Journal of materials and Environmental Sciences
ISSN : 2028-2508

JMES, 2017 Volume 8, Issue 1, Page 364-369

http://www.jmaterenvironsci.com/

Inactivation efficiency of total coliforms and *Escherichia coli* in doped natural water by heterogeneous Fenton: Effect of process factors

K. Castro-Ríos[1], E.J. Corpas[2], V. Cárdenas[1], G. Taborda[1*]

[1] *Grupo de Investigación en Cromatografía y Técnicas Afines, Departamento de Química, Universidad de Caldas, Manizales, Colombia*

[2]*Instituto de investigación en Microbiología y Biotecnología Agroindustrial, Universidad Católica de Manizales, Manizales, Colombia.*

Received 17 Oct 2014,
Revised 29 Aug 2016 ,
Accepted 03 Sept 2016

Keywords

- ✓ Advanced oxidation processes (AOP),
- ✓ Bacterial inactivation,
- ✓ Pillared clay,
- ✓ Natural water

gtaborda@ucaldas.edu.co
Tel:+5768781500

Abstract

The microbiological contamination of water is responsible for millions of deaths in the world annually, until now the disinfection of water has been based on the chlorination; however there is a growing concern for the formation of trihalomethanes, for this reason it was evaluated the inactivation of total coliforms and *Escherichia coli* (*E. coli*) by using the advanced oxidation process known as heterogeneous Fenton, catalysed with pillared clay, prepared by ultrasound and microwave in a semibatch reactor using as process factors the concentration of the oxidizing agent, catalyst load, pH, and the reaction time. The test of inactivation was made in natural water from "Pasto river", this water was doped with the microorganism until a concentration of 10^6 CFU/mL, the samples were analysed by the microbiological method of membrane filtration. The results showed that the factors evaluated, have a statistically significant effect ($p<0.05$) in the percentage of inactivation of total coliforms and *E. coli*, with 95% confidence, regardless of the treatment used in the preparation of the catalyst (ultrasound and microwave). The best conditions for the inactivation of microorganisms were pH of 3.7, catalyst load of 0.5 g/L, time among 180 - 240 min and H_2O_2 concentration between 0.12 and 0.18 mg/L, allowing inactivation efficiency of coliforms and *E. coli*between 42% to 75%.

1. Introduction

The lack of drinking water, as defined by the World Health Organization as one "... suitable for human consumption and for all usual domestic purposes, including personal hygiene" [1], remains a global problem linked to a deficiency of secure supply of this liquid [2], generating three million of deaths a year worldwide, of which two million are caused by diarrheal diseases with a high impact on child mortality [3]. There is also influence on the development of the individual, since it has been shown that the safety and health of the water, impact the physical and mental health and social and economic human development [4]. This reality and the implications of technological developments, pollution of water resources and global climate change, seem to indicate deficiencies in the safe supply of water.

The main disinfectant used in the potabilization treatment processes is chlorine, due to their availability, economics, oxidizing character and potential of eliminating pathogens, however is also considered a corrosive and potentially dangerous substance for human health because it can generate disinfection by-products such as trihalomethanes, which are formed by reaction with organic matter present in water and other conditions in the treatment[5-7], some trihalomethanes have been identified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, generating a growing awareness of the risk to health and for environmental conservation [8, 9].What has been discussed above allows the assessment of alternative methods, such as advanced oxidation processes (AOP), these involve the generation of highly reactive oxidants species, able to attack and degrade organic substances and microorganisms [10-12], these techniques have advantages, like the chemical transformation of the contaminants, high oxidant power, low or no-generation of sludge, the possibility of treating contaminants at low concentration and increased biodegradability [7].

Among the AOP are non-photochemical and photochemical technologies such as Fenton and photo-Fenton processes, respectively. These treatments seek the formation of hydroxyl radicals by the application of Fenton process (Fe^{2+}/H_2O_2) or the combination of this with irradiation with UV light (λ>300nm) [13]. The effectiveness

of these processes has been evaluated in the removal of pollutants from industry, pharmaceutical, hospitals, colorants, pesticides and elimination of pathogenic microorganisms [14-20].
In this study was evaluated the effect of process factors of heterogeneous Fenton treatment,with a pillared clay catalysts prepared by ultrasound or microwave, on the inactivation efficiency of total coliforms and *E. coli* in natural waterintended for human consumption.

2. Experimental

2.1. Catalysts

The pillaring solution was prepared according to a previous work developed in our research group [21], the solution was synthesized from $AlCl_3 \bullet 6H_2O$, $FeCl_3 \bullet 6H_2O$ and NaOH. The hydrolysis ratio OH/metal was 1.6. Once the hydrolysis ended, the solution was maintained at a constant temperature in a heating plate at 80°C, during seven hours, at the end of the heat treatment, the solution is left to room temperature (20°C) for 12 h. Montmorillonite clay was dispersed in three vehicles: water, ethanol and acetone, at three concentrations 2.0, 25 y 50 % (w/w). For the process of intercalation it was prepared a solution containing polycations to intercalate and the clay suspension, these were subsequently subjected to microwave or ultrasound, to get the corresponding oxides, in this way was obtained the catalyst prepared by ultrasound (CAT-US) and the catalyst prepared by microwave (CAT-MW).

2.2. Experimental procedure

For the inactivation experiments, were used samples of 450mL from the "Pasto river" (Nariño, Colombia) with a COD of 25 mgO_2/L, doped with total coliforms and *E. coli*until a concentration of 10^6 CFU/mL. These were deposited in a glass semibatch reactor of 1000 mL, with the catalyst prepared at constant agitation (300 rpm).The pH of the solution was adjusted with NaOH and H_2SO_4 (0.1 mg/L) and recording changes were measured with a potentiometer (Metrhom, Switzerland). The tests were conducted at an average temperature of 17±1°C.
After performing the tests of inactivation, microbiological analysis were done following the method of membrane filtration according to "standard methods"[22], where the sample is filtered through a membrane sterile of 0.45 μm, subsequently placed in a petri dish with Chromocult culture medium (Merck, Germany) and incubated at 35±2°C, for 24 to 48 h, for the subsequent counting of microorganisms.

2.3. Experimental design

Was applied a factorial experimental design that allow to evaluate the process factors: treatment time, pH, oxidant concentration and catalyst load,these parameters were selected based on previous research [21, 23]. The response variable was microorganism inactivation efficiency (%), and the levels for the factors studied are shown in Table 1. Ten experiments were performed in a random order, and the data were analyzed using the software Statgraphics®plus.

Table 1: Factors and levels used in the inactivation experiment.

Variables	Low Level	High Level
Time (min)	30	240
pH	3.7	7.3
Catalyst load (g/L)	0.5	5.0
Oxidant concentration (mg/L)	0.06	0.18

3. Results and discussion

Results from the statistical software Statgraphics®plus indicated that the process factors, concentration of H_2O_2, catalyst load, pH and treatment time, have a statistically significant effect ($p<0.05$) on the efficiency of inactivation of total coliform and *E. coli* with 95% of confidence, independent of the microwave or ultrasound employed in the catalyst preparation process. The impacts of each factor are described below.There are several studies suggesting that the formation of hydroxyl radicals in the Fenton process, affect the destruction of microbial cells [2, 10, 24-27]. Have proposed different effects on bacterial killing by hydroxyl radicals, such as oxidation, deformation and destruction of the membrane and cell wall, inactivation of enzymes and oxidative DNA damage [8, 11, 28].

3.1. Effect on treatment time on the efficiency inactivation of total coliforms and E. coli

In the Figure 1 is possible to observe an increase on the inactivation of total coliforms and *E. coli*, when is increased the treatment time, this coincides with the results presented by other authors in works developed with

Fenton and Photo-Fenton processes [25, 29]. The greater efficiency of inactivation of microorganisms was reached at 240 min, with inactivation efficiency between 59 and 75% using CAT-MW and CAT-US respectively. According to Fisher´s least significant difference (LSD) test, statistical differences were found in the average calculated between the times evaluated, except between 180 and 240 min for CAT-US.

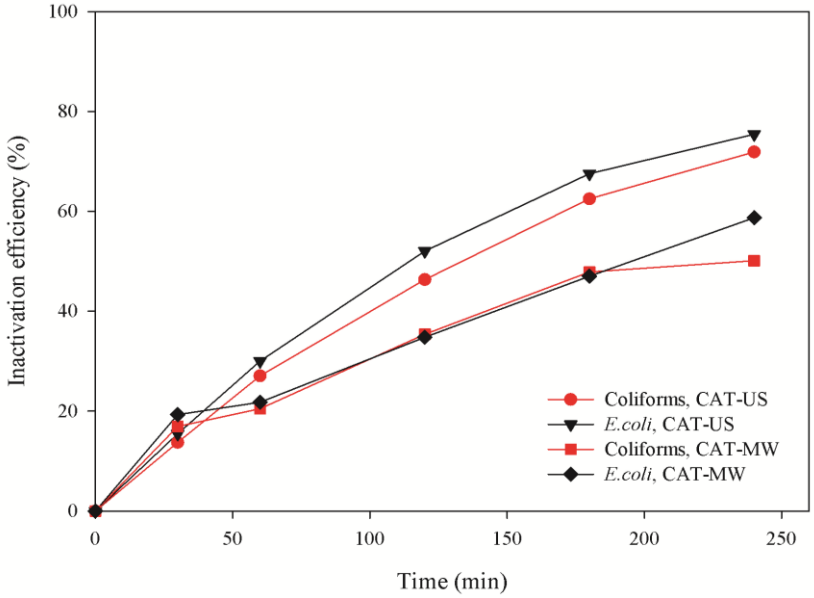

Figure 1: Inactivation efficiency of total coliform and *E. coli* as a function of time. [H_2O_2]= 0.06mg/L, [Catalyst load]= 0.5 g/L, pH = 7.3, [Microorganism] = 10^6 CFU/mL.

3.2. Effect of pH on the efficiency of inactivation of total coliform and E. coli

A statistically significant difference in the rates of inactivation of total coliforms and *E. coli* according to the pH was presented. The efficiency of inactivation of microorganisms was better when it was used a pH of 3.7 (Figure 2 and Figure 3), with an inactivation greater than 53%. When pH 7.3 was used, the values of inactivation were below 40% for CAT-US and 12% for CAT-MW. A lower efficiency of inactivation at neutral pH may be related to instability H_2O_2 at high pH, leading to the formation of iron hydrocomplexes, which negatively affect the formation of hydroxyl radicals [30, 31]. Moreover at acidic pH between 3 to 4, the Fenton reaction is enhanced, what could improve the solubility of iron on the catalyst and the interaction with H_2O_2, therefore there is an increase in treatment efficiency [25, 32]. The control was carried out in the "Pasto river"at pH 3.7, this value was selected because of the effect of the pH on the growth of microorganisms evaluated[33], however as shown in Figure 2 and Figure 3, the inactivation by effect of pH was less than 10%.

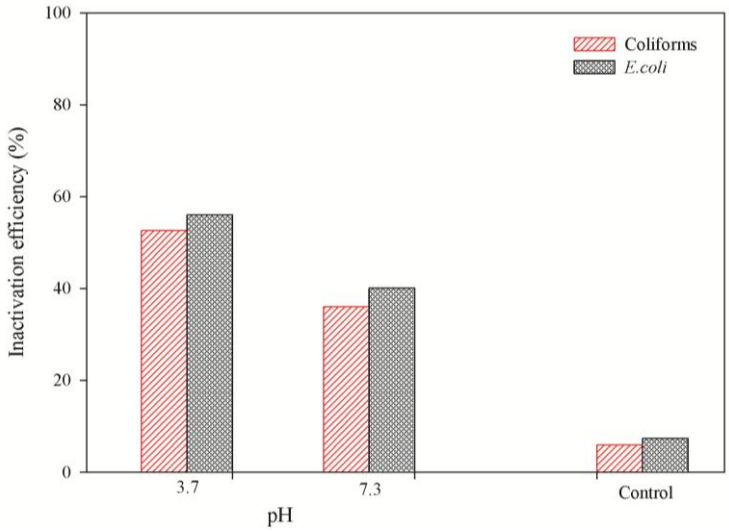

Figure 2: Inactivation efficiency of total coliforms and *E. coli* as a function of pH, using CAT-US.[H_2O_2]=0.06mg/L, [Catalyst load]= 0.5 g/L, [Microorganism]= 10^6 CFU/mL. Control: pH = 7.3.

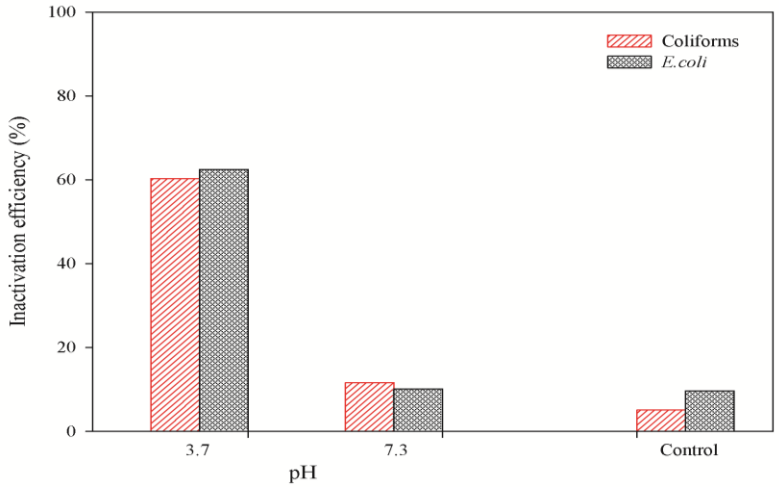

Figure 3: Inactivation efficiency of total coliforms and *E. coli* as a function of pH, using CAT-MW.[H_2O_2]= 0.06mg/L, [Catalyst load]= 0.5 g/L, [Microorganism]= 10^6 CFU/mL. Control: pH = 7.3.

3.3. Effect of catalyst load on the efficiency of inactivation of total coliform and E. coli.

Figure 4 shows an increase in the inactivation of microorganisms with CAT-US, at reduced load of catalyst (0.5 g/L) with values of 59% and 60% for coliforms and *E. coli* respectively. Statistical analysis found differences by using CAT-US, between 0.5-2.7g/L and 0.5-5.0g/L. For CAT-MW (Figure 5), there was an increase in the efficiency of inactivation of total coliform and *E. coli*, with the minimum and maximum load, with inactivation over 45%. Statistical analysis show differences in the inactivation, using catalyst concentrations between 0.5-2.7 g/L and 2.7-5.0 g/L. Overall the results show a better inactivation efficiency when low concentrations of catalyst are used, this may be due to a better relationship between the catalyst and the H_2O_2, favoring the production of hydroxyl radicals which have effect on the viability of microorganisms. A lower efficiency of inactivation of catalyst at higher doses, might be related to competitive reactions with the organic matter present on natural water, affecting the generation of hydroxyl radicals [4]. The control was performed at higher catalyst load, the results shows a removal below 13% showing slightly effect on the inactivation by the catalyst, this effect is likely related to adsorption of the microorganism in the catalyst.

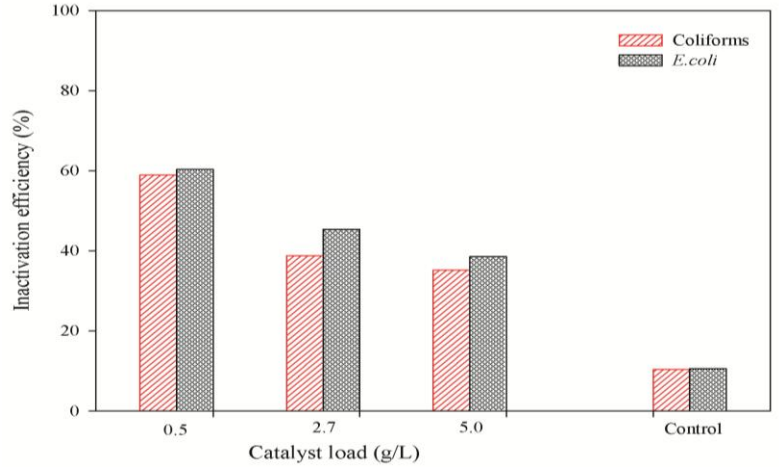

Figure 4: Inactivation efficiency of total coliforms and *E. coli* as a function of catalyst load, using CAT-US.[H_2O_2]= 0.06mg/L, pH= 7.3, [Microorganism] = 10^6 CFU/mL.Control: [Catalyst Load] = 5.0 g/L.

3.4. Effect of H_2O_2 concentration on inactivation efficiency of total coliforms and E. coli

The results of Figure 6 and Figure 7, show that the increase in the concentration of H_2O_2 has a positive effect on the inactivation of coliform and *E. coli*. Statistical analysis showed differences in the rates of inactivation of microorganisms, between 0.06-0.12 mg/L and between 0.06-0.18 mg/L. The control was at the higher concentration of H_2O_2, since this value has an effect on the growth of microorganisms evaluated, the results show inactivation efficiency between 18 and 22% caused by the oxidant. This would justify that at low concentration of H_2O_2, the inactivation is lower, although it is possible that insufficient H_2O_2 in the medium, reduce the formation of hydroxyl radicals, also affecting coliform removal and *E. coli*.

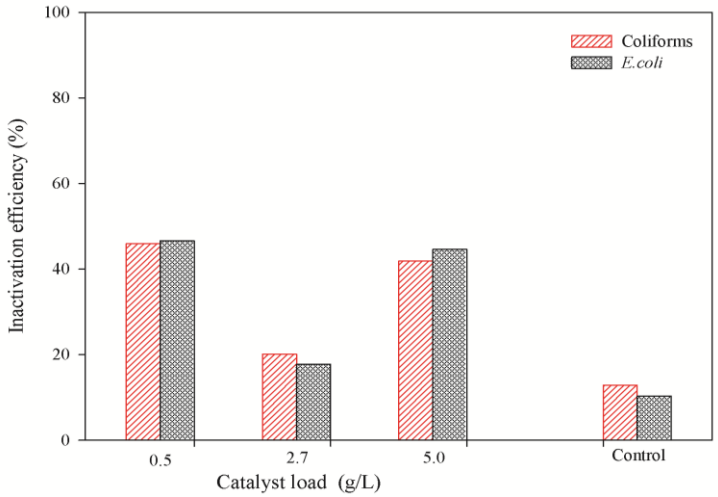

Figure 5: Inactivation efficiency of total coliforms and *E. coli* as a function of catalyst load, using CAT-MW.[H_2O_2]= 0.06mg/L, pH= 7.3, [Microorganism] = 10^6 CFU/mL. Control: [Catalyst Load] = 5.0 g/L.

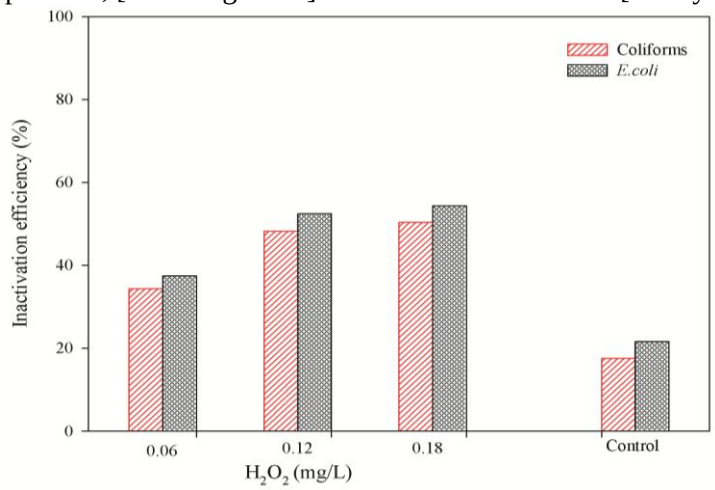

Figure 6:Inactivation efficiency of total coliforms and *E. coli* as a function of the dose of H_2O_2, using CAT-US.[Catalyst load]= 0.5 g/L, pH = 7.3, [Microorganism] = 10^6 CFU/mL. Control: [H_2O_2] = 0.18 mg/L.

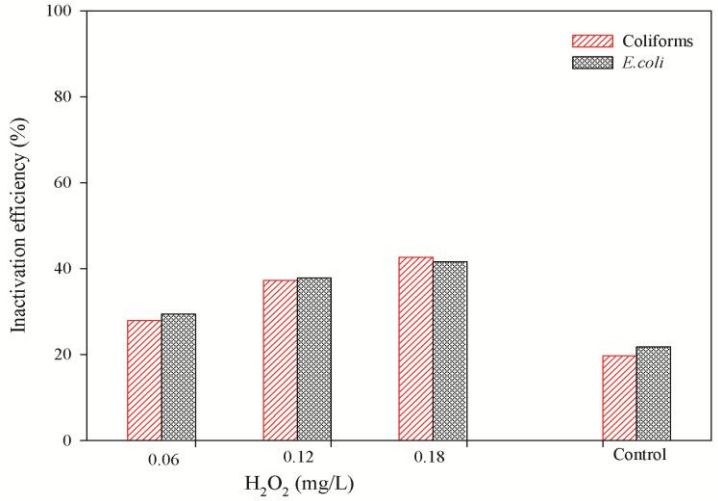

Figure 7:Inactivation efficiency of total coliforms and *E. coli* as a function of the dose of H_2O_2, using CAT-MW.[Catalyst load]= 0.5 g/L, pH = 7.3, [Microorganism] = 10^6 CFU/mL.Control: [H_2O_2] = 0.18 mg/L.

Conclusions

The main conclusions of this manuscript are:

It is possible to inactivate microorganisms using the process of heterogeneous Fenton, independent of the type of treatment used for the development of the catalytic converter (ultrasound or microwave).

Process factors evaluated in the heterogeneous Fenton: pH, catalyst load, H_2O_2 concentration and treatment time, showed a statistically significant effect on the inactivation of *E. coli* and total coliforms, allowing inactivation efficiencies between 42 and 75%.
The increase in the H_2O_2 concentration, an acidic pH, lower catalyst load and a treatment time of 240 min, were the best conditions for the inactivation of microorganisms. Heterogeneous Fenton process showed potential for reducing biological contaminants from raw water intended for human consumption.

Acknowledgements-The authors are grateful to "DepartamentoAdministrativo de Ciencia, Tecnología e Innovación" (Colciencias) for the financial support for this research.

References

1. WHO. Guidelines for drinking-water quality, World Health Organization, (2011).
2. Boateng M.K., Price S.L., Huddersman K.D., Walsh S.E. *J Appl Microbiol*. 111 (2011) 1533.
3. UNICEF. Handbook on Water Quality, UNICEF, (2008).
4. Spuhler D., Rengifo-Herrera J.A., Pulgarin C. *Applied Catalysis B: Environmental*. 96 (2010) 126.
5. Hrudey S.E. *Water Res.* 43 (2009) 2057.
6. Bach L., Garbelini E.R., Stets S., Peralta-Zamora P., Emmel A. *Microchem. J.* 123 (2015) 252.
7. Awaleh M.O., Soubaneh Y.D. *Hydrology: Current Research*. 5 (2014) 1.
8. Diao H.F., Li X.Y., Gu J.D., Shi H.C., Xie Z.M. *Process Biochem. (Amsterdam, Neth.)*. 39 (2004) 1421.
9. International Agency for Research on Cancer (IARC), IARC, 2016.
10. Pham T.T., Brar S.K., Tyagi R.D., Surampalli R.Y. *J Environ Manage*. 91 (2010) 1657.
11. Mamane H., Shemer H., Linden K.G. *J. Hazard. Mater.* 146 (2007) 479.
12. Aslani H., Nabizadeh R., Alimohammadi M., Mesdaghinia A., Nadafi K., Nemati R., Ghani M. *Journal of Environmental Health Science and Engineering*. 12 (2014) 1.
13. Osorio F., Torres J.C., Sánchez M. Tratamiento de aguas para la eliminación de microorganismos y agentes contaminantes, Ediciones Díaz de Santos, (2010).
14. Aguinaco A., Beltrán F.J., Sagasti J.J.P., Gimeno O. *Chem. Eng. J.* 235 (2014) 46.
15. Adish Kumar S., Sree lekshmi G.S., Rajesh Banu J., Tae Yeom I. *Water Qual. Res. J. Can.* 49 (2014) 223.
16. Barreca S., Velez Colmenares J.J., Pace A., Orecchio S., Pulgarin C. *Journal of Environmental Chemical Engineering*. 3 (2015) 317.
17. Ndounla J., Pulgarin C. *Sci. Total Environ.* 493 (2014) 229.
18. Haddad M.E., Regti A., Laamari M.R., Mamouni R., Saffaj N. *J. Mater. Environ. Sci.* 5 (2014) 667.
19. Alalm M.G., Tawfik A., Ookawara S. *J. Mater. Environ. Sci.* 6 (2015) 800.
20. Idrissi M., Miyah Y., Benjelloun Y., Chaouch M. *J. Mater. Environ. Sci.* 7 (2016) 50.
21. Cárdenas V. Preparación de Arcillas pilarizadas con Al-Fe y su actividad catalítica en el tratamiento de agua para consumo, optimizadas por medio de la superficie de respuesta, Universidad de Caldas, (2012).
22. APHA. Standard Methods for the Examination of Water and Wastewater, APHA, (1999).
23. Sun S.-P., Lemley A.T. *J. Mol. Catal. A: Chem.* 349 (2011) 71.
24. Moncayo-Lasso A., Sanabria J., Pulgarín C., Benítez N. *Chemosphere*. (2009) 296.
25. Moncayo-Lasso A., Torres-Palma R.A., Kiwi J., Benitez N., Pulgarin C. *Applied Catalysis B: Environmental*. 84 (2008) 577.
26. Blanco J., Torrades F., Varga M.D.l., García-Montaño J. *Desalination*. 286 (2012) 394.
27. Cengiz M., Uslu M.O., Balcioglu I. *Journal of Environmental Management*. 91 (2010) 2590.
28. Park S., Imlay J.A. *J. Bacteriol.* 185 (2003) 1942.
29. Kim J.Y., Lee C., Sedlak D.L., Yoon J., Nelson K.L. *Water Res.* 44 (2010) 2647.
30. Laiju A.R., Sivasankar T., Nidheesh P.V. *Environmental science and pollution research international*. 21 (2014) 10900.
31. Chen S.-h., Du D.-y. *Journal of Central South University*. 21 (2014) 1448.
32. Nogueira R.F.P., Trovó A.G., Silva M.R.A.d., Villa R.D., Oliveira M.C.d. *Quim. Nova*. 30 (2007) 400.
33. Hosein A.M., Breidt F., Jr., Smith C.E. *Appl. Environ. Microbiol.* 77 (2011) 889.