Environmental Engineering and Management Journal

May 2019, Vol. 18, No. 5, 1089-1095
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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DETERIORATION OF WATER QUALITY BY STAGNATION IN STORAGE TANKS

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Abstract

This study presents the influence of the storage recipients' material and of the use and the type of stirring on the drinking water quality. The kinetics of drinkable water quality alteration under stationary conditions and under magnetic and sonical stirring have been monitored for a two weeks period. The microbiological parameters (total number of germs developed at 37°C and 22°C, lactose-positive and lactose-negative bacteria, coliform bacteria and *Echerichia coli*), as well as the physico-chemical ones (turbidity and chlorine amount) have been determined on a daily basis, indicating different alteration degrees of the drinkable water, as a function of storage period and regime. It was found that glass not stimulate microbial growth while polyethylene recipients represents a high risk factor from the bacterial growth point of view. Mechanical stirring as well as sonication are able to significantly reduce the formation of the biofilm on the wall of the storage tanks, irregarding of the material from which the recipients are made of. Sonication has been proven to be inefficient for water storage in polyethylene recipients, due to the increase of the temperature and consequently of the planktonic bacteria activity.

Key words: microbiological activity, sonication, storage tanks, stirring, water quality

Received: March, 2016; Revised final: January, 2017; Accepted: June, 2017; Published in final edited form: May 2019

1. Introduction

Water is essential to human existence, and access to this resource and its sustainable management is the foundation for a sustainable development of the global community. Efficient usage of these resources has led to the development of the concept of "food, water, and energy nexus." Thus, water safety was one of the topics discussed at "The Word Economic Forum 2011, the Bonn 2011 Nexus Conference, the sixth World Water Week 2012". Therefore storage and distribution of drinking water to the consumer must be effective and safe for the human consumption.

The storage and distribution of drinking water is an area where permanent improvements are needed. A good example is the book of Brandt et al. (2017), where in Chapter 20 entitled "Treated Water Storage" indicates that safe drinking water storage tanks deserves all attention, because water is a perishable product.

Recently, Abokifa et al. (2016) and Gibellini et al. (2017) gave warn of drinking water distribution systems, and show that the action of disinfectants depending on the time of stagnation is reduced, allowing pathogens to redevelop. Also, Hannoun (1997) in his work "Optimizing storage distribution water quality: A hydrodynamic approach", stresses that the drinking water storage for long time period lead to the reduction of residual disinfectant. This means that relevant legal provisions in the field are severely violated.

Therefore it is evident that improving water storage, but also highlighting problems that occur in storage are issues of global concern.

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Consequently, water protection and management represent a domain of great importance (Ojomo et al., 2015; Olaru et al., 2010), so that solutions for improving water quality is a permanently task. The EU legislation regarding water has been transformed in 2000 by adopting the DCA 2000/60/EC directive, which aligns the approach regarding the management and protection of water quality to the international standards and trends in this domain. Also the EU directive promotes the sustainable use of the water resources, and along with directive 98/83/CE defines the essential quality standards for water destined for human consumption (Teodosiu et al., 2009). Among the principles of integrated management imposed by this directive is "the principle of unitary quantity-quality water management" in order to get the best technical and economic solutions, and the "solidarity principle" which requires the involvement of the state, local communities, users, water distribution companies and NGOs sites. Starting from these principles and recent studies on water storage problems, this paper highlights the vulnerability of drinking water quality during prolonged storage in reservoirs, while proposing a protective solution for storing potable water as an alternative.

In water storage tanks from the water distribution networks, the absence of a mass drive system favors the formation of a bacterial biofilm, as water flow rate is a key factor in microbial development (Manuel et al., 2007; Mohamed and Al Shehri, 2007; Walker et al., 2004). In search of the solutions to overcome these drawbacks, several studies have reported that sonication is able to reduce the microbial activity in the water from the public distribution network storage tanks (Hulsmans et al., 2010).

Starting from these principles, this study represents a comparison between three types of water storage (stationary, under mechanical stirring and sonication) from the microbiologically point of view. Due to the fact that the storage tanks material type is also an important factor to be taken into consideration in biofilm formation and development (Inkinen et al., 2014; Katsikogianni and Missirlis, 2004), the present study utilizes two types of storage recipients, namely from glass and polyethylene.

Apart from the monitoring of stored potable water degradation, the present study also presents a review of the benefits and disadvantages of different types of storage methods.

2. Experimental

On the laboratory scale, two types of water storage recipients (made of glass and polyethylene [HDPE], respectively), having 1 L volume, and three types of storage regimes were simulated (stationary, with stirring system and sonication).

The storage recipients were sterilized by washing with 70% ethanol, rinse well with sterile bidistilled water and then by autoclaving at 121°C for the HDPE recipients, and respectively, at 180°C for the glass recipients. The sterilised recipients were filled with a determined identical volume of potable water coming from Area I of distribution network of Brasov City and covered with sterile filter paper. The magnetic stirring was performed with a Heidolph MR Hei-Mix L magnetic stirrer, and the sonication with Elmasonic S 100 H ultrasound bath. During two weeks, the water treatment was performed for 20 min at 2h intervals. This interval was chosen based on certainty that the division of bacterial cells is well defined and notable after 1-2 h (Anderson and Lustbader, 1975). On a daily basis, water samples were withdrawn from the recipients for analysis.

The monitored parameters are: the total number of developed germs at 37 °C (NTG 37 °C), the total number of developed germs at 22 °C (NTG 22 °C), lactose-positive and lactose-negative bacteria, coliform bacteria and *E. coli*. The turbidity of the stored water was determined, as this factor is proportional to the total number of microorganisms in suspension. At the end of the storage period, the evaluation of the contamination degree of the recipient walls has also been assessed, by using different standardised tests. Evolution of free and total chlorine amount were also monitored, because we used, chlorinated water from the public distribution network. All determinations were performed in duplicate.

2.1. Materials

For drinkable water storage, transparent borosilicate glass Erlenmeyer flasks, according to ISO 1773 and transparent HDPE recipients with PP lids were used.

The water used in the experiments comes from the “Area I” of the potable water public distribution network of Brasov City, Romania (Tarlung reservoir).

2.2. Methods

2.2.1. Detection and counting of lactose-positive and lactose-negative bacteria

For the determination of lactose-positive bacteria, the filtering membranes method has been used (http://www.sartorom.ro/sites/default/files/produse/documente/control_microbiologic_ro.pdf, SR EN ISO 9308-1/2004 AC:2009). The principle of this method consists of filtration of determined volumes of water through membranes with 0.2-0.45µm porosity. The bacteria from the water sample remain on the surface of the membrane, and then the membranes containing the bacteria are placed on a selective culture medium (Tergitol TTC), which contains lactose, triphenyltetrazolium chloride (TTC) and sodium heptadecylsulphate (Tergitol). The culture media were purchased in a ready to use form (Nutri Disks Tergitol TTC from Dr. MÖLLER & SCHMETZ), which includes sterile Petri dishes with the culture medium and the corresponding filtering membrane, for each

Petri dish. The testing was performed in accordance with SR EN ISO 9308-1/2004 AC:2009. From each storage recipient, 50 mL of water was withdrawn and filtered through the membranes. The membranes were placed in the Petri dishes containing the hydrated culture media and incubated for 24 h on 36±2 ˚C. After the incubation period, the membranes were examined and all the yellow-orange lactose-positive bacteria and red lactose-negative bacteria colonies developed counted. Presumable coliform bacteria developed (yellow) were further analyzed in order to establish their type (coliform and/or *Escherichia coli*).

2.2.2. Detection and counting of coliform and Escherichia coli bacteria

From the culture media obtained, several strains were further inoculated on specific culture media such as Tryptone Soy Agar plates (a), Tryptophan Broth tubes (b) and TBXG plates (c) for oxidase, indole and β-glucuronidase tests:

a) The oxidase test were performed to confirm the presence of above-presumed coliform bacteria. For the culture media preparation, 40 g dry Tryptone Soy Agar (Sharlau), was dissolved under heating in 1000 mL distilled water and the pH adjusted to 7.2 ± 0.1 at 25 ˚C. The prepared culture media was further sterilized in an autoclave at 121 ˚C for 15 min and placed in the Petri dishes. Samples from the presumed coliform bacteria were thus inoculated on the Petri dishes with Tryptone Soy Agar and incubated at 36 ˚± 2 ˚C for 21± 2 h. After the incubation period, 2-3 drops of freshly prepared Oxidase Reagent (VWR PROLABO) were placed on a sterile filter paper. With a glass rod, samples from bacterial colonies developed on the Tryptone Soy Agar culture medium were placed on the filter paper. In 30 s, either an intense blue-violet coloring appeared, which confirms the presence of coliform bacteria (positive test), or no blue coloring appears, which confirms the presence of coliform bacteria-negative oxidase test.

b) For the indole test, lactose-positive bacteria were inoculated in Tryptophan Broth tubes, prepared as following: 16 g dry Tryptophan Broth culture (Sharlau) was dissolved in 1000 mL of sterilized distilled water under heating, cooled to room temperature, adjusted to pH 7.5±0.1 at 25 ˚C, and then placed in tubes sterilized in the autoclave at 121 ˚C for 15 m and cooled to room temperature. After inoculation, they were incubated at 44.0˚±0.5 ˚C for 21± 3 h. After incubation, indole presence has been verified by the addition of 0.2-0.3 mL of Kovacs reagent (Sharlau). Development of a rosewood-red color on the surface of the culture medium indicates the presence of *E. coli*.

c) Because some *Klebsiella oxytoca* strains also give positive indole results, β-glucuronidase test was also performed. In this way, *E.coli* will give positive results (development of green-blue colonies), and *Klebsiella* will give negative results (SR EN ISO 9308-1/2004 *AC:2009*). For the confirmation test, Tryptone Bile X-Glucuronide (TBXG) was prepared by weighing 31.6 g dry TBXG culture medium (Institut für Immupräparate und Nähr medien GmbH Berlin) and dissolving it under stirring in 1000 mL sterilized distilled water. After this step, the obtained dispersion was sterilized in an autoclave at 121 ˚C for 15 m, cooled to room temperature and the pH adjusted to 7.2±0.2 at 25 ˚C. *E. coli* bacteria existence was confirmed when the oxidase test is negative, indol test positive and gren-blue coloring of the growth medium.

2.2.3. NTG 37 ˚C and NTG 22 ˚C determination

The NTG parameter was determined according to SR EN ISO 6222/2004, and offers a general view on the water contamination level because it quantifies a large number of microorganisms, such as all the aerobic bacteria, yeasts and molds capable of forming colonies on agar-yeast extract culture media. The preparation of the culture media was performed as follows: 24g of dryed Tryptone Yeast Extract Agar (Sharlau) was dissolved in 1 L of sterilised distilled water.

The resulting dispersion was autoclaved at 121 ˚C for 15 min, cooled to 25 ˚C and adjusted to pH 7.2±0.2. On each Petri dish, 1 mL of analysed water has been added, following the addition of 15-20 mL sterile culture media, prepared as mentioned in section The Petri dishes have been incubated at 37 ˚C for 48 hours (NTG 37 ˚C) and respectively at 22 ˚C for 72 hours (NTG 22 ˚C). After the incubation period the results were expressed as colony-forming units/ mL "cfu/ mL", because yeasts and fungi were not identified.

2.2.4. Turbidity determination

Turbidity is a parameter strongly correlated with the microbian activity (Noumedem et al., 2013; Rojas et al., 2006; Tamokou et al., 2012). The water turbidity has been determined according to SR EN ISO 7027 / 01 by using a WTW 430 IR turbidimeter.

2.2.5. Free and total chlorine determination

The determination of the free and total chlorine provides useful information about the water sanitation degree. In the storage recipients, the chlorine reacts with the organic pollutants from water, which leads to the formation of the bound-chlorine, at the expense of the free chlorine amount. The free chlorine represents the active component, responsible for the water disinfection, and the total chlorine represents the sum between the bound and free chlorine.

For the spectrophotometric determination of free and total chlorine, SR EN ISO 7393-2/02 standard was used. For this determination an UV-VIS T60 spectrophotometer was used, and the calibration curve has been evaluated according to SR ISO 8466-1 / 1999.

2.2.6. Determination of the microbiological load of the recipient walls

The contact plates' method was used for the evaluation of the microbiological load of the recipient walls in which the water was stored. The test strips for the contact-plate method were purchased from Merck,

Germany. The two sides of the test strips consist of different sterile culture media: a pink-coloured one, for yeasts and molds and a bright-yellow one, for aerobic bacteria. The yellow part represents the GK – T culture medium: 15g casein peptone, 5g Soja peptone, 5g NaCl, 20 mg TTC, 15 g agar, and the pink part represents the GK – HS culture medium: 5g Peptone, 5g Tryptone, 40g Glucose, 50mg Rose Bengal, 30mg Gentamicin, 30mg Trimethoprim, 16g Agar. The control data of the sanitation degree could be interpreted according to Table 1 (http://www.productcatalogue.bode-chemie.com/products/equipment/product information/bode_dip_slides.pdf).

3. Results and discussions

No specific increase of bacteria on Tergitol TTC was observed in the case of the glass recipients. In the case of the initial water, 2 lactose-negative bacteria were registered, while for 24h stagnation, 0 cfu were recorded, for both water stirring methods (Fig. 1), which could mean that this type of treatment is unfavourable to bacterial development (Elvira et al., 2010).

In contrast with the glass recipients, for the HDPE recipients an increase in the number of lactose-negative bacteria was observed even after the first 24 hours (Fig. 2). As time passes microbial activity increases for all types of storage (Table 2).

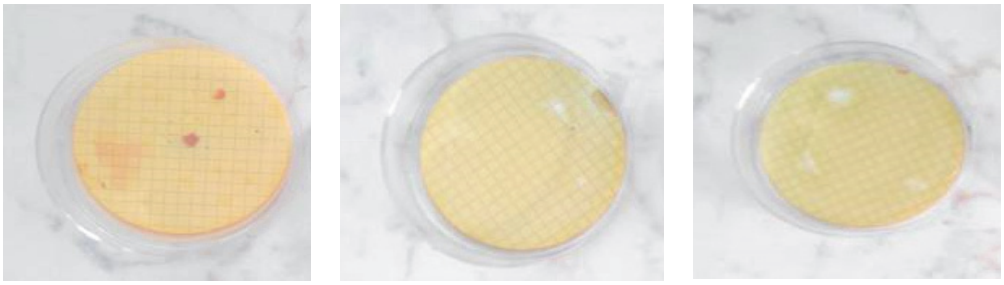
Unexpectedly, results indicate that ultrasonic agitation promotes microbial growth in comparasion with mechanical stirring for all microbiological parameters (Fig. 3). This means that sonication can lead to uncontrolled growth of bacteria and other micro-organisms. This trend is visible in the first 24 hours and monitoring for longer periods of time is not necessary.

Table 1. Interpretation of results obtained by the contact-plate method

Testing strain	*Colour of colonial bacteria on GK-T*	*Colour of colonial bacteria on GK-HS*
Escherichia coli ATCC 25922	red	-
Bacillus subtilis ATCC 6633	red	-
Enterococcus faecalis ATCC 19433	red	-
Aspergillus niger	white/black	white/black
Staphilococcus aureus 6538	pink-red	-
Candida albicans ATCC 10231	white-pink	white

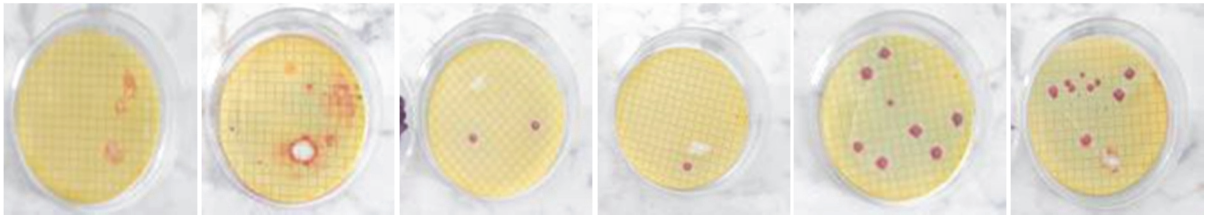
Table 2. Results for lactose-negative in the water stored in HDPE recipients

Days of stagnation	*Results for state A: without stirring (cfu/ 50mL)*				*Results for state B: magnetic stirring (cfu/ 50mL)*				*Results for state C: ultrasonic agitation (cfu/ 50mL)*			
	x_1	x_2	$\bar{x}$	*stdev*	x_1	x_2	$\bar{x}$	*stdev*	x_1	x_2	$\bar{x}$	*stdev*
1	5	8	6.5	2.12	2	1	1.5	0.71	8	11	9.5	2.12
2	60	56	58	2.83	48	45	46.5	2.12	78	74	76	2.83
7	> 300	> 300	-	-	> 100	> 100	-	-	> 100	> 100	-	-



Initial | After 24h of stirring | After 24h of sonication

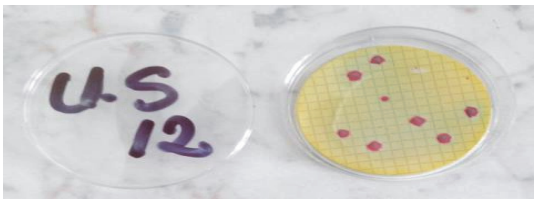
Fig. 1. Decrease in the number of lactose-negative bacteria after 24h for the water stored in glass recipients



1 day

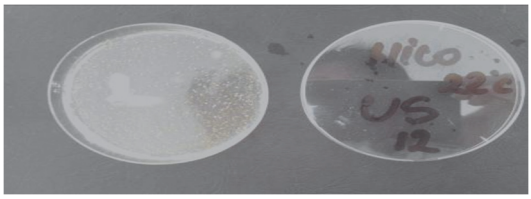
Duplicate samples without stirring | **Duplicate samples magnetic stirring** | **Duplicate samples ultrasonic agitation**

Fig. 2. Bacterial (lactose-negative) growth kinetic in the water stored in HDPE recipients

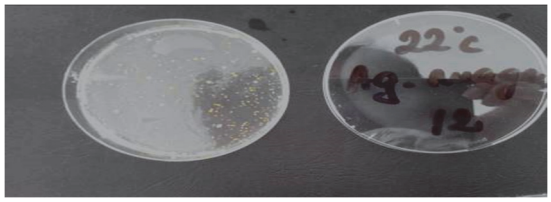


Lactose-negative bacteria results, after 24h of sonication

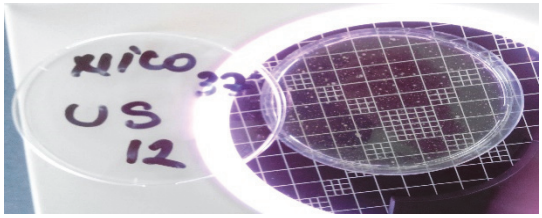
Lactose-negative bacteria results, after 24h of mechanical stirring



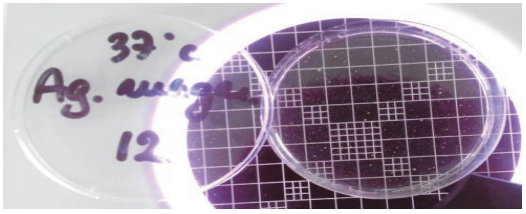
NTG 22 °C results after 24h of sonication



NTG 22 °C results after 24h of mechanical stirring



NTG 37 °C results after 24h of sonication



NTG 37 °C results after 24h of mechanical stirring

Fig. 3. Comparison between water sonicated and mechanically stirred for 24 h in HDPE recipients

After the first day of storage in the HDPE recipients, the water turbidity has increased with 95% in the case of sonication and with 80% for mechanical stirring and stagnation by comparing to the initial value (1.2 NTU). Turbidity indicates intensification of microbial activity (Bollela et al., 1999). The same conclusion results from microbiological determinations (Fig. 4). On longer time periods, the turbidity values increase slightly, after which they remain relatively constant.

In the case of the glass recipients no bacterial colonies have been determined by using Tergitol TTC, while in the case of the HDPE recipients presumable lactose-positive bacteria occurred (not confirmed as coliform or *E. coli*). According to the studies performed by LG SONIC, which comercialises sonication systems for drinkable water plants, ultrasound treatment reduces the number of cyanobacteria and filamentous algae, probably due to the disruption of the membranes and cell organelles (https://www.lgsonic.com/).

It was observed that ultrasound treatment favours bacterial development for the water stored in either the glass or HDPE recipients, probably due to the rise in the temperature of the water, explainable by taking into account the significant energy transfer called absorption. At the crossover between two media with different densities (water and storage recipient walls), absorption and reflexion occurs. Through absorption, a significant amount of the acoustic wave energy passes into the environment as heat. The velocity of the acoustic wave (c) depends only on the characteristics of the medium, being frequency independent. The square of the propagation velocity (c^2) is equal to the pressure variation (Δp) in respect with the density of the environment ($\Delta\rho$), at normal pressure (p_o). (Eq. 1).

$$c^2 = \frac{\Delta p}{\Delta \rho}\, p_o \qquad (1)$$

The velocity of the acoustic wave in solids (c_s) is higher than in liquids (c_l). The velocity of ultrasound waves' propagation in water is 1480 m/s, and in solids ranges from 3000 to 4100 m/s.

During the propagation into homogenous and isotropic environment, the energy of the ultrasound beam exponentially decreases due to absorption (releasing energy into the environment) (Eqs. 2 and 3).

$$I = I_0\, e^{-\alpha x} \qquad (2)$$

where: I_0 – initial intensity of the source;
I - intensity at distance "x" from the source;
α – absorption attenuation coefficient.

$$\alpha = [\ln I_0/I] / x \qquad (3)$$

where x – distance crossed.

The total attenuation coefficient varies directly proportional to the square of the ultrasound waves frequency (Mihailescu, 2015).

The contact plate tests have indicated that the ultrasounds do not allow the developing of the bacterial biofilm on the walls of the storage recipients, most probably due to the pressure exerted by the ultrasound waves. Thus, the bacterial adherence to the walls of the recipient could be eliminated (Fig. 5).

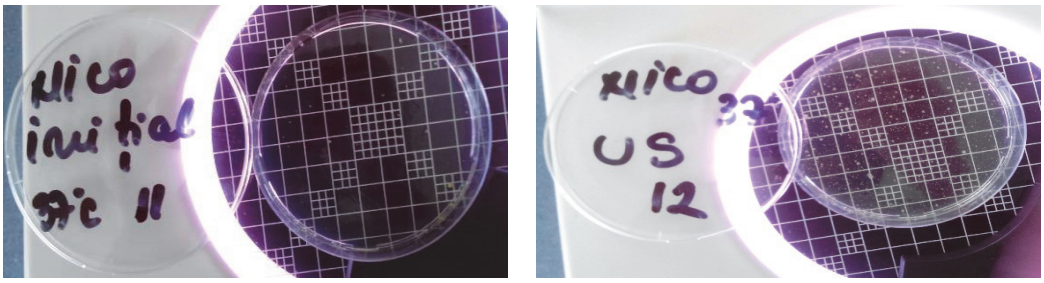


Fig. 4. Microbial load after 24h of storage in HDPE recipients

Fig. 5. HDPE storage recipients' walls microbial load

Also, it can be noted that the bacterial adherence is reduced in the case of the mechanical stirring but in a lower amount compared to the stationary water.

No yeast or mold development was observed in the case of all the studied water storage methods. These results are in agreement with the literature data (Luca, 2015) showing that water sonication alone is only partially effective in drinking water disinfection. Only combination of sonication with UV irradiation and water cooling systems could lead to the increase of biological safety of drinking water. Also Inacio et al. (2015), reported that sonication enables the growth of a high proportion of latent organisms encased in the biofilm. Regarding the free and total chlorine amount it has been determined that a significant decrease occurs after sonication, from 0.336 mg/L free Cl_2 and 0.395 mg/L total Cl_2, in the case of initial water, to bellow the detection limit after 24h.

4. Conclusions

This study demonstrates that the material from which the storage recipients are made of clearly influences the quality of the drinkable water.

Glass has been proven as the optimum material for the water recipients because it does not stimulate the microbial growth. The storage of the water in polyethylene recipients represents a high risk factor, from the bacterial growth point of view (either lactose-positive and negative, total number of germs at 22°C and 37°C). It has been proven that mechanical stirring as well as sonication are able to significantly reduce the formation of the biofilm on the wall of the storage tanks, irrespective of the material from which the recipients are made of.

Sonication has been proven to be inefficient for water conservation, as in the sonicated polyethylene recipients the most intense bacterial activity has been observed. This could be explained by either the more rapid consuming of the free chlorine due to the reaction of the organic material with the chlorine, as well as due to the rising of the temperature during sonication. So, when using ultrasound treatments is absolutely needed increased attention on all aspects (propagation environment, walls tanks, barriers found and facing, distance from source of ultrasound and so on), because it is possible to obtain negative effects.

Taking into account that in the large storage tanks from the public distribution network stirring is necessary in order to ensure the homogenous repartition of chlorine on all levels, the adaptation of the type of stirring in correlation with the material from which the storage tanks are made of could significantly improve the quality of the drinkable water.

Acknowledgements

This work was partially supported by the strategic grant POSDRU/159/1.5/S/137070 (2014) of the Ministry of National Education, Romania, co-financed by the European Social Fund – Investing in People, within the Sectoral Operational Programme Human Resources Development 2007-2013 and by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-II-RU-TE-2014-4-0173.

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