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BIODEGRADATION OF ANIONIC AND CATIONIC SURFACTANTS USING BACTERIAL STRAINS FROM ACTIVATED SLUDGE

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Abstract
Anionic and cationic surfactants are very common pollutants which could be biodegraded by microorganisms during the biological step of the Wastewater Treatment Plant (WWTP) process. During the biodegradation step, the microorganisms use the surfactant as as a source of nutrients breaking down their chemical structure into simpler chemical compounds. There have been data showing that certain surfactants can be completely biodegraded to CO2 and H2O, but there have been also data suggesting that some surfactants were extremely low biodegradable. Their low biodegradability could reside on surfactants negative effects on water's surface, such as reducing air/water oxygen transfer, lowering the water quality by foam introduction and sorption on solid particles which generate a toxic effect on microorganisms. For these reasons it is necessary to determine the individual biodegradability of each surfactant or each class of surfactants. In this study, we investigate de biodegradability potential of cationic and anionic surfactants and subsequently their effect on microorganisms. The cationic surfactants, quaternary ammonium salts are molecules with at least one long hydrophobic alkyl chain attached to a positively charged nitrogen atom. The properties of the cationic surfactants linked to surface activity, adsorption onto negatively charged solids, biocidal activity, and their reaction with anionic surfactants made them desirable for a large number of applications such as fabric softeners, disinfectants, demulsifies, emulsifiers, wetting agents, and processing aids. Unfortunately, quaternary ammonium salts can harm organisms from the aquatic systems. Alkyl sulfate and alkyl benzenesulfonate are two of the main anionic surfactants used and sold in high amounts because of their wide range utility in different fields such as personal care products, petrochemical production and foaming production. The excessively presence of cationic and anionic surfactants in the environment generates concern due the pollution effects on the ecosystems by affecting both aquatic systems (marine ecosystems and freshwater ecosystems). The study of their biodegradation potential was carried out for 28 days and the results showed a partial biodegradation of surfactants induced by the microbiological community from the activated WWTP sludge.

***Keywords**: Surfactants; Wastewater treatment; Biodegradability, Microbiological community*

Introduction

The high production and utilization of surfactants reached 13 million tons produced in 2008, but 44 billion tons have been preconized to be used in 2022 due to COVID pandemy

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which have had raised concerns about the pollution impact on the environment by discharging these amounts into the sewage systems. Based on electric charge, the surfactants can be classified in anionic, nonionic, cationic and zwitterion surfactants [1] (Fig. 1).

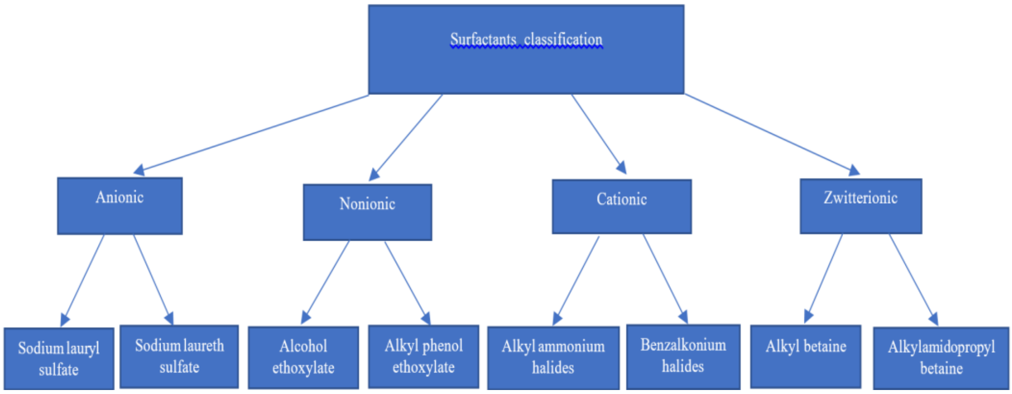

Fig. 1. Classification and most common type of surfactants used worldwide (adapted from K. Jardak et all [1])

Mixtures of anionic and cationic surfactants were ported in the aquatic environment because they are not largely removed from wastewater by biodegradation and absorption into wastewater biosolids and subsequently they could be discharged into surface water or soil where they bind to sediment or soil. Cationic surfactants and anionic surfactants can form ionic pairs, which could be responsible for reducing the concentration of cationic surfactants from wastewater treatment plant (WWTP) and for their accumulation into sediments [2].

The degradability assessment of surfactants could be performed through three methods: physical methods (precipitation, adsorption, flocculation, electrolysis, and sonication); chemical methods (oxidation with oxygen, ozone, H_2O_2/UV) and biological treatments (aerobic and anaerobic degradation). Physical methods are a good alternative to induce pollutants degradability, but they are quite expensive. The reagents used in chemical oxidation can conduct to a final product with a greater toxicity compared to the primary pollutant's compounds. Based on the above-described facts, the first two physical and chemical methods become less appealing than the biological treatments which generate a low cost and effective pollutants removal alternative [3]. The pollutants biodegradation is mainly carried out by bacteria strains consortium present in the activated sludge.

The surfactants biodegradation process occurs in two stages: primary biodegradation (when the main chain of the surfactant is degraded and surface-active properties are reduced or eliminated) and ultimate biodegradation (when the surfactants are totally assimilated by microorganisms and reduced to inorganic compounds - dioxide carbon, water and minerals) [4]. The surfactants biodegradation occures in two type of oxygen regime respectively in anaerobic and aerobic conditions.

The aerobic degradation is correlated with the rate of oxygen consumption and it offers advantages compared to anaerobic process due to a less retention time and a faster biodegradation process. Moreover, the anaerobic biodegradation has as secondary by-product, dihydrogen sulfide, which is a toxic pollutant [5].

The surfactants sulfate class is one of the most common anionic compounds used in household detergents and personal care products and this is related to the amount of ths type of surfactants found in wastewater influents (3148mg L^{-1}) [6].

Quaternary ammonium compounds (QACs), a major class of cationic surfactants, are emerging contaminants used in numerous comercial chemicals such as disinfectants, personal care products, food preservatives, fabric softeners [7, 8]. Due to their wide application, the majority of QACs are introduced into aquatic ecosystems through point source pollution through the discharged of effluents from wastewater treatment plants. The average concentration of QACs, worldwide, has been reported in domestic wastewater to approximately 500μg·L^{-1}, in treated effluents to approximately 50μg·L^{-1} and in surface water to approximately 40μg·L^{-1} [9]. The most used quaternary ammonium compound is benzalkonium chloride (BK) that is a mixture of alkylbenzyldimethylammonium chloride with a chain length of C12-C16.

During the biological wastewater treatment, both anionic and cationic surfactants adsorb onto biomass or are biodegraded by the microorganisms in the activated sludge. The microorganisms use surfactants as the carbon and energy source. The biodegradation of QACs takes place in the presence of microorganisms under aerobic conditions. The bacterial populations adapt very fast to various chemical structure pollutants, being very useful in biodegrading them [10-15].

The group of microorganisms capable of surfactant degradation include the Gram-negative bacteries which according to the literature in the case of anionic surfactants the major type of surfactants it can be *Acinetobacter sp*., *Pseudomonas sp.* [16], *Enterobacter sp. Serattia sp*. [17] and in the QACs degradation: *Pseudomonas spp.* [18-20], *Xanthomonas spp* [21], *Aeromonas hydrophila spp* [22], *Thalassospira spp*. and the Gram-positive bacteria *Bacillus niabensis* [23].

The biodegradation process depends on the structure of the compound, adsorption–desorption processes on sludge, type of microorganism consortia and the presence of anions [24-28].

In the present study, the biodegradability investigations were carried out of two important representative surfactants, namely dodecylbenzyldimethyl ammonium chloride (BK) and sodium dodecyl sulfate (LAS) when used as either single substance.

Experimental

Materials

Chemicals and Reagents. Sodium dodecyl sulfate (99.8%) and dodecylbenzyldimethyl ammonium chloride (99.1%) were purchased from Sigma Aldrich and methylene blue, disulphine blue, sulfuric acid, chloroform, were obtained from Merck.

Microbial inoculum. The activated sludge was collected from a WWTP biological tank then filtrated, washed and re-suspended (1.0g·L^{-1}) in a free chlorine tap water under aeration for 30min until use in the biodegradability experiments.

Culture media. The bacterial culture media was prepared according to the OECD 302 B where 1.0L of media contains 1500mg K_2HPO_4, 800mg NaH_2PO_4, 500mg NH_4Cl, 100mg NaCl, 500mg $MgSO_4$, 15mg $CaCl_2$, 40mg $MnCl_2$.

Methods

Biodegradation experiments. The experiments were performed in 1.5L glass reactors stirred with a Teflon-coated stirring bar and aerated with compressed air. All the experiments were carried out with a mixed bacterial aerobic culture from the activated sludge incubated in absence (control) or presence of 10mg·L^{-1} (S1) and 25mg·L^{-1} (S2); anionic surfactant and 5mg·L^{-1} (C1) and 10mg·L^{-1} (C2) cationic surfactant respectively was inoculated with 2mL microbial inoculum. The incubation was run for five days and aliquots were periodically taken for surfactants quantification and microbiological analyses.

Surfactants analyses. The anionic surfactant and cationic surfactant were analyzed on the supernatants collected from cultured glass reactors solution using two colorimetric methods.

The anionic quantification was performed according to methylene blue active method MBAS-SR ISO 903:2003. The anionic surfactant concentration was expressed in milligrams/liter.

The detection and quantification of cationic surfactants (dodecil benzyldimethyl ammonium chloride) was carried out in presence of disulphine blue active substance according to DIN 38409:1989 method. The cationic surfactant concentration was expressed in milligrams/liter.

Analyses of surfactants were performed using Specord BU 205 spectrophotometer (Analytic Jena, Germany).

Microbiological analyses. The densities of total coliform, fecal coliform and *Escherichia coli* (*E. coli*) bacteria from the sediment samples were performed by Most Probable Number (MPN) method (SR EN ISO 9308-2:2014), using Colilert-18 medium and incubate the samples at 36±2°C for 18-22 hours (total coliforms and *E. coli*) and 44.5°C for 18-22 hours (fecal coliforms). The positive control (*Escherichia coli ATCC25922*, *Citrobacter freundii* ATCC 8090 and *Enterobacter aerogenes ATCC 13048)* and the negative one (*Enterococcus faecalis ATCC 29212)* were tested. At the same time, a blank control with sterile distilled water was analyzed. The results were expressed in CFU/100mL for both water and sediment samples.

Results and Discussion

The monitoring of surfactants biodegradation by bacterial communities was performed up to 120 hours. The anionic surfactant extracted in organic phase (chloroform) was spectrophotometrically measured at 650nm where the detection limit was 0.03mg·L^{-1}, and the limit of quantification was 0.1mg·L^{-1}. The cationic surfactant was extracted in chloroform and spectrophotometrically quantified at 628nm, in presence of disulphine blue dye. The detection limit was 0.05mg·L^{-1} and the limit of quantification was 0.15mg·L^{-1}.

The results showed that both surfactants were stable during the experiment tipe (up to 120 hours) and their degradation rate was not significant compared to the biodegradation rate induced by bacterial communities (Fig. 2). The biodegradation rate of the anionic surfactants (S1, S2) was visible (up to 22% biodegradation) even after only 30 minutes incubation. The biodegradation rate was increased after 24 hours and no anionic surfactant (100% biodegradation) was detected after 48 hours, regardless of the initial concentration of the surfactant (S1 = 10mg·L^{-1} or S2 = 25mg·L^{-1}) (Fig. 2).

The degradation efficiency of anionic and cationic surfactants is shown in figure 2. In the case of cationic surfactants, the greater concentration (C2) had a very fast degradation in first 30 minutes (up to 40%) compared to 16% biodegradation for C1. A longer incubation time of cationic surfactant with the bacterial communities generated the same biodegradation profile as for the anionic surfactant, regardless of the initial concentration (Fig. 2). Overall, the anionic and cationic biodegradation profiles looked the same, with a slightly increading biodegradation rate for the cationic surfactant.

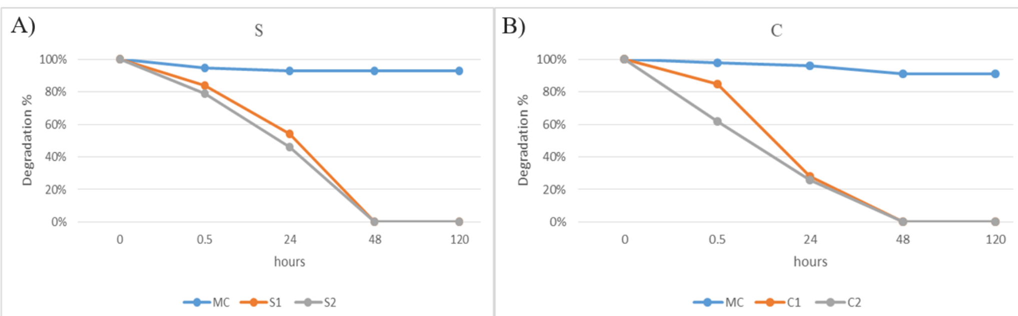

Fig. 2. The biodegradation of A) anionic and B) cationic surfactants. Controls were represented by anionic (MS) or cationic surfactant (MC) incubated without of bacteria. 10mg·L^{-1} (S1) or 25mg·L^{-1} (S2) anionic surfactant as well as 5.0mg·L^{-1} (C1) or 10mg·L^{-1} (C2) were incubated up to 120 hours in presence of the bacterial communities. Biodegradation experiments were carried out in duplicate.

The surfactants biodegradation results showed a clear implication of the bacterial communities, which possible used the surfactants as an energy source by metabolizing them.

If the metabolisation process took place, the number of colonies should increase compared to the control bacteria (incubated without surfactants) [29-31].

The quantification (CFU/100mL) of *total coliforms* (TC) in presence of surfactants showed an overall increasing of the bacterial growth pattern with a peak (10 folds higher) for the highes anionic concentration used, 10mg/L (AC2). The CFU/100mL ratio between bacteria incubated with surfactants and bacteria control (no incubation with surfactants) showed that higher concentrations of anionic or cationic surfactants stimulated the bacterial growth compared to lower concentrations (Fig. 3). The incubation time had a direct link to bacterial growth and to decrease in surfactants concentration.

The *faecal coliforms* (FC) are part of the total coliforms and the next assays showed more specific if the bacterial group faecal played a role in surfactants biodegradation (Fig. 4). The results showed the same growth activation pattern for higher concentrations, regardless of the surfactant type. Lower surfactants concentrations had the same values range as for controls.

An interesting situation happened on the analysis of how *Escherichia Coli* (E. coli*)* bacteria, part of total coliforms, behaved in presence of the surfactants. It seemed that cationic surfactants increased the bacterial growth in a direct linked with the concentration, AC2 (10 mg/L) increased the growth compared to AC1 (5.0mg·mL^{-1}) (Fig. 5). On the opposite, a higher anionic surfactant concentration (AS2) seemed to the toxic for *E. coli* and a lower concentration (AS1) induced an increased bacterial rate (Fig. 5).

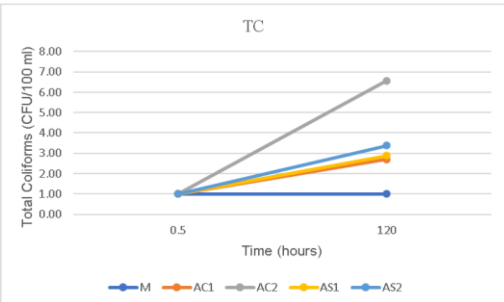

Fig. 3. Total Coliforms (TC) growth in presence of different types of surfactants

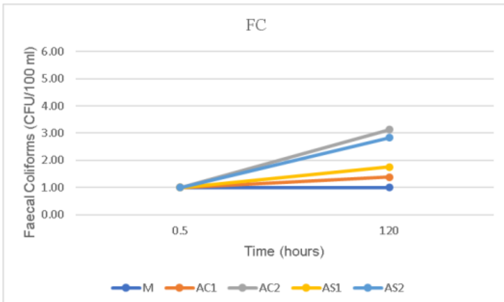

Fig. 4. Faecal Coliform (FC) growth in presence of different types of surfactants

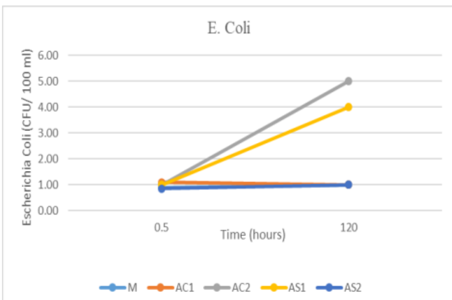

Fig. 5. Escherichia Coli (E. Coli) growth in presence of different types of surfactants

This result could be linked to the fact that the 22mg·L^{-1} cationic surfactant (benzalkonium chloride) reduced microorganisms sludge activity to half (EC50) as it was reported by was reported by *Zhang et al.*, 2011 [32].

Conclusions

This study has shown that dodecylbenzyldimethyl ammonium chloride (cationic surfactant) and sodium dodecyl sulfate (anionic surfactant) were not degraded by the abiotic conditions of the environment.

Their biodegradation was succesfuly carried out in presence of a microorganism consortia from activated sludge. *Total Coliform* (TC) bacteria cumminity and their subgroups *Escherichia Coli* (E. coli) and *Faecal Coliform* (FC) seemed to play an active role in anionic and cationic biodegradation. The biodegradation process was efficient when in 48 hours biodegraded almost all surfactants, used in this paper.

Acknowledgments

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References

[1] K. Jardak, P. Drogui, R. Daghrir, *Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes*, **Environmental Science and Pollution Research, 23**, 2016, pp. 3195–3216

[2] H. Sütterlin, R. Alexy, A. Coker, K. Kümmerer, *Mixtures of quaternary ammonium compounds and anionic organic compounds in the aquatic environment: Elimination and biodegradability in the closed bottle test monitored by LC–MS/MS*, **Chemosphere, 72**, 2008, pp. 479–484.

[3] Q. Wu, L. Zhao, R. Song, A. Ma, *Research progress of surfactant biodegradation*, **IOP Conference Series: Earth and Environmental Science, 227,** 2019, Article Number: 5.

[4] U. Merrettig-Bruns, E. Jelen, *Anaerobic Biodegradation of Detergent Surfactants*, **Materials, 2,** 2009, pp. 181-206.

[5] A. Garg, *Biodegradation of halogenated hydrocarbons*, **Environmental biotechnology assignment**, (Biodegradation of Organic Pollutants - slideshare.net).

[6] F. Aloui, S. Kchaou, S. Sayadi, *Physicochemical treatments of anionic surfactants wastewater: Effect on aerobic biodegradability*, **Journal of Hazardous Materials, 164,** 2009, pp. 353-359.

[7] M.F. Bergero, G.I. Lucchesi, *Degradation of cationic surfactants using immobilized bacteria: Its effect on adsorption to activate sludge*, **Journal of Biotehnology, 272,** 2018, pp. 1-6. DOI10.1016/j.jbiotec.2018.03.003.

[8] M. Chen, I. Zhang, Z. Wang, M. Liu, L. Wamg, Z. Wu, *Impacts of quaternary compounds on memgrane bioreactor performance. Acute and chronic responses of microorganisms*, **Water Research, 134**, 2018, pp. 153-161.

[9] P.I. Hora, W.A. Arnold, *Photochemical fate of quaternary ammonium compounds in river water*, **Environmental Science Processes and Impacts, 22**, 2020, pp. 1368-1381.

[10] M. Nita-Lazar, S. Gheorghe, A. Anghelache, A. Banciu, C. Stoica, I. Lucaciu, *Modulation of the bacterial defense mechanisms by various chemical structures*, **Revista de Chimie, 67**(8), 2016, pp. 1454-1457.

[11] M. Nita-Lazar, T. Galaon, A. Banciu, I. Paun, C. Stoica, I. Lucaciu, *Screening of various harmful compounds in a new bacterial biological model*, **Journal of Environmental Protection Ecology, 17,** 2016, pp. 237-247.

[12] V.M. Radu, P. Ionescu, G.Y. Deak, *Decreasing Nutrients Concentration From Aquatic Environment Using Aquatic Plants,* **Journal Of Environmental Protection And Ecology, 16**(2), 2015, pp. 610-619.

[13] M. Tudor, I.M. Tudor, O. Ibram, L. Teodorof, C. Nastaase, G.Y. Deak, *Analysis Of Biological Indicators Related To The Surface Water Quality In The Danube Delta Biosphere Reserve,* **Journal Of Environmental Protection And Ecology, 16**(2), 2015, pp. 443-452

[14] I.F. Burlacu, G. Deák, L. Favier, I.P. Serre, D. Balloy, *Advanced catalytic materials obtained from waste for wastewater treatment applications,* **UPB Scientific Bulletin, Series B: Chemistry and Materials Science, 83**(2), 2021, pp. 161-166

[15] C.I. Covaliu, A. Nedelcu, G. Deak, O. Stoian, N.M. Noor, *Biotechnology of water treatment based on algae cultures,* **IOP Conference Series: Earth and Environmental Science**, **616**(1), 2020, art. 012081

[16] F. Hosseini, F. Malekzadeh, N. Amirmozafari, N. Ghaemi, *Biodegradation of anionic surfactants by isolated bacteria from activated sludge,* **International Journal of Environment Science and Technology, 4**, 2007, pp. 127-132.

[17] M. Fedeila, Z. Hachaïchi-Sadouk, L. F. Bautista, R. Simarro, F. Nateche, *Biodegradation of anionic surfactants by Alcaligenes faecalis, Enterobacter cloacae and Serratia marcescens strains isolated from industrial wastewater,* **Ecotoxicology and Environmental Safety, 163,** 2018, pp. 629-635.

[18] A.S. Liffourrena, F.G. López, M.A. Salvano, C.E. Domenech, G.I. Lucchesi, *Degradation of tetradecyltrimethylammonium by Pseudomonas putida A ATCC12633 restricted by accumulation of trimethylamine is alleviated by additionof Al.+3 ions*, **Journal of Applied Microbiology, 104,** 2008, pp. 396–402.

[19] T. Nishihara, T. Okamoto, N. Nishiyama, *Biodegradation of didecyldimethylammonium chloride by Pseudomonas fluorescens TN4 isolated fromactivated slud*ge, **Journal of Applied Microbiology, 88,** 2000, pp. 641–647.

[20] S. Takenaka, T. Tonoki, K. Taira, S. Murakami, K. Aoki, *Adaptation of Pseudomonas sp. strain 7-6 to quaternary ammonium compounds andtheir degradation via dual pathways,* **Journal of Applied Microbiology, 73**, 2007, pp. 1797–1802.

[21] D. Dean-Raymond, M. Alexander, *Bacterial metabolism of quaternary ammonium compounds,* **Applied Environmental Microbiolology, 33,** 1977, pp. 1037–1041.

[22] M.A. Patrauchan, P.J. Oriel, *Degradation of benzyldimethylalkylammoniumchloride by Aeromonas hydrophila sp.*, **Journal of Applied Microbiology, 94**, 2003, pp. 266–272.

[23] D.E. Bassey, S.J.W. Grigson, D*egradation of benzyldimethyl heksadecylammo-nium chloride by Bacillus niabensis and Thalassospira sp. isolated from marinesediments*, **Toxicology and Environmental Chemistry, 93,** 2011, pp. 44–56.

[24] B. Brycki, M. Waligórska, A. Szulc, *The biodegradation of monomeric and dimeric alkylammonium surfactants,* **Journal of Hazardous Materials, 280,** 2014, pp. 797–815.

[25] E.I. Muresan, N. Cimpoesu, A. Cerempei, D. Timpu, I.G. Sandu, *Obtaining, Characterization and Using of Metallosilicate Beads for the Adsorption of Direct Red 95 Dye,* **Revista de Chimie, 66**(10), 2015, pp. 1663-1670.

[26] I. Stanescu, L.R. Manea, A. Bertea, A.P. Bertea, I.C.A. Sandu, *Application of the Taguchi Method in the Optimization of the Photo-Fenton Discoloration of Wastewater from Reactive Blue 19 Dyeing,* **Revista de Chimie, 67**(10), 2016, pp. 2082-2086.

[27] N. Barsan, D. Chitimus, F.M. Nedeff, I. Sandu, M.P. Lehadus, A.V. Sandu, O.I. Tartoaca, *Experimental Application of a Laboratory SBR Plant Used for Domestic Wastewater Treatment,* **Revista de Chimie, 70**(11), 2019, pp. 4098-4101.

[28] R. Romanescu, G.M. Cojoc, I.G. Sandu, A. Tirnovan, D. Dascalita, I. Sandu, *Pollution Sources and Water Quality in the Bistrita Catchment (Eastern Carpathians)*, **Revista de Chimie, 66**(6), 2015, pp. 855-863.
[29] N.T. Joutey, W. Bahafid, H Sayel, N.E. Ghachtouli, *Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms,* **Biodegradation - Life of Science**, Chapter 11, 2013. DOI: 10.5772/56194.
[30] V. Vasilache, M.A. Cretu, L.F. Pascu, M. Risca, E. Ciornea, C. Maxim, I.G. Sandu, C.I. Ciobanu, *Dehydrogenases activity in sludge samples of suceava river,* **International Journal of Conservation Science, 6**(1), 2015, pp. 93-98.
[31] V. Vasilache, C. Filote, M.A. Cretu, I. Sandu, V. Coisin, T. Vasilache, C. Maxim, *Monitoring of Groundwater Quality in Some Vulnerable Areas in Botosani County for Nitrates and Nitrites Based Pollutants,* **Environmental Engineering and Management Journal, 11**(2), 2012, pp. 471-479. DOI: 10.30638/eemj.2012.059.
[32] C. Zhang, U. Tezel, K. Li, D. Liu, R. Ren, J. Du, S.G. Pavlostathis, *Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge*, **Water Research, 45,** 2011, pp. 1238-1246.

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