THE GREEN ALGAE EXTRACT INFLUENCE ON BACTERIAL BIOFILMS FORMATION IN STATIC CONDITIONS

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ABSTRACT

Bacteria biofilms are important in aquatic environment because of ther role in surface colonization**.** In the seawater there are a series of factors that can influence biofilm formation such as nutrients concentration and other subtances presence in the water.

The aim of the this study was to test the effect of crude green algae extract on the first phases of bacterial biofilm formation in containers in laboratory static conditions.

In the presence of this organic extract bacterial cells had a higher density expecially in the case of the 7 mg and 9 mg additions per liter of seawater.

Key words: *biofilm, green algae, static conditions*

INTRODUCTION

Materials immersed in seawater are submitted to a series of physical, chemical and biological events which results in the formation of biofilms complex (layers of attached microorganisms). This biofilms form in fresh water, salt water, oil pipelines, in the human body, just in any kind of naturally occurring moisture interfaces (Fletcher, 1979, Lazar, 2003).

Bacteria usually multiply and are otherwise physiologically active in very dilute nutrient solutions fact manifest by the abundance of bacteria in seawater but all sow in fresh water ecosystems. The organic content of seawater is generally oligotroph with less than 5 mg/l, yet during the storage of seawater bacterial population increases and concentration of organic matter in high quantities does not influence bacterial cell growth, only low and very low concentration of 0.01 to 0.1 % of organic matter are important in the bacterial growth (Hoppe et al. 1988).

In the natural media biofilms are microcommunities formed by the attachment of different types of bacteria (bacilli, cocci, spirillum, filamentous and pedunculate forms), microalgae (chlorophyceae, diatoms,) and in late phase the invertebrates (barnacles, mussels) and microalgae adhere forming epibioses and fouling in harbor area (Costlow, 1984).

This report tested the hypothesis that bacteria usually grow associated with aquatic plants (green macroalgae) and they can be influenced by low concentrations of crude algae extract in laboratory conditions on the hydrophile surfaces.

MATERIAL AND METHODS

Biofilm formation was investigated in the experiments using 100 ml containers and seawater in static conditions. All the hydrophile surfaces (microscope slides) were previously degreased with 70% ethanol and sterilized (Lazar et al. 2004) by immersion in sulfochromic mixture (K2Cr2O7/H2SO4) for two days (Mercier-Bonin, 2004).

The crude green algae extract was prepare from three predominant fresh marine macroalgae *(Enteromorpha, Ulva, and Schytosiphon)* in 100ml distilled water, homogenized with a Potter Elvehjem devices, diluted and sterilized at 120ºC for 20 minutes (Marsollier, 2004).

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

The oligotroph seawater from Eforie Nord costal area was enriched with low concentrations of 0.1% of crude algae extract; the additions were 3 mg, 5 mg, 7 mg and 9 mg per liter of seawater used as culture medium. After harvesting the slides were stained by capillarity between slide and coverslip without fixation whit one drop of 0.1 % Methylene Blue.

In order to obtain data about the first phases in biofilms formation under environmental factors influences the biofilms were investigated for a period of 2 hours to 72 hours and analyzed under bright field light at the Hund Wetzlar Microscope with 100X objective and 10X ocular (Hulea, 1969). The number of bacteria was determined by means of the 10mm X 10mm micro-ocular grid (macroscopically), investigating 10 microscopic fields per harvested slide, three repetitions per analyzed time period (Fry, 1990).

RESULTS AND DISCUSSIONS

The analysis in bright field of the biofilms formed on the hydrophile surface of the glass slides collected from the containers with littoral seawater existence of successive phases for the formation of biofilms, which display an important increase of the bacterial density (Fig. 1) after a period of only three days (72hours) from the immersion of the substrate into seawater.

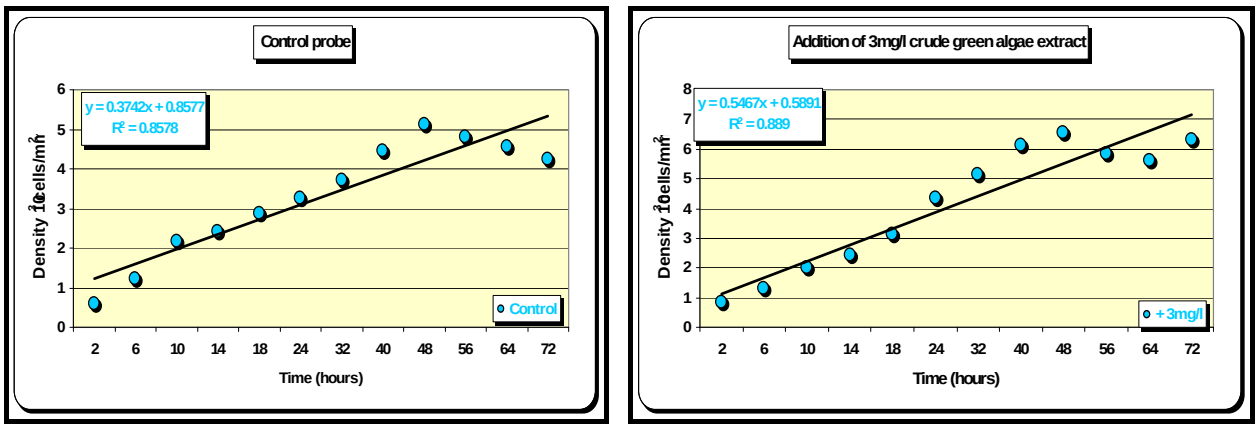

Fig.1 Bacterial cell growth in control probe (a) and after 3 mg/l of algae extract addition (b)

In the case of the slides collected from the containers without algae extract additions (control probe) there was initial cell density of $0.61 \cdot 10^3$ cells/mm^2 followed by a progressive increase of the cellular density after six hours at a value of $1.23 \cdot 10^3$ cells/mm^2 (double from the initial growth) and after 24 hours the cell density $3.25 \cdot 10^3$ cells/mm^2. This growth continues to 48 hours went the pick of $5.12 \cdot 10^3$ cells/mm^2 is achieved and after 72 hours the cell density decreases to a value of $4.23 \cdot 10^3$ cells/mm^2 (Fig.1a).

In figure 1.b the addition of 3mg/l of algae extract determined a higher initial density $0.83 \cdot 10^3$ cells/mm^2 followed by a progressive increase of the cellular density after six hours at a value of $1.33 \cdot 10^3$ cells/mm^2 (double from the initial growth) and after 24 hours the cell density $4.35 \cdot 10^3$ cells/mm^2. This growth continues to 48 hours went the pick of $6.55 \cdot 10^3$ cells/mm^2 is achieved and after 72 hours the cell density decreases to a value of $6.32 \cdot 10^3$ cells/mm^2.

The progression of cellular density growth was 0.37 for the control probe and 0.54, all sow the R coefficient was 0.86 for the control probe and growth and of 0.88 for the 3 mg/l addition.

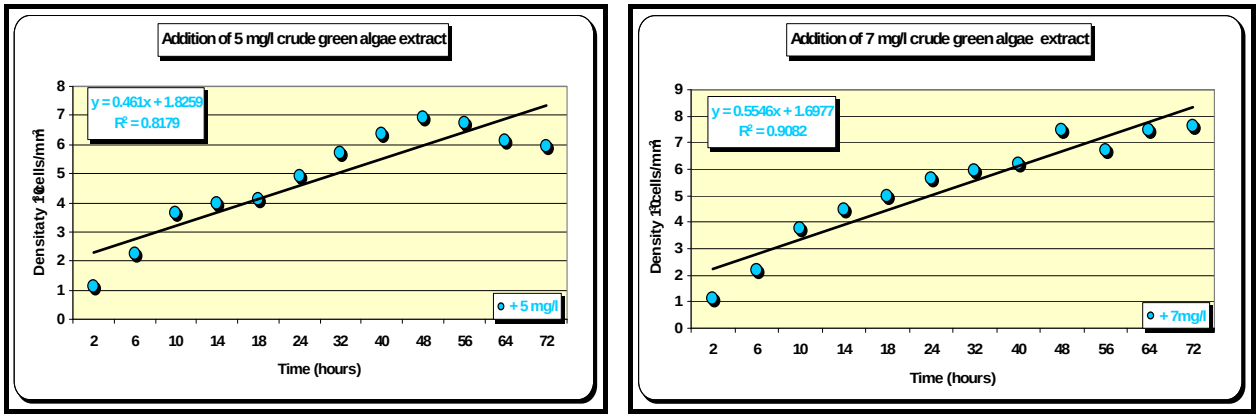

Fig.2 Bacterial cell growth after 5 mg/l (a) and 7mg/l (b) of algae extract additions

The addition of 5mg/l of algae extract determines a higher initial density $1.11 \cdot 10^3$ cells/mm^2 followed by a progressive increase of the cellular density after six hours at a value of $2.24 \cdot 10^3$ cells/mm^2 (double from the initial growth) and after 24 hours the cell density $4.93 \cdot 10^3$ cells/mm^2. This growth continues to 48 hours went the pick of $6.91 \cdot 10^3$ cells/mm^2 is achieved and after 72 hours the cell density decreases to a value of $5.96 \cdot 10^3$ cells/mm^2 (Fig.2a).

In figure 2.b the addition of 7mg/l of algae extract determines a higher initial density $1.12 \cdot 10^3$ cells/mm^2 followed by a progressive increase of the cellular density after six hours at a value of $2.19 \cdot 10^3$ cells/mm^2 (double from the initial growth) and after 24 hours the cell density $5.65 \cdot 10^3$ cells/mm^2. This growth continues to 48 hours went the pick of $7.46 \cdot 10^3$ cells/mm^2 is achieved and after 72 hours the cell density increase to a value of $7.61 \cdot 10^3$ cells/mm^2.

The progression of cellular density growth was 0.46 for 5 mg/l addition and 0.55 for the for the 7 mg/l one. The R coefficient was 0.81 in the case of the 5mg/l addition and even higher for 7 mg/l of 0.90.

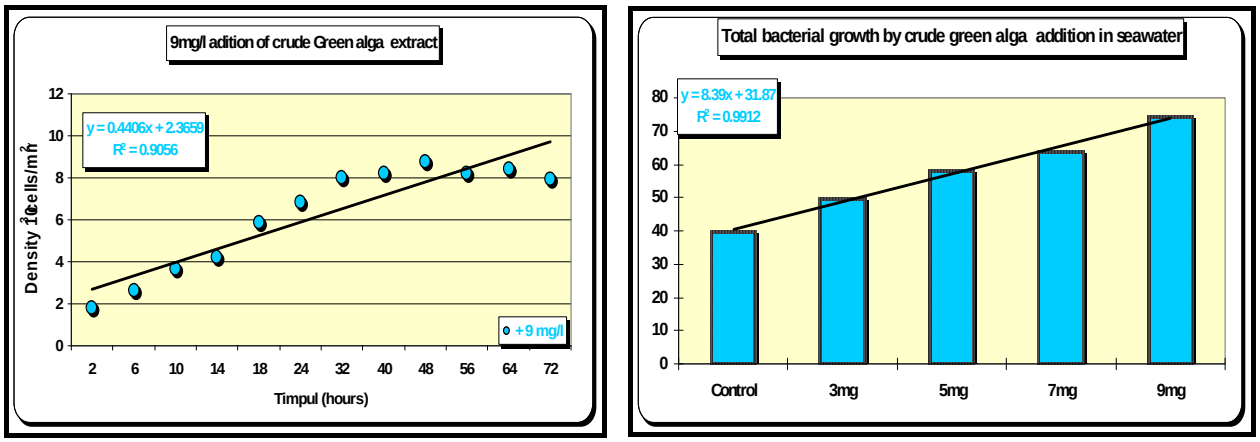

Fig.3 Bacterial cell growth after 9 mg/l of algae extract addition (a) and total cell growth (b)

The addition of 9mg/l of algae extract determines a higher initial density $1.77 \cdot 10^3$ cells/mm^2 followed by a progressive increase of the cellular density after six hours at a value of $2.65 \cdot 10^3$ cells/mm^2 (double from the initial growth) and after 24 hours the cell density $6.85 \cdot 10^3$ cells/mm^2. This growth continues to 48 hours went the pick of $8.76 \cdot 10^3$ cells/mm^2 is achieved and after 72 hours the cell density decreases to a value of $7.93 \cdot 10^3$ cells/mm^2 (Fig.3a).

In figure 1.b the control probes without 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 $49.7 \cdot 10^3$ cells/mm^2. A high concentration of 5 mg/l determined a bacterial cell density of $57.9 \cdot 10^3$ cells/mm^2. The use of even higher concentrations of 7 mg/l of tryptone reached the value of $63.6 \cdot 10^3$cells/mm^2 and at 9 mg/l was the bacterial cell density was $74.5 \cdot 10^3$cells/mm$^{2.}$

The progression of cellular density growth was 0.44 and R coefficient was 0.90 a high value for de 9 mg/l addition.

Table 1. The differences between control probe and the addition of crude algae extract

Differences	Crude algae extract
Control–3mg	$10.2 \cdot 10^3$ cells/mm^2
Control–5mg	$18.4 \cdot 10^3$ cells/mm^2
Control–7mg	$24.1 \cdot 10^3$ cells/mm^2
Control–9mg	$35 \cdot 10^3$ cells/mm^2

The differences between control probe in the case of tryptone additions were the 3mg addition this determine a bacterial growth of 25.8% from the initial cell growth, the addition of 5mg of tryptone determined a difference of 46.5% from the initial one. All sow the 7mg/l and 9mg/l additions determined a 61.0%, and respectively 88.6% from the initial cell growth of $39.5 \cdot 10^3$ cells/mm^2 (Tab.1).

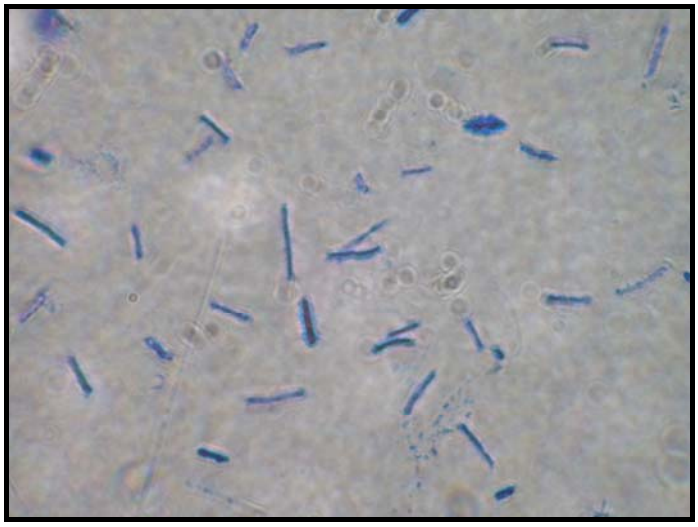

Fig.4 Bacterial cells attach under green algae extract influence

The late phases of bacterial biofilm formation do to the source of organic carbon provided by the green algae extract higher additions per liter of sea water, large bacteria adhere to the hydrophile surfaces submerged in static conditions (Fig.4).

CONCLUSIONS

Planktonic bacteria were influenced by the organic extract present in the sea water as a natural source of carbon.

High densities were obtain in the case of the 7 mg and 9 mg additions per liter of seawater.

Biofilms were formed by different bacterial cell forms attached on immersed surfaces but the bacilli forms were in high densities.

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