EFFECT OF Ag^{+}, Cu^{2+} AND Zn^{2+} CONTAINING HYBRID NANOMATRIXES ON THE GREEN ALGAE *CHLORELLA KEISSLERI*

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Summary. In the present work the algicidal effect of hybrid nanocomposites based on silicon precursor tetraethylortosilicate on the microalgae Chlorella keissleri was investigated**.** The inorganic-organic hybrid materials have been prepared by substituting part of the inorganic precursor with Ca alginate (5, 10, %). The antibacterial elements Ag, Cu or Zn was also included as nitrides (5 wt. %). Hybrid nanomatrix showed considerable decrease of algal cells growth. The results were estimated by the decrease of optic density (750 nm) and cells number /ml toward the control sample. The hybrid nanomaterials can be successfully applied as carriers for immobilization of different biomolecules, including whole bacterial cells. Thus created biocatalysts could be applied for different purposes, starting from ethanol production up to processes of bioremediation-treating of contaminated sites (waters and soils) polluted as a result from different industrial processes by microorganisms, mainly bacteria.

INTRODUCTION

Blooms of blue–green algae occurring in lakes, rivers are frequently

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toxic, producing a range of cyclic peptides known as microcystins. Unwanted algae growth in swimming pools is dilemma for pool owners worldwide. Over recent years a number of potable water purification systems have been developed to meet the needs of recreational and emergency use. As many lakes produce blooms, and an alternative water source may not be available, it is important to examine the performance of the potable purification systems.

The bioaccumulation and toxicity of heavy metals were reviewed with special reference to microalgae, the key component of the food web in aquatic ecosystems.

Many heavy metals (Fe, Cu, Mn, Zn) are essential micronutrients for algal metabolism and they may limit algal growth at low concentrations In contrast the heavy metal , Au, Ag, Pb and Cd have no known metabolic function, but may be toxic towards algae. The most commen overall toxcicity sequence to algae is Hg> Cu> Cd>, Ag > Pb. (Sorentino, 1979). Metal ions, either alone or in complexes, have been used for centuries to desinfect fluids, solids and tissues. Fundamentally bacteria and algae show low resistance to Ag, Cu and Zn ions which have been used for a long time in the biomedical field. Many new methods of purifying water from algae, fungi and bacteria include these metals. These results suggest that the metal ions caused stress in algal cells when enter inside either by means of active transport or by endocytosis and affect various physiological and biochemical processes of the algae. The toxicity primarily results from their binding to the sulphydryl groups in proteins or disrupting protein structure or displacing essential elements. Metals can break the oxidative balance of the algae, inducing antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and ascorbate peroxidase (APX) [1].

This materials prepared with the help of sol-gel chemistry have been applied to different research fields for years. The inorganic precursor mixed with a buffered aqueous solution containing different organic components in any ratio to formation hybrid organic-inorganic nanocomposites. The chemical composition is one of the most important parameters since its variation leads to formation of hybrid materials with distinctive physicochemical behaviors and profoundly different properties.

The aim of our work was to prepare hybrid nanomatrices with Cu^{2+}, Ag^{+} and Zn $^{2+}$ incorporated and study their algicidal effect.

MATERIALS AND METHODS

Preparation of matrixes

Using the sol-gel synthesis hybrid nanocomposites containing different quantity of caragiinan (5, 10 wt %) and the elements cooper, silver and zinc (5 %) have been prepared at room temperature as films. Silicon precursor tetraethylortosilicate (TEOS), "Sigma-Aldrich" purchased by "Merck" has been used.

A poly step sol – gel procedure is used for prepare the silica nanosol. Under strictly controlled pH conditions, in order to obtain the desired nanostructred materials. In all cases the ratio precursor /H2O is kept constant and equal to 1. A small amount of 0.1 N HCl is introduced to increase hydrolysis rate (pH~1.5). The hybrid nanomatrixes was poured into dishes to a layer thickness of approximately 1 mm. Gellation time of hybrid materials is about 60 minutes and was dried at room temperature and light.

Preparation of algal material

The green microalga Chlorella kessleri (initial density 0,5*106 cells/ml) was grown intensively with aeration (air+ 1 %CO2) on the Bold basal medium, pH 7,2 at light intensity 260 µEm-2s-1 and T 260C for 72 hours for 5 days to the late exponential growth phase, density 5. 10 ^{6}cells/ml. The algal cells were harvested by centrifugation .

Test the effect of nanomatrices on alga

An algal toxicity test has been developed specifically for assessment the effect of hybrid nano-matrices containing Ag^{+}, Zn^{2+}, or Cu^{2+} towards microalgae.

The nanomatrices were added to 5 ml suspension of Chl. kessleri

(2.10^6cell density).

The erlenmeyer flasks with the samples were allowed to remain undisturbed in luministate for 96 h. The algal growth was followed by cell count and by the decrease of optic density (750 nm) under Amplival light microscope. The influence of these antibacterial elements on free Chlorella cells was expressed by the dry weight, pigment contents reaction determination of absorbance spectra of the pigments (chlorophyll ”a”, chlorophyll ”b” and carotenoids after McKinney formula with Spectrophotometer (Carl Zeiss Iena, Germany). The exposure test involved post inoculation of algal cells in medium without matrix in order to determine wether the devices were algicidal or algistatic.

RESULTS AND DISCUSSION

The primary toxicity effect sought in this algal assay procedure is the algistatic responses of *Cl. kesslerii* to the tested materials - nanomatrices with incorporated ions of Ag^+, Zn^{2+} or Cu^{2+}. The influence of metal ions on Chlorella kessleri due to decreasing the algal growth and photosynthesis was in the turn: Cu> Ag> Zn. The considerable negative influence of Cu^{2+},

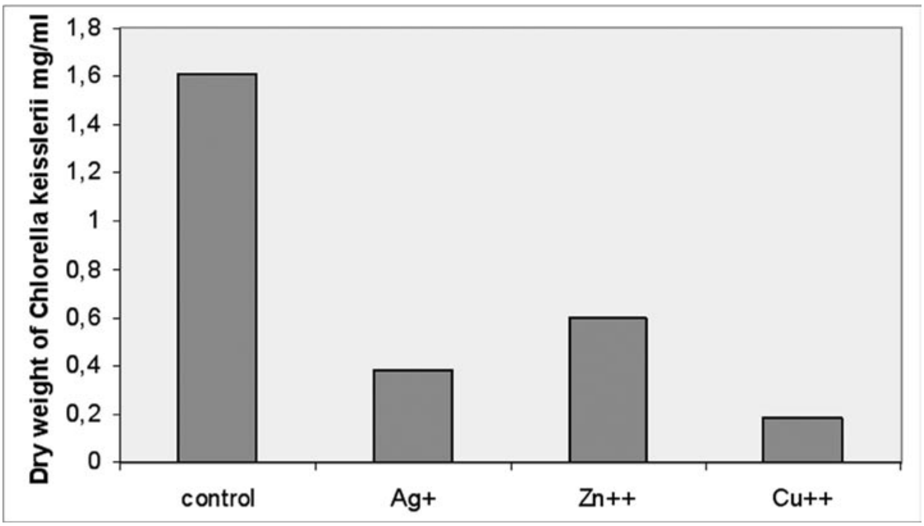

Fig. 1. Dry weight of *Chlorella keissleri.*

compared to the other ions, resulted with 2.6 – 2.8 fold decrease of cell number and 8 fold lower dry weight (Fig.1). The cells of Chlorella loose their green pigmentation after 96h contact with this matrix as a result of decreasing the content of green pigments chlorophyll "a" 1.4 fold ; chlorophyll "b" 1.9 fold and carothens –1.5 fold (Fig. 2).The addition of alginate (10 % or 5 %) in preparation the hybrid nanomatrix increased the algistatic effect caused by Cu^{2+} , lowering 2-2.8 fold the number of algal cells and between 1.5 and 1.6 fold the algaestatic effect caused by Ag^{+}, Zn^{2+} (Table 1).

Algae exhibit a variety of responses towards heavy metals which may determine growth and survival. The toxicity of metal ions toward representatives of the type Chlorophyta is different and depended on the metal examined as well as species properties of the algal strains (7T). It is considered that Chlorella vulgaris is more sensitive than Scenedesmus acutus to Cd, Zn and Chr, although Chlorella free cell system support Zn concentration until 600 mg/ L^{-1}.(7) , The Ag toxcitity to Chlorella reinhardtii is a direct result of intracellular accumulation, rather than surface interactions (1T). According to copper the discovery, that many algae are highly susceptible to copper, led to use of copper ions, alone or in

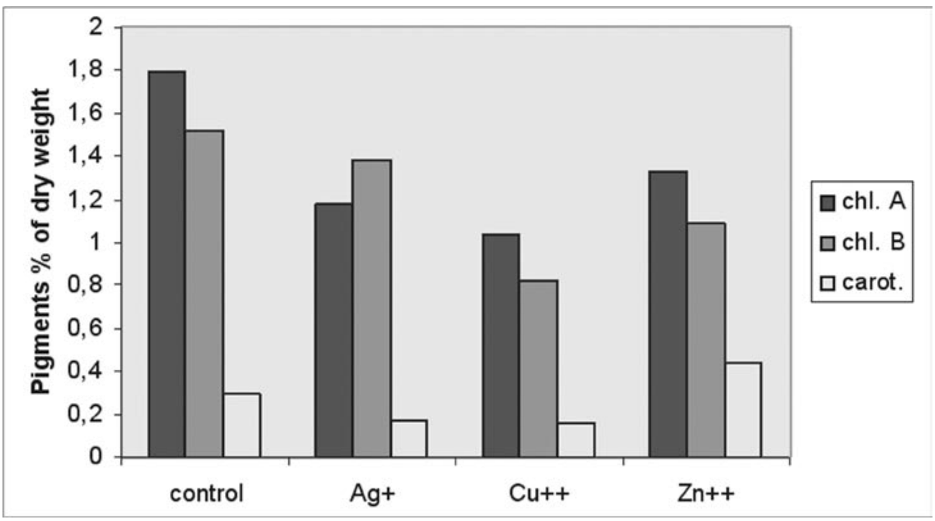

Fig. 2. Pigments of different microalgae, % of dry weight.

complex for centuries to prevent the development of algae in potable water reservoirs, Today copper is used as a water purifier, algaecide, fungicide, anti-bacterial, antifouling agent and others. Our results on tested in vivo strain Chlorella kesslerii with hybrid nanomatrix (TEOS + alginate) and Ag, Cu, Zn ions confirmed that the metal ions retained their algistatic effect to this representative of Chlorophyta even after their incorporation in nano – matrix.

Our results confirmed the suggestion that hybrid nanomaterials can be successfully applied as carriers for immobilization of different heavy metals for purify of water (Bottcher al., 2004). They are .promising for further utilization of the nano-matrices containing Ag, Cu, Zn in cleaning water

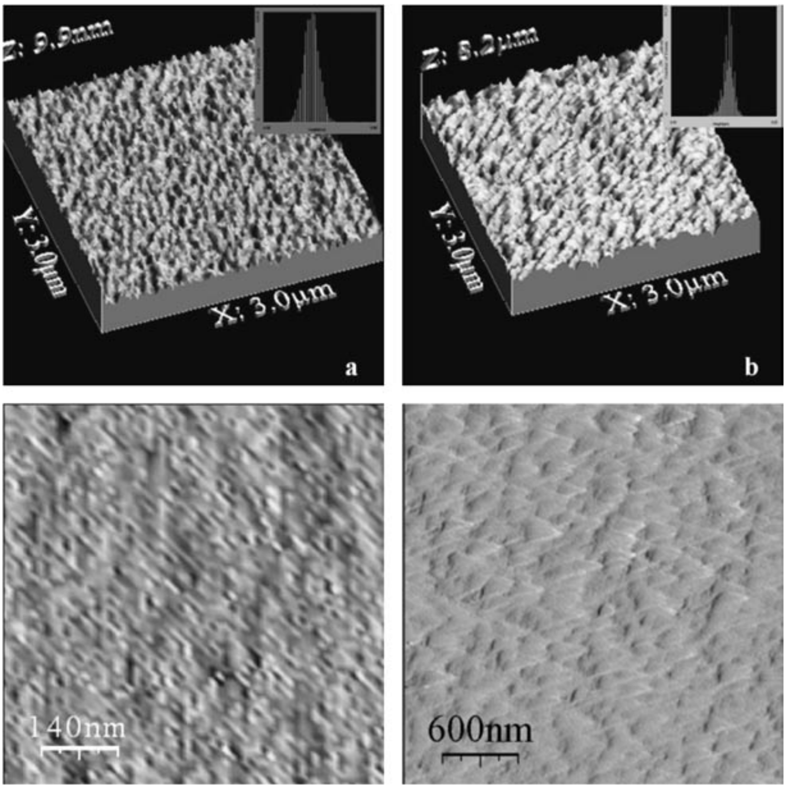

Fig. 3. AFM image and height distribution profile of surface roughness of hybrid materials containing 5 % (a); 10 % (b) carrageenan.

Table 1. Influence of different nanomatrices on the number cells of *Chlorella keissleri.*

Carageenan	Cu^{++}	Zn^{++}	Ag^{+}	Control
5 %	2.0*10^{6}	3.0*10^{6}	3.1*10^{6}	5*10^{6}
10 %	1.8*10^{6}	3.3*10^{6}	3.3*10^{6}	

basins from algae, especially because incorporated in non-biodegradable nanomatrices it can be avoid their toxicity to fish insects ,humans and others , when there are apply directly .

Recently in Nevada based, one of the leader in nanotechnology, Altair Nanotechnologies, uses 40 nm particles of a lanthanum based compound which absorbs phosphates from the water and prevents algal growth.

Nanostructure with well-defined nanounits and their aggregates, formed by self-organizing processes, was observed by AFM studies. The size of nanoparticles was from 6 to 12 nm and the dimensions of their self-assembled aggregates were about 25 – 86 nm (Fig. 4). In the same figure the height distribution profiles of surfaces roughness were shown. The histograms of the surface height distribution profiles, obtained from AFM images, showed that all of the inorganic-organic hybrid samples had surfaces with irregularities of quite small height. From AFM observation, the presence of periodically distributed nano-units (~10nm) was a verification of existence of Nanoscale Building Blocks through hybrid synthesis. Combined with XRD results and FT-IR spectra analysis, it was easy to deduce that although the hybrids had an amorphous nature from XRD, the observed Si-O-C and Si-C bonds in FT-IR spectra proved the presence of strong chemical bonds in the hybrid materials. These structures with ordered state in short range were better described as “self-organized”, as can be confirmed from AFM analysis.

The surface morphology and structure of nanobuilding blocks in each synthesized hybrid was different and depended on its chemical composition. In all samples the nanoparticles were well distributed in the entire hybrid matrix with a lower degree of aggregation. Although all being amorphous, quite different self-organized structures could be observed in these hybrids.

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