SUPORT CERAMIC POROS CU MICROORGANISME ÎNCORPORATE FOLOSIT ÎN PURIFICAREA APELOR NATURALE
POROUS CERAMIC SUPPORT EMBEDDED WITH MICROORGANISMS USED IN WATER PURIFICATION

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The aim of this study was to synthesize and characterize a ceramic material with a high porosity, which can be used as a support for the microorganisms' colonies for water purification. Given the target application, in order to obtain a composition of the ternary system MgO - Al_2O_3 – SiO_2, there were selected two natural materials: kaolin and talc powders. The chosen processing method involves mixing the ceramic powder with a porogen organic compound, in order to achieve a homogeneous suspension. Sintered ceramic samples were characterized in terms of ceramic properties, microstructure and phase composition using laser granulometry, Hg porosimetry, X-ray diffraction and scanning electron microscopy. The growth and activity of biofilm was investigated.

Scopul lucrării a fost acela de a sintetiza și caracteriza un material ceramic cu porozitate mare, care ar putea fi utilizat ca suport pentru colonii de microorganisme utilizate în purificarea apelor. Datorită aplicației țintite, pentru a obține o ceramică în sistemul ternar MgO - Al_2O_3 – SiO_2, au fost selectate pentru sinteză două materiale naturale: caolinul și talcul. Metoda de procesare aleasă implică amestecarea pulberilor ceramice cu un material organic porogen pentru a obţine o suspensie omogenă. Ceramicile poroase sinterizate au fost caracterizate în ceea ce privește proprietăţile ceramice, microstructura şi compoziţia fazală utilizând tehnici precum granulometria laser, porozimetrie cu mercur, difracţie de raze X şi microscopie electronică de baleiaj. Creşterea şi activitatea biofilmului au fost de asemenea investigate.

Keywords: *silicates, environment, biomaterials, ceramic filter, microorganisms*

1. Introduction

Water is a natural heritage essential to the human health. This is also an important compound used in a variety of industries like electronics, pharmaceutical and food industry. The world is facing formidable challenges in satisfying the requirements of increased quantities of clean water, due to the lower freshwater resources available caused by population growth, prolonged droughts, or more stricter regulations regarding health.

A new method of purification for lakes and freshwater was studied, using consortia of microorganisms embedded in porous ceramic substrates that are capable of removing the organic compounds from water and sediments. These ceramics ensure a good stability and allow the microorganisms to be placed effectively in the area of interest.

For various biotechnological applications, immobilization of microorganisms in a ceramic template represents an advantage, thus making an efficient use of their physiological capacity (for example in obtaining the secondary metabolites or in the biotransformation/biocatalysis reactions). The primary benefits of the bacteria immobilization are prevention of culture removal, easy separation of the formed products and the protection of incorporated cells from shear forces, pH etc. [1-4].

Water treatment using immobilized microorganisms inside ceramic matrix is characterized by cheap bioremediation costs since this system can be used several times without any significant loss, therefore it represents a new in situ or ex situ treatment for polluted waters.

It is also important to know the size of the microorganisms that can be used, varying between 100 nm to 50 mm, depending on their type. Given this fact, it is necessary to achieve a controlled microstructural design of porous materials.

Over the years there has been a significant growth of interest in the manufacture and use of porous ceramic materials which have specific properties such as high specific surface area, high permeability, low density, low specific heat and low heat transfer coefficient. These features are essential for technological applications such as ceramic filters, ceramic membranes or porous implants used as biomaterials.

Foamed ceramics have porosities between

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40 and 90%, and are characterized by open or closed spherical pores with different sizes. They can be obtained from different ceramic oxides (aluminum oxide, spinel– combination of magnesium and aluminum oxides, mullite– combination of aluminum and silicon oxides, cordierite– combination of aluminium, silicon and magnesium oxides).

Materials porosity can be obtained in a well-defined and homogeneous manner or heterogeneously. It can be oriented, separated, or interconnected. From these possibilities pores of different shape, size, and interconnectivity arise.

Inert ceramic foams combine a very high (geometric) surface area with a large and open pore structure, which allows water to flow with limited resistance. They can be used as a compact filter structure, which efficiently converts toxic wastes into non-toxic compounds and reducing the level of organics and algae [5,6].

2.Materials and methods

2.1. Ceramic component synthesis

The ceramic composition was chosen in the primary crystallization field of the cordierite [7], in an area where the melting of raw materials in the system is carried at low temperatures. This composition is characterized by the following percentages of oxides: 21% MgO, 17% Al_2O_3 and 62% SiO_2.

By choosing this compound, it is ensured that it is not affecting chemically the natural environment in which the materials will be placed in and, of course neither the microorganisms colonies that we want to incorporate. Also, it should have a high mechanical resistance and a low price. The specified composition can be obtained from natural raw materials, which is an important advantage. Homogenization of the precursor's powders was performed in a planetary ball mill using a FRITSCH Pulverisette, which was operated at a speed of 150 rpm/min for 30 minutes in ethanol. After this stage, the mixture was dried and after that subjected to calcination in an electric oven at a temperature of 1000°C for 2h, followed by powder grinding. Calcination temperature was determined according to the thermo gravimetric analysis performed (Figure 1).

As a result of the thermal analysis of the raw materials mixture, at 1000°C there is a total weight loss of 10.61% (Figure 1), according to TGA analysis. At temperatures of 508°C and respectively 927°C are two major endotherm effects, which are attributed to the dehydration of kaolin and talc.

In order to obtain a porous structure in the ceramic samples, it is necessary to introduce a porogen organic agent that will release a certain amount of gas, resulting in the formation of porosity. The conditions of the decomposition

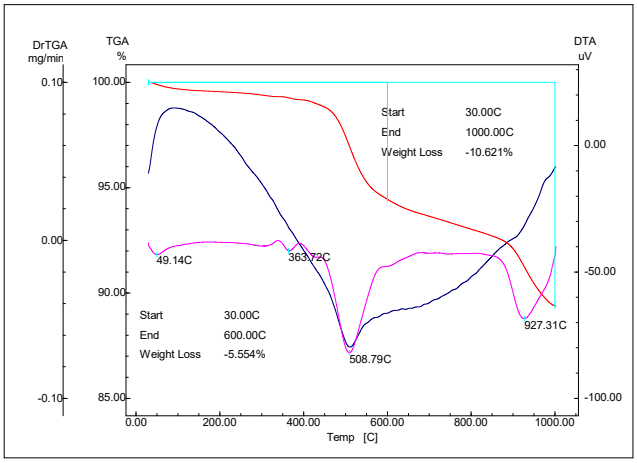


Fig. 1- Thermal analysis of kaolin/talc mixture / *Analiza termică a amestecului caolin/talc.*

reaction (in terms of temperature and reaction speed) are very important, this determining the microstructural and mechanical properties of the porous ceramic material.

2.2 Particle size distribution

Ceramic powder obtained by heat treatment at 1000°C was characterized in terms of particle size distribution using a Mastersizer 2000 laser diffraction particle size analyzer with a measuring area of 20 nm – 2 mm, that has a semiautomatic dispersion unit Hydro2000MU and an automatic air release dispersion unit SCIROCCO. Size distribution diagram is shown in Figure 2. There are present particles with sizes between 1 and 100 microns. Specific surface of the powder is of 1.23 m^2/g and the average particle size of 7.59 microns.

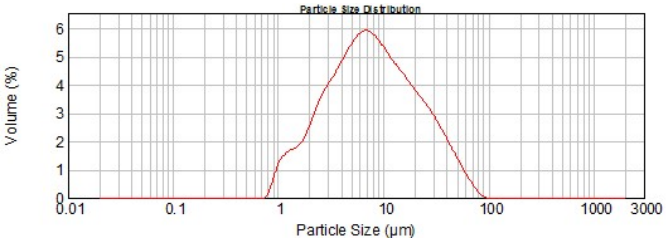


Fig.2- Granulometric distribution of ceramic powder heat treated at 1000°C / *Distribuţia granulometrică a pulberii ceramice tratată termic la 1000°C.*

2.3 Experimental procedure

To achieve a porous ceramic with a 60-90% porosity, it was chosen an organic additive from the carbohydrate class – anhydrous glucose *D(+) R.P.NORMAPUR* [8]. For ensuring a good homogeneity, additives were added, each with a specific role. Darvan C and ammonium citrate dispersants were added for stabilizing the ceramic suspension.

Mixing the ceramic component with the organic porogen agent was carried in an

environment in which the organic additive is soluble, water, thus obtaining an advanced structural homogeneity. Therefore, the first step consists in dissolving the organic additive in water, then the dispersants and ceramic powder were added. Mixing was realized first with with a magnetic stirrer finishing with a magnetic one and the temperature was maintained constant at 60°C. The recipient containing the mixture was immersed in a water bath to maintain the temperature stable and uniform through the entire volume of suspension. After obtaining a cvasi-dried powder, it was transferred into a drying oven where it was left at 45°C for 24 hours, followed by the sintering treatment.

Therefore, the choice of the porogen organic compound has a major importance, it is responsible for the obtained material characteristics and the heat treatment that allows it to be removed. Removal process should develop slowly to prevent cracking, expansion or hollow struts that may influence the mechanical strength of the final material. Hence, in the case of slow heating, the released gases have enough time to expand under the heat influence [9,10].After uniaxial pressing, samples were heat treated individual in crucibles because they have the tendency to lose their shape.

The heat treatment necessary for obtaining porous ceramic templates was carried in two main steps, one intended for the removal of organic compound and one step of sintering. The first stage involves heating the samples up to 150°C with a speed of 50°C/h, followed by a calcination treatment increasing the temperature up to 500°C with a speed of 10°C/h and a maintenance time of 2h at 500°C. The second stage involves the sintering treatment of the three samples at temperatures of 1100, 1200 and respectively 1300°C with a speed of 120°C/h and a maintenance time of 2h for all of these samples. The last phase is cooling the samples with a speed of 120°C/h.

2.4. Microorganisms immobilization

From the three sediment samples taken from three points of Lake Snagov, Antena Tăncăbești Complex Peace and Santu` Floreşti, about 10 g of sludge was diluted with 100 ml distilled water. The suspension was stirred for 2 hours and then was allowed to sediment for 2 hours (or the suspension was filtered through a fluted filter twice). From the supernatant (clear) a sample of 20 ml was taken and inoculated in a nutrient solution sample of known concentration according to SR EN ISO 14581:2005 [11].

To obtain the nutritional solution which will simulate a natural environment, it was used a standardized medium. This medium consists of several solutions that will serve as nutrients for the microorganisms.

Solution A was obtained by dissolving 1.7 g of anhydrous potassium di-hydrogen phosphate KH_2PO_4, 4.35 g anhydrous di-potassium hydrogen phosphate K_2HPO_4, 6.68 g of disodium dehydrate $Na_2HPO_4 \cdot 2H_2O$, 0.1 g of ammonium chloride NH_4Cl in distilled water up to 200ml in a flask. The solution B was obtained by dissolving 2.25 g of hepta-hydrate magnesium sulfate $MgSO_4 \cdot 7H_2O$ in distilled water up to 100 ml in a flask. Solution C was obtained by dissolving 3.65 g of di-hydrate calcium chloride $CaCl_2 \cdot 2H_2O$ in water to 100 ml. Solution D is obtained by dissolving 0.025 g of hexa-hydrate iron (III) $FeCl_3 \cdot 6H_2O$ in distilled water 100 ml.

To make one liter of test medium there were added to 500 ml of distilled water 10 ml of solution A, 1 ml of solution B, C and D, and the flask was filled with water up to 1000 ml.

Fig. 3- Porous ceramic supports in simulation medium / *Simulare în mediu cu suportul ceramic poros.*

The porous support is inserted to the simulation medium and air is bubbled at a known rate (Figure 3). The microorganisms' film formed on the support is analyzed.

3. Results and discussions

The resulting ceramic powder was investigated in terms of ceramic properties, microstructure and phase composition using laser granulometry, Hg porosimetry, X-ray diffraction (XRD) and scanning electron microscopy (SEM).

3.1. Mineralogical composition

Mineralogical composition was determined using X-ray diffraction, analysis was carried with an X-ray diffractometer XRD SCHIMADZU 6000. XRD patterns obtained on ceramic powder sintered at 1100, 1200 and 1300°C are shown in Figure 4. By applying the sintering heat treatment it can be observed that with increasing the temperature, the intensity of diffraction interferences increases. However, starting with 1200°C besides mineralogical phases present at 1000°C (enstatite $MgSiO_3$ [ASTM 84-0652] and

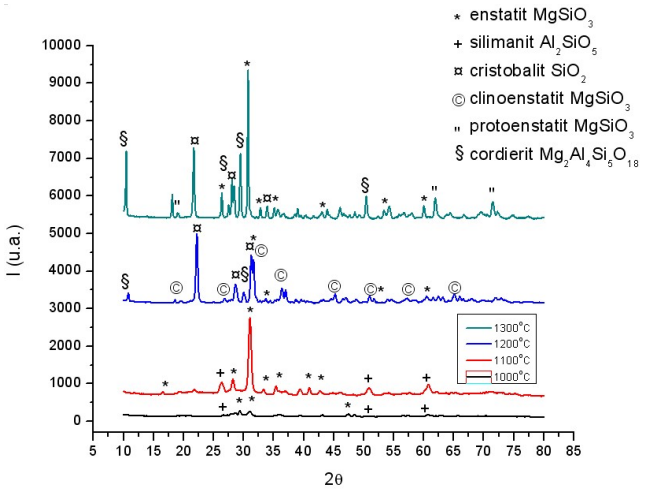


Fig.4- X-ray diffraction spectra of calcined and heat treated ceramic powder at 1100-1300°C / *Difractograme de raze X obţinute pe pulberile ceramice calcinate şi tratate termic la temperaturi cuprinse între 1100 – 1300°C.*

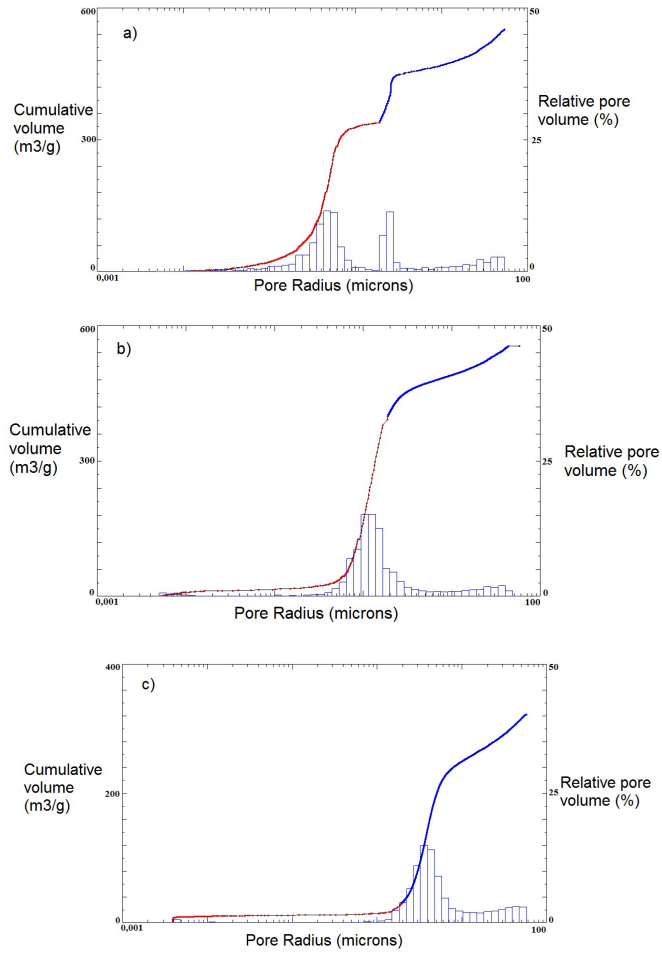


Fig. 5 - Pore size distribution of sintered materials at: a) 1100°C, b) 1200°C and c) 1300°C / *Distribuţia granulometrică a porilor pentru materialele sinterizate: a) 1100°C, b) 1200°C, c) 1300°C.*

silimanite Al_2SiO_5 [ASTM 01-0626]), it can be observed the formation of clinoenstatite $MgSiO_3$ [ASTM 84-0652] and cristobalite SiO_2 [ASTM 82-1406] phases. At 1300°C sample contains cordierite $Mg_2Al_4Si_5O_{18}$ [ASTM 84-1221], cristobalite SiO_2 [ASTM 75-1544], protoenstatite $MgSiO_3$ [ASTM 76-1806] and enstatite $MgSiO_3$ [ASTM 03-0519].

3.2. Porosity

There were performed porosity measurements on sintered porous ceramics using a Hg porosimeter Pascal 240/120. Porosity values obtained are shown in Table 1, and the pore distribution is shown in figure 5, for all sintering temperatures.

High values of porosity is observed for the sample sintered at 1100°C – 80%, this percent decreasing to 42%, as the degree of densification increases with increasing heat treatment temperature up. Simultaneous, the medium pore size is increasing, from 0.568 μm to 4.17 μm. This fact can be explained by the disappearance of smaller size pores during the densification treatment.

By analysing the distribution of pores is observed mono-modal distribution of it for sintering temperatures of 1200 and 1300°C, while at 1100°C the distribution is bimodal due to the presence of smaller pore size fraction.

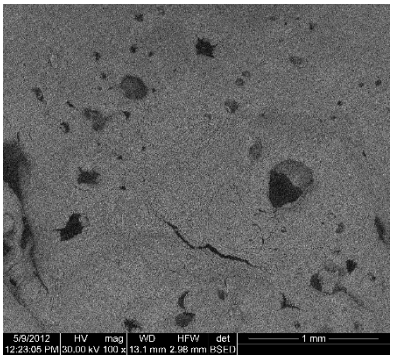
3.3 Microstructure

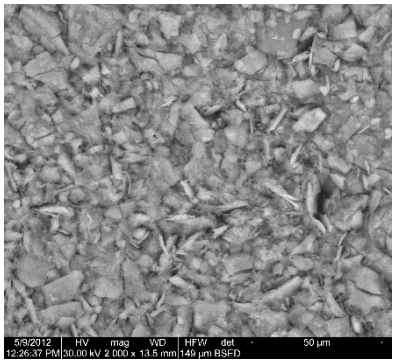
Investigation of porous ceramic samples was performed with a scanning electron microscope (SEM) QUANTA INSPECT F, equipped with a field emission electron gun FEG with a 1.2 nm resolution and an energy dispersive X-ray spectrometer (EDS), with 133eV resolution at MnK.

Scanning electron microscopy images are shown in Figure 6 for section and surface samples of the porous ceramic materials treated at temperatures between 1100 and 1300°C.

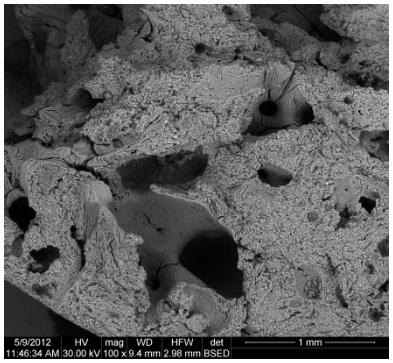
Depending on the sintering heat treatment (1100°C, 1200°C and 1300°C) is observed that samples containing 50% glucose have different microstructure.

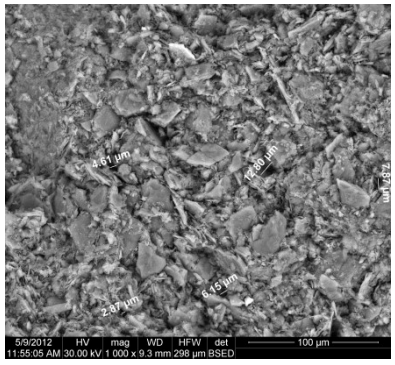
With increasing the heat treatment temperature, porosity becomes more uniform, micro-sized pores disappear and the macro-sized ones become relatively spherical in shape. In the detailed image is rendered homogeneous size distribution as a result of good sintering. Elongated grains are predominant and a better densification of the ceramic material is observed.



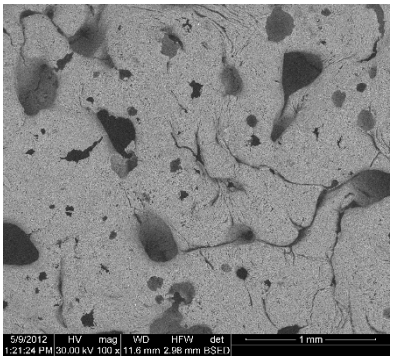


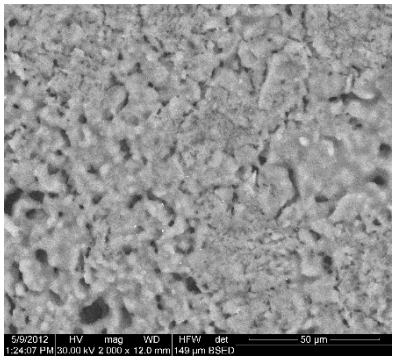
A1) SEM images of ceramic sample surface obtained at 1100ºC */ A1) Imagini MEB obţinute pe suprafaţa probei ceramice sinterizate la 1100°C.*



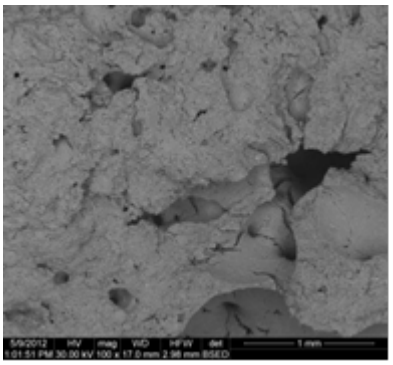


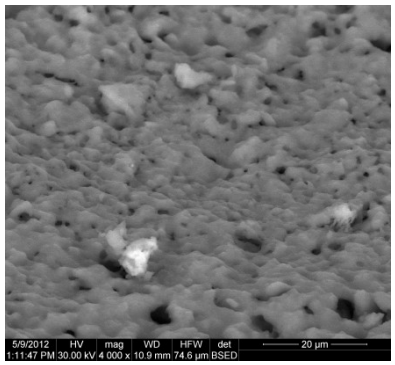
A2) SEM images of ceramic sample in fracture obtained 1100ºC / *A2) Imagini MEB obţinute în fractura probei ceramice sinterizate la 1100°C.*



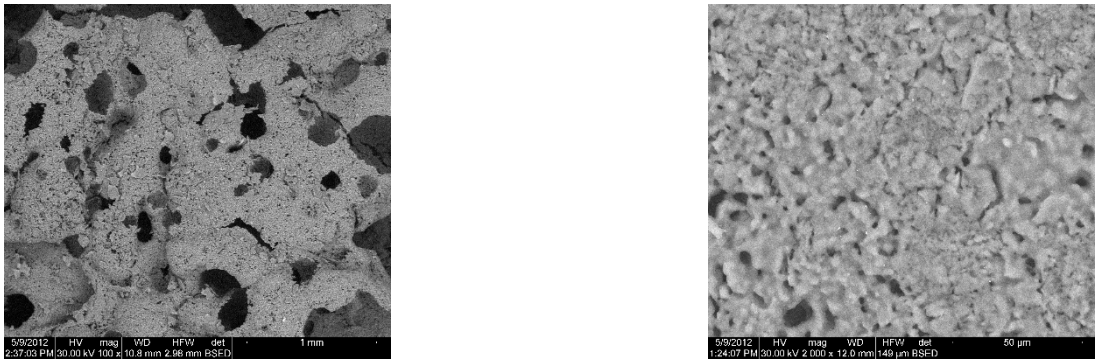


B1) SEM images of ceramic sample surface obtained at 1200°C / *B1) Imagini MEB obţinute pe suprafaţa probei ceramice sinterizate la 1200°C.*

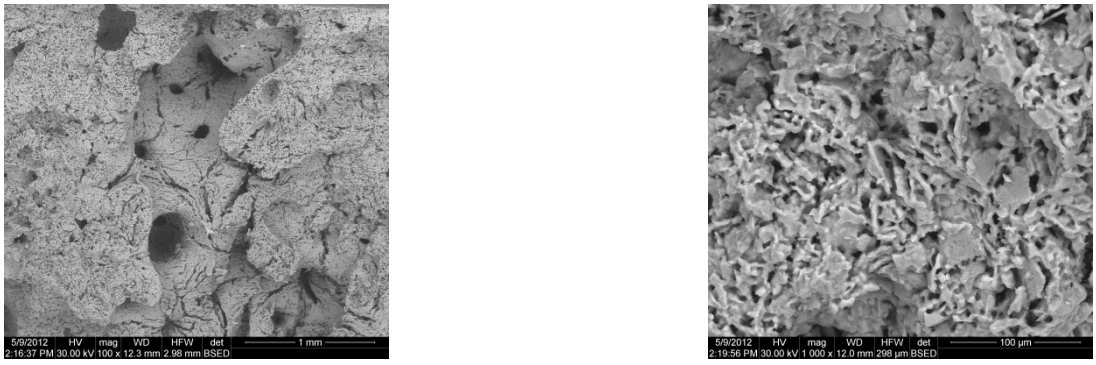




B2) SEM images of ceramic sample in fracture obtained at 1200°C / *B2) Imagini MEB obţinute în fractura probei ceramice sinterizate la 1200°C.*



C1) SEM images of ceramic sample surface obtained at 1300°C / *C1) Imagini MEB obţinute pe suprafaţa probei ceramice sinterizate la 1300°C*



C2) SEM images of ceramic sample in fracture obtained at 1300°C / *C2) Imagini MEB obţinute în fractura probei ceramice sinterizate la 1300°C*

Fig. 6- SEM images of sintered samples at: a) 1100°C, b) 1200°C and c) 1300°C / *Imagini MAB obţinute pe ceramicile sinterizate la: a) 1100°C, b) 1200°C and c) 1300°C.*

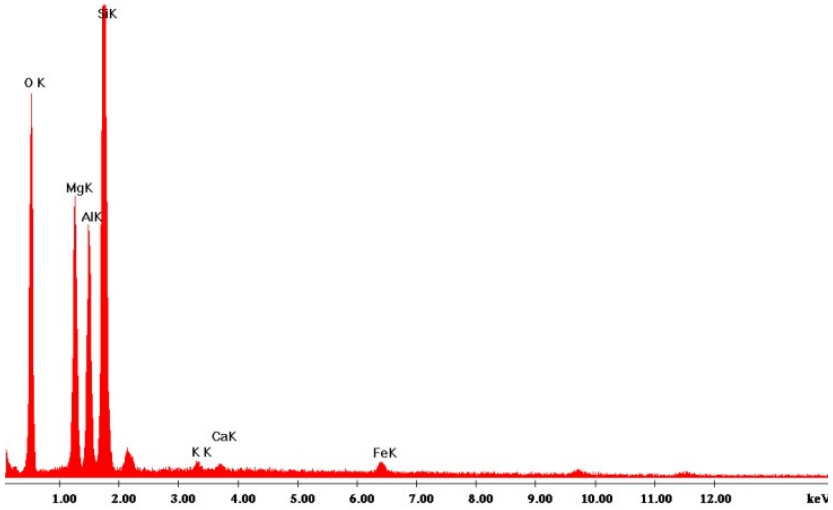


Fig. 7- EDX spectrum associated with ceramic sample sintered at 1100°C / *Spectru EDX obţinut pe proba ceramică sinterizată la 1100°C*

Energy dispersive X-ray spectrum (EDAX) reveals the main phases present in the sample, indicating a good homogeneity of components (Figure 7). However, there are three minority phases that occur due to impurities existing in kaolin – Fe, Ca and K.

4. Proteolytic activity

Based on the previously conducted SEM analyzes, we concluded that the best sintering temperature for ceramic supports was of 1300^0C, due to crack disappearance and more homogenous porosity.

Proteins are considered nutrients for microorganisms, although they are not assimilated directly due to their large size, therefore they need to be decomposed into peptides and then into amino acids using extracellular enzymes [12,13]. Bacterial cultures with highest enzymatic activity that can decompose nitrates, nitrites and organic

compounds were grown from the water samples. This enzymatic activity was determined using two methods: casein hydrolysis and gelatin hydrolysis.

In order to highlight the proteolytic microorganisms', from the hydrolysis of casein colonies leave around them a clear zone of lysis in the culture medium. Measuring the diameter of each lysis and colony, we established the protolithic indices of every strain.

Data obtained from casein hydrolysis suggest that the highest values of proteolytic indices are within 21 hours after inoculation. Over time, the colonies size increases, which may lead to lower proteolytic indices at the final reading.

Table 1

Proteolytic activity of bacteria colonies by casein and gelatin hydrolysis */ Activitatea proteolitică a coloniilor de bacterii determinată prin hidroliza cazeinei și gelatinei*

Strain	Casein hydrolysis	Gelatin hydrolysis
LSF1		-
LSF2		-
LSF3	+	-
FSF1	+	-
FSF2	+	-
FSF3		-
LAT1		-
LAT2	**+**	**+**
LAT3		+
FAT1	+	-
FAT2	+	+
FAT3		+
LCP1	+	-
LCP2		-
LCP3	**+**	**+**
FCP1		+
FCP2	+	+
FCP3	**+**	**+**

Microorganisms producing extracellular proteases can hydrolyze gelatin culture medium which it loses its ability to solidify at low temperatures.

Based on enzymatic activity, we observed that strains with the best ability to hydrolyze casein and gelatin are strains LAT2, LCP3 and FCP3. These strains were grown on porous ceramic supports in order to observe the development of biofilm.

4.1. Biofilm growth

Investigation of biofilms samples was performed also with a scanning electron microscope (SEM) QUANTA INSPECT F, equipped with a field emission electron gun FEG with a 1.2 nm resolution and an energy dispersive X-ray spectrometer (EDS), with 133eV resolution at MnK.

On all three substrates the biofilm development it is present. The coating of the samples is heterogeneous, films having thicknesses between 50 nm and 1 μm. The presence of chain bacilli and fungal matter it is confirmed from these SEM images (Figure 8).

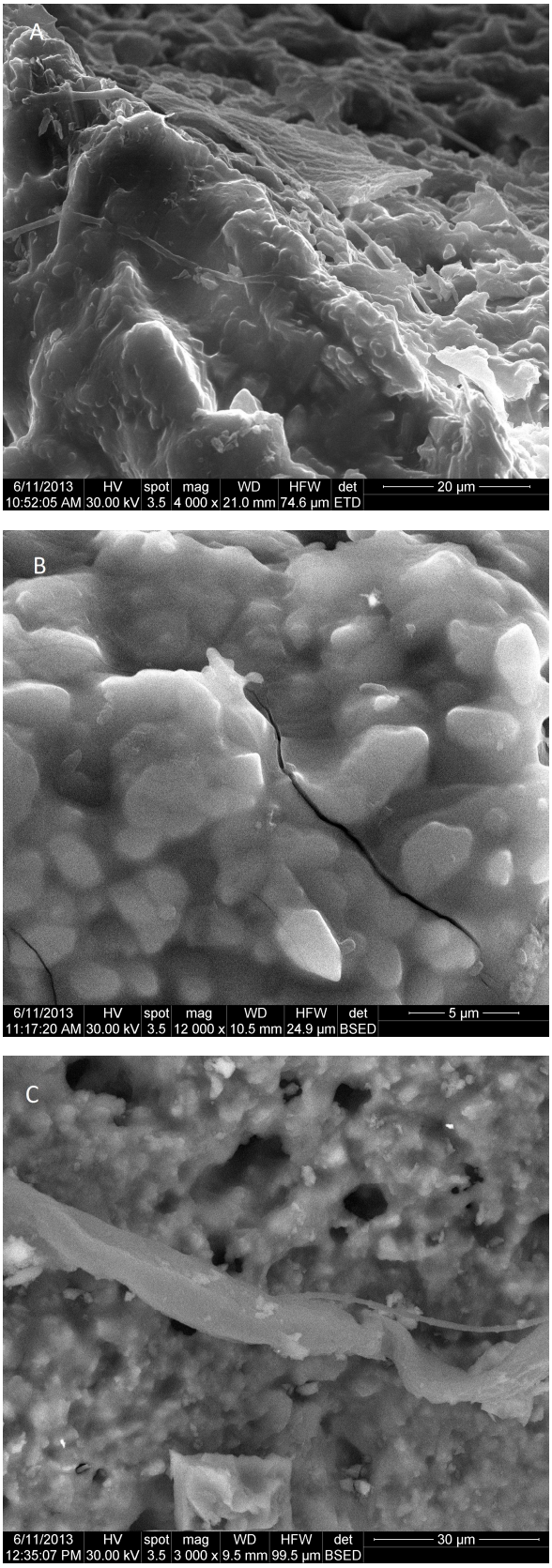


Fig. 8- SEM images of biofilm samples: A) LCP3, B) FCP3 and C) LAT2 / *Imagini MEB pe probele pe care a fost crescut biofilm: A) LCP3, B) FCP3 and C) LAT2.*

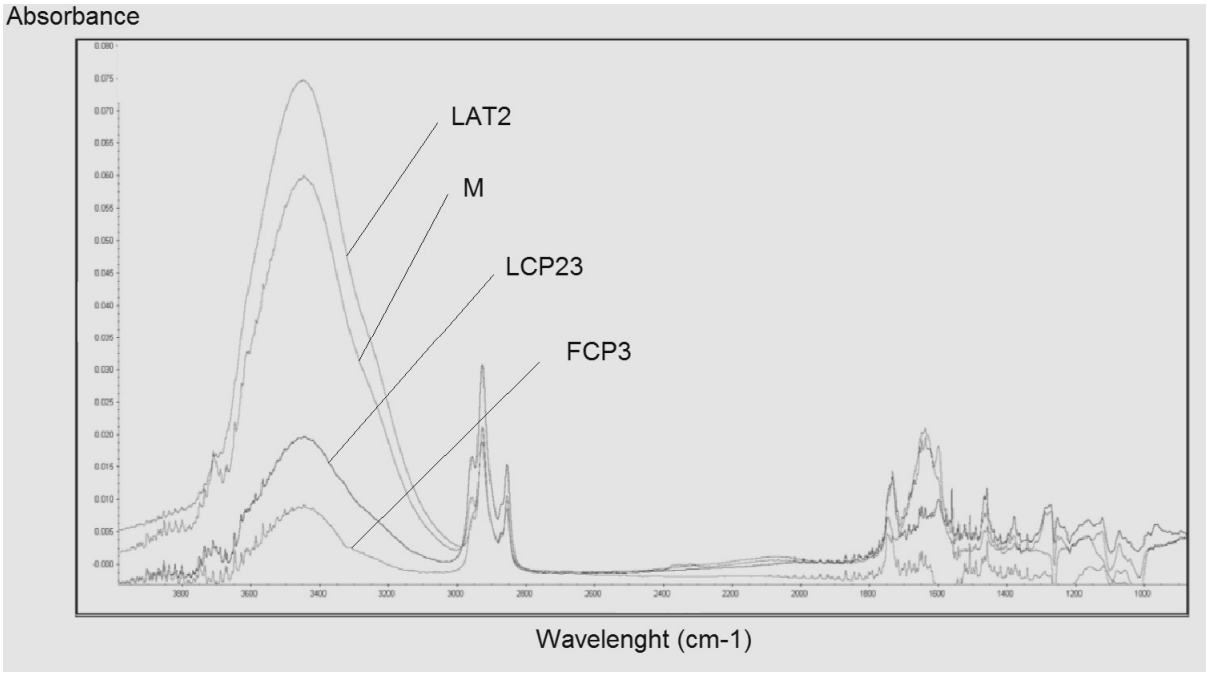


Fig. 9- FT-IR absorption spectrum of biosupport samples and control sample / *Spectru de absorbţie FTIR pentru probele cu biofilm şi proba control.*

4.2. Supports activity

Medium activity was investigated using a Nicolet 6700 FT-IR spectrophotometer (Thermo Nicolet, Madison, WI), connected to the working Omnic software (version 7.0. Thermo Nicolet) used to determine the content of organic substances present in the samples. For this analysis there have been created biosupport/ water samples systems, results are shown below.

By comparison (Fig. 9), it can be observed an increase in the intensity of bands from 2956 cm^{-1} to 2855 cm^{-1} corresponding to symmetric υCH3, symmetric υCH2 and asymmetric υCH2 groups for A) LCP3, B) FCP3 and C) LAT2 samples than the intensity peaks of the of the control sample which is coloured in red. This can be attributed to an increase in the amount of organic matter by micro-organisms accumulation.

Also, the intensity of specific N-H group bands found at 1638-1588 cm^{-1} wavelength have a tendency to decrease, possible due to decomposition of organic ammonium ions by microorganisms and their conversion into inorganic nitrogen. The largest decrease occurred for LCP3 sintered sample [14].

5. Conclusions

The goal of this study was to obtain a porous material based on kaolin and talc, corresponding to the desired properties that can be used as a ceramic support in water purification. To create the porosity, it was used a porogen organic compound method, which involves mixing ceramic powder with an organic agent that generates pores by combustion- glucose. Mixing the two components was performed in suspension.

X-ray diffraction spectra revealed different mineralogical compositions depending on sintering temperatures, at a temperature of 1300°C the main phases present are cordierite, enstatite and cristobalite.

Hg porosimetry results indicate lower porosity value of the sample with increasing heat treatment temperature, but also an increase in the pore size. Pores are interconnected, with sizes between 0.56 and 4.2 microns.

Scanning electron microscopy showed a heterogeneous structure with crates and large pores- up to 600 μm, but also the presence of smaller pores in the ceramic matrix forming that form bridges between cells.

Based on the enzymatic activity of the colonies, there were selected only those with high proteolytic activity, obtained by the hydrolysis of casein and gelatin. These resulting strains (LCP3, FCP3 and LAT2) were grown on porous ceramic supports.

Development of biofilms was successful on all samples, although the morphology and distribution were different for each kind strain.

FT-IR spectroscopy has shown a decrease in the intensity of peaks corresponding to organic ammonia in the samples. FCP3 sample had the best activity in this regard.

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MANIFESTĂRI ȘTIINȚIFICE/ SCIENTIFIC EVENTS

Topics:

- Computational modeling, simulation and design of new materials and processes
- Fibers and preforms
- Interfaces and interphases
- Innovative Design, Advanced Processing, and Manufacturing Technologies processing in composites : Oxides & Geopolymers (incl. short fibers)
- Innovative Design, Advanced Processing, and Manufacturing Technologies processing in non-oxide composites : SiC/SiC, C/SiC, hybrid CMCs (incl short fibers)
- Additive manufacturing of CMCs : 3D printing, laser sintering, etc …
- Materials for Extreme Environments: Ultrahigh Temperature Ceramics (UHTCs) and Nano-laminated Ternary Carbides and Nitrides (MAX Phases)
- Advanced Thermal and Environmental Barrier Coatings: Processing, Properties, and Applications
- Polymer Derived Ceramics and Composites (incl. Reinforced foams)
- Carbon/carbon composites
- Thermomechanical behavior and performance of Composites
- Nondestructive Testing and Health Monitoring of Ceramic Composites
- Joining & integration
- CMC Applications in Space Transportation
- CMC Applications in Terrestrial Transportation and Industrial Systems
- CMC Application in aeronautic engines
- Advanced materials for sustainable energy (incl. nuclear fission and fusion, industrial gas turbines)

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