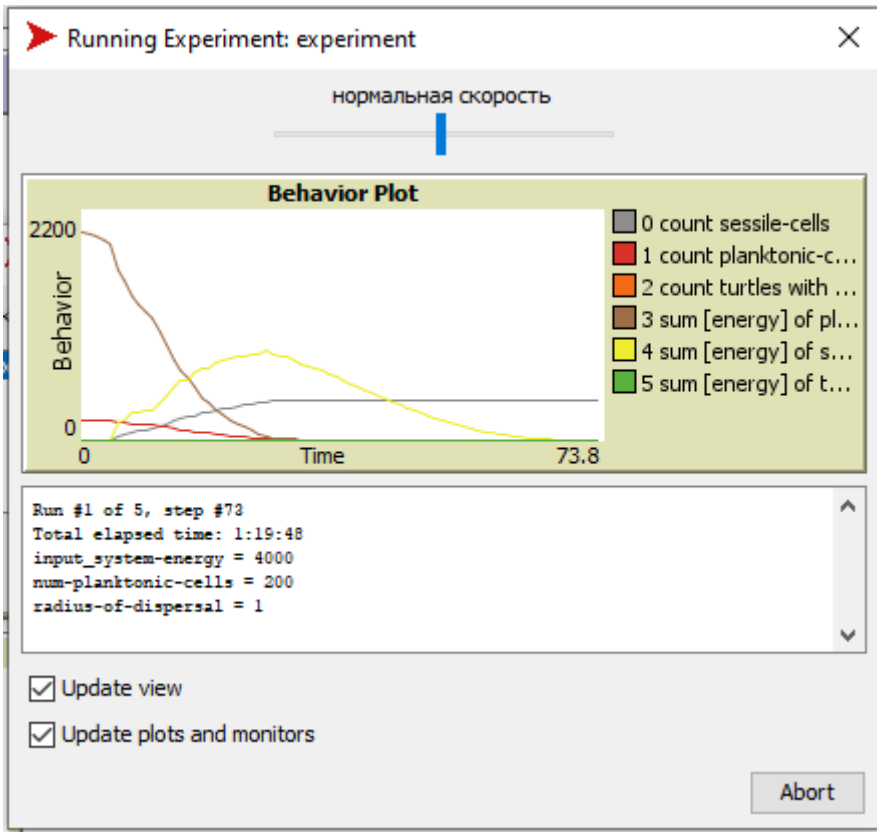

Figure 8. Representation of BehaviorSpace tool

In this project, the number and total energy of planktonic, sessile and newly-created planktonic cells were assessed by varying the initial number of planktonic cells (10, 20, 50, 100, 200) and radius of dispersal (1-10 patches) parameters. The BehaviorSpace tool was set to stop the simulation in two cases: when the time reaches 1000 ticks and when the total energies of sessile and planktonic cells equal to 0. The ticks represent the passage of simulated time in NetLogo software.

According to the obtained data, the initial number of planktonic cells plays a crucial role in triggering the biofilm formation and dispersal processes. As can be seen from figures, 10 initial planktonic bacteria cannot initiate the biofilm formation. The energy provided to the cells is entirely spent on the movement of bacteria and thus, the full cycle of biofilm formation is not achieved in the system. These observations are true regardless of the values of the second variable parameter- radius of dispersal. The radius of dispersal indicates the distance within which the cells are converted from sessile to new planktonic cells. When there are enough initial planktonic cells for provoking them for further transformations to sessile cells, the sessile cells with enough energy and quantity in a specified radius are converted to new planktonic cells.

These new bacteria after gaining threshold c-di-GMP concentration are transformed into sessile cells at the surface of the simulation box wall, bringing the full biofilm growth cycle. This feature of the model can be observed in the constructed graphs (Figures 9-14). As can be seen from these graphs, the number of planktonic and sessile cells varies depending on time steps, depicting the constant transformation of one bacteria to another. Sessile cells have wider fluctuations in numbers and total energies compared to planktonic cells, due to their relatively low energy gain values and consistent transformations to planktonic cells, preventing their energy and number increases. By comparing two sets of graphs with r=6 and r=10, it can be deduced that a higher radius of dispersal is characterized by a higher number of sessile cells and a higher number of planktonic cells. Figures below demonstrate the results of the simulation. The ABM code and other outputs of the simulation (Figures 15-20) are shown in the Appendix section of the report.

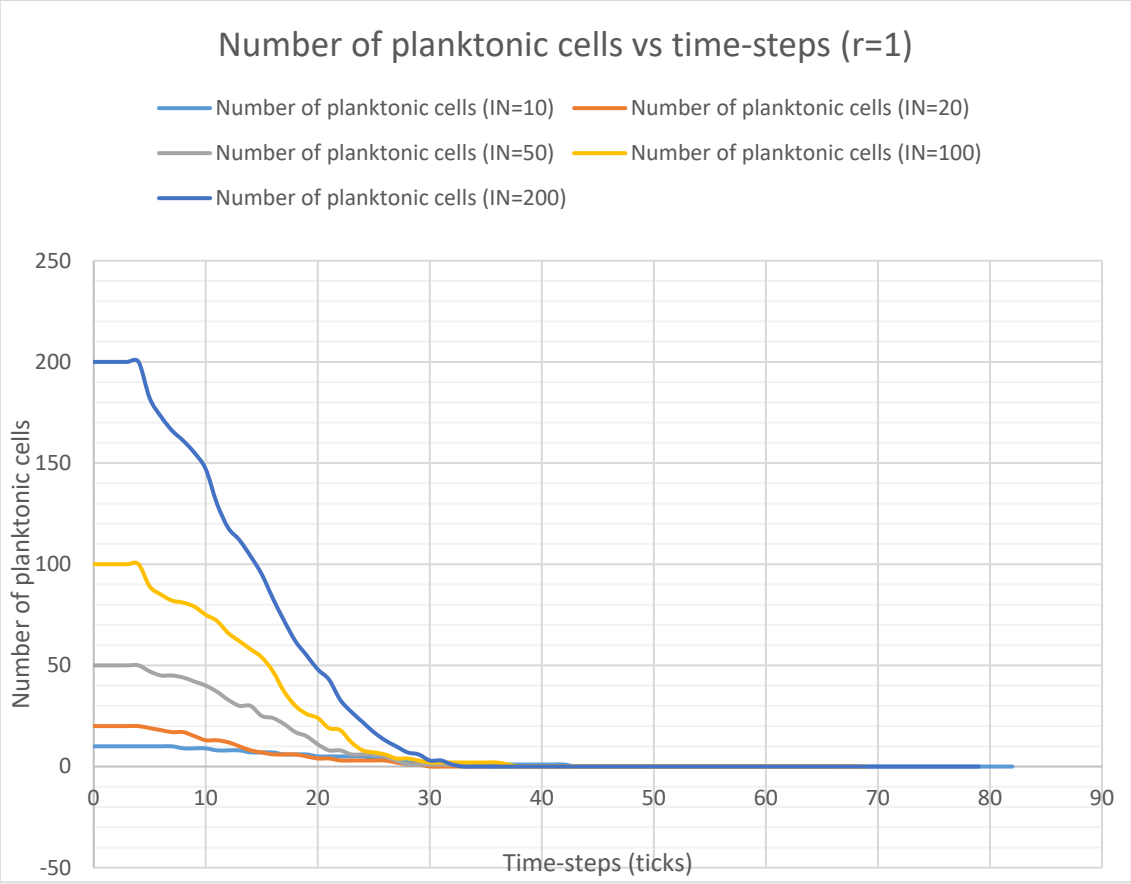

Figure 9. The results of the simulation (r=1)

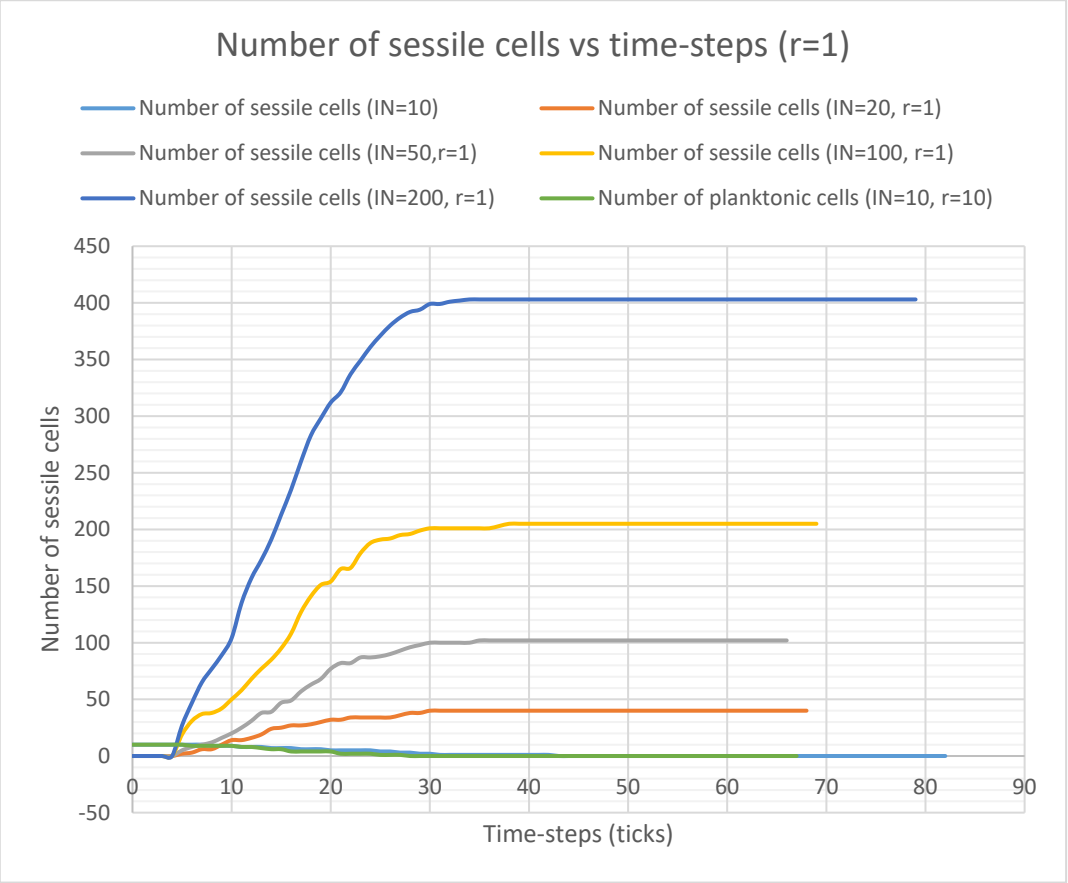

Figure 10. The results of the simulation (r=1)

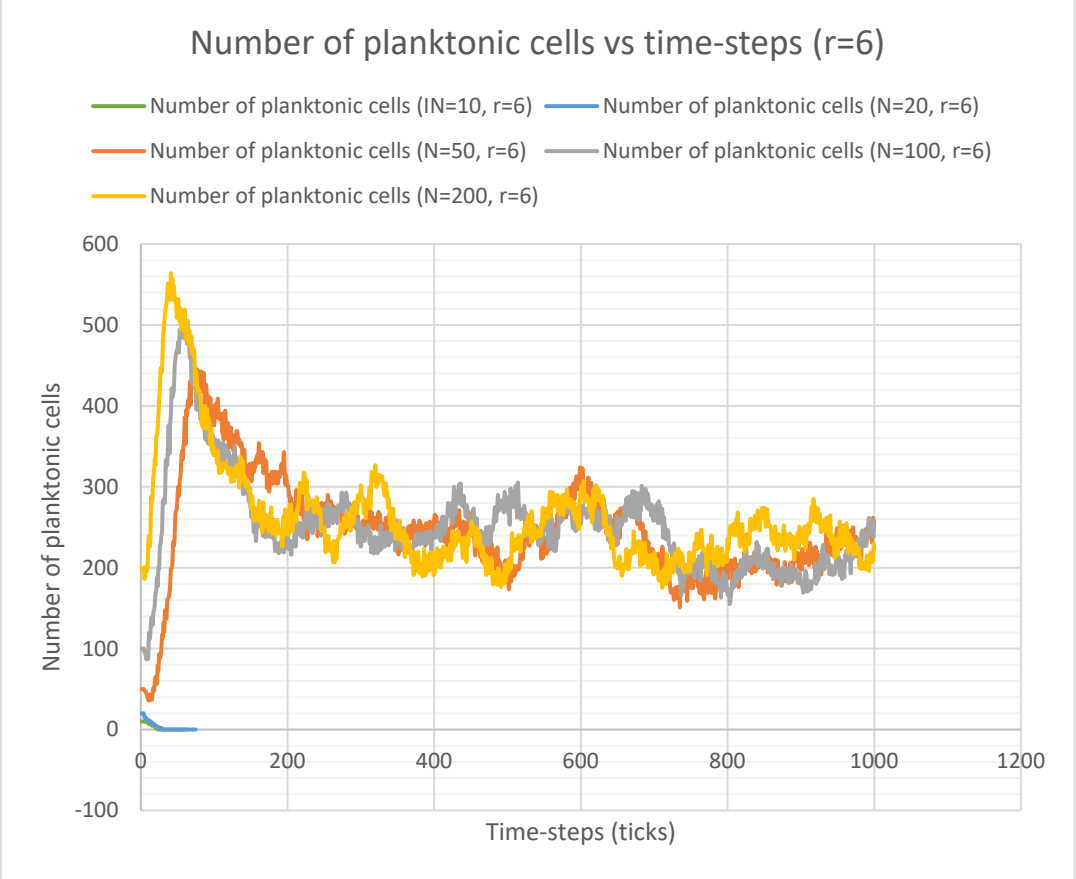

Figure 11. The results of the simulation (r=6)

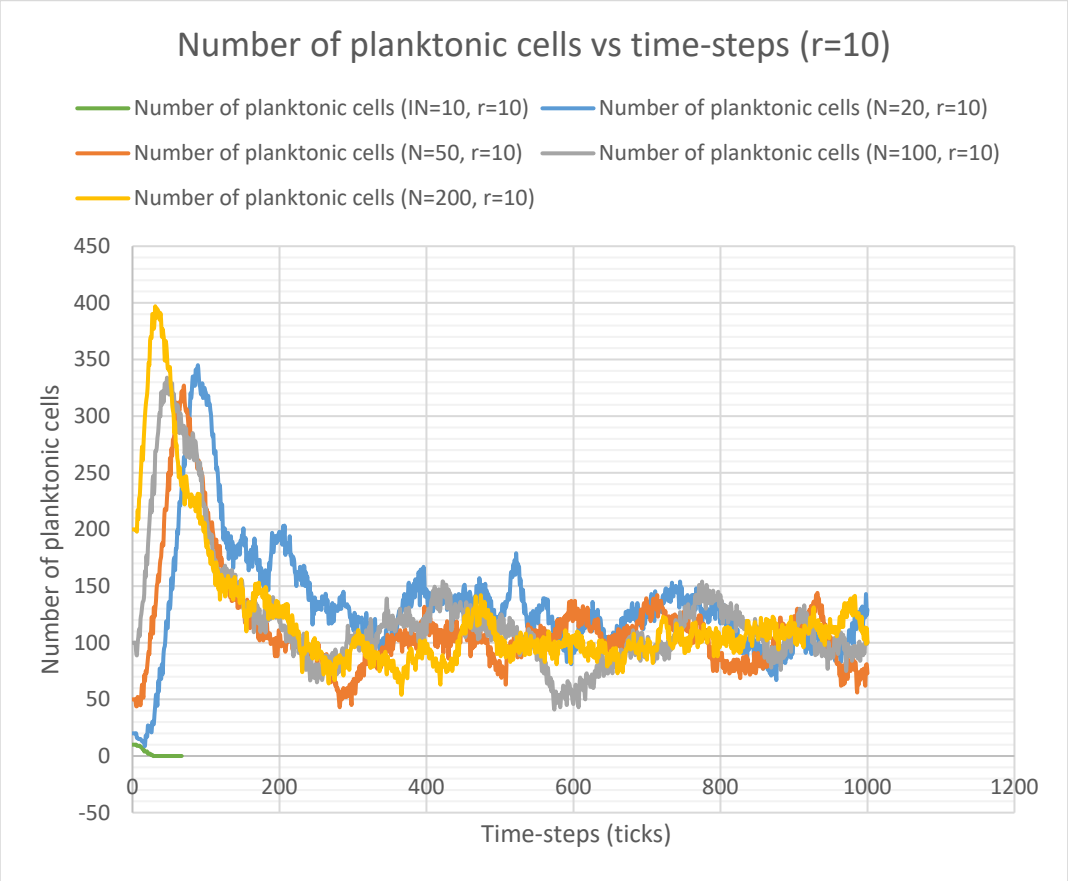

Figure 12. The results of the simulation (r=10)

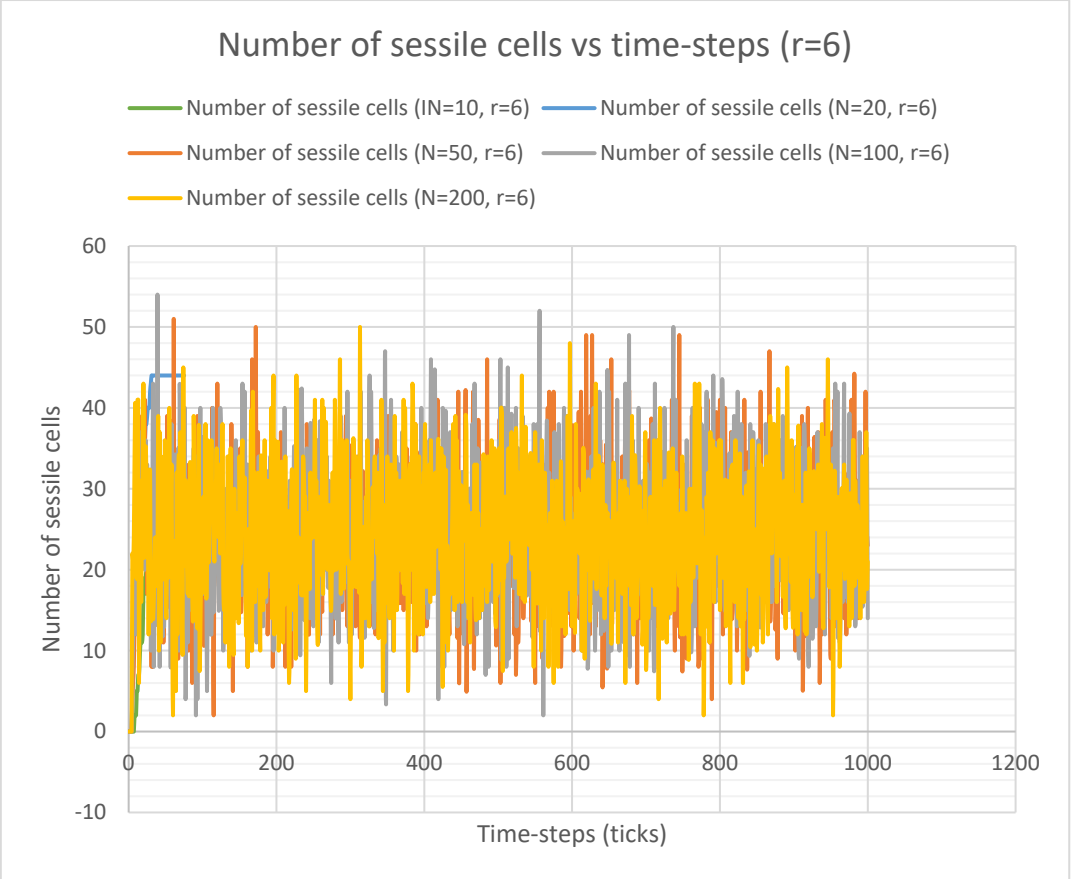

Figure 13. The results of the simulation (r=6)

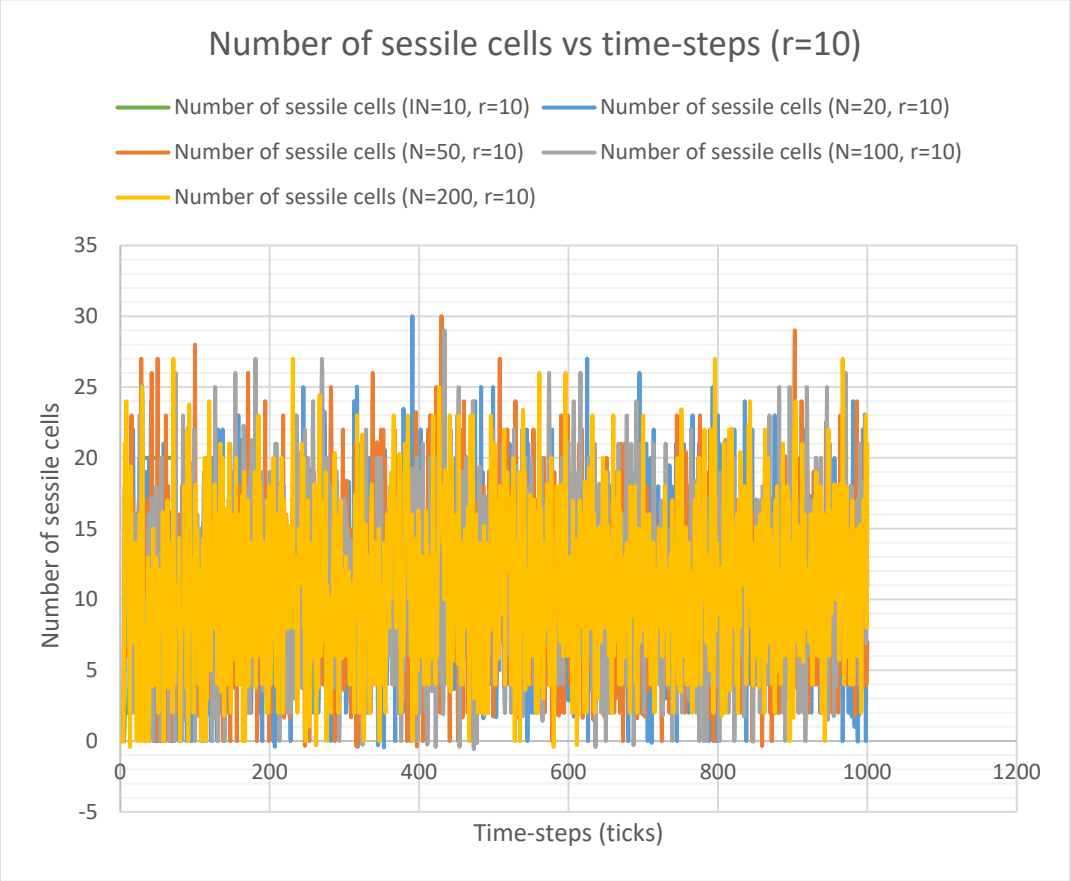

Figure 14. The results of the simulation (r=10)

This biofilm growth model is simplified and accomplished assuming zero fluid flow and bacterial viscosity characteristics. Despite the fact that, NetLogo is a simple ABM programming environment, it is capable of creating comprehensive interacting 3D agents. For future work, it is projected to add additional parameters to the model, in order to create a more complete model, capable of predicting the biofilm growth in a more precise and accurate way. The future simulation is projected to run at high-performance computing equipment (HPC-NU).

5. Chapter 5-Conclusion

The interactions of the biofilm with the surrounding environment are complex. This causes problems in creating accurate computational models of them. In my Master's thesis work, I have contributed to the understandings of the biofilm development mechanisms along with their predictions in nature by developing a biofilm growth prediction programming algorithm in the NetLogo programming environment. Despite the relative simplicity of this ABM model, it is one of the first models in the research field serving to predict the behavior of microorganisms

in the biofilm. This work has a great potential for further improvements and applications for research and analysis purposes. In order to create a more comprehensive ABM model, this 3D NetLogo model can be used further extended and then used as an example for generating a code in more complex programming languages. The results then should be validated with the laboratory experiments.

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Appendix

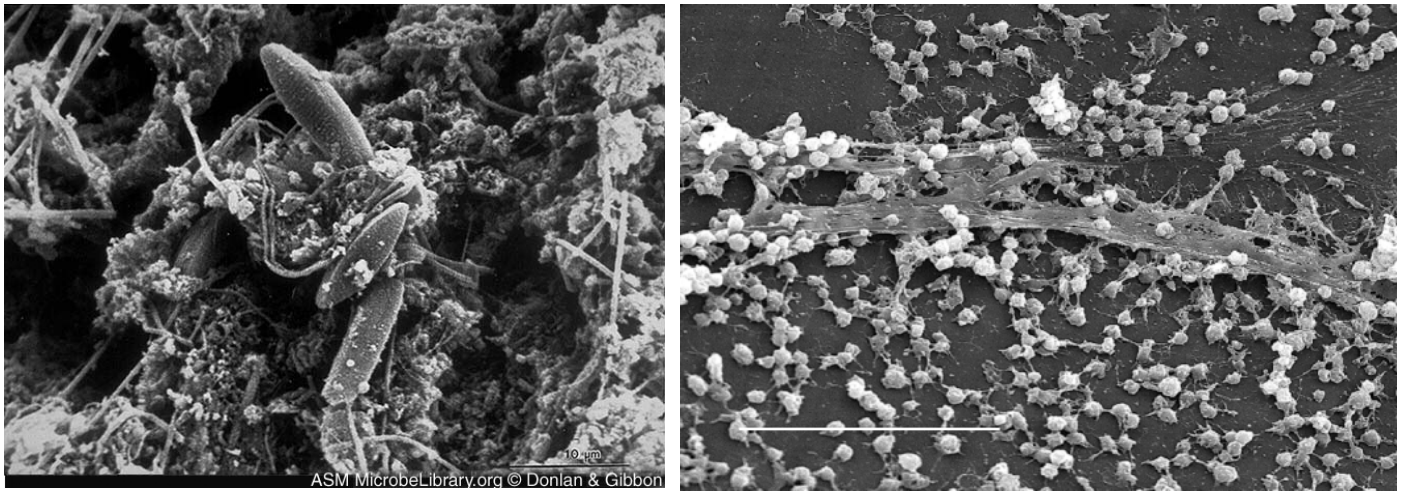

Figure 1. Scanning electron micrograph of a native biofilm [2]

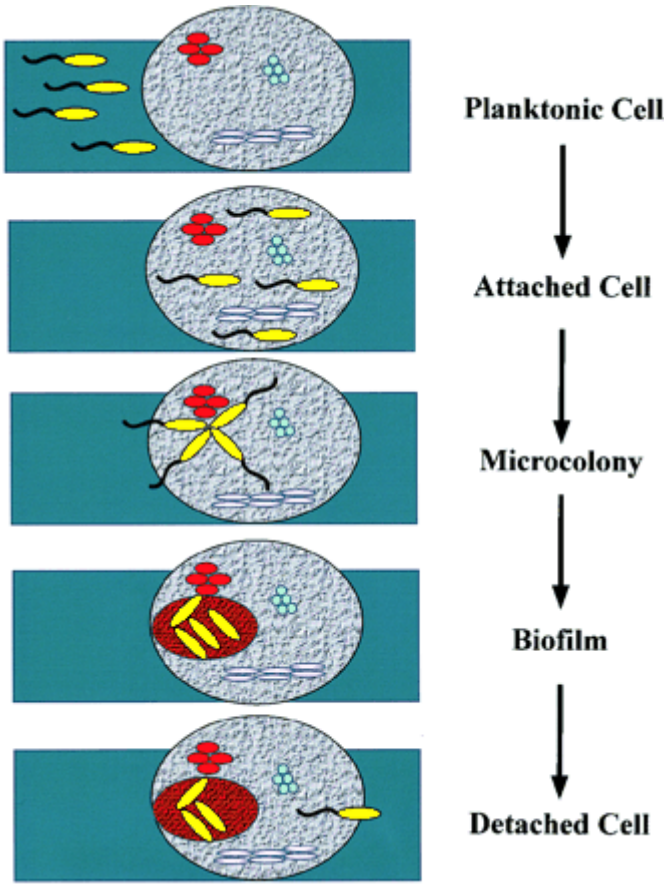

Figure 2. Steps a new bacterial species takes in forming a biofilm [3]

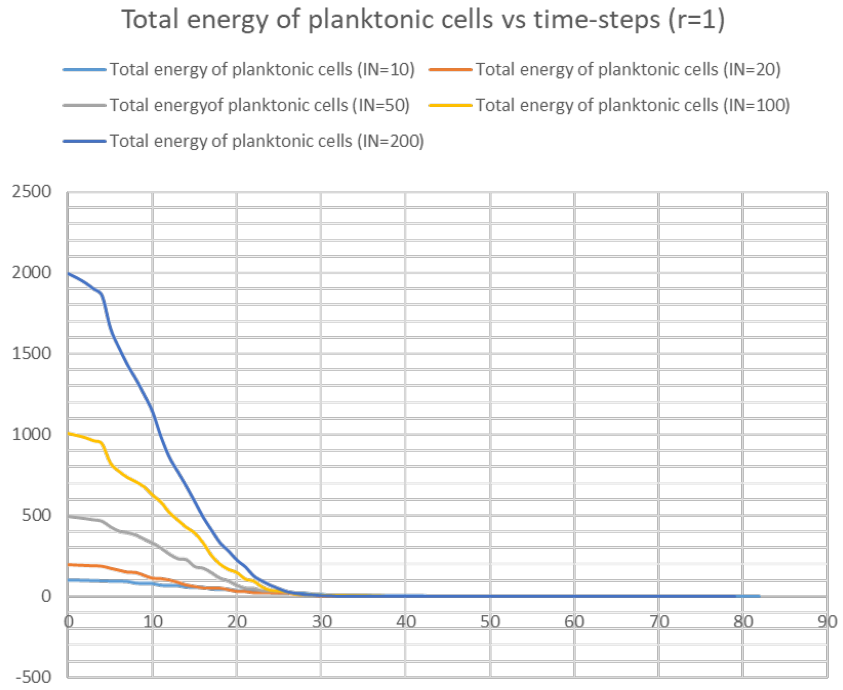

Figure 15. The results of the simulation (r=1)

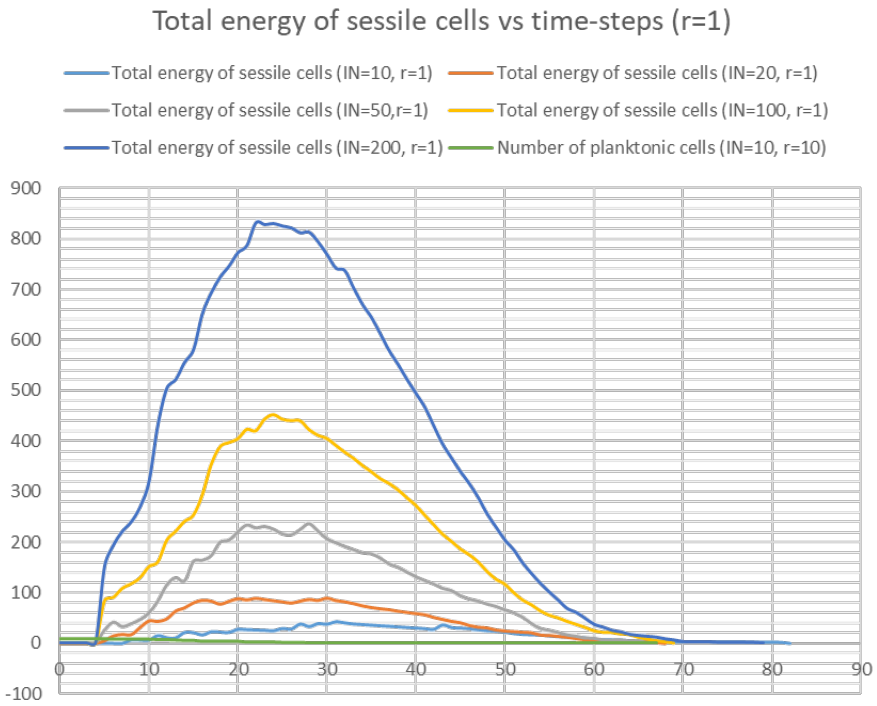

Figure 16. The results of the simulation (r=1)

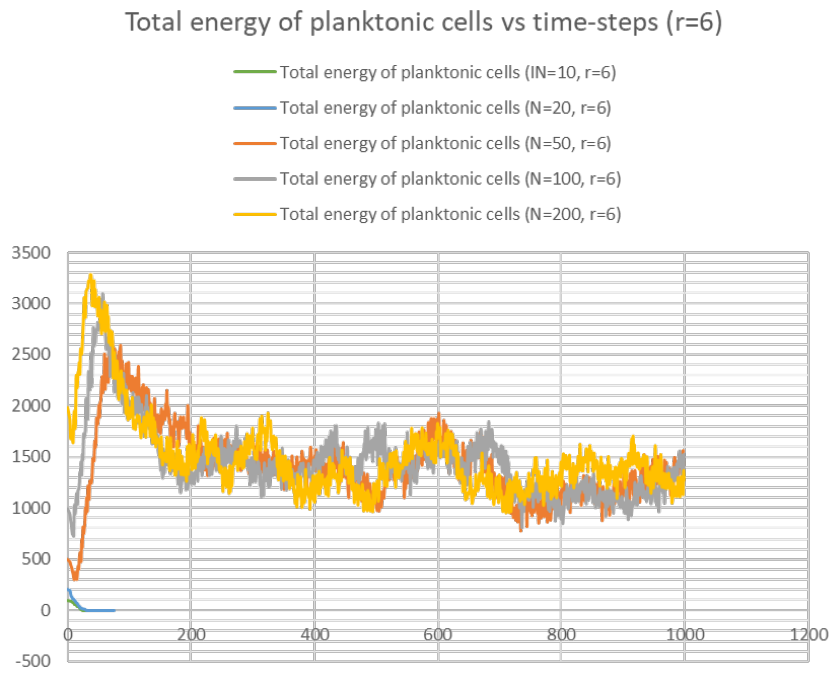

Figure 17. The results of the simulation (r=6)

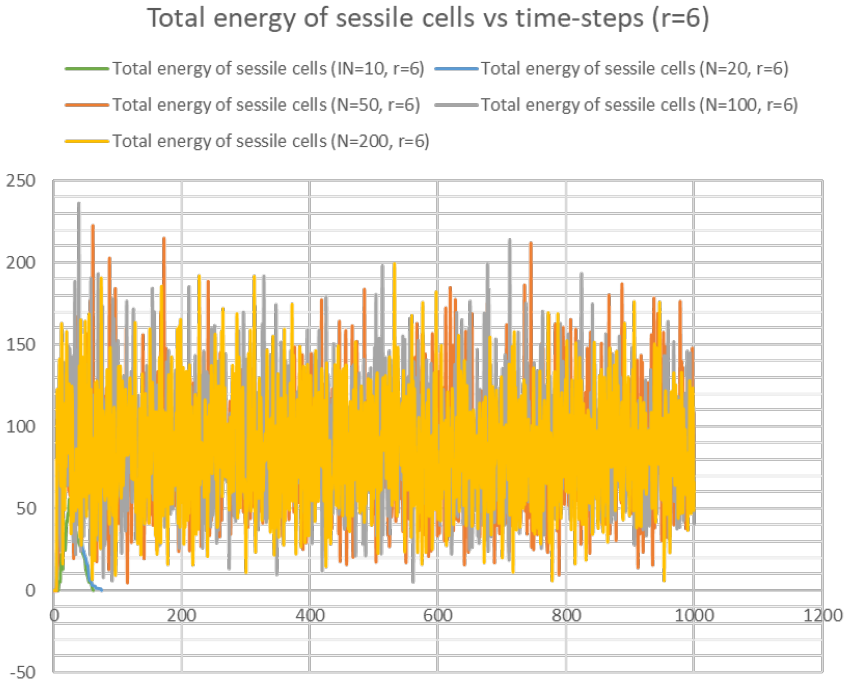

Figure 18. The results of the simulation (r=6)

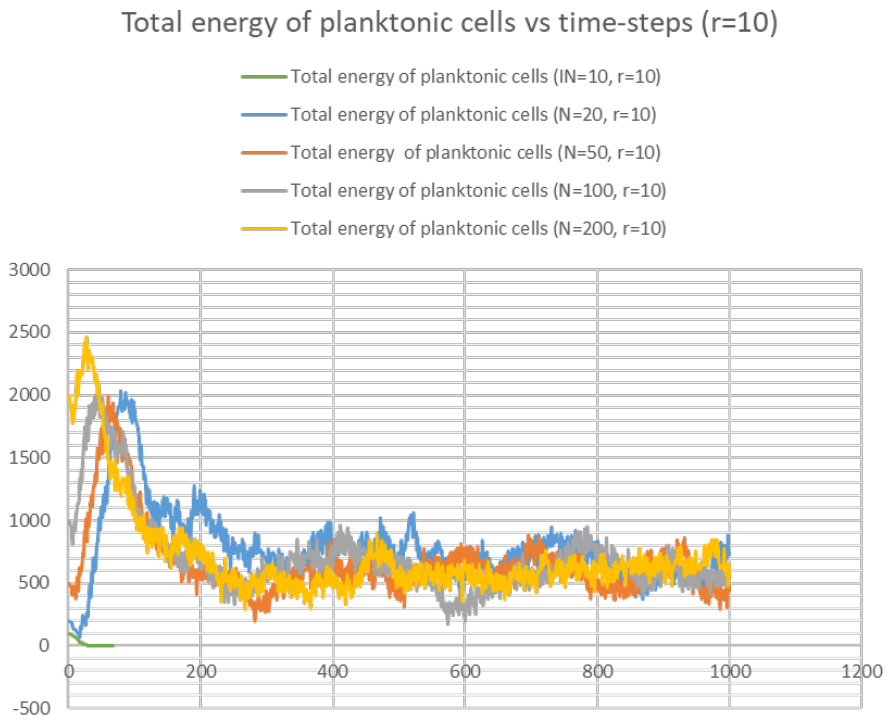

Figure 19. The results of the simulation (r=10)

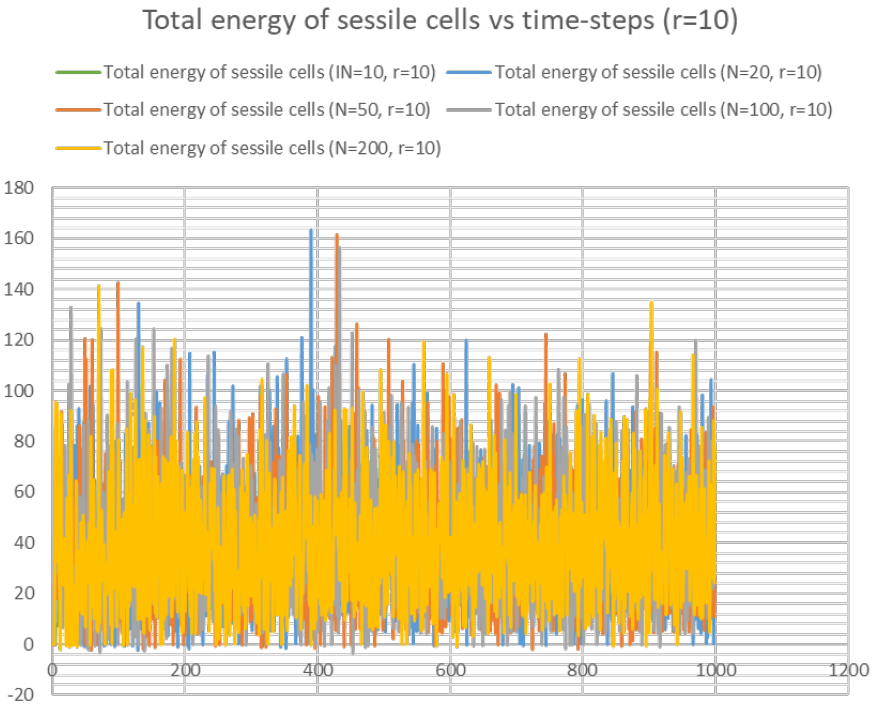

Figure 20. The results of the simulation (r=10)

The code of the simulation

```
breed [planktonic-cells planktonic-cell]
breed [sessile-cells sessile-cell]
globals
  [total_energy plankt_cell_energy_mean plankt_cell_energy_sd]
patches-own [surface?
  AHL-level
  patch_energy
]
planktonic-cells-own[
  planktonic-concentration
  speed
  in-the-AHL-zone?
  energy
  threshold
  cost
  energy-gain]

sessile-cells-own[
  sessile-concentration
  energy
  threshold
  cost
  energy-gain
  in-the-AHL-zone?]
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;; initial setup procedures  ;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
to setup
  clear-all
  reset-ticks
  settings-of-patches
```

```
  settings-of-planktonic_cells
  settings-of-borders
end

to settings-of-patches
ask patches
    [ set surface? false
    set AHL-level 0
    set total_energy input_system-energy
        set patch_energy ( total_energy / count patches)]
end

to settings-of-planktonic_cells
   create-planktonic-cells num-planktonic-cells [setxyz random-xcor random-ycor random-zcor

  set plankt_cell_energy_mean 10
  set plankt_cell_energy_sd 1

  set energy random-normal plankt_cell_energy_mean plankt_cell_energy_sd

  set threshold 4
  set cost 4

  set energy-gain 1

  set shape "circle"
  set size 0.5
  set color blue
  set speed 2
  set planktonic-concentration 0
  set in-the-AHL-zone? [false]
  ]
end
```

```
to settings-of-borders
  ask patches with [ pxcor = 0 AND pycor <= 10 AND pycor >= 0] [ set surface? true]
   ask patches with [ pxcor = 50 AND pycor <= 10 AND pycor >= 0] [ set surface? true]
   ask patches with [ pycor = 10 AND pxcor <= 50 AND pxcor >= 0] [ set surface? true]
   ask patches with [ pycor = 0 AND pxcor <= 50 AND pxcor >= 0] [ set surface? true]
   ask patches with [ pzcor = 0 AND pxcor <= 50 AND pxcor >= 0] [ set surface? true]
   ask patches with [ pzcor = 10 AND pxcor <= 50 AND pxcor >= 0] [ set surface? true]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;; runtime procedures  ;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to go
  ask planktonic-cells
   [long-range-movement;;
   increase_concentration-by-hitting-the-sessile-cells
   increase-conc-by-hitting-the-wall
   transform
   stick-to-the-wall
   ;reproduce  CAN BE ENABLED
   live
   eat
    if energy <= 0
      [die]]
  ask sessile-cells [
    move_on_the_surface
    stick-to-the-AHL-zone
    stick-to-sessile-cells
    reproduce
    live
    eat
    disperse
```

```
    if energy <= 0
   [set energy 0
  stop]]
  tick
end


to move_on_the_surface ;; turtle procedure
  face one-of patches with [surface? = [true] of myself]
  let ahead [AHL-level] of patch-ahead 1
  let myright [AHL-level] of patch-right-and-ahead 30 1
  let myleft [AHL-level] of patch-left-and-ahead 30 1
  ifelse (myright >= ahead) and (myright >= myleft)
  [ rt 30 ]
  [ if myleft >= ahead
    [ lt 30 ] ]
  fd 0.01
  increase-AHL-level
  if energy <= energy-gain [set energy energy - energy]
  if energy > energy-gain [set energy energy - energy-gain * 0.05]
end


to long-range-movement

  right random 1
  left random 1
  tilt-up random 90
  tilt-down random 90
  roll-right random 90
  roll-left random 90
  fd speed
  if energy <= energy-gain [set energy energy - energy]
```

```
  if energy > energy-gain [set energy energy - energy-gain * 0.75]

end



to increase-conc-by-hitting-the-wall
  if [surface? = true] of patch-here
  [set heading heading - 180
       set planktonic-concentration planktonic-concentration + 1 ;; ;;
    increase-AHL-level]
end

to increase_concentration-by-hitting-the-sessile-cells
    if any? other sessile-cells-here
     [set heading heading - 180
      set planktonic-concentration planktonic-concentration + 1 ;; ;;
      increase-AHL-level]

end

to stick-to-the-wall
  ask planktonic-cells[
  if planktonic-concentration >= 49 and [AHL-level > 4] of patch-here and [surface? = true] of
patch-here and not any? other sessile-cells-on patch-here
      [stop]]
end



to stick-to-the-AHL-zone
  if any? neighbors with [AHL-level > 4]
  [set in-the-AHL-zone? [true]
    stop]
end
```

```
to stick-to-sessile-cells
  if count sessile-cells with [AHL-level = 5] in-radius 1 > 3
[fd 0.01]
end


to increase-AHL-level
  ask patch-here [
    set AHL-level AHL-level + 1
    if AHL-level >= 4 [ set AHL-level 5]]
end


to transform
  if planktonic-concentration >= 5[
    set breed sessile-cells
    set shape "circle"
    set size 0.5
    set color red
    set in-the-AHL-zone? [false]
    set sessile-concentration 6
    set energy random-normal plankt_cell_energy_mean plankt_cell_energy_sd

  set threshold 4
  set cost 4
  set energy-gain 1
    ]

end


to eat
```

```
  let number random 100
  if number <= 100 * patch_energy / ( ( input_system-energy / count patches ) * 0.5 ) [



  if patch_energy > energy-gain [
 set energy energy + energy-gain
 set patch_energy patch_energy - energy-gain
  ]

  if patch_energy < energy-gain [
  set energy energy + patch_energy
 set patch_energy patch_energy - patch_energy ]
  ]
end



to reproduce
  if any? sessile-cells [
  ask one-of sessile-cells[
  if energy >= 4 and any? patches with [count sessile-cells-here = 0] in-radius 1 [
  let empty-patch one-of patches with [count sessile-cells-here = 0] in-radius 1
  set energy (energy - 4) / 2
  hatch 1 [
   set energy energy
    ]
  ]]]
end



to live
 set energy energy - energy-gain * 0.02
end
```

```
to disperse
  if (count sessile-cells in-radius radius-of-dispersal with [ patch_energy < 10 and energy < 10
] > 10 ) [
    ask sessile-cells in-radius radius-of-dispersal [
    set breed planktonic-cells
    set color green
    set size 0.5
    set shape "circle"
    set speed 2
    set in-the-AHL-zone? [false]
    set plankt_cell_energy_mean 10
    set plankt_cell_energy_sd 1
    set energy random-normal plankt_cell_energy_mean plankt_cell_energy_sd
    set threshold 4
    set cost 4
    set energy-gain 1
    set planktonic-concentration 0
    long-range-movement
   increase_concentration-by-hitting-the-sessile-cells
   increase-conc-by-hitting-the-wall
   live
   eat
   stick-to-the-wall
   if planktonic-concentration >= 5
      [set breed sessile-cells
    set shape "circle"
    set size 0.5
    set color red
    set in-the-AHL-zone? [false]
    set sessile-concentration 6
    set energy random-normal plankt_cell_energy_mean plankt_cell_energy_sd
  set threshold 4
  set cost 4
```

```
  set energy-gain 1]]]
end
```