BACTERIAL BIOFILMS UTILIZATION OF LOW CONCENTRATIONS OF ORGANIC MATTER ON HYDROPHILE SURFACES SUBMERGED IN SEAWATER

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Abstract: A series of experiments were designed to determine the effect and the metabolic rate utilization of various types of organic matter in low concentration by heterotrophic marine bacteria using as Henrici slide technique as culture method and “in vitro” static conditions in sterile containers in order to obtain bacterial biofilms on the hydrophile surface of glass. The bacteria attachment and biofilm formation was analyzed for a period from 2 hours to 72 hours in order to observe de first phase of biofilm formation in condition of seawater supplied with organic matter and noninvasive optic microscopy analysis. The utilization of five different types of organic substances (amino-acid mixture, yeast extract, tryptone, glucose and starch) reveled that bacteria multiply and are otherwise physiologically active in this very dilute nutrient solutions of 0.1% and also the results revealed that the bacterial growth was considerable in the case of the substances like amino-acid mixture with a total density of $30.9 \cdot 10^3$ cells/mm^2 and tryptone with a total density of $28.85 \cdot 10^3$ cells/mm^2 comparable to the other types of organic matter used to supply the seawater.

INTRODUCTION

Bacterial growth and biomass in aquatic ecosystems are controlled by several factors, such as organic and inorganic nutrients, predation, and viral lysis. The finding of relatively low variability in bacterioplankton abundance implies that bacterial abundance is rather tightly regulated by the different gain and loss factors (Matz and Jürgens, 2003; Satheesh, 2010).

The growth of the marine proteolytic, amilolytic, lipolytic or heterotrophic bacteria can be influenced by the amount of dissolved organic matter (DOM) present as 80 - 90 % and the particulate organic matter (POM) which is an important component present at the surface interface. Seawater and nutrients represent very important environmental factor because the bacteria chemotaxie and adherence toward the solid surfaces is determined by organic matter and microstructures know as biofilms are formed (Kjellberg et al.1982; Costerton, 2007).

Biofilms are composed of very diverse microorganisms that are involved in multiple ecological processes, such as primary production, nutrient recycling and organic compound decomposition. Also biofilm bacteria, due to their high abundance and diverse metabolic capabilities, dominate the assimilation and flux of dissolved organic matter (DOM), the dominant form of organic carbon in aquatic systems (Zarnea 1994; Lazar, 2003).

Research has determined that seawater contains sufficient organic matter in solution necessary for bacterial population growth, but it must be determined what types of organic matter are more influent on bacterial multiplication and organic decomposition. Microbiologists have studied the effect of organic substances from simple to complex nature, which can be added to sea water in order to observe a bacterial cell forms response and determine the total population number that could be quantified by various types of methods (Münster and Chrost, 1990; Azua, 2003).

The fact that many bacteria multiply and are otherwise physiologically active in very dilute nutrient solutions is manifest from the abundance of bacteria in fresh and seawater. The organic content of seawater is generally less than 5 mg/l due to the oligotroph characteristics of this water, yet during the storage of seawater in the laboratory the bacterial population usually increases also is considered that the concentration of food does not influence the rate of growth of bacteria except when the concentration is very low of 0.01 to 0.1% of organic matter (Hoppe et al. 1988).

This study was realized in order to determine bacterial cell quantification and differences in bacterial cell growth under the influence of various types of dissolved organic matter in seawater and also to observe the first phase of biofilms formation on the artificial hydrophile surfaces in laboratory static conditions using a noninvasive optic microscopy method.

MATERIAL AND METHODS

The bacterial biofilms were obtained on the smooth surfaces of hydrophile glass surfaces (microscope slides) using fresh seawater from the Black Sea (Eforie Nord area) as culture medium in sterile containers of 100 ml containers whit static conditions in vitro. Prior to the experiment all surfaces were degreased with 70% ethanol (Lazar et al. 2004), sterilized by immersion in sulfochromic mixture ($K_2Cr_2O_7/H_2SO_4$) for two days and washed with double

distilled water to avoid contamination with microorganisms and organic matter before the experimental protocols (Mercier- Bonin, 2004).

In order to observe organic matter influence on the marine bacteria attach to the artificial surfaces, seawater was enriched with low concentrations of 0.1% organic matter of five different types: amino-acid mixture, yeast extract *Difco*, bactotryptone *Difco*, glucose *Merck* and starch *Merck*. The organic solutions were prepared by adding 100 mg of organic matter in sterile containers 100 ml distillate water and after mixture low quantities of 3 mg, 5 mg, 7 mg and 9 mg were added to the containers with fresh seawater. All the subtances were filtered before use with a 0.22µm Millipore membrane filters.

The *Henrici Slide Technique* was used as culture method in order to obtain biofilm on the artificial surfaces of glass as shown by www.BiofilmsONLINE.com, 2008, with the help of containers and microscope slides as solid surfaces immerse in a oblique position as an adaptation for avoiding debris and excess of bacterial cells attachment on the surfaces, as suggested by Kuman and Prasad in 2006 and shown in previous experiments by Moldoveanu, 2010).

After harvesting the slides were immediately stained by capillarity whit one drop of 0.1% Methylene Blue in order to observe the natural growth and attachment on the surfaces whit minimal quantification errors due to slides preparation and storage.

The slides were immerse for a period of 2 hours up to 72 hours necessary to observe the first phases of biofilm formation and afterwards were analyzed whit bright field microscopy at the Hund Wetzlar Microscope with 100X objective and 10X ocular (Hulea, 1969). Bacterial quantification was realized by means of the 10mm X 10mm micro-ocular grid (macroscopically), investigating 10 microscopic fields per harvested slide whit three repetitions per analyzed time period (Fry, 1990).

RESULTS AND DISSCUSIONS

Sea water analysis

The Black Sea waters are oligotroph do vertical currents limitations and in order to determine the influence of organic matter on bacterial biofilms formation and growth on the hydrophile surface of glass slides chemical analysis of the seawater were accomplished in the Chemistry Laboratory within the "George Antipa" Institute for Marine Research of Constanta to observe the differences between the standard parameters for the romanian coastal waters and the one from the Eforie area used as culture medium in the experiments (Table 1).

Table 1. The values of the chemical parameters of seawater (liquid culture medium).

Principal chemical parameters	**a. Seawater (Mamaia area – standard probe)**	**b. Seawater (Eforie area)**
salinity	15 – 17 g/l	13.75 g/l
pH	8,2 – 8,4 units	8.24 units
$P\text{-}PO_4$	0,2 – 0,5 µmoli/dm^3	0.35 µmoli/dm^3
$N\text{-}NO_2$	2 – 5 µmoli/dm^3	0.13 µmoli/dm^3
$N\text{-}NH_4$	2 – 4 µmoli/dm^3	1.24 µmoli/dm^3
$N\text{-}NO_3$	1 – 3 µmoli/dm^3	2.04 µmoli/dm^3

$Si\text{-}SiO_4$	0,2 – 0,3 μmoli/dm^3	9.60 μmoli/dm^3
Organic matter (CCO-Mn)	2 – 5 mg O_2/l	2.04 mg O_2/l

The chemical analysis of the seawater used in the experiments determinate some differences from the standard chemical parameters of seawater (salinity, pH, concentration of organic and inorganic substances) and the experimental sea water used as liquid culture medium. The results were analyzes by comparing them to the normal values recorded in the previous years (Cociaşu et al., 2008). The values of the seawater from the Eforie area are considerably under the normal limits, displaying a decrease of salinity of over 1.25 g/l and a decrease and a pH in normal limits, compared to the standard values of littoral seawater on the Romanian shore due to the particularities of this area as water clarity and rocky shores that imply a low organic and inorganic concentrations. Also this area is not as expose to pollution as the Mamaia seawater area.

Inorganic substances had a lower concentration compared to normal the values of seawater the phosphates are in normal limits with a value of 0.35 μmoli/dm^3, the amount of azotizes level was 0.13 μmoli/dm^3, two to three times lower than the normal value, while the amount of nitrates was 1.24 μmoli/dm^3 lower the organic matter was lower than normal values for standard probes, the silicates value was 9.60 μmoli/dm^3, higher than the normal values which are very low 0,2μmoli/dm^3. The existence of these differences in the culture medium used may determine changes in the formation and the temporal dynamics and generation of bacterial biofilms in liquid medium.

The organic matter was low of 2.04 mg O_2/l and this value was supply with the 3 mg, 5 mg, 7 mg and 9 mg per litter of protein and carbohydrates in order to observe bacterial growth in low concentrations organic addition in oligotroph seawater.

Organic matter influences

The analyses in bright field of the biofilms formed on the hydrophile surface of the glass slides collected from the containers with littoral seawater enriched with proteins and carbohydrates emphasized the existence of successive phases in biofilms formation and an important increase of the bacterial density after the period of 72 hours of substrate immersion in seawater.

Planktonic bacteria in marine systems can be found either free in the aqueous phase or attached to particles. These two bacterial communities have been comparatively characterized in terms of size, growth rate, hydrolytic activities, incorporation of low molecular weight compounds and taxonomic composition. It has been reported that some taxonomic groups are selected in the particles, and attached bacteria are often bigger and more active than free-living ones.

The proteins are one of the main organic subtances metabolized by marine bacteria but for their degradation enzymes are required and essential amino acids are more available than de protein complexes with high organic weight (Figure 1).

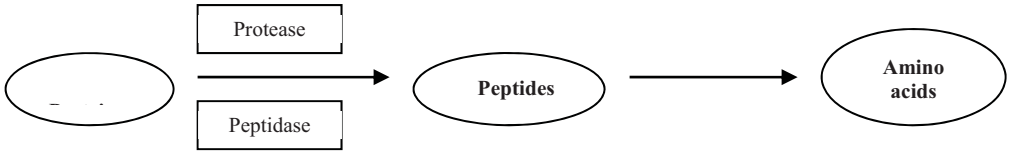

Figure 1. Proteins utilization by marine bacteria

At fist an amino acid mixture formed of essential elements and a very accessible source of organic matter for bacterial biofilms was used. Also carbohydrates and other polypeptides that are considered the two main fractions of organic matter in the sea due to glycosidase and peptidase activities and play a key role in bacterial growth were used to enrich the experimental seawater.

The use of the amino acid mixture determined in the control probes without any organic matter supply a bacterial cell attachment of $41.4 \cdot 10^3$ cells/mm^2. After the seawater supply with a low quantity of amino acid mixture of only 3 mg/l, the bacterial cell attachment increased to a value of $50.6 \cdot 10^3$ cells/mm^2. The use of a higher concentration of 5 mg/l of amino acid mixture determines a bacterial attachment of $56.3 \cdot 10^3$ cells/mm^2. The growth was even higher in the cases of 7 mg/l of mixture when the bacterial cell attachment reach densities of $69.4 \cdot 10^3$ cells/mm^2 and the pick was observed at the value of $83.2 \cdot 10^3$ cells/mm^2 for the 9 mg/l addition (Figure 2.a)

In table 2.a the differences between control probes for the amino acid mixture additions are observed. On the case of the 3mg addition determined a difference of $9.2 \cdot 10^3$ cells/mm^2 and bacterial growth of 22.3% of the initial value of cell density on the surfaces, the addition of 5mg/l determine a difference of $14.9 \cdot 10^3$ cells/mm^2 this determines a value of 36.2% from the initial value, for de 7mg additions the difference was $28 \cdot 10^3$ cells/mm^2 a value of 68.12% from the initial growth and for 9mg/l the difference was 41.4 this determine a bacterial cell growth of over 101.7% from the initial value of $41.4 \cdot 10^3$ cells/mm^2.

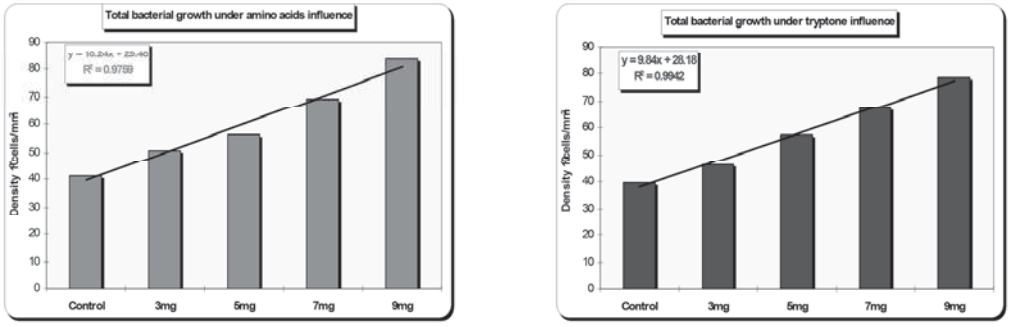

Figure 2. Total bacterial growth under amino acid mixture **(a)** and tryptone **(b)** influence

Another protein mixture used was tryptone due to the casamino acids contained in it that can be produced by acid hydrolysis and typically only have free amino acids and few peptide chains, propertied that makes it very accesible for marine bacteria.

In figure 2.b the control probes whit out organic matter supply determined a bacterial cell density of $39.5\cdot 10^3$ cells/mm^2 on the artificial surfaces. After the supply of sea water with 3 mg/l of amino acid mixture the bacterial cell attachment reached the value of $46.4\cdot 10^3$ cells/mm^2. A high concentration of 5 mg/l determined a bacterial cell density of $57.1\cdot 10^3$ cells/mm^2. The use of even higher concentrations of 7 mg/l of tryptone reached the value of $69.4\cdot 10^4$cells/mm^2 and at 9 mg/l was the bacterial cell density was $78.3\cdot 10^3$cells/mm^2.

Table 2. The differences between control probe and the addition of amino acid **(a)** and tryptone **(b**

Differences	**a. Amino acids**	**b. Tryptone**
Control–3mg	$9.2\cdot10^3$ cells/mm^2	$6.9\cdot10^3$ cells/mm^2
Control–5mg	$14.9\cdot10^3$ cells/mm^2	$17.6\cdot10^3$ cells/mm^2
Control–7mg	$28\cdot10^3$ cells/mm^2	$27.7\cdot10^3$ cells/mm^2
Control–9mg	$41.8\cdot10^3$ cells/mm^2	$38.8\cdot10^3$ cells/mm^2

The differences between control probes in the case of tryptone additions were at first of $6.9\cdot 10^3$ cells/mm^2 for the 3mg addition this determine a bacterial growth of 17.4% from the initial cell growth, the addition of 5mg of tryptone determined a difference of $17.6\cdot 10^3$ cells/mm^2 a value of 44.5% from the initial one. Also the 7 mg/l and 9 mg/l additions determined difference of $17.6\cdot10^3$ cells/mm^2 (70.12%), and respectively $38.8\cdot 10^3$ cells/mm^2 98.22% from the initial cell growth of $39.5\cdot 10^3$ cells/mm^2 (Table 2.b).

The progression of cellular density growth was 10.24 for the amino acid mixture growth and 9.84 for the tryptone The R coefficient was 0.99 higher for the tryptone growth and of 0.97 for the amino acid mixture use. This determines a higher bacterial growth in the case of amino acid mixture compare to tryptone (Figure 2).

Glucose as a simple sugar (monosaccharide) was an important a source of energy for cell metabolism and respiration, usually bacteria use glucose because many bacteria possess the enzymes required for the degradation and oxidation of this sugar and in some deep-sea bacteria glucose is produced by chemosynthesis due to its important roles (Figure.3).

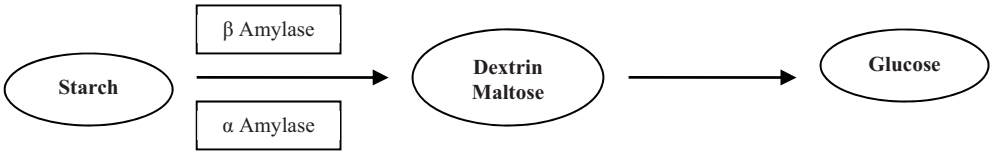

Figure 3 Starch utilization by marine bacteria

In the control probes without organic matter supply the bacterial growth was of 39· 10^3 cells/mm^2. After the supply of sea water with 3mg/l of glucose determined a bacterial cell attachment of 45· 10^3 cells/mm^2. A high concentration of 5 mg/l determined a bacterial cell density of 51· 10^3 cells/mm^2 and the growth was even higher in case of 7 mg/l addition of 64· 10^3 cells/mm^2 and the pick was observed at 9 mg/l were the bacterial cell attachment reached the value of 70· 10^3 cells/mm^2 (Figure 4.a).

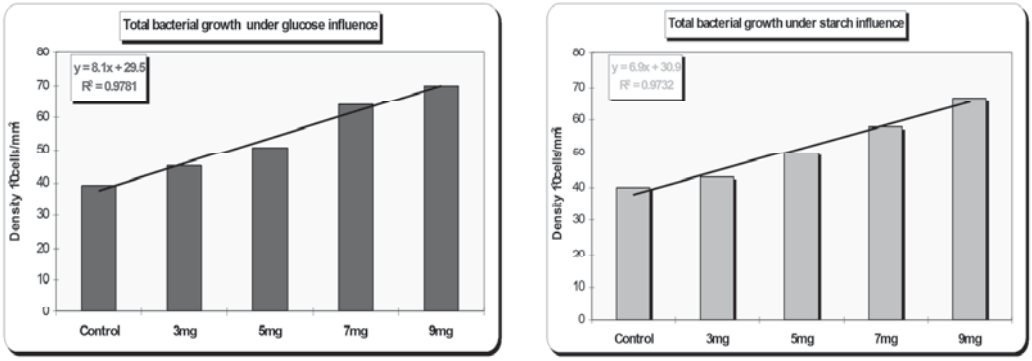

Figure. 4 Total bacterial growth under amino acid glucose **(a)** and starch **(b)** influence

In the control probes without organic matter supply the bacterial growth was of 40· 10^3 cells/mm^2. After the supply of sea water with 3 mg/l of amino acid mixture the bacterial growth was of 43· 10^3 cells/mm^2. The high concentration of 5 mg/l the bacterial cell density was 50· 10^3 cells/mm^2. The growth was high in case of 7 mg/l of 58· 10^3 cells/mm^2 and the pick was observed at 9 mg/l were the bacterial growth was of 67· 10^3 cells/mm^2 (Figure 4.b).

The progression of cellular density growth is over 8.1 for the glucose and 6.9 for the starch for the formed biofilms and the R coefficient was the same of 0.97. The progression of cellular density growth was over for the formed biofilms a higher growth in the glucose case (Figure 4).

Fewer bacteria are able to use complex carbohydrates like polysaccharides (starch) this are simple sugars that are linked by glycoside bonds, bacteria must produce enzymes to cleave these bonds such that the simple sugars that result can be transported into the cell. The starch after enzyme cleavage can be use very quickly by marine cells and determine a high bacterial cell growth and attachment but not as quickly as glucose.

The yeast extract as a water-soluble portion of autolyzed yeast is carefully controlled to preserve naturally B-complex vitamins and is prepared and standardized filtered clear and dried into a powder by spray drying for bacteriological use for this reason was an excellent stimulator

of biofilm bacterial growth, but the values obtain were much lower compared to the other types of organic matter used as water supply.

In the control probes without organic matter supply the bacterial growth was of 39.7· 10^3 cells/mm^2. After the supply of sea water with 3 mg/l of amino acid mixture the bacterial growth was of 42.2· 10^3 cells/mm^2. The high concentration of 5 mg/l the bacterial cell density was 48.9· 10^4 cells/mm^2. The growth was high in case of 7 mg/l of 53.3 ·10^3 cells/mm^2 and the pick was observed at 9 mg/l were the bacterial growth was of 66.5· 10^3 cells/mm^2 (Figure 5.a).

The progression of cellular density growth was over 6.49 for the formed biofilms and the R coefficient was 0.93.

In table 3.a the differences between control probes were in the case of glucose additions of 6.9·10^3 cells/mm^2 for the 3mg/l addition this determines a bacterial growth of 15.3% from the initial cell growth, for the 5mg/l addition the differences were 12· 10^3 cells/mm^2 and the growth was of 30.7%. In the case of the higher addition of 7mg/l the differences was 25· 10^3 cells/mm^2 (64.1%) and for the 9mg/l addition the difference was of 17.6· 10^3 cells/mm^2 (79.4%) from the initial cell growth of 39· 10^3 cells/mm^2.

Table 3. The differences between control probe and the addition of glucose **(a)** and starch **(b).**

Differences	**a. Glucose**	**b. Starch**
Control – 3mg	6· 10^3 cells/mm^2	3· 10^3 cells/mm^2
Control – 5mg	12· 10^3cells/mm^2	10· 10^3 cells/mm^2
Control – 7mg	25· 10^3 cells/mm^2	18· 10^3 cells/mm^2
Control – 9mg	31· 10^3cells/mm^2	27· 10^3 cells/mm^2

The differences between control probe in the case of yeast extract additions were of 2.5· 10^3 cells/mm^2 for the 3mg addition this determine a bacterial growth of 6%, for the 3mg/l addition the difference was 2.5· 10^3 cells/mm^2 22.2%, for the 5mg/l addition the difference was 13.8· 10^3 cells/mm^2 and for the 7mg/l addition was of 33.3%, for the 9 mg/l addition the differences was of 2.5· 10^3 cells/mm^2 64.7% (Table 4.a)

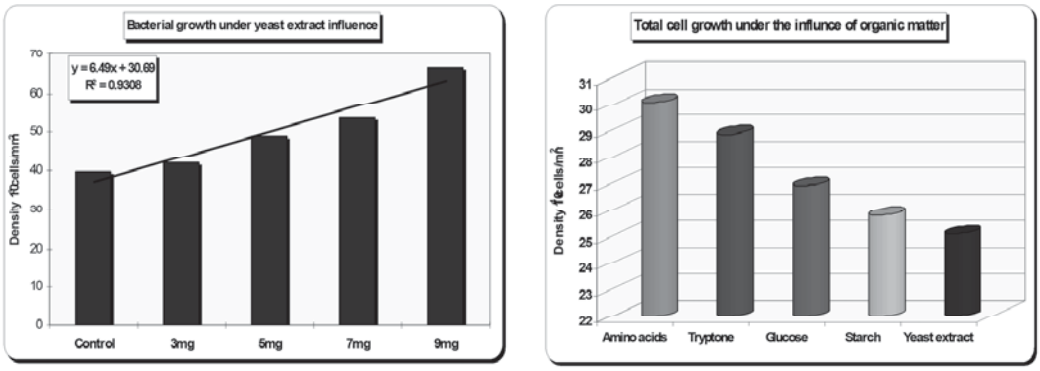

Figure. 3 Total bacterial growth under amino acid yeast extract influence **(a)** and total bacterial growth **(b)**

Total bacterial number under organic matter supply a high growth protein substances of $30.9 \cdot 10^4$ cells/mm^{2}for amino acid mixture $28.85 \cdot 10^4$cells/mm^2 in tryptone case and the bacterial growth was of $25.8 \cdot 10^4$ cells/mm^2. In carbohydrates case the growth was of $26.9 \cdot 10^4$ cells/mm^2. The growth was high in case of 7 mg/l of $69.4 \cdot 10^4$ cells/mm^2 and the pick was observed at 9mg/l were the bacterial growth was of $25.8 \cdot 10^4$ cells/mm^2 (Figure 5.b).

Table 4. The differences between control probe and the addition of yeast extract **(a)** and total bacterial number **(b).**

Differences	**a. Yeast extract**	**Differences**	**b. Total bacterial number**
Control – 3mg	$2.5 \cdot 10^3$ cells/mm^2	Control – Amino acids	$25.8 \cdot 10^3$ cells/mm^2
Control – 5mg	$9.2 \cdot 10^3$ cells/mm^2	Control – Tryptone	$24.9 \cdot 10^3$ cells/mm^2
Control – 7mg	$13.8 \cdot 10^3$ cells/mm^2	Control – Glucose	$23.0 \cdot 10^3$ cells/mm^2
Control – 9mg	$26.8 \cdot 10^3$ cells/mm^2	Control – Starch	$21.8 \cdot 10^3$ cells/mm^2
		Control – Yeast extract	$21.1 \cdot 10^3$ cells/mm^2

The differences between the control probes and the total number of bacteria after the bacterial growth was of $25.8 \cdot 10^3$ cells/mm^2 for de amino acid mixture, of $24.9 \cdot 10^3$ cells/mm^2 for the tryptone, of $23.0 \cdot 10^3$ cells/mm^2 for the glucose , of $21.8 \cdot 10^3$ cells/mm^2 for the starch and $21.1 \cdot 10^3$ cells/mm^2 for the yeast extract. This results show that bacteria are very influenced by the organic matter with low organic weight.

The differences between control probe in the case of starch additions were of $3 \cdot 10^3$ cells/mm^2 for the 3 mg/l addition this determine a bacterial growth of 7.5%, for the 5 mg/l

addition was $10 \cdot 10^3$ cells/mm^2 25%, for the 7 mg/l de differences was $18 \cdot 10^3$ cells/mm^2 45% and for the 9 mg/l the difference was $27 \cdot 10^3$ cells/mm^2 67.5% (Tab.4.b).

A series of studies reveled indeed that bacteria are very influenced by the type of organic matter used to supply seawater but also by the inorganic nutrient and water properties.

Some of the first researchers in this field of marine bacteria, Penfold and Norris in 1912 observed that effect of adding small amounts of glucose in tubes with bacteria and peptone. It was first expected that with low peptone concentrations the generation-time would be reduced by this procedure and this proved to be the case. The amount of glucose added was 0-1.75 %. The additions of glucose to tubes containing 1.0 % and 0.1 % peptone respectively. In the case of the 1.0 % peptone the rate of growth is not much increased. Only the later part of the growth curve were affected, the generation-time being decreased from 39 minutes to 34 minutes. In the other case, however, the rate of growth is greatly increased by the small amount of glucose added and the generation-time decreased from 111 minutes to 50 minutes or about 50 %. This results show that indeed the low concentration of 0.1% of organic matter are more influent on the bacterial cell metabolism and growth, data determined in the present study as well.

Hagstrom et. al in 1984 determine how bacterial metabolism and growth is related to the steady-state concentrations of total carbohydrates and amino acids in et al. measure the components of the DOM in both the inflow and the outflow at two dilution rates. This measurements of DOM components in both filtered and unfiltered inflow samples showed that nearly all were indeed dissolved or smaller than 0.2 pm. The outflow should represent steady-state concentrations in the culture medium. Several observations were made: first a dilution rate of D = 0.11 h^{-1}, bacteria removed 63 % of dissolved carbohydrate and 37 % of dissolved TAA from the inflow. The amount of carbohydrate utilized 1.7 pM glucose equivalents was equivalent to $1.2 \cdot 10^{-4}$ g C 1^{-1}. If it is assumed that $5 \cdot 10^{-14}$ g C 1^{-1} (bacteria here were larger than in natural populations) and 50 % assimilation efficiency for carbohydrates, then $1.4 \cdot 10^{-4}$ g C 1^{-} carbohydrate carbon would be required to sustain growth at D = 0.11 hp' (6 h doubling time). Thus the rate of carbohydrate utilization was rapid enough to provide nearly all of the energy and carbon for bacterial growth. The nitrogen in TAA, however, even if assimilated with 100 % efficiency, could have provided only 21 % of the bacterial protein nitrogen (assuming a bacterial C : N ratio of 3). Therefore other sources of nitrogen must have been used. At a dilution rate of D = 0.018 h^{-1} (generation time 39 h) there was a much greater accumulation of cells. Here, even if all TCHO (total carbohydrates) in the inflow had been used (at 50 % efficiency) it could account for only 41 % of the carbon required for growth. Therefore, other sources of DOC must have been utilized. Similarly, the amount of TAA (total amino acids) utilized (at 100 % efficiency) could have supported a maximum of ***5*** % of cell growth.

Incubating seawater with high levels of glucose or peptone (100 mg/l^{-1}) for 1 h did not increase % motile but it changed bacterial behavior, prolonging the percentage of time bacteria were motile. The fraction of bacteria that were motile for 80% of the 5 min observation time increased from 27% (control) to 62% (glucose) and 72% (peptone) Enrichment (3.7 g l–1 ZoBell 2216 E) led to an increase in motile bacteria (>80%, 12 h) approximately equal to total increase in bacteria. Finally, enrichment with inorganic nutrients or trace metals did not change % motile, even after 16 h as Grossart et. al. observed in 2001 study.

Also marine bacteria cell growth can be determined in vitro by making microcosms as Azua et al. in 2003 observed that different bacterial density by using carbohydrates and amino acids as dissolved organic matter sources for bacteria. So in the type M1 the bacterial abundance in ambient water ranged from $0.84 \cdot 10^6$ to $5.69 \cdot 10^6$ cells ml^{-1} and in aggregates ranged from 1.34

$\cdot 10^9$ to 6.15 $\cdot 10^9$ cells ml-1. In microcosms type M2 the bacterial abundance in ambient water ranged from 1.09 $\cdot 10^6$ to 9.26 $\cdot 10^6$ cells ml^{-1} and in aggregates ranged from 1.12 $\cdot 10^9$ to 7.75 $\cdot 10^9$ cells ml^{-1}. The Compare to these results the values of the current study very lower due to the oligotroph characteristics of the sea water and to the low organic enrichment.

In both types of microcosms, the concentrations of total carbohydrates, total amino acids and TOC (Total organic carbon) were two to three orders of magnitude higher in the aggregates than in the surrounding water. The average concentrations of carbohydrates, amino acids and TOC in both types of aggregates and in ambient water were not significantly different between the two types of microcosms M 1 and M 2.

CONCLUSIONS

Bacterial biofilms formation in laboratory condition is influenced by organic matter enrichment due to planktonic bacteria tend that adhere rapidly on the liquid - solid interfaces.

The oligotroph properties of the sea water determents that a utilization of low quantities of organic matter is most appropriate for the marine waters.

The organic substances with low concentrations are more rapidly degraded by marine bacteria and due to this fact the density values obtain are more realistic than the utilization of higher concentrations of organic matter which determines very high bacterial density similar to organic pollution.

Marine heterotrophic bacteria prefer the substances with a low molecular weight like amino acids and glucose due to their rapid availability for bacterial cell metabolism.

The substances with a high molecular weight are use more slowly and by a low number of bacteria due to their low availability and the necessity to use enzyme in their degradation.

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