"OVIDIUS" UNIVERSITY CONSTANZA
FACULTY OF NATURAL AND AGRICULTURAL SCIENCE
DOCTORAL SCHOOL – BIOLOGY
MICROBIOLOGY

THE ABSTRACT OF
Ph. D. THESIS

„BIOFILMS FORMED BY MARINE PROKARYOTES"

Scientific Coordinator,

Prof. Ph. D. Ardelean Ioan

Ph. D. Student,

Moldoveanu Aurelia-Manuela

Constanta 2013

ACKNOWLEDGEMENTS

To **Prof. Ph.D. Ioan Ardelean** the coordinator of this thesis for the scientific and moral support provided over the research years.

To **Assoc. Prof. Ph.D. Skolka Marius** for the scientific support, advices and access to the Marine Biodiversity laboratory.

To **Assoc. Prof. Ph.D. Onciu Teodora** for the scientific support, technical, clerical advice and access to laboratory Hydrobiology.

To **Assoc. Prof. Ph.D. Aonofriesei Florin** for the scientific support and bibliographic offer.

To **Ph.D Asist. Researcher Tudor Marian** for the technical support in conducting the experiments.

To **Ph.D Researcher Dan Vasiliu** for the chemical seawater analiyis samples and scientific support.

And lastly I thank my family for the moral support offered in both my professional fulfillment and in the realization of this thesis and in my previous activities.

CONTENTS

RESULTS AND DISCUSSION

Key words: biofilm, marine bacteria, microbial adhesion, bioreactor, microcomos, environmental factors, organic matter, hydrophile, hydrophobe, bacterioneuston.

INTRODUCTION

The surfaces that are immersed in the marine environment are almost immediately a suitable support for the attachment of a polymer coating in the environment, resulting in the phenomenon of adherence of microorganisms and the formation of communities of microorganisms, algae and other organic forms (Parsek and Fuqua, 2004; Cho et al., 2007).

The importance of the thesis is due to the study of prokaryotic presence on the surfaces by determining the total number of cells attached to the substrate or the interface between the air - liquid cell with altered cellular membrane structure and function and cell capable of biofilm growth and multiplication, under normal conditions and under the influence of environmental factors (temperature, salinity, nutrients, pollutants, and the differebt types of substrate).

CHAPTER I

MARINE PROCARYOTES

In general, prokaryotes have maximum numbers in coastal regions (especially in the neighboring large urban communities) in estuarine and pelagic zones progressively decreases as the distance from the shore and with depth (Zarma, 1963, 1965). This number is also increased in the area of upwelling in the first few centimeters of the seabed where it can reach high values. It decreases dramatically with depth due to anaerobic sediments and lack of nutrients in these areas (Zarnea 1994).

Microorganisms play an essential role in marine ecosystems with a key role in the trophic relations of this type of ecosystem, they are both microorganisms (microbiota) that break marine snow, but recovered and dissolved organic matter and simultaneously helps to build epibiosies (Sleigh, 1987; Zarnea, 1994).

Marine bacteria grow optimally at salt concentrations between 33-35‰ and do not grow or grow poorly in the absence of NaCl, are oligotrophic, or psychrophilic, psychrotrophe except tropical surface waters. There are, depending on the habitat, barophobe (sea surface), barotolerant or barophile. Presents a pronounced pleomorphism caused probably oligotrophic and elevated hydrostatic pressure effects. Most of them (80% - 95%) are Gram negative, mobile (75% - 85%), aerobic, facultativ anaerobic and anaerobic forced to live in the bottom sediments (Zarma, 1959[a]; Zarnea, 1994).

CHAPTER II

ENVIROMENTAL FACTORS INFLUNCES ON MARINE PROCARYOTES

a. Salinity

Natural environments offer very different conditions of salinity for organisms, from those with very low concentrations (water rivers and lakes) to those with high concentrations such as salt lakes and seas, or even those that are true solutions saturated (Zarnea, 1994).

Salinity is an important variable in marine microorganisms in biofilms are influenced by this center according to their degree of tolerance to NaCl concentrations (Stanley and Morita, 1968; Zarnea, 1994).

b. Temperature

The growth and multiplication of microorganisms is the result of a coordinated series of metabolic reactions whose normal development is ensured by a suitable temperature (Zarnea, 1994; Eddlemann, 1998, Hinrichsen, 2011).

The effect of temperature on the growth of microorganisms is related to its action on their enzymatic reactions. As a result, the speed of the two processes gradually increases as the temperature rises until it reaches an optimal level. Beyond this limit, their speed decreases until the enzyme is denatured by the high temperature (Zarnea, 1994; Hinrichsen, 2011).

c. Water dynamics

Have a great importance because it determines one of the most important properties of marine ecosphere that is relatively uniform environmental conditions. By making, according to their direction (vertical or horizontal) and the level at which they appear (surface or deep regions), the mixture of large masses of water, they also produce nutrient mixture, as well as a redistribution of the microbiota (Zarnea, 1994; Lewandowski and Stoodley , 1995).

If current presence influences biofilm accessible nutrient components and facilitates exchanges between cells (Pedersen, 1982[a], Zarnea, 1994; Stepanovic et al., 2001).

d. The response to the lack of nutrients

Nutrition information requirements of microorganisms derived from artificial environments rich in nutrients readily available. However little is known about the chemistry

of microhabits in nature because of the exploring difficulty (Heukelekian and Heller, 1940; Zarnea, 1994).

Natural environments are very heterogeneous in terms of their nutrient content. Although biosphere contains an infinite variety of ecosystems, a very large proportion are inhospitable habitats whose nutrient content is close to the lowest possible limits for the growth of microorganisms (Penfold and Norris, 1912; Hagstrom et al., 1984).

e. The influence of the interfaces on microorganisms

In natural environments, in appropriate physico-chemical conditions, any surface exposed to aqueous solutions containing small amounts of nutrients are covered by a film of microorganisms as a single cell layer, of microcolonies or biofilms (Marshall, 1986, Zarnea, 1994).

This phenomenon was confirmed by numerous observations that revealed accumulation and microbial growth even at the interface represented by the atach phase when microorganisms are found on the solid surfaces (Marshall, 1986; Zarnea, 1994; James et al., 1995).

CHAPTER III

MICROBIAL ADHESION

This grip is of fundamental importance in biology, fixation of prokaryotes on different surfaces, is a necessary precursor of most biological processes, physiological or pathological microorganisms (Senet, 1995, cit. Lazăr, 2003).

Prokaryotes have some of the properties of colloidal particles so that their adhesion to various substrates has been studied experimentally as a physicochemical phenomenon applying the principles of colloid chemistry, somewhat simplistic because prokaryotes are not inert colloidal particles, modifying ther properties in time, depending on changes in environmental conditions. The phenomenon has been studied most in terms of solid-liquid interfaces (van Loosdrecht, 1990, cit. Zarnea 1994, Marshall, 1992; Cooksey and Wigglesworth-Cooksey, 1995; Moldoveanu, 2010c).

CHAPTER IV
BIOFILMS FORMATION

Phases of biofilm formation:

1) The transport phase, that the movement of the microorganisms in the environment to the substrate surface which in turn can be achieved by three different mechanisms (Lazăr, 2003)

2) The initial adhesion phase is reversible, in the sense of a deposit on the surface of the backing, the maintenance of a Brownian motion and flagellation and the possibility of removal, either by mobility, or by gentle stirring (Lazăr, 2003).

3) The secondary adhesion phase or permanent cessation is characterized by Brownian motion and the possibility of removing adherent prokaryotes under the action of powerful forces stirring. This is achieved in a very short distance from the cell wall, and the remote interactions are dominated by attractive forces (Sutherland, 2001).

4) The colonisation phase. After tying firm, prokaryotes begin to grow and multiply rapidly. The cells are irreversibly bound to the substrate, but not to each other to form a continuous monolayer covering the entire surface of the substrate, and the substrate and linked to each other and form microcolonies and biofilms (Lazăr, 2003).

CAPITOLUL V
BACTERIONEUSTON

In the seawater are two types of interfaces particularly important: the solid-liquid where biofilms are formed and the air-liquid classical where the bacterioneuston is formed (microstratul surface) (Zook, 1992; Henk, 2004; Agogué et al., 2004; Hakvåg et al., 2008).

Air-liquid interface (ALI) is a ubiquitous environment that occurs on the surface of any type of aquatic ecosystem and is a trophic niche for microorganisms. Thus, any body of water, be it small or big, natural or man-made type has an ALI is formed between the atmosphere and hydrosphere (Goldacre, 1949 Garrett and Duke, 1980; Burchardt and Marshall, 2003 Marshall and Burchardt, 2005). Through this interface there is a dynamic and continuous gas exchange, water and inorganic substances. Also, the level of this interface there is a series of processes involved in transformation of energy and the formation of aerosol. The specific properties of existing molecules at this level differ from those of the same molecules when water table is

below the interface. These properties provide training in aquatic ecosystems tension (Harvey, 1966; Parker and Barsom, 1970; Zaitsev, 1997; Henk, 2004).

AIM AND OBJECTIVES OF THE THESIS

The *aim* of this thesis is to study the dynamics of biofilm formation and marine prokaryotes bacterioneuston in laboratory (containers) under controlled conditions of temperature, salinity and organic supply.

Objective 1: The dynamics of the cells in biofilms formed at different temperature conditions (6 °C to 18 °C) and at different salinities (5 g/L, 10 g/L and 15 g/L).

Objective 2: Dynamics of the number of cells in biofilms formed under static and dynamic conditions on hydrophilic or hydrophobic surfaces.

Objective 3: The dynamics of the number of cells and the type of cells/colony in biofilms in seawater supplementation with organic substances (amino acids, tryptone, yeast extract, glucose, starch).

Objective 4: The dynamics of the cell number in bacterioneuston made in terms at different salinities (5 g/L, 10 g/L, 15 g/L and 25 g/L) and temperature (6 ºC and 18º C and 23º C).

Objective 5: Influence of organic substances (peptone and gasoline) on the dynamics of cell number bacterioneuston in container conditions.

CHAPTER VI

MATERIALS AND METHODS

6.1 Methods use to obtain biofilms at the liquid – solid interface

Container type systems are advantageous because they can be controlled within certain parameters (temperature, salinity, nutrient concentration and/or pollutants, if at the beginning of the experiment, and so on), thus achieving more streamlined experimental models compared the natural ecosystem. This simplification allows, as a rule, clearly the interpretation of the results compared with those obtained *in situ*.

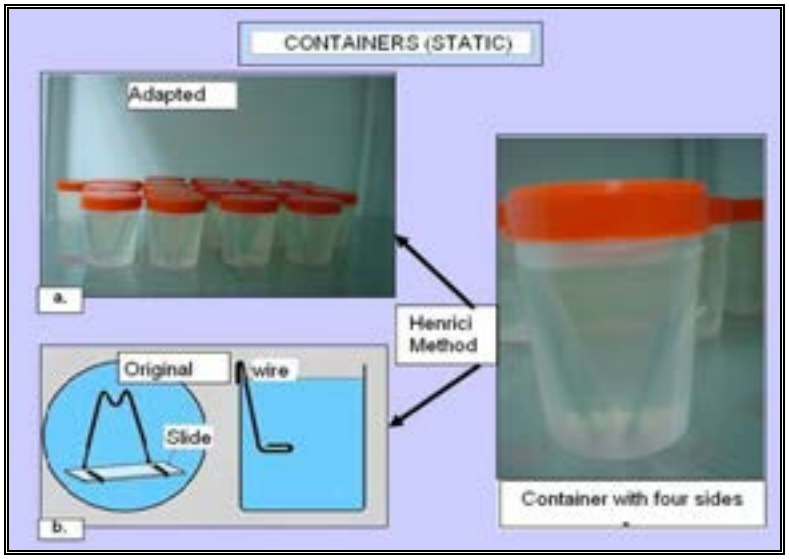


Figure 6.1 Containers with seawater for biofilm formation. Henrici method adapted (original), b) how classical Henrici (www.biofilmsonline.com, 2008)

Unfiltered seawater is a favorable culture medium for biofilms due to the keeping concentration of organic matter and marine bacteriovors that influence biofilm formation *in situ*.

Henrici technique is anchored in the natural or laboratory of sterile glass slides by means of cables or wires in a horizontal position in order to allow the formation of biofilm on the surface (Henrici, 1933) – Figure 6.1.

Thus to conduct experiments in dynamic conditions containers with a circuits were used to achieve these conditions in vitro, according to the model suggested by Harsen et al. (2007). Thus, we use PVC boxes storage capacity of 15 L, which has been attached to the pump tank of 200 L/h to ensure continuous flow of the water in the experimental surfaces.

In these containers with a circuit were used 2.5 L of seawater were placed high above the angle by which the blades 12 of the glass for microscopy by two plastic rods, located on a top of the culture medium. Glass slides were in contact with seawater that seeps onto the surface, the method containers with a circuit and method *Microbial fishing* (Pedersen, 1982b, Hansen and Sørensen, 2007; Burmølle et al., 2007) v. Figure 6.2 .

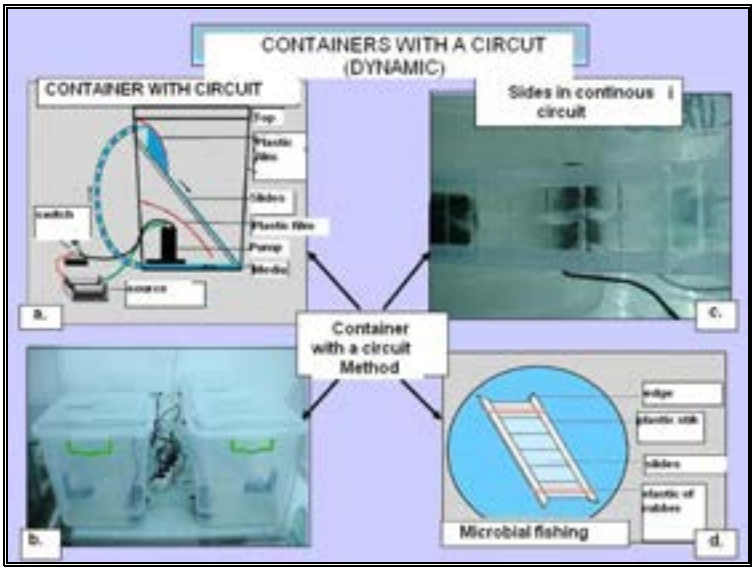


Figure 6.2 Getting biofilm under dynamic, d) scheme to achieve biorecatoarelor (www.biofilmsonline.com, 2008) and b, c) layout used in laboratory containers with a circuits (original)

6.1.1 Bacterial biofilm formation under the influence of temperature and salinity

The experiment was conducted in a room thermostatically controlled at a constant temperature of 18 ºC in Laboratory Investigation of Biodiversity, University Ovidius Constanta in a refrigerator at a constant temperature of 6 ºC, using 144 microscope slides heat-sterilized and placed in time T_0 in containers (Moldoveanu and Ardelean, 2010).

Salinity change was made of coastal seawater from 15 g/L normal value for the Black Sea have created two experimental variables with a salinity of 10 g/L (to make a mixture of 666 ml seawater osmosis with 333 ml of water) and a salinity of 5 g/L (the mixture of 333 ml of seawater with 666 ml of water osmosis), using the 216 microscope slides heat sterilized seawater salinity which amended (Moldoveanu and Ardelean, 2010).

6.1.2 Formation of biofilm under static and dynamic conditions

Slides were used in a containers with a circuit using the *microbial fishing* with two plastic rods, 288 microscope slides where used as sample surfaces (Moldoveanu and Ardelean, 2010b).

Sterilization microscope slides, sampling and desalting were performed according to the protocol presented in the previous experiment.

Static conditions were obtained in containers according to the method glass slides of Henry, that as the experiment described above (Moldoveanu and Ardelean, 2010b).

6.1.3 Biofilm dynamics under variation of the amount of organic matter

The study was carried out using an adapted form of the Henrici method in sterile plastic containers with a capacity of 100 ml, which was introduced seawater supplemented with pure organic substances. There has been great water per liter of a number of changes in the amount of organic matter with a mixture solution of 0.1% of amino acids (Difco), tryptone (Difco), yeast extract (Difco), glucose (Merck) and starch (Merck) - (Moldoveanu, 2011c), water and chemical analysis showed a number of differences, shown in Table 6.3, in particular if the amount of the organic substance used in the experiment.

6.1.4 Formation of biofilm on hydrophilic and hydrophobic surfaces

Experiments performed under static and dynamic variable temperature on different hydrophilic or hydrophobic surfaces, using a method adapted form Henrici and method which allowed us to obtain biofilm containers with a circuit under dynamic *in vitro* (Moldoveanu and Ardelean, 2012c).

The surfaces were sectioned on microscope slides of dimensions 75 × 25 mm subjected to a sterilization process to minimize possible contamination with micro-organisms, such parafilm and Teflon was immersed in 70% ethanol (Lazăr et al., 2004) and glass blend sulfochromate for two days (Moldoveanu, 2010b).

6.2 Methods use to obtain biofilms at the air – liquid interface (Bacterioneuston)

Collecting samples from the surface layer has long been a problem for researchers. In laboratory conditions, the chemical structure of this layer and the effect of surface tension on organic molecules has become a field of study of great importance because of the way these molecules are organized at the air interface - water (Henk, 2003).

For these studies I used 15 PVC rectangular box with a capacity of 1.5 L, in which there were added 600 mL of the unfiltered seawater, and modifying various environmental factors - figure 6.3

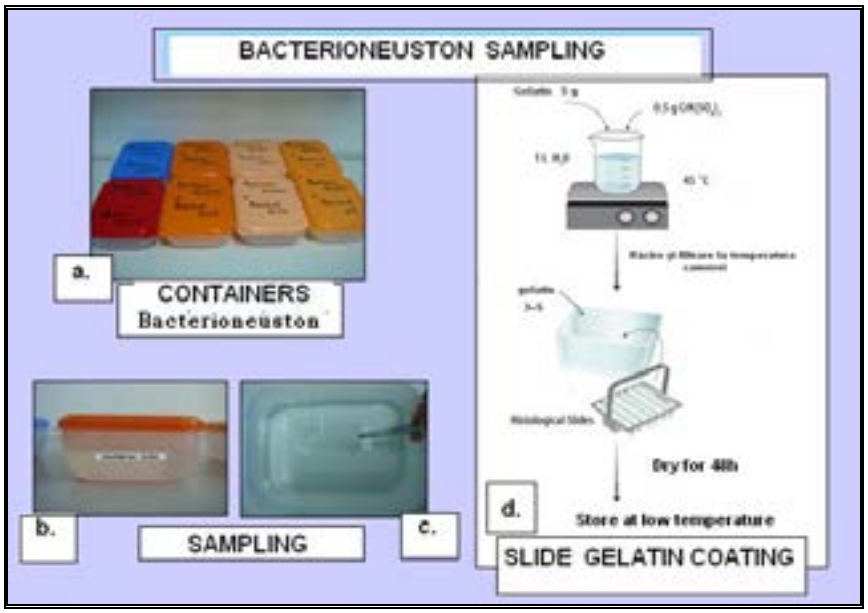


Figure 6.3 Containers used in experiments - control samples and experimental samples (original), b), c) Sampling (original), d) scheme to achieve the gelatin-coated slides (R & D Products & Protocol Guide, 2012)

6.2.1 Dynamics of marine bacterioneuston under the influence of temperature and salinity

The salinity of the medium was first analyzed factor of four different variants were chosen: a low value of 5 g/L, both average values of 10 g/L and 15g/L and a high value of 25 g/L (Moldoveanu and Flo 2012).

Temperature values were chosen similar to seasonal variations in the Black Sea: a low value of 6 ºC, an average and constant 18 ºC and a high value of 23 ºC (Moldoveanu and Ardelean, 2012).

6.2.2 Dynamic of marine bacterioneuston in variable nutrient conditions

In this case we made an additional seawater with organic substances of varying concentrations of 0.1%, 0.5%, 1% peptone to simulate organic matter pollution, and similar concentrations to simulate a gasoline pollution of the baterioneustonului (Moldoveanu and Ardelean, 2012b).

6.3 The microscopic analysis of the surfaces

6.3.3 Optical microscopy in biofilm research

Brightfield microscopy is an essential method for direct observation of the biofilm or histological sections, as long as biofilms can be included in paraffin and sectioned by conventional histology techniques (Yuehu and Friedman, 1997). Microscopy allows

examination of the entire structure of the biofilm and allows understanding its spatial organization and their relations to surfaces, especially in the early stages of their development (Surman et al., 1996).

6.3.2 Epifluorescence microscopy to study biofilm

This is extremely useful in analyzing properties of microorganisms and organic or inorganic substances using the phenomena of fluorescence instead of refraction and absorption used by optical microscopy (Lawrence et al., 1991; Sonak and Bhose, 1995, Suci et al., 1997; Moldoveanu, 2010).

CHAPTER VII

THE DYNAMICS OF BIOFILMS FORMATION AT THE SOLID - LICHID INTERFACE

7.1. Salinity

Salinity is an important factor contributing to the formation of a specific profile for the communities of microorganisms in shallow waters. Because the Black Sea is considered at large an atypical sea, if you were to compare it with other seas the organisms are expected to present some peculiarities. In inland waters where there is permanent fluctuations of salinity, microorganisms present spatio-temporal changes of their activity. Osmotic stress created by abrupt changes in salinity may affect cellular metabolism and bacterial density (Gauthier et al., 1990, 1991). Adaptation of the microorganisms to conditions of osmotic stress is due to the protective enzyme may increase the acquisition of osmoprotector compounds of the bacterial cells (Lim et al., 1996).

In the figure 7.1 are the experimental data on the dynamics of biofilm formation under different salinity. The slides immersed into seawater with a high salinity of 15 g/L show an increase in bacterial cell density at $12 \cdot 10^2$ cells/mm^2 after one hour immersion, to a value of $25 \cdot 10^2$ cells/mm^2 fafter eight hours.

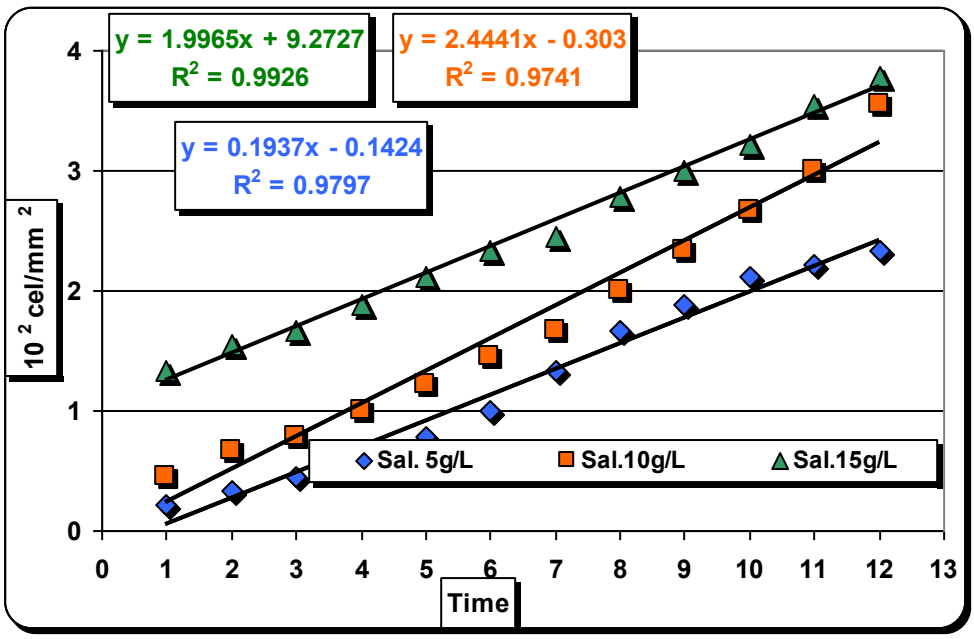


Figure 7.1. The dynamics of bacterial density during the formation of biofilm in the containers with various salinity (5 g/L, 10 g/L and 15 g/L)

The density of cells in biofilms formed at the different salinity showed an increase in the rate of change, thus a salinity of 5 g/L (slope of 0.19) the ratio is less than 1 in the case of biofilms formed salinity of 15 g/L (slope is 2.44) and 10 g/L (slope is 1.99), the values for these experiments demonstrating that microorganisms quickly colonize surfaces at higher salinities. Also, the coefficient values (R) were between 0.99 and 0.97 which shows a linear increase.

In the international literature there is data on biofilm formation under different salinity. To study the colonization of surfaces by bacteria *Bdellovibrio bacteriovorus*, Kelley et al. (1997) have made experiments under the influence of different salinity was observed there was a trend for colonization and biofilm formation on values between 34 g/L and 35 g/L. The salinity influenced biofilm formation even at 5 g/L or 4 g/L, there was a decrease in the number of cells from $3.5 \cdot 10^6$ CFU/cm^2 at 3, $8 \cdot 10^4$ CFU/cm^2 after 5 days of immersion in water areas.

Some experiments on the role of salinity (from 12 g/L and 80 g/L) in corrosion surfaces were made by Franca et al. (2000), which was used as stainless steel substrates have shown an increase in cell density with the salinity of seawater samples. Subsequently a maximum of corrosion occurring in 35 g/L of bacterial cell density values between $1.7 \cdot 10$ CFU/cm^2 and $2.1 \cdot 10^9$ CFU/cm^2 aerobic and anaerobic species analyzed in the experiments.

7.1.2 Temperature

Water temperature can affect the rate of about 55% of the temporal variation of bacterial abundance (Sommaruga and Conde, 1997 cit. Aonofriesei, 2004). Other studies indicate a positive correlation between temperature and bacterial growth in planktonic phase and their abundance (Tibes, 1996 Wikner et al., 1999 cit. Aonofriesei, 2004).

The temperature effect on bacterial growth is dependent on the nutrients, thus, nutrient-rich environment and ecosystems have a low temperature, but in a lower concentrations of nutrients (oligotrophic) temperature has a clear effect (Philip et al., 1996).

In the figure 7.2 are shown the dynamics of the bacterial cell density in the formation of biofilm on microscope slides at a temperature of 18 °C or 6 °C.

For large water containers kept at 6 ºC in the refrigerator there is a progressive increase in the amount of 0,5 $\cdot 10^3$ cells/mm^2 after one hour and a doubling of its immersion after seven hours at 1,0 $\cdot 10^3$ cells/mm^2, and a threefold increase from 1,5 $\cdot 10^3$ cells/mm^2 after eight hours.

Data analysis revealed the existence of a dynamic biofilm formation, so that if biofilm formed 18 º C can be observed in one hour from immersing slides a cell density value of 1,2 $\cdot 10^3$ cells/mm^2, value is doubled after eight hours 2,5 $\cdot 10^3$ cells/mm^2 and increase gradually until there is a trend to triple cell density to a value of 3,7 $\cdot$ 10^3 cells/mm^2 after 11 hours.

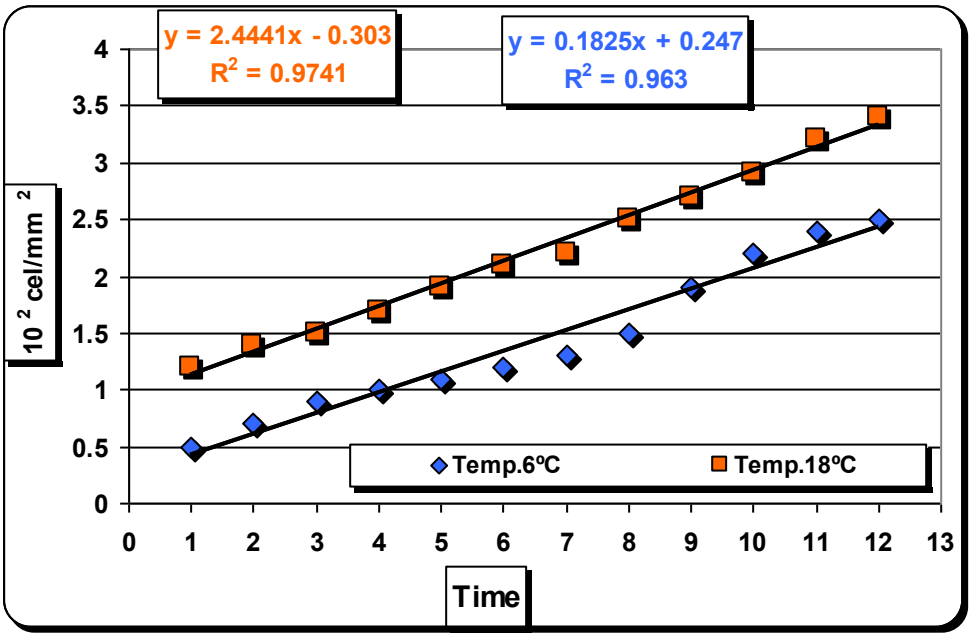


Figure 7.2. Dynamics of bacterial density during biofilm formation in seawater containers at variable temperatures (6 ºC and 18 ºC)

In 1994, Rogers et al. analyzed on different types of surfaces (copper, PVC and polybutylene) phenomenon of adhesion at different temperatures: 20 ºC, 40 ºC, 50 ºC and 60 ºC using the

species *Legionella pneumophila* (species with wide limits of tolerance of temperature (between 5.7 and 63 ºC) and other strains of *non-Legionella* for a period of 21 days, after which it was found that the strains used are shown in a logarithmic growth phase growth impairs the density values of 1,3 $\cdot 10^4$ cells/cm^2 and 7,56 $\cdot 10^4$ cells/cm^2 on the surface of PVC polybutylene and non-Legionella strains at 20 °C and 4,25 $\cdot 10^4$ cells/cm^2 polybutylene surface at 60 °C. It is obvious that a higher colonization hydrophobic surfaces at 20 ºC, 60 ºC there is a decrease in prokaryotes are beyond the optimum temperature for the development of microorganisms.

Kelley et al. (1997) conducted experiments on the colonization of surfaces by bacteria Bdellovibrio bacteriovorus under the influence of different temperatures (4 ºC and 29 ºC), using as substrate shell valves, glass and polystyrene. They found a positive correlation for the factor and temperature during the formation of the biofilm, with a joint maximum of cells in biofilms at 18 °C and a minimum of 14 °C as well as a significant decrease in density at temperatures below 5 °C, over a period of 24 hours after this period is followed by a progressive increase of the density values up to 120 hours after the start of the experiments.

The data presented in the literature reveals an increase in bacterial density on the time of exposure of the surface to the aquatic environment contiţiile to increase the temperature of the culture. If personal data observed at 18 º C have high bacterial cell density recorded within the tolerances, bacteria generally having significant growth up to temperatures of 25 ºC - 30 ºC. Subsequently the temperature rise above 35 ºC, 40 ºC, 50 ºC affects biofilm formation because it exceed the optimum survival of bacterial species (Di Bonaventura et al., 2007).

7.1.3 Influence the dynamic conditions

Biofilms were analyzed in different hydrodynamic conditions such as laminar or turbulent flow and showed that they react differently to the altered dynamic conditions. Speed water flow or hydrodynamic influences biofilm structure and may have a number of effects on the type of biofilm formed. The amplified circuit is formed are fragmented and the rough looking cell aggregates separated by interstitial spaces. Biofilms formed under turbulent circuit are also fragmented but diverse and elongate forms that arrive in the fluid surrounding the biofilm (Kokar, 2009).

Glass slides where harvested from flasks and containers with a circuit were analyzed after counting microscopic fields observed a progressive increase in cell density for biofilms formed in seawater in bottles at 1,2 $\cdot$ 10^3 cells/mm^2 at a hour after immersion in a doubling of

the value of the cell density after eight hours in $2,5 \cdot 10^3$ cells/mm^2 and its value tripled after 16 hours of immersion of the surface, as shown in figure 7.3.

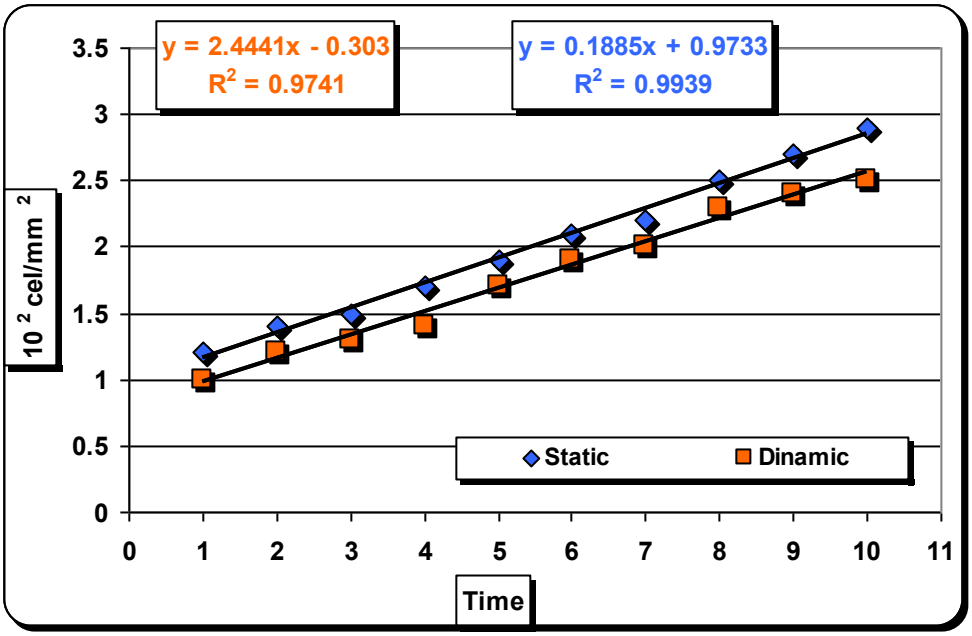


Figure 7.3. Dynamics of bacterial density in containers during biofilm formation under static and dynamic

Changes in cell density in biofilms were determined by a faster growth rate of the marine prokaryotes, the slope is 2.44 for microscope slides immersed in containers (static) compared itwith the value of 0.18 obtained for containers with a circuit, in dynamic .

The values of cell density obtained in the experiments are lower than those obtained by Rijnaarts et al. (1993) that, under comparable conditions have seen an increase in bacterial density between $5 \cdot 10^4$ cells/cm^2 - $1,6 \cdot 10^7$ cells/cm^2 under static conditions and $5 \cdot 10^4$ cells/cm^2 - $3,6 \cdot 10^7$ cells/cm^2 dynamic conditions large difference may be due to the recirculation of water in a slower pace allows the intake of substances for biofilm cells, and a better fit through the mechanisms of adhesion to the surface of the sample.

Such experiments in static and dynamic conditions have been achieved and in-situ by Casse and Swan (2006), which obtained a higher density of 80% in the case of bacterial cells formed on surfaces submerged under static conditions compared to cell densities achieved submerged surfaces without applying antifuling substances under dynamic marine environment.

7.4. Effect of organic substances on the total growth of the cells in biofilms

Planktonic marine prokaryotes can have a number of changes, they can attach to solid surfaces and can incorporate organic particles, some even with a high degree of selectivity to these components, so that many of them sessile stage becomes larger than their planktonic form (Münster and Chrost, 1990).

From the data obtained in the control sample without additional organic matter resulting total bacterial density of 41,4 · 10^3 cells/mm^2. Subsequently supplementation of seawater with a small amount of mixture of amino acids of 3 mg/L, has influenced the the bacterial metabolism and the value of cell density was of 50,6 · 10^3 cells/mm^2. The use of a higher concentration of 5 mg/L of a mixture of amino acids results in a total density of 56.3 cells/mm2 · 103. The increase was even greater in the case of supplementation with 7 mg/L of a mixture, such that the bacterial density was 69,4 · 10^3 cells/mm^2, so that at a higher amount of 9 mg/L density to 83,2 · 10^3 cells/mm^2 (figure 7.4).

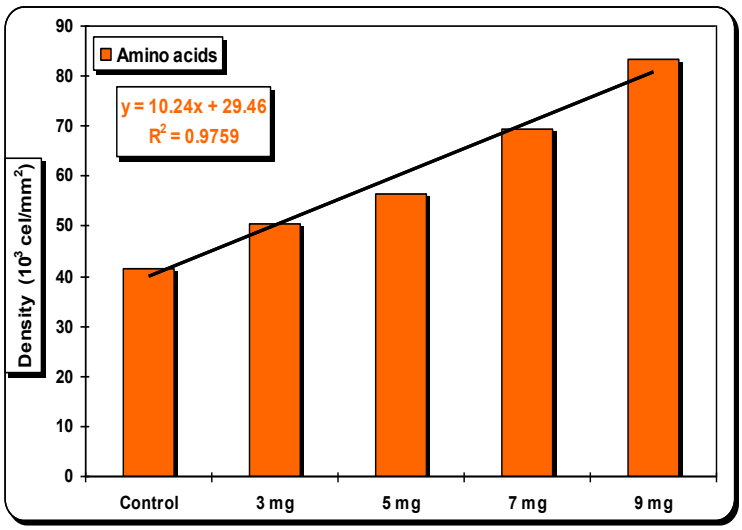


Figure 7.4. Maximum bacterial density after 72 hours in containers with different concentrations of the amino acid mixture

Another source of protein used was tryptone, which due to the amino acid content of casein (which can be produced by acid hydrolysis) make it an accessible source for marine prokaryotes.

In the control probe, without additional with organic matter, was determined a density bacterial total of 39,5 · 10^3 cells/mm^2. Subsequently supplementation of seawater with a small amount of mixture of amino acids of 3 mg/L, has influenced the the bacterial metabolism, and the value of cell density was of 46,4 · 10^3 cells/mm^2. Using a concentration of 5 mg/L

tryptone resulted in a total density of 57,1 · 10^3 cells/mm^2. The increase was even higher in the case of supplementing with 7 mg/L of the mixture, the bacteria density was 69,4 · 10^3 cells/mm^2, respectively to the amount of 9 mg/L, the density is 78,3 · 10^3 cells/mm^2 (figure 7.5).

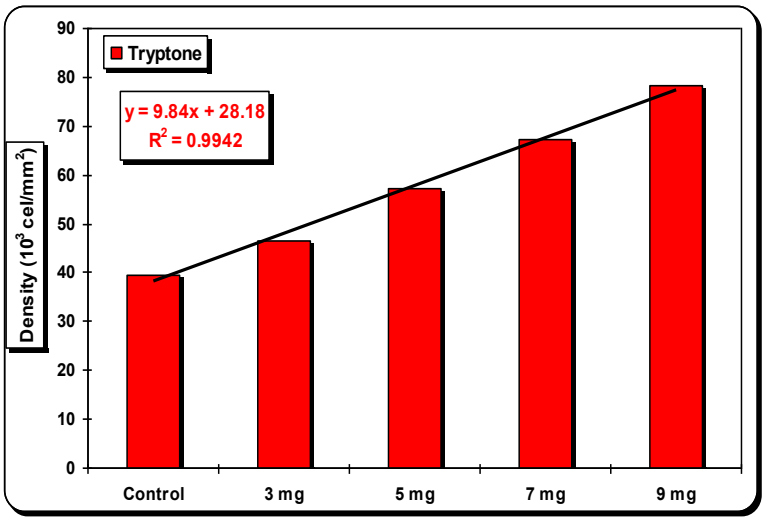


Figure 7.5. Maximum bacterial density after 72 hours in containers with different concentrations of tryptone

On the basis of the values of cell density differences can be observed between the control sample and the mixture supplemented with amino acids, such that with at least 3 mg/L resulted in a difference of 9,2 · 10^3 cells/mm^2, and that the prokaryotic being 22.3% of the initial cell density on the surface, while the addition of 5 mg/L resulted in a difference of 14,9 · 10^3 cells/mm^2, with a value of 36.2% of the original value. For samples supplemented with an amount of a mixture of 7 mg/L difference was 28 · 10^3 cells/mm^2, with an increase in the amount of 68.12% of the initial and 9 mg/L, the difference was 41,4 · 10^3 cells/mm^2, which resulted in an increase in cell density than 101.7% of the initial value of 41,4 · 10^3 cells/mm^2.

Glucose is a simple sugar (monosaccharide) and is the most important source of energy for cellular metabolism and cellular respiration. Glucose is produced by deep-sea prokaryotes in important processes of chemosynthesis.

The control sample without additional organic matter resulted in a total of 39 · 10^3 cells/mm^2. Subsequently seawater supplemented with glucose a small amount of 3 mg/L, and the amount of influence the metabolism of the bacterial cell density was 45 · 10^3 cells/mm^2. Using a higher concentration of 5 mg/L of glucose causes an overall density of 51 · 10^3 cells/mm^2. The increase was even greater in the case of supplementing with 7 mg/L of

glucose, the bacteria density was 64 · 10^3 cells/mm^2, so that at a quantity of 9 mg/L to be 70 · 10^3 cells/mm^2 (figure 7.6).

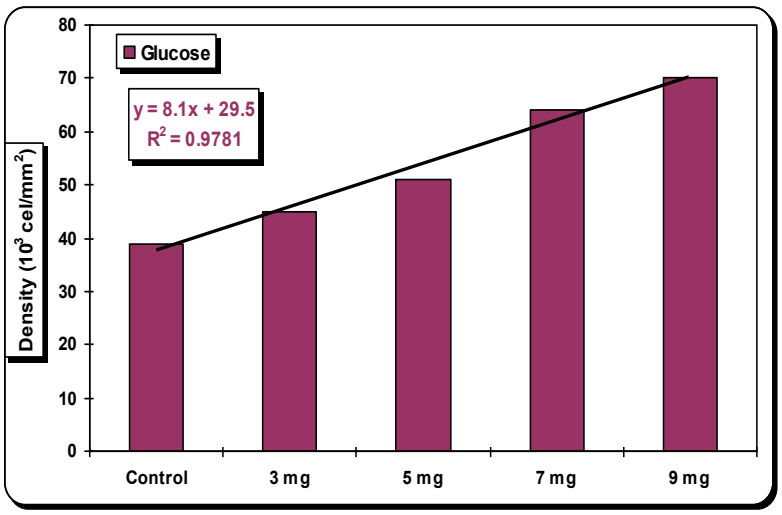


Figure 7.6. Maximum bacterial density after 72 hours in containers with different concentrations of glucose

A small percentage of marine prokaryotes are able to use complex carbohydrates such as polysaccharides (starch), prokaryotes are enzymes (polizaharidaze) in order to divide these compounds, as starch source of energy is not as fast as glucose microorganisms .

The control sample without additional starch total bacterial density was 40 · 10^3 cells/mm^2. Supplementing the seawater with a small amount of starch of 3 mg/L, and the amount of influence the metabolism of the bacterial cell density was 43 ·10^3 cells/mm^2. The use of a higher concentration of 5 mg/L of starch resulted in a total density of 50 · 10^3 cells/mm^2. The increase was even greater in the case of supplementing with 7 mg/L of starch, such that the bacterial density was 58 ·10^3 cells/mm^2, so that at increasing amount of 9 mg/L to 67 · 10^3 cells/mm^2 (figure 7.7).

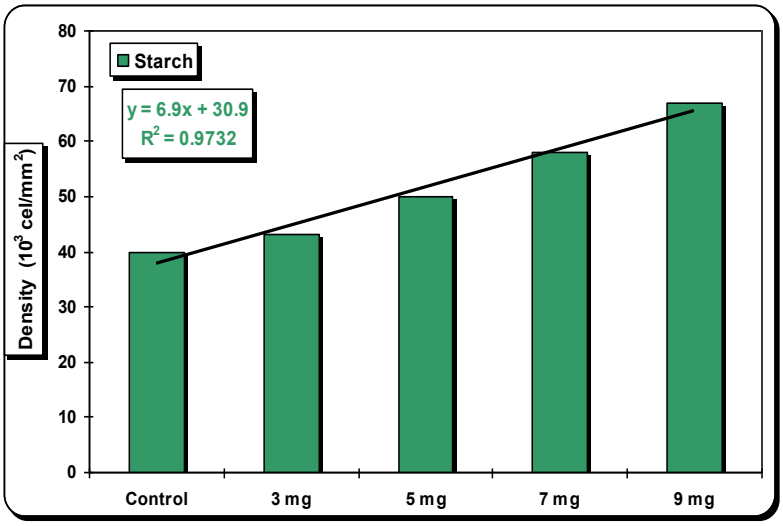


Figure 7.7. Maximum bacterial density after 72 hours in containers with different concentrations of starch

On the basis of the values of cell density differences can be observed between the control sample and supplemented with glucose as an additional 3 mg/L resulted in a difference of 6,9 · 10^3 cells/mm^2 and prokaryotic increase of 15.3% from The initial value of cell density on the surface, addition of 5 mg/L results in a difference of 12 · 10^3 cells/mm^2 in an amount of 30.7% of the original value. For samples supplemented with an amount of glucose to 7 mg/L difference was 25 · 10^3 cells/mm^2, representing 64.1% of the initial growth when added to 9 mg/L, the difference was 17,6 · 10^3 cells/mm^2 which resulted in an increase in cell density over 79.4% of baseline at 39 · 10^3 cells/mm^2.

The slope of the increase in cell density was over 8.1 for glucose and 6.9 for starch biofilms formed and the ratio R was as 0.97.

Yeast extract is a water-soluble product containing a high proportion of B vitamins in addition to the other components and is used as an excellent bacteriological stimulating bacterial growth, but we obtained values were much lower than those obtained with other types of organic matter in seawater added.

The control sample without additional yeast extract, total bacterial density is 42,8 · 10^3 cells/mm^2. Subsequently seawater supplemented with a small amount of 3 mg/L of yeast extract, influence the bacterial metabolism, and the density value was 42,8 · 10^3 cells/mm^2. The use of a higher concentration of 5 mg/L results in the total density of 48,9 · 10^3 cells/mm^2. The increase was even greater in the case of supplementing with 7 mg/L of extract of yeast, such as bacterial density was 53,5 · 10^3 cells/mm^2, so that at a quantity of 9 mg/L density is from 83,2 · 10^3 cells/mm^2 (figure 7.14).

On the basis of the values of cell density differences can be observed between the control sample and supplemented with yeast extract, so an additional 3 mg/L resulted in a difference of 2,5 · 10^3 cells/mm^2 and an increase in prokaryotes by 22.3% of the The initial value of cell density on the surface, while the addition of 5 mg/L resulted in a difference of 9,2 · 10^3 cells/mm^2 in an amount of 36.2% of the original value. For samples supplemented with a large amount of 7 mg/L, the difference was 13,8 · 10^3 cells/mm^2, with an increase in the amount of 68.12% of the original, and in the case of the addition of 9 mg/L, difference to reach 31 · 103 cells/mm2, this leading to increased cell density than 101.7% of the initial value of 26,8 · 10^3 cells (figure 7.8).

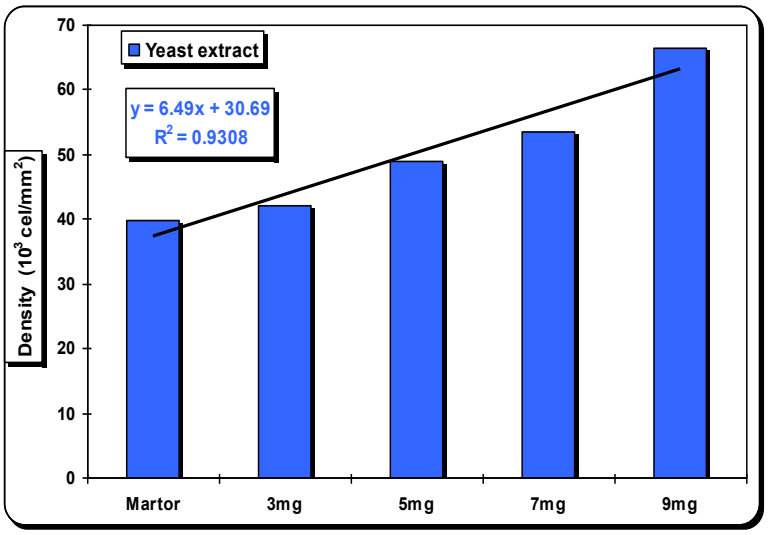


Figure 7.8. Maximum bacterial density after 72 hours in containers with different concentrations of yeast extract

The progression of the cell density increase was more than 6.49 to biofilms formed and the ratio R was 0.93.

The data revealed that statistically total bacterial density following supplementation with organic substances was 25,8 · 10^3 cells/mm^2 in the mixture of amino acids, 24,9 · 10^3 cells/mm^2 for tryptone and 21,1 · 10^3 cells/mm^2 for yeast extract. If the addition of carbohydrates bacterial density was 23 · 10^3 cells/mm^2 for glucose and starch 21,8 · 10^3 cells/mm^2.

7.3 The influence of the surfaces

Generally any material in direct contact with various liquids containing bacteria may become a substrate for biofilm formation. The surfaces may have some chemical and physical characteristics that affect the rate and extent of adhesion of microorganisms to the

substrate. These properties include the available space, hydrophobicity, surface energy, the toxicity of the compound or nutrient character detached from the substrate and a series of physical defects such as grain structure of the surface, recesses, elevations, cracks (Donlan and Costerton 2002). In the biofilm developed on inert substrates, the bacterial cells are more hungry than those fixed to the substrate cell, so death and basal cell autolysis, biofilm thickness gas production contributes to its destabilization and detachment of fragments responsible for the dissemination distance (Lazăr, 2003).

Marine prokaryotes were fixed on the different types of artificial hydrophilic and hydrophobic surfaces under varying conditions of temperature, static or dynamic form biofilms.

The first type of experiment was conducted in order to track changes in cell adhesion to a sampling period of one hour at room temperature (23 °C). Initially hydrophilic glass surface just one hour immersion increase is $1,67 \cdot 10^3$ cells/mm^2, followed three hours bacterial density reached the value of $3,05 \cdot 10^3$ cells/mm^2 and after a six-hour increased to reach a value of $4,86 \cdot 10^3$ cells/mm^2 (figure 7.25).

On the surface hydrophobic parafilm initial bacterial density reached the value of $2,59 \cdot 10^3$ cells/mm^2 after a four-hour period leading to the value of $5,07 \cdot 10^3$ cells/mm^2, and the interval of six hours harvest, reaching a value of $6,08 \cdot 10^3$ cells/mm^2 (figure 7.9).

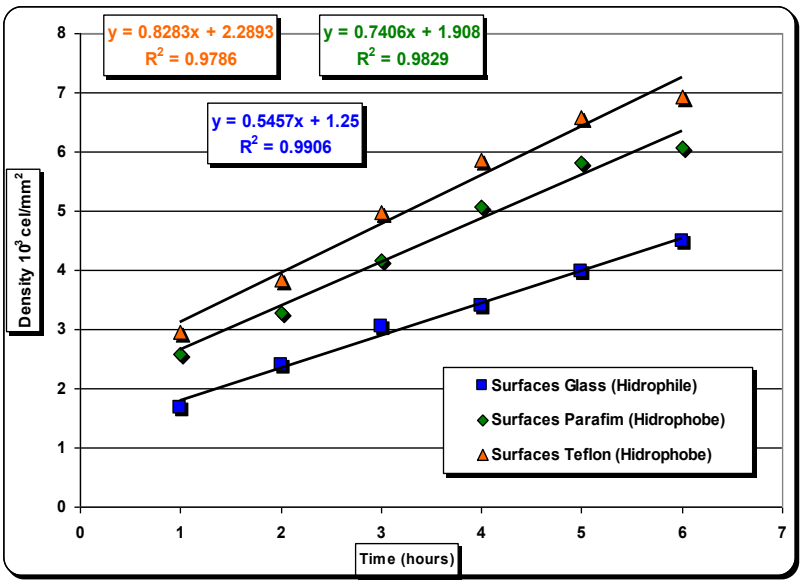


Figure 7.9. Dynamics of bacterial density during biofilm formation in containers (containers with a circuits) on hydrophilic surfaces (glass) and hydrophobic (parafilm, Teflon)

In the case of bacterial density PTFE film has reached the initial value of $2,96 \cdot 10^3$ cells/mm^2, so that after a four-hour period to experience a doubling of the bacterial growth in the amount of $4,5 \cdot 10^3$ cells/mm^2.

The linearity coefficient values (R) were between 0.99 and 0.97, which means a progressive linear increase, the slope was 0.54 in the case of glass, of 0.74 to 0.82, respectively parafilm and for PTFE, the values showing a variation of the density values of the three kinds of surfaces.

To observe the effect of temperature was performed a second experiment at a constant temperature lower than 18 °C, and the period of harvest was up to eight hours after the immersion of the hydrophilic and hydrophobic surfaces.

In the case of prokaryotes glass surface has a first density of $1,11 \cdot 10^3$ cells/mm^2, so that after a four-hour period appears to double the number of bacterial cells adhered to $2,89 \cdot 10^3$ cells/mm^2 and after a six-hour shows a threefold increase from baseline to $3,88 \cdot 10^3$ cells/mm^2, followed by eight hours a density reaches $4,86 \cdot 10^3$ cells/mm^2. This point is close to the value obtained at 23°C, the period of six hours (figure 7.10).

Parafilm surface temperature under constant initially observed an increase in density of $1,79 \cdot 10^3$ cells/mm^2 after an interval of three to four hours when there is a doubling of the bacterial growth reaches $4,12 \cdot 10^3$ cells/mm^2 to show a threefold increase in the amount of $5,85 \cdot 10^3$ cells/mm^2, and the value obtained by the eight range $6,28 \cdot 10^3$ cells/mm^2 again be close to that of the period of six hours for experiments at room temperature (figure 7.10).

Teflon film presented the highest density values after the initial value of $2,14 \cdot 10^3$ cells/mm^2 in a doubling of it after an interval of four hours at the value of $4,83 \cdot 10^3$ cells/mm^2 and tripled in a span of seven hours of harvest to the value of $6,57 \cdot 10^3$ cells/mm^2, followed eight hours after bacterial density to reach $7,18 \cdot 10^3$ cells/mm^2, close to the range of six hours $5,86 \cdot 10^3$ cells/ mm^2 at 23 ºC (figure 7.10).

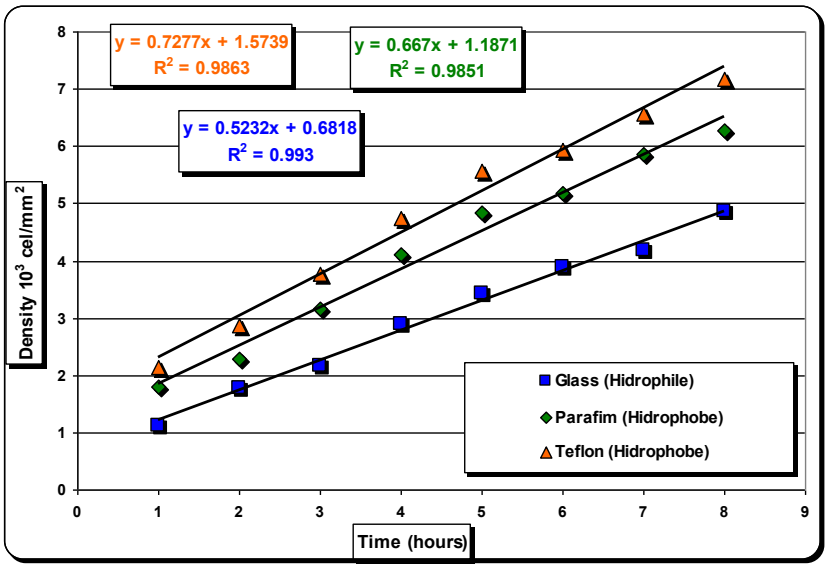


Figure 7.10. Dynamics of bacterial density in the biofilm formation at 18 ° C in containers (storage boxes) on hydrophilic surfaces (glass) and hydrophobic (parafilm, Teflon)

Under conditions of constant temperature, the linearity coefficient (R) was between 0.98 and 0.99 which shows a linear increase gradual slope being 0.52 for glass, 0.66 for respectively parafilm 0.72 for Teflon, these values showing steady slope between the three types of surfaces, a factor influencing the temperature.

Araujo et al. (2004) investigated anaerobic biofilms formed on the surface of the hydrophobic and hydrophilic polypropylene bottle within the modified Robbins Device (DRM).

After the first day of growth the bacterial biofilm is formed on the surface of $4{,}6 \cdot 10^4$ cells/mm^2 polypropylene, compared with the glass surface where the biofilm formed had a density of $8{,}2 \cdot 10^3$ cells/mm^2. However, after nine days, the values were similar at $6{,}3 \cdot 10^5$ cells/mm^2 for polypropylene and $7{,}2 \cdot 10^5$ cells/mm^2, where glass. The data obtained show that biofilms are not influenced by the hydrophobicity major where to obtain them by using the Robbins (DRM) - (Araujo et al., 2004).

Colonization experiments were performed on surfaces with Bdellovibrio bacteriovorus using as substrate shell valves, glass and polystyrene (Kelley et al.., 1997). These authors found a positive correlation for Factor temperature and the formation of biofilms, with a joint maximum of cells in biofilms at 18 ° C and a minimum of 14 ° C as well as a significant decrease in the density of below 5 ° C, over a period of 24 hours , followed by a progressive increase in the density value after 120 hours from the start of the experiments. The values obtained showed an increase in cells adherent to the value of $,1 \cdot 10^5$ CFU/cm^2 la $1{,}4 \cdot 10^5$

CFU/cm^2 the shell valves, 1,7 · 10^3 CFU/cm^2 şi de 1,8 · 10^4 CFU/cm^2 for glass and 5,4 · 10^3 CFU/cm ^{2} şi de 1,0 · 10^4 CFU/cm^2 for polyethylene (Kelley et al.., 1997).

Chavez et al. (2002) analyzed the influence of different types of surfaces using MCDB 202 medium as a supplement and obtained values of 10^7 CFU/cm2 after two hours of contact with the surfaces studied prokaryotes. Initially, at 37 ° C on the surface of stainless steel was obtained from 0.2 to 0.6 log units, and the surface of the Teflon at 20 ° C values were increased from 0.4 to 0.5 unit. log, except the temperature of 8 ° C on Teflon surface, the number of prokaryotic was increased (from 0.4 to 1.4 units. log). The maximum values of the adhesion have been attained by one, two, five or seven days for the two surfaces at 37 ° C, 20 ° C and 8 ° C. At 37 ° C and 8 ° C there is a growing evident from the teflon surface of stainless steel.

In the first 48 hours at 20 ° C was observed exactly the opposite, and after this time the teflon prokaryotic population decreases and that of the stainless steel increased slightly. At lower temperatures there is a reduction in the population of prokaryotes obvious adherent Teflon surface (Chavez et al., 2002).

In terms of inoculation of prokaryotic or stationary phase lag at varying temperatures, no differences were observed in the ability of colonization, but there are clear differences between hydrophilic and hydrophobic surfaces. Bacterial density was 108 CFU/cm2 for stainless steel at a temperature from 15 ° C to 37 ° C, and after 48 hours the increase is not below 107 CFU/cm2. Teflon surface density is higher than CFU/cm2 108 stainless steel at a temperature between 18 ° C and 30 ° C, for 24 hours to four days. After this period appear slightly lower values for these temperatures (Cerca et al., 2005).

With the marine environment, water dynamics is an extremely important phenomenon influencing bacterial adhesion. To achieve dynamic conditions in vitro and are used containers with a circuits with water recirculation system is the most appropriate large type of apparatus for laboratory experiments.

Surface analysis showed a progressive bacterial growth, but density values obtained were much lower than those obtained under static conditions at room temperature due to continuous flow of seawater.

On the surface of the glass, initially, there is a density value of 0,85 · 10^3 cells/mm^2, followed after an interval of two to three hours may experience a doubling of growth in the amount of 1,87 · 10^3 cells/mm^2 and after four hours interval appears to triple the original amount to 2,74 · 10^3 cells/mm^2, as six hours between bacterial density value is 3,76 · 10^3 cells/mm^2 (figure 7.11).

If there is an initial increase in parafilm 1,64 · 10^3 cells/mm^2, after a three-hour interval there is a doubling in the amount of 4,01 · 10^3 cells/mm^2, and after the period of six hours to reach the value of 5,02 · 10^3 cells/mm^2 (figure 7.11).

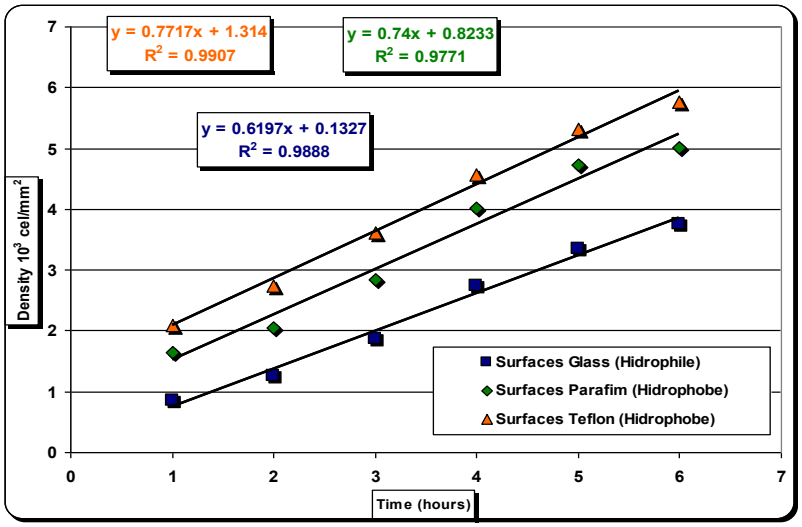


Figure 7.11. Dynamics of bacterial density during biofilm formation in containers (containers with a circuits) on hydrophilic surfaces (glass) and hydrophobic (parafilm, Teflon)

Teflon film presented the highest values of density from an initial value of 2,1 · 10^3 cells/mm^2 in a doubling of it after an interval of four hours at the value of 4,56 · 10^3 cells/mm^2 and tripled in a span of seven hours of harvest to the value of 5,76 · 10^3 cells/mm^2 (figure 7.11).

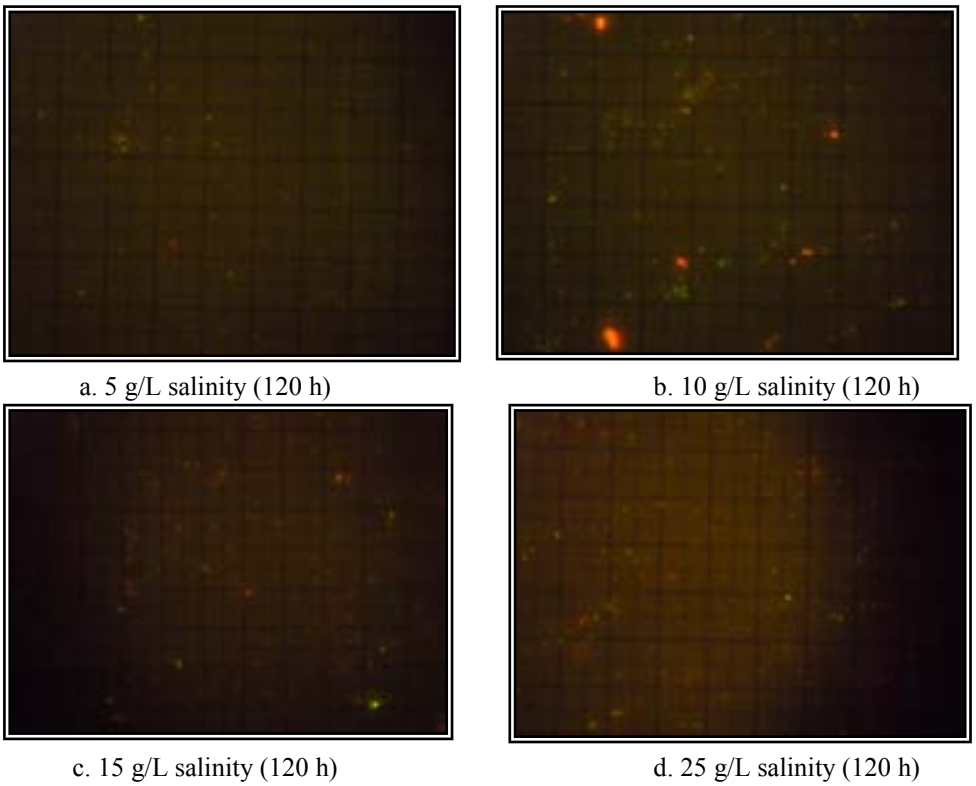
In dynamic conditions (containers with a circuit) linearity coefficient (R) was between 0.98 and 0.99 which shows a linear increase progressively as the slope of 0.61 for glass, 0.74 for parafilm and 0.77 respectively for PTFE, these values showing steady slope between the three types of surfaces.

THE DYNAMICS OF BIOFILMS FORMATION AT THE AIR - LICHID INTERFACE

Air-liquid interface provides different experimental conditions, this is a hydrophilic and hydrophobic properties interface, surface tension, ventilation high concentrations of certain nutrients and often there are large amounts of radiation. Organisms that live in this niche have adapted to these conditions and characteristics which allow to populate these areas (Henk, 2003).

8.1.1 The influence of salinity on bacterioneuston

The biofilms formed were analyzed in monolayer structure, ideal for epifluorescence microscopy analysis and this bacterioneuston formation in vitro is a complex process that can be affected by the modification of physico-chemical parameters with major influence on the migration of planktonic bacteria to the interface liquid air.



a. 5 g/L salinity (120 h) b. 10 g/L salinity (120 h)

c. 15 g/L salinity (120 h) d. 25 g/L salinity (120 h)

Figure 8.1 Epifluorescence microscopy images concerning bacterioneuston formed in different salinity conditions (Large square 50μm^2)

The analysis of the sample surface an increase in bacterial cell density of the bacterioneuston structure in the early stages after experimental salinity and temperature variation under laboratory containers.

In figure 8.1 are shown some pictures of the bacterioneuston formed after 120 hours from the experiment initiation in laboratory containers with different salinities.

In the beginning of our experiments with low salt concentration in the sample after two hours of immersion in the number of bacteria is reduced and the period of 120 hours after the bacteria have a high density. The cell density was high when the salinity of 25 g/L and the temperature of 23 °C.

Dynamics of cell density is shown in figure 8.2 ascertaining the following: At low salinity 5 g/L of the seawater after two hours the density was $0,82 \cdot 10^2$ cells/mm^2 and after 24 hours the density was $3,27 \cdot 10^2$ cells/mm^2, while a peak is observed cell growth of $4,12 \cdot 10^2$ cells/mm^2, 72 hours after the density was $6,24 \cdot 10^3$ cells/mm^2 (figure 8.2).

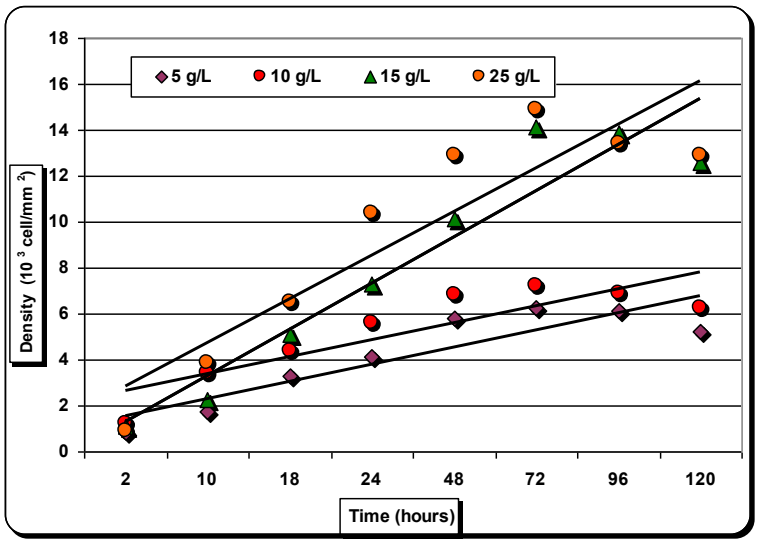


Figure 8.2. The dynamics of bacterial density during the formation of the containers bacterioneuston (storage box) with seawater at a salinity variable (5 g/L, 10 g/L, 15 g /L and 25 g/L)

At the microscopic level with an average salinity of 10 g/L of seawater density of $1,22 \cdot 10^3$ cells/mm^2 (after two hours), the density was $4,37 \cdot 10^3$ cells/mm^2 (after 18 hours) of $5,62 \cdot 10^3$ cells/mm^2 (24 hours), you can see a peak of cell growth, then the density was $7,24 \cdot 10^3$ cells/mm^2 (72 hours) (figure 8.2).

The linearity coefficients were lower at 48 hours salinity of 0.98 to 5 g/L, and in case it changes the value of 10 g/L ratio was 0.97. The value of y was lower slope of 1.15 to 2.59.

At another average value of salinity of 15 g/L of seawater density of $1,12 \cdot 10^3$ cells/mm^2 (after two hours), the density was $5,11 \cdot 10^3$ cells/mm^2 (after 18 hours) , when cell growth observed peak $7,29 \cdot 10^3$ cells/mm^2 (24 hours), the density is from $7,24 \cdot 10^3$ cells/mm^2 (72 hours) (figure 8.2).

At high salinity of 25 g/L of seawater is observed after two hours a density of $0,92 \cdot 10^3$ cells/mm^2, 18 hours density was $6,4 \cdot 10^3$ cells/mm^2, while a peak is observed cell growth of $10,39 \cdot 10^3$ cells/mm^2, 72 hours later density was $14,90 \cdot 10^3$ cells/mm^2 (Figure 8.2).

Linearity coefficients R were lower by 0.96 to changing salinity at 15 g/L The value of y slope was low (2.62) to (2.88) to salinity of 25 g/L in 48 hours.

8.1.2 The influence of temperature on bacterioneuston

The seawater is observed after two hours a density of 0,67 · 10^2 cells/mm^2, 18 hours density was 2,46 · 10^2 cells/mm^2, you can see a peak of cell growth 2,99 · 10^2 cells/mm^2, 72 hours later density was 4,07 · 10^2 cells/mm^2 (figure 8.3).

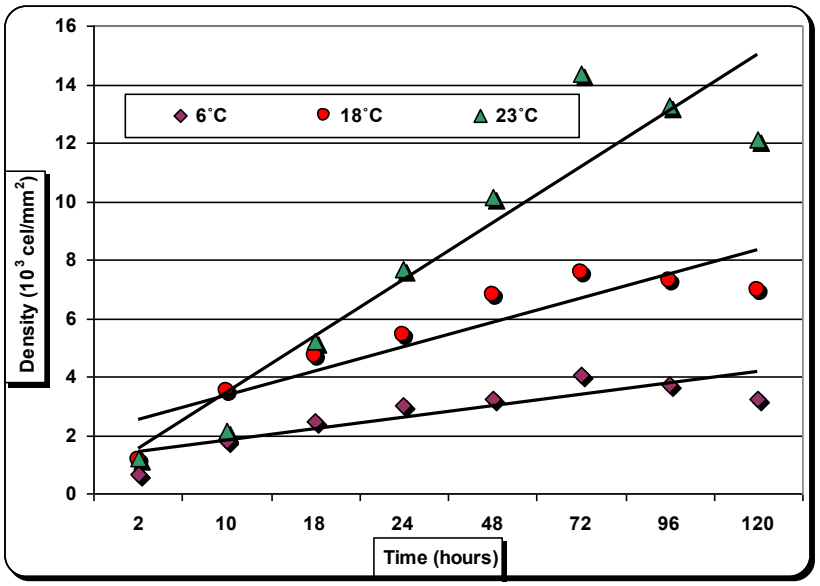


Figure 8.3. The dynamics of bacterial density during the formation of the containers bacterioneuston (storage box) with seawater at a salinity variable (5 g/L, 10 g/L, 15 g/L and 25 g/L)

At the average temperature seawater of 18 ºC is observed after two hours a density of 1,7 · 10^3 cells/mm^2, 18 hours density was 4.73 · 103 cells/mm2, you can see a peak of cell growth 5,42 · 10^3 cells/mm^2, 72 hours later density was 5,42 · 10^3 cells/mm^2 (figure 8.3).

For the high seawater temperature of 23 ºC is observed after two hours a density of 1,20 · 10^3 cells/mm^2, after 18 hours the density was of 5,23 · 10^3 cells/mm^2, you can see a peak of cell growth 7,69 · 10^3 cells/mm^2, after 72 hours density was 14,33 · 10^3 cells/mm^2 (figure 8.3).

Linearity coefficients R were lower at 48 hours of 0.84 and under changing temperature 6 º C, compared with values of 0.97 and 0.96 for temperatures of 18 º C and 23 º C. The value of y was lower slope of 0.37, compared to 1.21 and 2.63 respectively.

Under natural conditions (Mediterranean) Agogué et al. (2005) observed the waters surface area of Barcelona a Microstream (SML) with a density of 1,43 · 10^6 and 1,30 · 10^6 cells ml^{-1}, respectively (n = 10). The area Banyuls sur Mer was observed values of 0.93 · 10^6 cells ml^{-1}respectively (n = 13). These values demonstrate the existence of differences with higher values to stop Barcelona from Banyuls-sur-Mer between the analyzed layer in the immediate

vicinity of the interface layer of seawater. Determining the number of viable cells in agar culture showed a higher capacity at the upper interface. The water from the Barcelona this proportion was 4.16% and 2.51%, while in the Banyuls sur Mer 1.58% and 0.34%, thus the percentage of prokaryotic cultivation was increased in the surface layer Barcelona and both parts of the study (Agogué et al., 2005).

Table 8.1. The dynamic density of dead cells (membrane degraded) of bacterioneuston formed at different salinities and temperatures (10^3 cells/mm^2)

T (ore)	5 g/L	10 g/L	15 g/L	25 g/L	6ºC	18ºC	23ºC
2	0,12	0,5	0,2	0,3	0,2	0,45	0,21
10	0,31	0,71	0,77	0,87	0,5	0,77	0,77
18	0,57	1,37	1,11	1,14	0,9	1,14	0,98
24	0,62	1,62	1,32	1,32	1,12	1,32	1,49
48	0,81	1,89	2	1,85	1,39	1,85	1,15
72	1	2,04	2,14	2,21	1,07	1,14	2,33
96	1,12	2,5	2,42	2,45	1,42	1,28	2,29
120	1,33	2,8	3	3,16	1,62	1,56	2,89

Consistent with experiments in the literature (Agogué et al., 2005) we determined the number of propidium iodide-permeable cells, cells with impaired plasma membrane structural and functional considered dead cells. The following table shows the format bacterioneuston dead cell densities at different salinities and at different temperatures.

The personal data of the densities of dead cells (degraded membrane permeable to propidium iodide) in the experimental samples at different salinity and temperature are presented in table 8.1 for different experimental periods. These values are lower than those in Figures 8.1 - 8.8, where the total density values shown include both live cells and dead ones. Higher density of dead cells after 72 hours compared to values obtained in early experiments suggesting the importance of this type of cell in the structure of marine bacterioneuston.

8.2. The influence of organic substances on bacterioneuston

Supplementing the seawater with organic substances has thermodynamically predictable effect is affected by the temperature and the thermal conversion in the interface. The types of

organic materials used can be set depending on the concentration and quantity. Possible effect of organic substances can be analyzed based on the phenomenon of capillarity, by disrupting and stopping the transfer of gas (CO2, O2, N2) at the interface level, the suspension of bubbles forming and generating organic particles aggregate. The formation of these aggregates may influence the presence of metal ions at the interface (Lion and Leckie, 1981 Carlucci et al., 1991).

Regardless of the type of material used, the organic components of the air-liquid interface has a unique chemical composition and are substances with a high concentration that allows to form metal compounds such as organic matter. Of the metal ions present in such compounds may be mentioned those of Cu, Ni, Pb and Fe (Lion and Leckie, 1981, Wurl and Holmes, 2008).

Bacterial growth showed the influence of organic matter compared with the control. Thus, at the beginning of the experiment the control sample, two hours after the bacteria have a lower density compared with data obtained at 120 hours, the bacteria are of higher density than the most significant increase was observed with the addition of high concentrations of peptone.

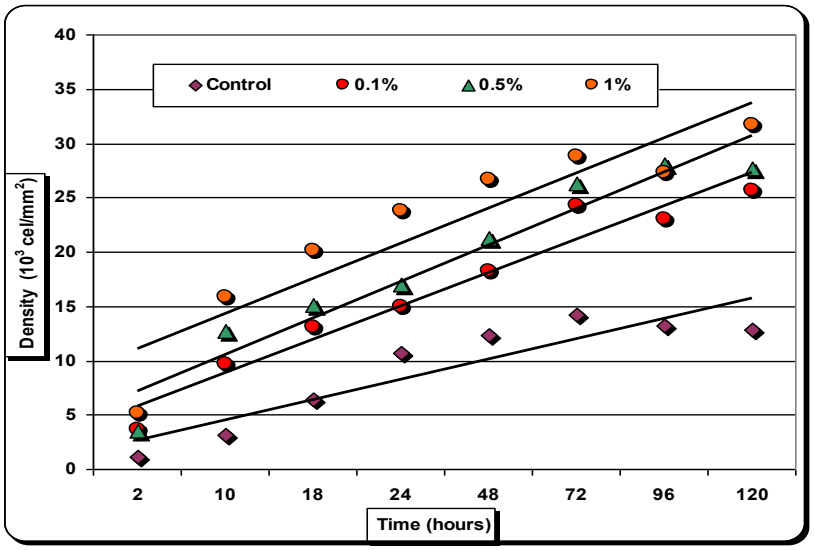


Figure 8.4. Dynamics of bacterial density in containers during training bacterioneuston (storage boxes) seawater supplemented with peptone (0.1%, 0.5%, 1%)

The dynamics of cell density bacterioneuston supplementation formed in the seawater with varying amounts of peptone is shown in the figure 8.5 observing the following:

In the control sample (without addition of organic matter) after two hours of harvesting bacterial density was 1,12 · 10^3 cells/mm^2, 18 hours reaches 6,40 · 10^3 cells/mm^2 after 24

hours from 10,70 · 10^3 cells/mm^2, so that at 72 hours the cell density reaches to 14,20 · 10^3 cells/mm^2 (figure 8.5).

In the case of the addition of low concentrations of peptone, 0.1%, after two hours of harvesting bacterial density was 3,60 · 10^3 cells/mm^2, 18 hours reaches 13,10 · 10^3 cells/mm^{2}and after 24 hours at 15,00 · 10^3 cells/mm^2, followed 72 hours to reach a value of 15,00 · 10^3 cells/mm^2 (figure 8.5).

In the container with peptone 0.5%, bacterial density was 3,50 · 10^3 cells/mm^2 (after two hours), reaching a value of 15,10 · 10^3 cells/mm^2 after 18 hours and at 17,70 · 10^3 cells/mm^2 (after 24 hours), followed 72 hours to reach a value of 27,65 · 10^3 cells/mm^2 (figure 8.5).

In container with peptone 1%, after harvest bacterial density was 5,20 · 10^3 cells/mm^2 (after two hours), reaching a value of 20,10 · 10^3 cells/mm^2 after 18 hours and at 23,80 · 10^3 cells/mm^2 (after 24 hours), followed 72 hours to reach a value of 28,80 · 10^3 cells/mm^2 (figure 8.5). The coefficient was low of 0.83 at 48 hours, in the case of addition of organic substances 0.95. Also, the slope of y was 3.06 compared to control sample of 1.86.

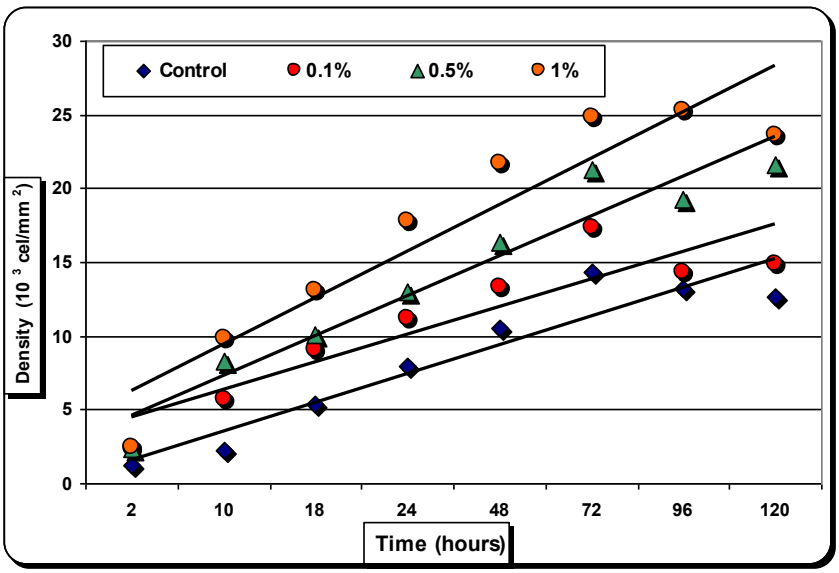


Figure 8.5. Dynamics of bacterial density in containers during training bacterioneuston (storage boxes) seawater supplemented with gasoline (0.1%, 0.5%, 1%)

The linearity coefficients R were lower at 48 hours of 0.97, and for 0.1% peptone supplementation compared with values of 0.94 and 0.91 for supplementation with 0.5% and 1%. The value of y was lower slope of 3.74, compared to 4.04 and 4.41 respectively. As can be seen in figure 8.6 are differences between the structure of the dynamics of cell density in the containers bacterioneuston varying concentrations of gasoline.

The control sample (without addition of organic matter) after two hours of harvesting

bacterial density was 1,24 · 10^3 cells/mm^2, 18 hours reaches 5,40 · 10^3 cells/mm^2 after 24 hours to 7,90 · 10^3 cells/mm^2, followed 72 hours to reach a value of 14,30 · 10^3 cells/mm^2 (figure 8.6).

In the case of the addition of low levels of gasoline of 0.1%, after two hours of harvesting bacterial density was 9,10 · 10^3 cells/mm^2, 18 hours reaches 13,10 · 10^3 cells/mm^2 and after 24 hours at 11,22 · 10^3 cells/mm^2, followed 72 hours to reach a value of 17,30 · 10^3 cells/mm^2 (figure 8.6).

In the container of gasoline of 0.5% bacterial density was 2,30 · 10^3 cells/mm^2 after two hours of harvesting, reaches 10,1 · 10^3 cells/mm^2 after 18 hours at 13,0 · 10^3 cells/mm^2 after 24 hours, so that at 72 hours to reach a value of 21,30 · 10^3 cells/mm^2 (figure 8.6).

In the case of the addition of high concentrations of gas of 1% after two hours of harvesting bacterial density was 2,51 · 10^3 cells/mm^2, 18 hours reaches 13,10 · 10^3 cells/mm^2 and after hours at 24 de ore la 17,80 · 10^3, followed 72 hours to reach a value of 24,80 · 10^3 cells/mm^2 (figure 8.6).

The linearity coefficients were lower than 0.99, and in the case of supplementing fuel by 0.1% compared to values of 0.97 and 0.98 for the supplementation of 0.5% and 1%. The value of y was lower slope of 2.85, compared to 3.78 and 4.33 to 48 hours respectively (figure 8.7).

Cunliffe et al. conducted experiments in 2009 in containers with water from the fjord, which was supplemented with determinable ingredient (16mm $NaNO_3$ and 1 mM KH_2PO_4) for a period of 11 days. Monitoring of the samples was made every day, but significant samples were collected at the beginning of the experiment in five days and ten days after the end of the experiment.

Sample analysis was performed by two methods, flow cytometry and PCR assay (Q-PCR) of bacterial 16S gene Rarn. Samples were stained with SYBR Green, and bacterial density analysis of samples older than five days immersed in seawater was between 2,8 · 10^5 and 8,0 · 10^5 cells mL^{-1} (ie 5.5 · 10^5) in samples from control containers and between 1,1 · 10^5 and 9,6 · 10^5 cells mL^{-1} (ie 6.6 · 10^5) in containers with added nutrients. On the tenth day there was a significant increase in the coefficient containers with organic extra (P = 0.004) for cells in containers with added nutrients, with a value of 7.6 · 10^5 to 1.8 · 10^6 cells mL^{-1} (ie 1.4 · 10^6), but not in the control sample with a value of 5.1 · 10^5 to 8.9 · 10^5 cells mL^{-1} (ie 6.9 · 10^5) (v. Cunliffe et al., 2009).

Bacterial density in the surface layer is the same way as the increase of the samples taken from the immediate vicinity of the surface layer. Changing the density of bacterial cells from the surface layer more older than five days immersion in seawater was between 5 · 10^5 –7,1 ·

10^5 cells mL^{-1} (ie $6 \cdot 10^5$) in the control samples container and between $8 \cdot 10^5$ –$1{,}9 \cdot 10^6$ cells mL^{-1} (ie $7.7 \cdot 10^5$) in containers with nutrient supplement. With the growth of bacteria in the samples after the tenth day there was a significant increase in the coefficient additional containers with organic ($P = 0.001$) for cells in containers with added nutrients, worth $8 \cdot 10^5$ –$1{,}9 \cdot 10^6$ cells mL^{-1} (i.e. $1.5 \cdot 10^6$), but not in the control sample with a value of $4 \cdot 10^5$–$1 \cdot 10^6$ cells mL^{-1} (i.e. $6.1 \cdot 10^5$). Bacterial density in the surface layer was less than $1{,}4 \cdot 10^5$ cells mL^{-1} (Cunliffe et al. 2009).

The number of heterotrophic bacteria from the surface layer (CFU 22 ˚ C) was 2.0 - 13.3 times greater than the number of bacteria in the adjacent layer ($P < 0.001$), where the number of cells was between 1.48 and $12.52 \cdot 10^3$ cells ml^{-1}, while CFU ranged between 0.35 and 0.94 $\cdot 10^3$ cells ml^{-1} according to the observations of Kalwasińska and Donderski (2005).

Studies have shown that the number of bacteria in the surface layer is higher than that of the adjacent layer was observed that bacteria of Apine (1989) as well. The Kalwasińska and Donderski (2005), Maki and Herwig (1991) and Donderski et al. (1998), Mudryk et al. (1999). The increased number of bacteria in the surface layer is favored by increased nutrient concentration in this layer which acts as a stimulant for bacteria at this level (Kalwasińska and Donderski, 2005).

Walczak of a study carried out in 2008 on the bacterial cell density in the surface layer and the adjacent layer by analyzing samples of water from the sterile recipinte samples were collected from 10 ml. Analysis of samples targeted total number of bacteria (TNB), the number of metabolically active bacteria (TNAB) dehydrogenase activity, the rate of protein and DNA synthesis. Samples were filtered, colored with Acridine orange and analyzed by direct quantification methods epiluorescenţă microscope

Change values (TNAB) after 24 hours were higher than those obtained for (NTB). May average values of TNAB, during the day, the surface was microstratul $8{,}35 \cdot 10^6$ cells cm^{-3}, while nocturia during reached a value of $17{,}14 \cdot 10^6$ cells cm^{-3}. Cellular metabolism values were higher during the month of July (Walczak, 2008).

CONCLUSIONS

1. Biofilm formation on the glass in seawater is rapidly at the salinities of 10 g/L and 15 g/L similar to natural salinity medium (17 g/L) than at 5 g/L. After 12 hours the cell density in

the biofilm formed from 5 g/L, 10 g/L and 15 g/L were 13,4 · 10^2 cells/mm^2, cells/mm2 18,7 ·10^2 cells/mm^2 and 26,7 · 10^2 cells/mm^2 respectively.

2. Biofilm formation on glass is fastest at the temperature of 18 ºC than at 6 ºC, after 12 hours the cell density in biofilms were 26,7 · 10^3 cells/mm^2 and 17,2 · 10^2 cells/mm^2 respectively.

3. Biofilm formation on glass is faster when seawater is kept under static conditions compared to the experimental water circulation by pumps, the cell density in biofilms after 12 hours were 21,2 · 10^2 cells/mm^2 and 17,4 · 10^2 cells/mm^2 respectively.

4. Supplementing the seawater with pure organic substances in a concentration of 0.1% after 72 hours led to an increase in cell density at 30,9 · 10^4 cells/mm^2 biofilm, in the case of mixtures of amino acids, 28,85 · 10^4 cells/mm^2, in the case of tryptone, 26,9 · 10^4 cells/mm^2 in the case of glucose, 25,8 · 10^4 cells/mm^2, in the case of starch and 25,8 · 10^4 cells/mm^2, , in the case of yeast extract compared with the control probe to which the cell density was 39,5 · 10^3 cells/mm^2 respectively.

5. Biofilm formation on the hydrophilic support (glass) is slower than the hydrophobic support (parafilm and Teflon) after eight hours of the initiation of the experimental density of cells in the biofilm are 1,70 · 10^5 cells/mm^2 (glass) as compared to the density of three, 3,35 · 10^5 cells/mm^2 (parafilm) and 3,88 · 10^5 cells/mm^2 (Teflon).

6. The bacterioneuston formation is faster in seawater with salinity closer (15 g/L and 25 g/L) similar with the natural salinity of the Black Sea (17g/L) than 10 g/L and 5 g/L. After 72 hours the cell density in the biofilm formed at 15 g/L (14,10 · 10^3 cells/mm^2) and 25 g/L (14,90 · 10^3 cells/mm^2) are higher than the results obtained in the salinity of 5 g/L (66,24 · 10^2 cells/mm^2) and 10 g/L (7,24 · 10^3 cells/mm^2).

7. The bacterioneuston formation is faster at 23 ºC or 18 ºC than at 6 °C after 72 hours the cell density in biofilms was 14,33 · 10^3 cells/mm^2, 7,56 · 10^3 cells/mm^2 respectively 0,40 · 10^3 cells/mm^2.

8. Dead cell density increases with time (from 24 - 72 hours) in both the bacterioneuston formed on the salinity of 5 g/L ($1 \cdot 10^3$ cells/mm^2), 10 g/L ($2{,}04 \cdot 10^3$ cells/mm^2) 15 g/L ($2{,}14 \cdot 10^3$ cells/mm^2), 25 g/L ($2{,}21 \cdot 10^3$ cells/mm^2) and the bacterioneuston formed at different temperatures 6 °C ($1{,}07 \cdot 10^3$ cells/mm^2) 18 ºC ($1{,}14 \cdot 10^3$ cells/mm^2) and 23 ºC ($2{,}33 \cdot 10^3$ cells/mm^2).

9. Peptone supplementation in concentrations of 0.1%, 0.5% and 1% determined after 72 hours of initiation of experiments an increase in cell density of 103 cells/mm2 · bacterioneuston of $24{,}30 \cdot 10^3$ cells/mm^2, $26{,}30 \cdot 10^4$ cells/mm^2, respectively $24{,}30 \cdot 10^3$ cells/mm^2, $26{,}30 \cdot 10^4$ cells/mm^2 compared to the control probe in the cell density after 72 hours is $14{,}30 \cdot 10^3$ cells/mm^2.

10. The addition of gasoline in concentrations of 0.1%, 0.5% and 1% determined after 72 hours from the start of the experiments an increase in cell density up to values $17{,}30 \cdot 10^3$ cells/mm^2, $21{,}30 \cdot 10^3$ cells/mm^2 and respectively $24{,}80 \cdot 10^4$ cells/mm^2, compared with the control probe in which cell density after 72 hours was $14{,}20 \cdot 10^3$ cells/mm^2.

RESEARCH PERSPECTIVES

- Determination of the taxonomic structure of marine prokaryotes obtained in vitro biofilm.
- Analysis of biofilms by electron microscopy.
- Use of substances involved in preventing and combating biofilm formation on surfaces.
- Analysis of enzymatic activity in vitro biofilm formed.
- Chemical analysis of exopolysaccharides from biofilm structure formed in vitro.
- The use of physically or chemically modified surfaces for biofilm formation in vitro.
- Carried out experiments on biofilm formation in situ conditions

SELECTIVE REFERENCES

1 . Agogué H., Casamayor E. O., Bourrain M., Obernosterer I., Joux F., Herndl G. J. and Lebaron P., 2005[a] - A survey on bacteria inhabiting the sea surface microlayer of coastal ecosystems, FEMS Microbiology Ecology, 54, pp. 269–280.

2 . Agogué H., Casamayor E. O., Joux F., Obernosterer I., Dupuy C., Lantoine F., Catala P., Weinbauer M. G. Reinthaler T., Herndl G. J. and Lebaron P., 2004 - Comparison of samples for the biological characterization of the sea surface microlayer, Limnology and ***Oceanography***: Methods, 2, pp. 213–225.

3 . Aonofriesei F., 2004 – Studii asupra comunităţilor bacteriene din zona litorală românească (traducerea in limba engleză a tezei de doctorat), Editura ExPonto, pp. 1- 83.

4 . Araujo J. C., Mortara R., Campos J. R. and Vazoller R. F., 2004 - Development and analysis of anaerobic biofilms onto hydrophobic and hydrophilic surfaces, Environmental Technology, 25 (7), pp. 809 – 17.

5 . Burchardt L. and Marshall H. G., 2003 - Algal composition and abundance in the neuston surface micro layer from a lake and pond in Virginia (U.S.A.), Journal of Limnology, pp. 139-142.

6 . Carlucci A. F., Craven D. B. and Wolgast D. M., 1991 - Microbial populations in surface films and subsurface waters: amino acids metabolism and growth, Marine Biology, 108, pp. 329 - 339.

7 . Casse F. and Swain G.W., 2006 - The development of microfouling on four commercial antifouling coatings under static and dynamic immersion, International Biodeterioration and Biodegradation, 57, pp. 179–185.

8 . Cerca N., Pier G.B., Vilanova M., Oliveira R. and Azeredo J., 2005 - Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of Staphylococcus epidermidis, Research in Microbiology, 156(4), pp. 506–514.

9 . Chavant P., Martinie B., Meylheuc T., Bellon-Fontaine M. N. and Hebraud M., 2002 - Listeria monocytogenes LO28: Surface Physicochemical Properties and Ability to Form Biofilms at Different Temperatures and Growth Phases, Applied and Environmental Microbioloy, 68(2), pp. 728–737.

10 . Cho H. J, Jönsson H., Campbell K., Melke P., Williams J. W., Jedynak B. Stevens A. M., Groisman A. and Levchenko A., 2007 - Self-Organization in High-Density Bacterial Colonies: Efficient Crowd Control, Plos Biology, 5(11), pp. 2614-2623.

11 . Cooksey K. E. and Wigglesworth - Cooksey B., 1995 - Adhesion of bacteria and diatoms to surfaces in the sea: a review, Aquatic Microbial Ecology, 9**,** pp. 87- 96**.**

12 . Cunliffe M., Salter M., Mann P. J., Whiteley A. S., Upstill - Goddard R. C. and Murrell J. C., 2009 - Dissolved organic carbon and bacterial populations in the gelatinous surface microlayer of a Norwegian fjord mesocosm, FEMS Microbiol Letters, 299, pp. 248 –254.

13 . De Franca F. P., Ferreira C. A. and Lutterbach M. T. S., 2000 - Effect of different salinities of a dynamic water system on biofilm formation, Journal of Industrial Microbiology and Biotehnology, 25, pp. 45 – 48

14 . Di Bonaventura G., Piccolomini R., Paludi D., D'Orio V., Vergara A., Conter M. and Ianieri A., 2007[a] - Influence of temperature on biofilm formation by Listeria monocytogenes on various food-contact surfaces: relationship with motility and cell surface hydrophobicity, Journal of Applied Microbiology, 104, pp. 1552–1561**.**

15 . Donderski W., Mudryk Z. and Walczak M., 1998 - Utilization of low molecular weight organic compounds by marine neustonic and planktonic bacteria, ***Polish Journal*** of Environmental Studies, 7, pp. 279-283.

16 . Donlan R. M. and Costerton J. W., 2002 - Biofilms: survival mechanisms of clinically relevant microorganisms, Clinical Microbiology Reviews, 15, pp. 167-193.

17 . Eddleman H., 1998 - Optimum Temperature for Growth of Bacteria, Indiana Biolab, 14045 Huff St., Palmyra IN 47164.

18 . Filip M., Pace M. L. and Cole J. J., 1996 - Regulation of planktonic bacterial growth rates: the effect of temperature and resources, Microbial Ecology, 31, pp. 15-28.

19 . Garrett W. D. and Duce R. A., 1980 - Surface microlayer samplers, In: F. Dobson, L. Hasse, R. Davis (Eds), Air-Sea Interaction Plenum, New York, pp. 471- 490.

20 . Gauthier M. J., Flatau G. N. and Clément R. L., 1990 - Influence of phosphate ions and alkaline phoshatase activity of cells on the survival of Escherichia coli in seawater, Microbial, 20, pp. 245-251.

21 . Gauthier M. J., Flatau G. N., Le Rudulier D., Clément R. L. and Combarro Combarro M. P., 1991 - Intracellular accumulation of potassium and glutamate specifically enhances survival of Escherichia coli in seawater, Applied Environmental Microbiology, 57(1), pp. 272- 276.

22 . Goldacre R. J., 1949 - Surface films on natural bodies of water, ***Journal of Animal Ecology***, 18, pp. 36-39.

23 . Hagstrom A., Ammerman J. W., Henrichs S. and Azam F., 1984 - Bacterioplankton growth in seawater: II. Organic matter utilization during steady-state growth in seawater cultures, Marine Ecology Progress Series, 18, pp. 41 – 48.

24 . Hakvåg S., Fjærvik E., Josefsen K. D., Ian E. , Ellingsen T. E. and Zotchev S. B., 2008 - Characterization of Streptomyces spp. Isolated from the Sea Surface Microlayer in the Trondheim Fjord, Norway Marine Drugs, 6, pp. 620 – 635.

25 . Hansen M. L. H. and Sørensen S. J., 2007 - Establishment and early succession of a multispecies biofilm composed of soil bacteria, Microbial Ecology, 54, pp. 352 – 362.

26 . Hansen S. K., Rainey P. B., Haagensen J. A. and Molin S., 2007 - Evolution of species interactions in a biofilm community, Nature, 445, pp. 533-536.

27 . Harvey G. W., 1966 - Microlayer collection from the sea surface: a new method and initial results., Limnology and ***Oceanography***, 11, pp. 608 - 613.

28 . Henk M. C, 2004 - Method for Collecting Air-Water Interface Microbes Suitable for Subsequent Microscopy and Molecular Analysis in Research and Teaching Laboratories, Applied Environmental Microbiology, 70 (4), pp. 2486 – 2493.

29 . Henk M. C., 2003 - Microscopy of natural and experimental biofilms in the undergraduate laboratory: A simple collection kit with multiple applications. Pages 59-75, in Tested studies for laboratory teaching, Volume 24 (M. A. O'Donnell, Editor). Proceedings of the 24th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), pp. 334

30 . Henrici A. T., 1933 - Studies of Freshwater Bactria: I. A Direct Microscopic Technique, Journal of Bacteriology, 25, pp. 277 – 286.

31 . Heukelekian, H., and Heller A., 1940 - Relation between food concentration and surface for bacterial growth, Journal of Bacteriology, 40, pp. 547 – 558.

32 . Hinrichsen E., 2011 - Effect of Temperature on Salinity, The Hub for bright minds, Marine Engineering, pp. 1 – 3.

33 . James G. A., Beaudette L. and Costerton J. W., 1995 - Interspecies bacterial interactions in biofilms, Journal of Industrial Microbiology, 15, pp. 257–62.

34 . Kalwasińska A. and Donderski W., 2005 - Neustonic versus plantonik bacteria in eutrophic lake, Polish Journal of Ecology, 53(4), pp. 571 – 577.

35 . Kelley J. I., Turng B. F, Williams H. N., and Baer M. L., 1997 - Effects of Temperature, Salinity, and Substrate on the Colonization of Surfaces in situ by Aquatic Bdellovibrios, Applied Environmental Microbiology, 63(1), pp. 84 – 90.

36 . Kokare C. R., Chakraborty S., Khopade A. N. and Mahadik K. R., 2009 – *Biofilm*: *Importance and applications*, Indian Journal of Biotechnology, 8, pp. 159-168.

37 . Lawrence J. R., Kotber D. R., Hoyle B. D., Costerton W., Caldwell D. E., 1991 - Optical Sectioning of Microbial Biofilms, Journal of Bacteriology, 173(20), pp. 6558 -6567.

38 . Lazăr V., 2003 - Aderenţa microbiană, Editura Academiei Române, Bucureşti, pp. 1 – 216.

39 . Lewandowski Z. and Stoodley P., 1995 - Flow induced vibrations, drag force, and pressure drop in conduits covered with biofilm, International IAWQ Conference Workshop on Biofilm Structure, Growth, and Dynamics, Noordwijkerhout, The Netherlands.

40 . Lim C. H., Oykanca R., Flint K.P, 1996 - The effect of osmotic stres son survival and alkaline phosphatase activity of Aeromonas hydrophila, FEMS Microbiology Letters, 137, pp. 19-24.

41 . Lion W. L. and Leckie J. O., 1981 - The biogeochemistry of the air-sea interface, Annual Reviews Earth Planet Science, 9, pp. 449- 486.

42 . Maki J. and Herwig R., 1991 - A diel study of neuston and plankton bacteria in an Antarctic ponds, ***Antarctic Science***, 3, pp. 47- 51.

43 . Marshall H. G. and Burchardt L., 2005 - Neuston: Its definition with a historical review regarding its concept and community structure, Archiv für Hydrobiologie, 164, pp. 429–448.

44 . Marshall K. C., 1986 - Adsorption and adhesion processes in microbial growth at interfaces, Advances in Colloid and Interface Science, 25, pp. 59–86.

45 . Marshall K. C., 1992 - Planktonic versus sesile life of prokaryotes. In The Prokaryotes. A Handbook on the Biology of Bacteria. Ecophysiology, Isolation, Identification, Applications. 2nd Edn. (A. Balows, H.G. Tr・er, M. Dworkin, W. Harder and K.H. Schleifer), pp. 262 – 275.

46 . Mudryk Z. and Skórczewski P., 1998 - Utilization of dissolved amino acids by marine bacteria isolated from surface and subsurface layers of the Gdańsk Deep. ***Polish Journal*** of Environmental Studies, 7, pp. 289 - 293.

47 . Müster U. and Chrost R. J., 1990 - Origin composition and microbial utilization of dissolved organic matter. In Overbeck J. and Chrost R. J. Aquatic Ecology, Biochemical and Molecular Approaches, Springer, New York, pp. 8 – 46.

48 . Parker B. and Barsom G., 1970 - Biological and chemical significance of surface microlayers in aquatic ecosystems, BioScience, 20, pp. 87- 93.

49 . Parsek M. R. and Fuqua C., 2004 - Biofilms 2003: Emerging Themes and Challenges Surface Associated Microbial Life, Journal of Bacteriology, pp. 4427– 4440.

50 . Pedersen K., 1982[a] - Factors Regulating Microbial Biofilm Development in a System with Slowly Flowing Seawater, Applied and Environmental Microbiology, 44(5), pp. 1196 -1204.

51 . Pedersen K., 1982[b] - Method for Studying Microbial Biofilms in Flowing - Water Systems, Applied and Environmental Microbiology, 43, pp. 6 – 13.

52 . Penfold W. J and Norris D., 1912 - The relation of concentration of food supply to the generation –time of bacteria , Biology of Bacteria, Journal of Hygiene, pp. 527 – 531

53 . Rijnaarts H. M., Norde W., Bouwer J., Lyklema J., Zehnder A., 1993 - Bacterial Adhesion under Static and Dynamic Conditions, Applied Environmental Microbiology, 59(10), pp. 3255 – 3265.

54 . Rogers J., Dowsett A. B., Dennis P. J., Lee J. V. and Keevil C. W., 1994 - Influence of Temperature and Plumbing Material Selection on Biofilm Formation and Growth of Legionella pneumophila in a Model Potable Water System Containing Complex Microbial Flora, Applied Environmental Microbiology, 60 (5), pp. 1585 – 1592.

55 . Sleigh A. M, 1987- Microbes of the sea, John Wiley and Sons, Inc., New York., pp. 241.

56 . Sonak S. and Bhosle N., 1995 - A simple method to assess bacterial attachment to surfaces, Biofouling, 9 (1), pp. 31 – 38.

57 . Stanley S. 0. and Morita R. Y., 1968 - Salinity Effect on the Maximal Growth Temperature of Some Bacteria Isolated from Marine Environments, Journal of Bacteriology, 95 (1), pp. 169 – 173.

58 . Stepanovic S., Vukovic D., Jezek P., Pavlovic M. and Svabic-Vlahovic M., 2001 - Influence of dynamic conditions on biofilm formation by Staphylococci, European Journal of Clinical Microbiology & Infectious Diseases, 20, pp. 502-504.

59 . Suci P. A., Siedleki K. J., Palmer R. J., White D. C. and Geesey G. G., 1997 -Combined Light Microscopy and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy for Integration of Biofilm Structure, Distribution, and Chemistry at Solid-Liquid Interfaces, Applied and Environmental Microbiology, 63 (11) , pp. 4600 – 4603.

60 . Surman S. B., Walkerb J. T., Goddard D.T. and Mortond L. H. G., 1996 - Comparison of microscope techniques for the examination of biofilms, Journal of Microbiological Methods, 2 (5), pp. 57 – 70.

61 . Sutherland I.W., 2001- The biofilm matrix - an immobilized but dynamic microbial environment, Trends in Microbiology, 9, pp. 222-227.

62 . Walczak M., 2008 - Day – to – night activity of bacteria in the surface microlayer eutrophic lake, Polish Jurnal of Ecology, 56 (3), pp. 379 – 389.

63 . Wurl O. and Holmes M., 2008 - The gelatinous nature of the sea-surface microlayer, Marine Chemistry, 110, pp. 89–97.

64 . Yuehuei H. A. and Friedman R. J., 1997- Laboratory methods for studies of bacterial adhesion, Journal of Microbiological Methods, pp.141-152.

65 . Zaitsev Yu. P., 1997 - Neuston of seas and ocean, In: P. Liss, R. Duce (Eds), The Sea Surface And Global Change, Cambridge, University Press, New York, pp. 371- 382.

66 . Zarma M., 1959[a] – Microbiologie marină, Cercetării sovietice în perioadă 1946 – 1957, Ed. Academiei R.P.R., Bucureşti.

67 . Zarma M., 1963 – Les bacteries sulfato reducrices denitrifiantes de la Mer Noire, Rapport et proces – verbaux des reunions CISMM, Paris, 17, pp. 679 – 687.

68 . Zarma M., 1965 – Quelques donnes concernant le nombres des bacteries et de la biomass bacterienne dans l' eau de la plat – forme continentale de la Mer Noire a niveau de la ville Constantza, Rapport et proces – verbaux des reunions CISMM, Paris, 18, 3, pp. 635 – 643.

69 . Zarnea Gh., 1994 - Tratat de Microbiologie generală. Bazele teoretice ale Ecologiei microorganismelor, Microorganismele şi mediile lor naturale, Editura Academei Române, Bucureşti, pp. 1- 1078.

70 . Zook D., 1992 - The Microcosms Curriculum Guide to Exploring Microbial Space, Kendall Hunt Publishing Company, Dubuque**,** pp. 480**.**

PERSONAL ANEXX

I. SCIENTIFIC PAPERS:

Publications included in the thesis

1. Scientific papers published in the journals of internaţional conferences ISI Proceedings:

- **Moldoveanu A. M., Ardelean I. 2012** – The dynamics of marine bacterioneuston in laboratory containers: 1. The influence of salinity and temperature, *vol. SGEM Conference Proceedings*, III, pp. 1001 - 1008
- **Moldoveanu A. M., Ardelean I. 2012** – The dynamics of marine bacterioneuston in laboratory containers: 2. The influence of organic matter, *vol. SGEM Conference Proceedings***,** III, pp. 1009 - 1016

2. Scientific papers published in the naţional conferences journals BDI

- **Moldoveanu A. M., 2010** – Analysis of biofilm temporal dynamic on the hidrofile and hidrofobe surfaces in containers with a circuit conditions, *Annals of RSBC*, vol. **XV (2)**, pp. 235 – 242
- **Moldoveanu A. M., Ardelean I. 2010** - The formation of bacterial biofilms on the hidrofile surface of glass in laboratory static conditions: the effect of temperature and salinity , *Ovidius University Annals of Natural Sciences, Biology - Ecology Series, **14*****,*** p. 147-156.
- **Moldoveanu A. M., 2011** – Bacterial biofilms utilization of low concentrations of organic matter on hidrofile surfaces submerged in seawater, *Scientific annals of Al. I. Cuza University Jassy, Section II, Genetics and Molecular Biology*, tom. **XII (4),** pp. 165 – 175

3. Scientific papers published in the naţional conferences journals CNCSIS – category B+:

- **Moldoveanu A. M., Ardelean I. 2010** – Studies regarding the formation and temporal dynamics of bacterial biofilms on the hidrofile surfaces of glass in static and dynamic condition, *Journal of Science and Arts*, vol. **XIII (2)**, pp. 313 – 318
- **Moldoveanu A. M., 2010** – Preliminary studies regarding cell density and temporal dynamic of bacterial biofilms formed at liquid- sediment interface using bright field and epifluorescence microscopy, *Scientific Study and Research – Biology*, vol. **XIX,** pp. 75 -81
- **Moldoveanu A. M., 2011** – The influence of organic matter on bacterial colonies and cell forms in bacterial biofilms, *Scientific annals of Al. I. Cuza University Jassy, Animal biology*, vol. **LVII,** pp. 167 - 175
- **Moldoveanu A. M., 2012** – The influences of *Mytilus* extract on biofilm cells attachment , *Annals of RSBC*, vol. **XVII(1)**, pp. 111 - 117
- **Moldoveanu A. M., 2012** – Environmental factors influences on bacterial biofilm formation, *Annals of RSBC*, vol. **XVII (1)**, pp. 118 - 126

4. Scientific papers published in the naţional conferences journals CNCSIS – other caterories:

- **Moldoveanu A. M. 2011** – The use of methods to obtain and investigate bacterial biofilms in the studies of surface colonization , *Ovidius University Annals of Natural Sciences, Biology – Ecology Series* , vol. **XV,** pp. 81-93

5. Scientific papers published in the naţional journals :

- **Moldoveanu A. M., 2007** – Microbiology reasearches and the microorganisms role in the Black Sea ecology, *Ecological education in the romanian costal area good practice protocols*, pp. 68 - 73
- **Moldoveanu A. M., 2010** – Bacterial biofilm formation in laboratory conditions, quality and monitoring of the marine environment, *UMC Nautica press*, vol. **I ,** pp. 93 – 101

- **Moldoveanu A. M., 2011** – Bacterial biofilms implications in marine microfouling fomation, Quality and monitoring of the marine environment, *UMC Nautica press*, vol. **II,** pp.19 – 29

Publications not included in the thesis

1. Scientific papers published in the journals of internaţional conferences:

- **Moldoveanu A. M., 2011** – The influence of green algae extract on bacterial biofilms in static conditions, *Science & Technology*, vol. **I (2)**, pp. 7-11
- **Moldoveanu A. M., 2011** – The temporal dynamic of bacterial biofilms in intermittent and continuous conditions in containers with a circuits, *Science & Technology*, vol. **I (2)**, pp. 12 – 16

2. Scientific papers published in the naţional conferences journals BDI

- **Moldoveanu A. M., 2012** – The influences of *Mytilus* extract on biofilm cells attachment , *Annals of RSBC*, vol. **XVII(1)**, pp. 111 - 117
- **Moldoveanu A. M., 2012** – Environmental factors influences on bacterial biofilm formation, *Annals of RSBC*, vol. **XVII (1)**, pp. 118 - 126

II. PARTICIPATION AT SCIENTIFIC MEETINGS:

1. National scientific meetings

- The scientific meetings „ **ECOLOGICAL EDUCATION IN THE ROMANIAN COSTAL AREA GOOD PRACTICE PROTOCOLS"**, NOG Mare Nostrum, 31 Octomber 2007, CONSTANŢA
- The scientific meetings **„ A DOUĂ SESIUNE DE NATIONALĂ DE ŞTIINŢE APLICATE ÎN STUDIUL MEDIULUI ÎNCONJURĂTOR AND MATERIALELOR"**, Valahia University, 28-30 April 2010, TÂRGOVIŞTE
- The scientific meetings **„ BIODIVERSITATE- PREZENT AND PERSPECTIVE"**, Ovidius Universitaty, 7-8 June 2010, CONSTANŢA
- The scientific meetings **„ A XXVIII-A SESIUNE STIINTIFICĂ A SOCIETAŢII ROMANE DE BIOLOGIE CELULARĂ**", Ovidius University and RSBC, 9-12 June 2010, CONSTANŢA
- The scientific meetings **„ INTERACŢIUNII MOLECULARE ÎN LUMEA VIE",** Al. I. Cuza University**,** 15-16 Octomber 2010, IAAND
- The scientific meetings **„ BIODIVERSIATATE AND DEZVOLTARE DURABILĂ"**, Al. I. Cuza University**,** 15-16 Octomber 2010, IAAND
- The scientific meetings **„ CALITATEA AND MONITORINGUL MEDIULUI ÎNCONJURATOR - DEZVOLATAREA DURABILĂ ÎN MEDIUL NATURAL",** Maritim University**,** 31 Octomber 2010, CONSTANŢA
- The scientific meetings „ **A XXI-A SESIUNE DE COMUNICĂRI ŞTIINŢIFICE A CADRELOR DIDACTICE AND STUDENŢILOR",** Universitatea Ovidius**,** 25-26 March 2011, CONSTANŢA
- The scientific meetings „ **MANAGEMENTUL ECOLOGIC AL MARII NEGRE IN MILENIUL III",** INCDM**,** 29-30 Octomber 2011, CONSTANŢA
- The scientific meetings „ **IMPACTUL UMAN ASUPRA BIODIVERSITATII ZONELOR MARII NEGRE",** Agigea Biological Station**,** 29-30 Octomber 2011, CONSTANŢA
- The scientific meetings **„ BIODIVERSIATEA IN SITU AND EX SITU – BCIS ",** 21- 25 martie 2012, CONSTANŢA

2. International scientific meetings

- The scientific meetings „**A 21^{A} CONFERINŢĂ INTERNAŢIONALĂ**", Tracia University – Asociation of Scientific People , 2-3 June 2011, STARA ZAGORA, BULGARIA
- The scientific meetings „**AL 3 ^{LEA} CONGRES INTERNAŢIONAL SNBC**", 8-12 June 2011 Vasile Goldis University, Arad, Romania – Biological Center, SZEGED, UNGARIA
- The scientific meetings **„CONGRESUL MULTIDISCIPLINAR SGEM"**, 17 - 23 June 2012, SGEM, ALBENA, BULGARIA

III. COMUNICĂRI ORALE:

- **"Cercetarile microbiologice and rolul microorgismelor marine în ecologia Mării Negre",** Moldoveanu Aurelia Manuela, Sesiunea Mare Nostrum, 31 October 2007
- **"Formarea biofilmelor în condiţii de laborator",** Sesiune Marea Neagara UMC, 31 October 2010
- **"The influence of green algae extract on bacterial biofilms în static conditions"**, Moldoveanu Aurelia Manuela, Sesiune Bulgaria, 2-3 June 2011
- **"The temporal dynamic of bacterial biofilms in intermittent and continuous conditions in containers with a circuits"**, Moldoveanu Aurelia Manuela, Sesiune Bulgaria, 2-3 June 2011
- **"The influences of *Mytilus* extract on biofilm cells attachment",** Moldoveanu Aurelia Manuela, Sesiune Arad, 8-12 June 2011
- **"Environmental factors influences on bacterial biofilm formation",** Moldoveanu Aurelia Manuela, Sesiune Arad 8-12 June 2011
- **"Marine biofilms temporal dynamics from formation to detachment phase",** Moldoveanu Aurelia Manuela, Sesiune BCIS 21-25 March 2012
- **"The dynamics of marine bacterioneuston in laboratory containers: 1. The influence of salinity and temperature",** Moldoveanu Aurelia Manuela, Sesiune SGEM 17 - 23 June 2012

IV. POSTERS:

- **„Studii privind dinamica temporală and formarea biofilmelor bacteriene pe suprafaţa hidrofilă a sticlei în condiţii statice and dinamice",** Moldoveanu Aurelia Manuela, Ioan I. Ardelean, Valahia University, 28-30 April 2010, TÂRGOVIŞTE
- **„Formarea biofilmelor bacteriene pe suprafaţa hidrofilă a sticlei în condiţii statice de laborator: efectul salinităţii and temperaturii",** Moldoveanu Aurelia Manuela, Ioan I. Ardelean, Ovidius University, 7-8 June 2010, CONSTANŢA
- **„Analiza dinamicii temporale a biofilmelor bacteriene formate pe suprafeţe hidrofile and hidrofobe în condiţii de containers with a circuit",** Moldoveanu Aurelia Manuela, Ovidius University and RSBC, 9-12 June 2010, CONSTANŢA
- **„Utilizarea concentraţiilor scăzute de substanţă organică de către procariotele marine fixate pe suprafeţele hidrofile imersate în apa marină",** Moldoveanu Aurelia Manuela, Al. I. Cuza University, 15-16 October 2010, IAAND
- **„Influenţa substanţelor organice asupra formării coloniilor bacteriene and tipurilor de cells prezente în biofilme",** Moldoveanu Aurelia Manuela, Al. I. Cuza University, 15-16 October 2010, IAAND
- **„Dinamica formării biofilmelor marine pe suprafeţe hidrofile and hidofobe în condiţii de laborator" ,** Moldoveanu Aurelia Manuela, Ioan I. Ardelean, Ovidius University, 25-26 March 2011, CONSTANŢA

- **„The influence of interfaces on marine biofilms in static conditions”,** Moldoveanu Aurelia Manuela, Ioan I. Ardelean, Ovidius University, 20 - 22 October 2011, AGIGEA – CONSTANŢA
- **„The dynamics of marine bacterioneuston in laboratory containers: 2. The influence of organic matter”,** Moldoveanu Aurelia Manuela, Ioan I. Ardelean, Ovidius University, 17 - 23 June 2012, SGEM, ALBENA, BULGARIA

V. ABSTRACTS:

- **„The influence of interfaces on marine biofilms in static conditions”,** Moldoveanu Aurelia Manuela, Ioan I. Ardelean
- **„Marine biofilms temporal dynamics from formation to detachment phase”,** Moldoveanu Aurelia Manuela

VI. POSTGRADUATE COURSES :

- **Postuniversitar Course** Institutul de Biologie al Academiei Române a organizat de studii avansate “ **Realizări and perspective în Biologie ”**, ediţia 2009, cu tema: “Impactul factorilor de mediu asupra Biodiversităţii ”, 5 – 7 noiembrie 2008
- **Postuniversitar Course** Institutul de Biologie al Academiei Române a organizat de studii avansate “ **Realizări and perspective în Biologie** ”, ediţia 2009, cu tema: “Complexitatea biologică sub aspect macro, micro, nano”, 8 – 10 iulie 2009
- **Training Session** în Autorat Ştiinţific din cadrul proiectului " **Doctoratul în Scoli de Excelenţa** “, 27 - 28 August 2009