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EFFECT OF NATURAL ZEOLITE FUNCTIONALIZED WITH TiO_2 FOR *ENTEROCCOCUS FAECALIS* REMOVAL FROM WATER

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The aim of this paper was the syntheses of natural zeolite modified with undoped TiO_2 / nitrogen doped TiO_2 by calcination method in order to remove *Enterococcus faecalis* from water. The obtained materials were characterized by X-ray diffraction (XRD), UV-VIS diffuse reflectance spectroscopy (DRUV-VIS), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The antibacterial tests showed that the antibacterial activity of hybrid materials is excellent against *Enterococcus faecalis*.

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1. Introduction

Microbial decontamination of water is usually the final stage in the water treatment process. This step is necessary to prevent the introduction of pathogenic microorganisms in natural water bodies. Recently the use of chlorine for microbial decontamination has been widely discussed [1]. Concerns exist over the conversion of organic compounds to chlorinated carcinogenic molecules [2, 3]. Previous health and epidemiological studies by the US Environmental Protection Agency (US EPA) have demonstrated that colony-forming unit (CFU) densities of the bacterial genus Enterococcus in both marine and freshwater samples are directly correlated with gastroenteritis illness rates in swimmers exposed to these waters [4]. In the last decade however, enterococci have become recognized as leading causes of nosocomial bacteremia, surgical wound infection, and urinary tract infection [4]. The photocatalytic activity of titanium dioxide doped with metallic ions, has been extensively studied over the last 25 years by the researcher from different prestigious researches institutes. The photocatalysis are usually used for his degradative effect on the organic compoud that contaminates air and water.

In the case of nano-materials we can point out that while their particles size decrease, the rate of photo catalytic reactions and their redox potential increases [5]. Photo catalysis refers to chemical reactions that take place when radiations focus on chemical components. In the case of TiO_2 chemical reactions repeat and the effect is obtained very close to the substratum, causing microorganism degradation [6]. In 1985, Matsunaga et al. carried forward that microbial cells from

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the species Escherichia Coli present in water have been destroyed when they made contact with TiO_2-Pt that was exposed to UV radiations for 60-120 minutes [7]. Some years later, the same group of researchers made a dispositive intended for a photo catalytic experiment which immobilised ashes of TiO_2 on a membrane of acetylcellulose. Introducing in the interior of the device a suspension of E. coli, demonstrating that all the microbial cells have been killed [8]. These inventions have opened a new era for sterilization methods of water through photocatalytic technology.

Photocatalysts based on TiO_2 have been intensively investigated in order to achieve better photocatalytic efficiency for different applications. Recently, studies on the doping of transition metal and non-metal ions into TiO_2 and the immobilization of TiO_2 on supports have become attractive in the area of photocatalysis [9-13]. Various substrates have been used as a catalyst support for the photocatalytic degradation of polluted water, such as glass and stainless steel, alumina beds, activated carbons, MCM-41 and zeolites [14-17]. The results revealed that supports possess good pollutant adsorption, diffusion properties and absence of light absorption, which exhibited the enhancement of photocatalytic activity of dispersed TiO_2 [18]. Zeolites, synthetic or natural, are aluminosilicates that have a framework structure with enclosed cavities and tunnels which are occupied by freely moving hydrated cations that make ion exchange possible. Clinoptilolite is the most abundant and cosmopolitan zeolite and it has been widely exploited for its ion-exchange capabilities since it can easily exchange its interstitial sodium for external cations in solution [19].

This paper reveals the synthesis of hybrid materials based on natural zeolite modified with undoped TiO_2 / nitrogen doped TiO_2 by calcination method. X-ray diffraction, DRUV-VIS spectroscopy and SEM/ EDX analysis were used for the structural and morphological characterization of hybrid materials. The performances of the photocatalytic activities of the hybrid materials were determined comparatively for the removal of *Enteroccocus faecalis* species from water.

2.Materials and methods

2.1. Hybrid materials synthesis

Romanian zeolitic mineral from Mirsid, used as support for doped TiO_2 loading, was supplied by Cemacon Company, Romania. The mineral was powdered and sieved with a Multilab sieve shaker. The diameter of grains size selected to carry out the experiments was between 0.8-1.2 mm with the mass composition 62.20% SiO_2, 11.65% Al_2O_3, 1.30% Fe_2O_3, 3.74% CaO, 0.67% MgO, 3.30% K_2O, 0.72% Na_2O, 0.28% TiO_2.

The hybrid materials based on natural zeolite and undoped and N-doped TiO_2 were synthesized by calcination method. Consequently, Na forms of clinoptilolite are expected to remove other cations easily in ion-exchange applications. For that reason, Na forms are the forms most frequently prepared for research in clinoptilolite literature [20]. Therefore, the preparation of the chemically modified zeolite presumes two stages to reach acid form (H form) by using 2M HCl solution and sodium form (Na form) with 2M $NaNO_3$ solution for a more efficient ion exchange.

For the hybrid materials synthesis it was used N-doped TiO_2 nanocrystals previously synthesized by sol-gel method [21]. An amount of ethanol was stirred with 5 mL TTIP and after 10 minutes, 30 mL of distilled water was added in dropwise. The pH of the initial solution was 5.5 and before adding the doping precursor (uree) the pH was adjusted to 8 with NH_3 solution. The solutions were filtered, washed and dried to 60ºC for 5 hours. For crystallization the materials were annealed into an oven at different temperatures.

The next step was the mixing of 5g of natural zeolite as Na form with 30 mL ethanol and undoped TiO_2 (Z-TiO_2), respectively N-doped TiO_2 (Z-TiO_2-N) nanocrystals under continuous stirring for 4 hours. The solutions were filtered, washed and dried to 60ºC. The thermal treatment was performed for 1 hour in an oven to 400ºC.

2.2. Structural and morphological characterization

The crystallinity of the prepared samples was measured by X-Ray diffraction (XRD) using PANalytical X'PertPRO MPD Difractometer with Cu tube. A scanning electron microscopy (SEM) using Inspect S PANalytical model coupled with the energy dispersive X-ray analysis detector (EDX) was used to characterize the external surfaces of the hybrid materials, using catalyst powder supported on carbon tape. The light absorption properties of the hybrid materials were studied by UV-VIS diffuse reflectance spectroscopy (DRUV-VIS), performed under ambient conditions using Lambda 950 Perkin Elmer in the wavelength range of 200-600 nm. The blank board was used as the reference.

2.3. Photocatalytic antibacterial procedure and analytical methods

The determination hybrid materials efficiency modified with undoped and doped TiO_2 was realized on microbial suspensions of *Enterococcus faecalis* ATCC 29212. This kind of suspensions were used because they were considered advantageous from the point of view that enterococcus can be considered indicators of water contamination.

Microbial suspension was obtained from hydration for 30 minutes of a pastille from the body of an *Enterococcus faecalis* ATCC 29212 Epower TM in a 99 ml flask of distilled water buffered at the temperature of 36^0C. After 5 minutes of stiring it was obtain a suspension of concentration $65x10^3$ CFU. 1 ml is taped from this suspension and it is distributed in the 99 ml of distilled water buffered at the temperature of 36^0C. Stirring was used for uniform distribution of bacterial cells in all the solution volume. The number of viable germs can vary between 30 and 80 ml per suspension. In our case there were evolved 34 germs/ml.

For precise determination of CFU/ml of the reviewed assay the technique from standard method for intestinal enterococcs isolation was followed. This way, 1 ml is taped from bacterial suspension obtained and it was inseminated through inglobulation in the culture medium pellet – aesculin – triazo-compound (Produced by Scharlau Chemie S.A. Spain). Inglobulation was realized by sedimentation of the bacterial suspension in a Petri pallet with the diameter of 9 mm and pouring over this culture medium permanently unifying, so there can exist a uniform repartition of bacterial cells in the medium. After few minutes the plates were incubated for 24 hours at 44^0C. Fecal streptococcs are capable to reduce the chloride 2,3,5 – triphenyltetrazolium at formazan and can hydrolyse aesculine at 44^0C on the culture medium. The final product – 6,7 dihydroxycoumarin, is combined with ions of Fe(III) to give a compound of black color which diffuses into the culture medium, giving a characteristic aspect, observable on Petri dishes. The measurements were made at 24 hours [22].

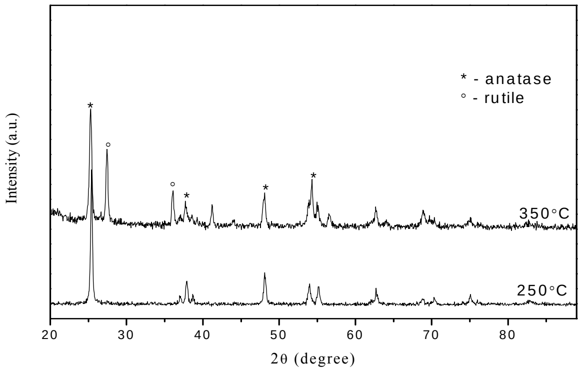
For the detection of the bactericide or bacterostatic effect of hybrid materials (Z-TiO_2), respectively N-doped TiO_2 (Z-TiO_2-N), there has been taped 10 ml of microbial suspension of *Enterococcus faecalis* ATCC 29212 obtained through the technique described in which there were added quantities of 0.05 and 0.1 g of hybrid materials ashes and were radiated with visible light for 60 minutes, 120 minutes and 180 minutes. After ilumination 1 ml from this bacterial suspension was taped and inseminated through inglobulation in the culture medium pellet – aesculin – triazo-compound. After 30 minutes the inseminated Petri plates were incubated for 24 hours at 44^0C. After 24 hours the reading of the pellets was made, counting the colonies evolved.

3. Results and discussions

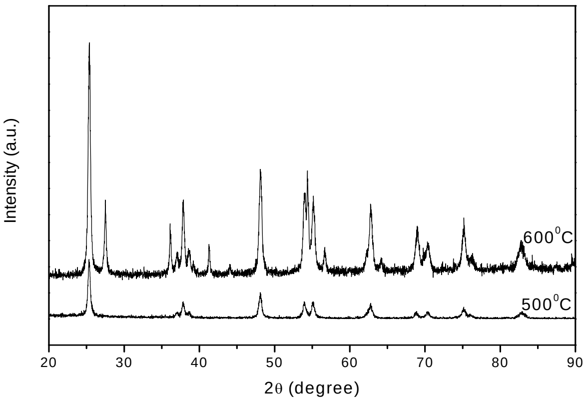
3.1. XRD analysis

In Fig. 1 are presented the XRD patterns of undoped TiO_2 (a) and N-doped TiO_2 (b) nanocrystals synthesized by sol-gel method. It is known that annealing improved the crystallization of TiO_2 powders and accelerated the transformation from amorphous phase to anatase or rutile phase. It can be seen that for a temperature of 250°C the crystalline phase of undoped TiO_2 nanocrystals reveals anatase form and by temperature increasing at 350°C the phase transition from anatase to rutile occurred (Fig. 1a). which become metastable at higher temperature and it is converted into stable rutile phase. The N-doped TiO_2 nanocrystals exhibited a different behavior under the same annealing temperature range. The pure anatase form was noticed until to 500°C temperature, without the appearance of the rutile form (Fig. 1b).Thus, the diffraction peaks of anatase TiO_2 corresponding to 2θ: 25.3°, 37.8°, 48°, 54°, 55° [23] and of rutile phase to 2θ: 27.3°, 35.9°, 41.1°, 54.1° were noticed [24].

Crystallite size was calculated by the use of Scherrer formula i.e. $D=k\lambda/ \beta cos\theta$. The average particle size obtained for anatase from XRD data for undoped and doped nanocrystals were about 20 nm.



a)



b)

Fig. 1. XRD patterns of undoped TiO_2 (a) and N-doped TiO_2 (b)

Taking into account that in general, the anatase form exhibited an improved photocatalytic activity in comparison with rutile form, for the further experiments regarding obtaining zeolite-based hybrid materials were selected the TiO_2 materials crystallized as anatase form, i.e., undoped TiO_2 annealed at 250°C and N-doped TiO_2 annealed at 500°C. Anatase TiO_2 exhibits higher photocatalytic activity than rutile TiO_2 due to its conduction band position which demonstrates stronger reducing power [25].

Fig. 2 described XRD patterns of hybrid materials based on natural zeolite and undoped/ N-doped TiO_2 nanocrystals, *i.e.*, Z-TiO_2 and Z-TiO_2-N. For comparison, the XRD pattern of the natural zeolite in Na form is also shown in Fig. 2 (spectra a). The results presented in Fig. 2 revealed that the natural zeolite used is mostly clinoptilolite (2 theta: 10°; 22.5°; 30°) [21]. The main peak positions of natural zeolite (clinoptilolite) are unchanged, indicating that the structure of natural zeolite has a good thermal stabilization, after thermal treatment. In comparison with the XRD pattern of Z-Na, the specific peaks corresponding to anatase TiO_2 form recorded at 2 theta= 25.3° was noticed for Z-TiO_2.

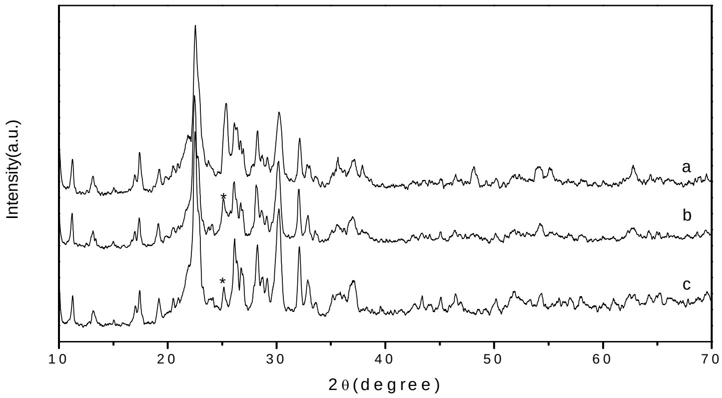


Fig. 2. XRD patterns of Z-Na (a), Z-TiO_2 (b) and Z-TiO_2-N (c)

3.2. DRUV-VIS spectroscopy

DRUV-VIS spectra are examined to determine the light absorption quantification and absorption wavelength range correlated with band gap energy. Fig. 3 presents an intense absorption maximum at ~250 nm, which can be assigned to isolated titanium with tetrahedral coordination. Another absorption range found in the range of 300-370 nm indicates that some Ti^{4+} is also in an octahedral environment [26].

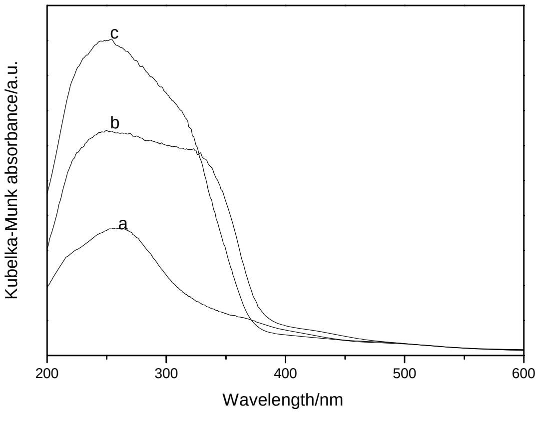


Fig. 3 DRUV-VIS spectra of Z-Na (a), Z-TiO_2-N (b) and Z-TiO_2 (c)

For the Z-TiO_2 (Fig. 3, spectra c) the absorption bands intensity is higher in UV domain, because the anatase form of undoped TiO_2 with the band gap energy about 3.2eV strongly adsorbs in this domain [26]. The doping with N of the hybrid material led to a slight shifting of the absorption band to the visible range (Fig. 3, spectra b).

3.3. SEM / EDX analysis

The SEM images (Figs. 4a, c and e) presents the lamellar texture of clinoptilolite, according to the literature data [27]. The TiO_2 particles are distributed randomized and form cluster agglomerate groups on the surface and in site of zeolite channels. At 12.000x magnification, it is obvious that the TiO_2 nanocrystals (spherical form) are non-uniformly distributed on the zeolite surface, forming the porous surface (Figs. 4c and e). EDX microprobe provided a semiquantitative elemental analysis of the surface indicating that Ti and N were present on the zeolite surface (Figs. 4d and f). Also, this natural zeolite contain the major elements such as Na, Si, Al, O, Fe, K, Mg as can be seen from the EDX spectra (Fig. 4b).

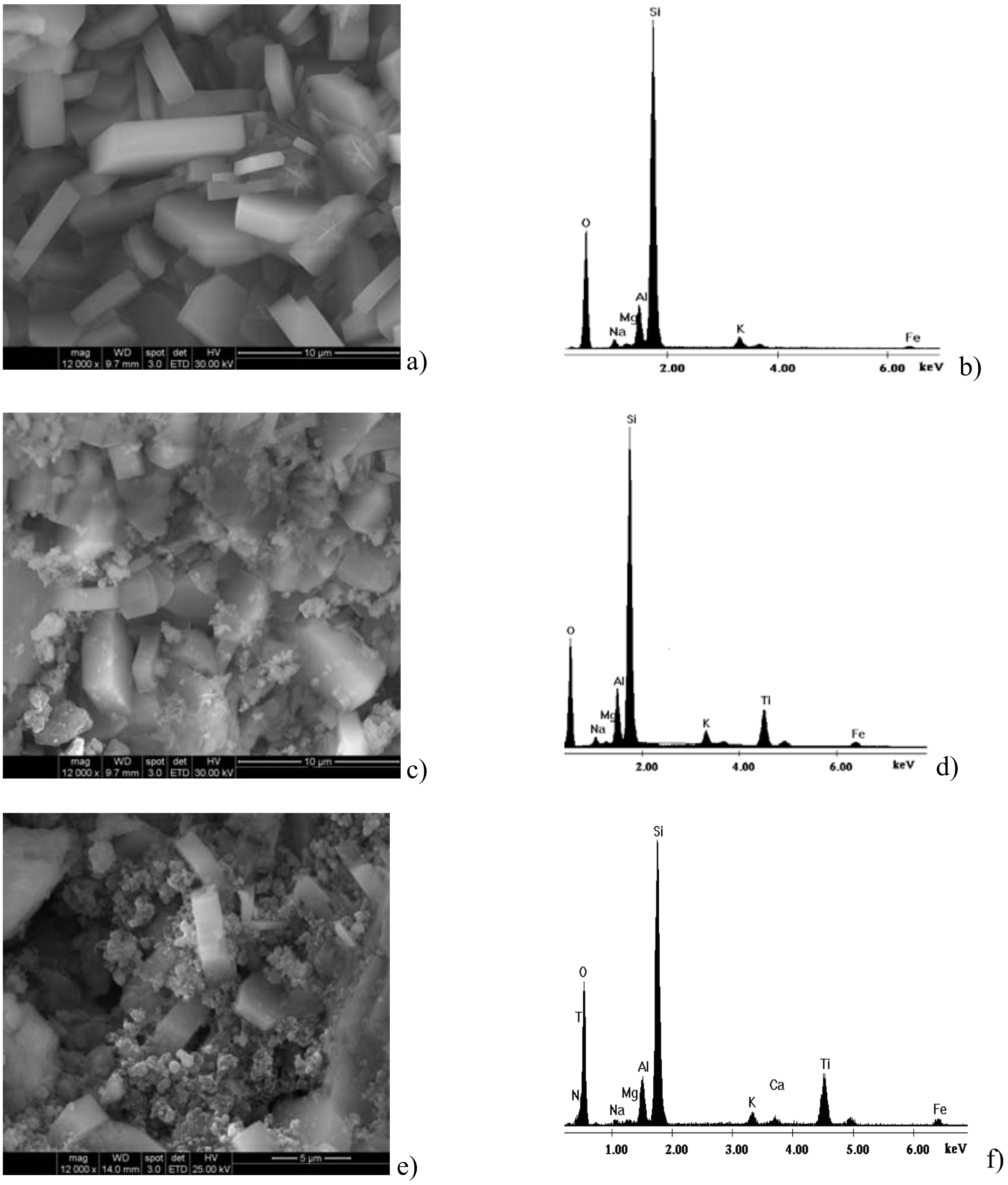
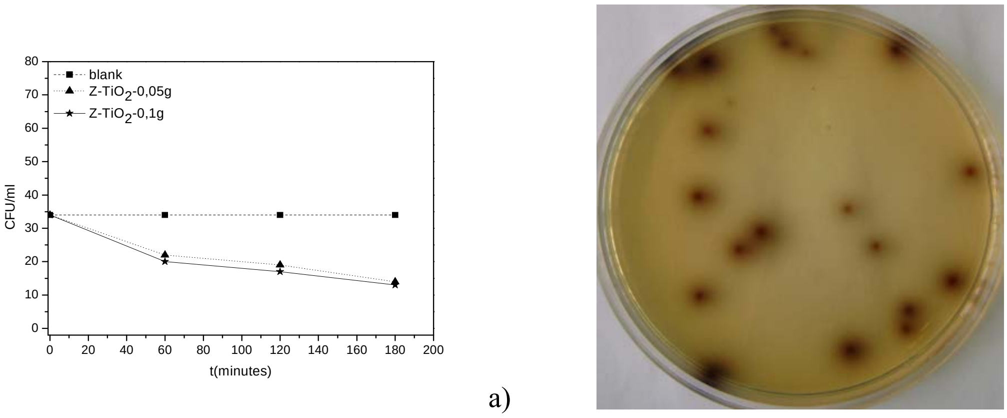


Fig. 4. SEM morphology for a) Z-Na, c) Z-TiO_2, e) Z-TiO_2-N, and EDX spectra for b) Z-Na, d) Z-TiO_2, f) Z-TiO_2-N

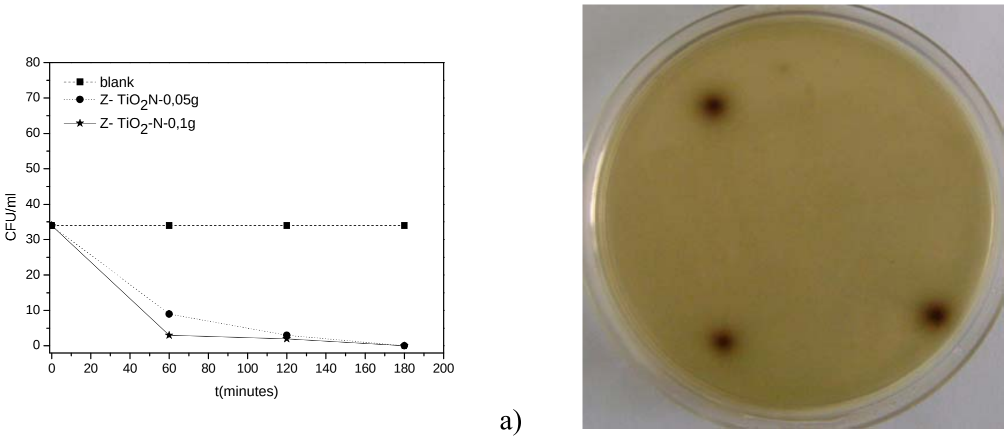
3.2. The antibacterial activity of hybrid materials for Enterococcus faecalis removal

CFU value in Petri plates only with inoculum without the hybrid materials and also samples containing inoculum and Z-Na, unirradiated / irradiated was about 34 germs. Thus was evidenced that simple irradiation of samples did not produced bacterial cell death and there was no changes in CFU plate compared to blank non-irradiated samples. Also it was noticed that sodium form of natural zeolite has not bactericidal effect. These aspects are very important, because otherwise it would have been difficult to assess whether and to what extent only bactericidal material used or only illumination are responsible for cell death. From the experimental data was observed that bactericidal efficiency is different depending on materials used, illumination doses and times. The lowest values of bactericidal efficiency were obtained by using hybrid materials based on natural zeolite and undoped TiO_2 nanocrystals where the number of colonies developed are significantly closer to the CFU value presented by the blank sample. Even if material doses and illumination time were increased the best results were obtained by using 0.1 g of Z-TiO_2 at the irradiation time of 180 minutes (Fig. 5.a. and 5.b). At a dose of 0.05 g of Z-TiO_2-N the bacteria viability decreases progressively with the irradiation time, such after 180 minutes are obtained sterile samples, the bactericidal efficiency beeing 100%. By increasing the bactericide dose is sufficient an lower illumination time (120 minutes) for the obtaining of 100% efficiency (Fig. 6.a and 6.b).



a) b)

Fig. 5. Inactivation of Enterococcus faecalis using Z-TiO_2



a) b)

Fig. 6. Inactivation of Enterococcus faecalis using Z-TiO_2-N

4. Conclusions

The hybrid materials based on natural zeolite and undoped / N-doped TiO_2 were successfully synthesized by calcination method. The optimum annealing temperature was set up at 500°C for undoped TiO_2 and N-doped TiO_2 to obtain pure anatase TiO_2 form. XRD results proved the presence of anatase form of undoped and N-doped TiO_2 onto zeolite. The average particle size obtained for anatase form calculated by Scherrer's equation was about 20 nm. Also the SEM images confirmed the anatase TiO_2 particle size on the natural zeolite surface. Nitrogen presence and material purity was confirmed by EDX analysis. The DRUV-VIS spectra indicated that for N-doped TiO_2 nanocrystals onto natural zeolite the absorption bands were slightly shifted to the visible range, and led to a stronger visible light response than undoped TiO_2 onto zeolite. The N-doped TiO_2 onto zeolitic matrix exhibited an enhanced photocatalytic activity for *Enterococcus faecalis* removal under visible light irradiation in comparison with undoped TiO_2 onto zeolitic matrix.

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