Poll Res. 40 (1) : 27-33 (2021)

ISSN 0257–8050

WATER DISINFECTION FROM MICROORGANISMS USING CHITOSAN

M.N. SAPRYKINA, E.S. BOLGOVA, L.O. MELNYK AND V.V. GONCHARUK

**A. V. Dumansky Institute of Colloid and Water Chemistry, 42, Vernadsky Blvd, Kyiv, Ukraine, 03680*

(Received 28 May, 2020; accepted 20 August, 2020)

ABSTRACT

It is established that the effectiveness of the process of water disinfection depends on both the molecular weight of chitosan, the degree of its deacetylation, and the type of microorganism *(Escherichia coli*, *Candida albicans)*. The optimal pH value of the medium for inactivating the culture of *Candida albicans* was determined. For the first time, a significant contribution of the flocculation of microorganisms with chitosan to the total effect of water disinfection is shown. It is also shown that the presence of organic and inorganic impurities in water reduces both the disinfecting and flocculating effects of the polysaccharide on the microbiological object. The impact of temperature on the antimicrobial effect of chitosan was revealed. Thus, the results obtained convincingly indicate that chitosan can be used to effectively purify water from a number of microorganisms. The use of investigated dependencies will improve the quality of water purification.

KEY WORDS : Bactericidal effect, Chitosan, Flocculating effect, Water disinfection.

INTRODUCTION

The problem of clean water is closely related to the quality of water sources used in water treatment systems. The main source of centralized water supply is increasingly becoming artificial reservoirs and freshwater seas, which are formed owing to the riverbed blocking, flooding of large areas of land. As a result of human activity in the aquatic environment soil microscopic fungi, i.e., micromycetes, have appeared (Goncharuk *et al.*, 2004).

Micromycetes are widespread in the environment. They are an integral part of the human environment. Recently, however, micromycetes often become the cause of infectious and inflammatory diseases. Besides, it is known that micromycetes are capable of producing mycotoxins (Zabolotnyi *et al.*, 2019). When systematically injected into the human body, mycotoxins even in small doses are capable of provoking oncological diseases. They also have neuro- nephro- and hepatotoxicity, show mutagenic activity, and have an immunosuppressive effect (Naresh and Olsen 2004).

Nowadays, the studies on the disinfection and purification of water using chitosan (CTN) and materials made on its base are being intensively carried out in the world, which is associated with the need to improve the environmental friendliness of water treatment processes, as well as make the requirements for drinking water quality stricter (Li *et al.*, 2008; Zeng *et al.*, 2008; Fabris *et al.*, 2010; Abebe *et al.*, 2016; Yang *et al.*, 2016; Unuabonah *et al.*, 2017; Kangama *et al.*, 2018). Chitosan obtained from chitin (a natural polymer) by deacetylation in an alkaline medium due to the variety of physicochemical and biological characteristics is widely used in the food industry, cosmetology, biomedical engineering, and agriculture (Kong *et al.*, 2010; Pontius 2016). The papers (Kong *et al.*, 2008; Tymchuk and Grubnyak 2017) note the important advantages of chitosan, such as biodegradability and high disinfecting ability against gram-positive and gram-negative bacteria, while there is no toxicity towards mammalian cells. This creates prospects for the use of chitosan as an antibacterial agent alone or in a mixture with other natural polymers.

As it was shown in (Chung and Chen, 2008; Eaton *et al.*, 2008; Raafat *et al.*, 2008; Goy *et al.*, 2009), one of the most likely mechanisms of chitosan antibacterial effect is the electrostatic interaction of protonated amino groups of its molecules with a negatively charged microbial cell membrane, mainly due to competition with Ca^{2+} ions for electronegative sites on the cell membrane surface. This leads to a double effect. Firstly, the permeability of the membrane wall changes, which causes an internal osmotic imbalance and, therefore, inhibits the growth of microorganisms. Secondly, due to the hydrolysis of peptidoglycans in the wall of microorganisms, intracellular electrolyte (i.e., potassium ions, low molecular weight protein components: proteins, nucleic acids, glucose and the lactate dehydrogenase enzyme) leaks. Chitosan and its various derivatives are also considered as potential environmentally safe coagulants and flocculants for water treatment, given their environmental safety and high efficiency at relatively low doses (Li *et al.*, 2013). Thus, according to (Pontius, 2016), the optimal doses of aluminum sulfate, ferric chloride and chitosan in the process of coagulation of lake water, determined by the turbidity of the settled water, are 30, 30 and 8 mg/dm^3, respectively. However, in work (Pontius 2016) it is noted that chitosan has not been studied enough as a coagulant for drinking water.

The purpose of this work was to study the disinfecting effect of high molecular weight and low molecular weight CTN with different degrees of deacetylation on *Escherichia coli* (*E.coli)* and *Candida albicans* (*C.albicans)* microorganisms depending on the physicochemical parameters of the water and determine the contribution of flocculation of microorganisms with chitosan to the total effect of water disinfection in order to meet the drinking water quality requirements.

MATHERIALS AND METHODS

Water disinfection was carried out using a 0.1% chitosan working solution in a 0.1 mol/dm^3 solution of acetic acid. High molecular weight CTN_1 (with the molecular weight (Mw) constituting 100–300 kDa) and low molecular weight CTN_2 (with a Mw constituting 50-60 kDa) with a degree of deacetylation of 95 and 75-85%, respectively, were investigated.

The culture of sanitary indicative test microorganism *E. coli* 1257 was obtained from the collection of Tarasevich State Institute of Standardization and Control of Biomedical Preparations (Moscow). Suspension of *E. coli* 1257 bacteria (10^7 colony-forming unit (CFU)/cm^3) was prepared according to (Potapchenko *et al.*, 2001). Yeast-like fungus *C. albicans* 10231 was received from L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine. Cultivation of yeast-like fungi was carried out in a liquid nutrient medium Saburo, which was prepared according to (Grigorieva, 1981). The preparation of a suspension of yeast-like fungi was carried out similarly to (Goncharuk *et al.*, 2008).

Conducting the study of the disinfecting effect of CTN, the cups of 100 cm^3 were filled with water contaminated with microorganisms, the necessary amount of CTN was added, so that the concentration of the latter was 0.1, 0.5, 1.0, 5.0 or 8.0 mg/dm^3. After a certain period of time, an aliquot of the treated water was taken and sown on a nutrient medium with further counting of the colonies.

Studying the joint bactericidal and flocculating effect of CTN on the process of water disinfection, the 100 cm^3 volume cups were filled with water contaminated with microorganisms and the necessary amount of CTN, so that the concentration of the latter was 0.1, 0.5, 1.0, 5.0 or 8.0 mg/dm^3. At certain intervals the treated water was passed through paper filters (i.e., a white tape – Whatman Grade 40 filter paper 8 μm, which simulates filtration through a sand filter (Kong *et al.*, 2010), an aliquot of the filtrate was collected and sown on a nutrient medium with further counting of the colonies.

The survival of microorganisms was determined by the presence of CFU when sowing water samples taken on Saburo agar medium for *Candida albicans* and Endo agar medium for *Escheriñhia coli.* Microorganisms were cultured for 14-18 hours at 37 °C. The result was expressed as the logarithm of the ratio of the test microorganism concentration in the solution after treatment with CTN (N_t) to the concentration of the microorganism in the initial solution (N_0).

Conducting the research, distilled water (DW), tap water (TW), and water after ultrafiltration treatment with an UPM-20 membrane (UFW) were used. The physico-chemical characteristics of the types of water in question are presented in Table 1.

Studying the effect of water pH on the efficiency of the disinfection process, a 0.1% NaOH solution or a 0.1% HCl solution was used to adjust the pH value. The pH value was determined using an I-

Table 1. Physico-chemical composition of the investigated waters

Type of water	Name of indicators					
	Conductivity at 18°C, μS/cm	Color, degrees	Turbidity, mg/dm^3	pH	Sulfates, mg/dm^3	Chlorides, mg/dm^3
DW	2	< 2	< 0.3	5.3	0.5	0.02
TW	382	18	0.9	7.2	57.6	26.0
UFW	298	8	0.1	7.3	14.6	16.0

160M ionomer.

Studying the effect of temperature on the disinfection of water with CTN, the investigated water samples were thermostated at 42, 37, 27, 17 and 7 ° C.

RESULTS AND DISCUSSION

Table 2 shows that the efficiency of distilled water disinfection from gram-negative bacteria *Escherichia coli* with two types of chitosan (concentration 0.1 mg/dm^3) practically does not depend on the molecular weight of the polymer and the degree of its deacetylation. Already after an one hour of contact of *Escherichia coli* (initial concentration 500000 CFU/cm^3) with CTN, the degree of culture inactivation constitutes two orders of magnitude, and CTN has a pronounced bactericidal effect, since there is no growth of culture over time.

In the case of disinfection of the yeast-like fungus *C.albicans* at its initial concentration of 2400000 CFU/cm^3 under similar conditions, it was found (see Table 2) that the degree of inactivation of micromycete by chitosan depends on the molecular weight and degree of deacetylation of the latter. A low molecular weight CTN with a degree of deacetylation amounting to 75-85% inactivates only slightly more than one culture order after an one hour of contact, while high molecular weight CTN with a deacetylation degree constituting 90% inactivates over a similar period of more than two and a half culture order. It has been established that *C. albicans* is more stable in comparison with *E. coli* to the effect of the disinfecting agent, which is especially noticeable with an increase in the time of contact of the culture with CTN. Obviously, this effect is associated with a different cell structure. The yeast cell is surrounded by a rather thick cell wall.

The results obtained confirm our conclusion in previous studies (Bolgova *et al.*, 2016) that *C. albicans* is a more suitable test object for evaluating the effectiveness of disinfection processes compared to *E. coli*. Based on the obtained results, CTN_1 was selected for further research in this work.

The results of the study of the influence of pH on the efficiency of disinfection of water from *C. albicans* with CTN_1 are presented in Figure 1.

Figure 1 shows that the highest degree of inactivation of the culture is achieved in a weakly acidic medium (pH 5.0), whereas at pH 8.5 the disinfecting effect is insignificant. Comparison of Figure 1a and 1b indicates a significant contribution of flocculation of microorganisms with chitosan to the total effect of water disinfection, which is especially noticeable at relatively short periods of contact of culture with chitosan. Thus, the concentration of *C. albicans* with a contact duration of an one hour decreases by about 4 and 6 orders of magnitude, respectively, with the bactericidal (see Figure 1a) and the joint bactericidal and flocculating action (see Figure 1b) of chitosan. It is important to note that the degree of retention of *E.coli* and *C.albicans* when filtered through paper in the absence of chitosan did not exceed 0.1-0.3 order of the initial concentration.

The higher efficiency of the disinfecting and

Table 2. Disinfection of distilled water from *E. coli* and *C. albicans* with chitosan (0.1 mg/dm^3) with different molecular weights (pH – 6.6; t – 13°C)

t, h	CFU/cm^3			
	E. coli		*C. albicans*	
	CTN_1	CTN_2	CTN_1	CTN_2
0	500000	500000	2400000	2400000
1	5000	2900	13000	60000
168	0	0	20000	10000
504	0	0	130	18000

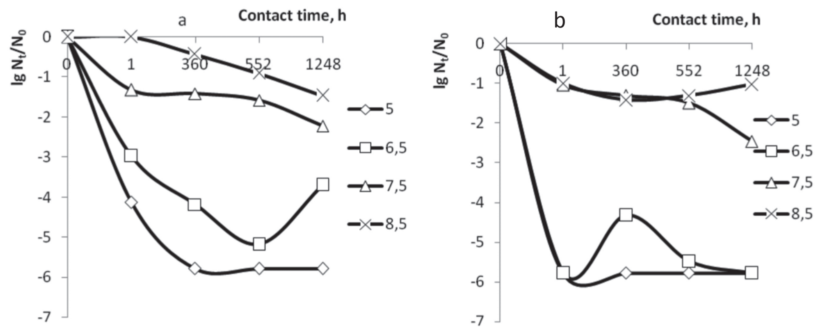

Fig. 1. The kinetics of disinfection of distilled water with CTN_1 (0.1 mg/dm^3) from *C. albicans* at different pH values (characters indicated on the curves): a) the bactericidal effect of CTN_1; b) joint bactericidal and flocculating effect of CTN_1.

flocculating effect of chitosan in a weakly acidic environment is associated with a high degree of protonation of the polymer amino groups under these conditions, which contributes to the electrostatic interaction between negatively charged microorganisms and positively charged molecules of chitosan, causing, on the one hand, changes in the permeability of the cell membrane and its violation functioning, and, on the other hand, cell aggregation and flocculating effect (Raafat *et al.*, 2008; Goy *et al.*, 2009; Yang *et al.*, 2016).

As the further studies have shown, the presence of various impurities in water significantly impairs the efficiency of its disinfection with chitosan (see Tables 3 and 4). Figures 3 and 4 show that the bactericidal effect of chitosan as well as its joint bactericidal and flocculating effect on *C.albicans* micromycetes is most pronounced in distilled water – in the experimental conditions the decrease in the concentration of the microorganism constitutes 4-5 orders of magnitude (The initial amount of culture varies from 8.5×10^4 to 6×10^5). At the same time, in tap water and tap water after ultrafiltration treatment, the degree of disinfection of *C. albicans* under similar conditions does not exceed 1-2 orders of magnitude, being higher in water that underwent additional membrane cleaning.

Table 3. The kinetics of disinfection of various types of water from *C. albicans* with CTN_1 (0.1 mg/dm^3) at pH 5.0

τ, h	Type of water		
	TW	UFW CFU/cm^3	DW
1	1.2 × 10^5	2.0 × 10^4	4.5 × 10^1
360	4.3 × 10^4	3.9 × 10^3	1
720	1.2 × 10^4	2.3 × 10^3	1

Table 4. The kinetics of joint bactericidal and flocculating effect of CTN_1 (0.1 mg/dm^3) on various types of water disinfected from *C. albicans* at pH 5.0

τ, h	Type of water		
	TW	UFW CFU/cm^3	DW
1	7.8 × 10^4	1.2 × 10^3	1
360	4.3 × 10^4	3.2 × 10^2	1
720	2.0 × 10^4	7.5 × 10^1	1

The results obtained can be explained by the presence in the TW and UFW of a whole range of organic and inorganic impurities that compete with microorganisms for interaction with CTN molecules, reducing both the disinfecting and flocculating effect of the polysaccharide on the microbial object.

Thus, as arises from the results presented above, the use of chitosan at a concentration of 0.1 mg/dm^3 for disinfection of tap water as well as ultrafiltration-treated tap water, is ineffective. To obtain the desired result, it is obviously necessary to increase the dose of chitosan during water treatment.

Figures 2 and 3 show the results of disinfection of tap water as well as tap water treated using ultrafiltration method from *C. albicans* (with the concentration in the source water constituting 1×10^4 – 1×10^5 CFU/cm^3) at various concentrations of chitosan in the solution.

As can be seen from Figure 2a and Figure 3a, an increase in the concentration of chitosan in tap water from 0.5 to 8.0 mg/dm^3 significantly increases the degree of its disinfection from *C.albicans*, moreover, as in the patterns shown in Figure 1, a significant contribution of the flocculating effect of chitosan to this process is found. Almost complete disinfection of tap water from *C.albicans* is observed after an one

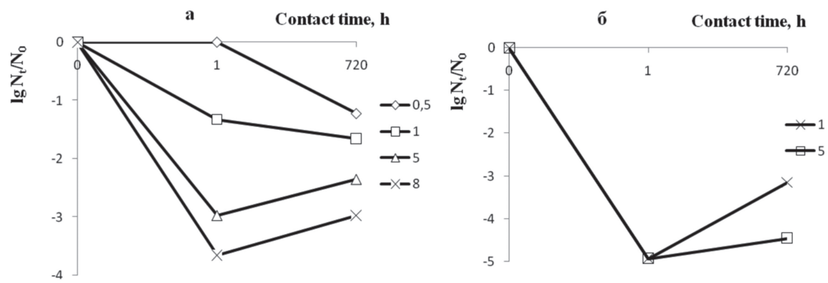

Fig. 2. The kinetics of disinfection of water from *C. albicans* at various concentrations of CTN_1 (characters indicated on the curves, mg/dm^3): a) tap water (pH = 7.1); b) tap water after ultrafiltration (pH = 7.1)

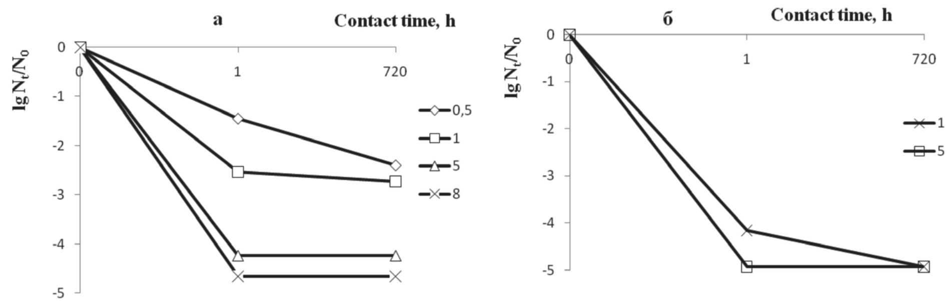

Fig. 3. The kinetics of water disinfection from *C.albicans* with the joint bactericidal and flocculating effect of different concentrations of CTN_1 (characters indicated on curves, mg/dm^3): a) tap water (pH = 7.1); b) tap water after ultrafiltration (pH = 7.1)

hour of contact of the culture with chitosan at a concentration of the latter constituting 8.0 mg/dm^3 and additional filtration of the chitosan treated water through a paper filter. Effective disinfection of water that has passed the preliminary ultrafiltration treatment is observed at a lower concentration of chitosan (5.0 mg/dm^3), which was obvious considering the results presented above.

It is established that the antimicrobial effect of chitosan in relation to the selected microorganisms significantly increases with increasing temperature from 7 to 42°C (Figure 4 a, b). A decrease in the degree of inactivation of the culture with a decrease in temperature may be associated with a decrease in the number of reactive groups on the cell surface for binding to chitosan. Such changes in the cell arise because of the stress factor acting thereon, namely, a low temperature.

CONCLUSION

Experimental studies conducted lead to the following conclusions

The degree of inactivation of the *E.coli* culture does not depend on the type of chitosan studied in the work (a high molecular weight CTN_1 (with molecular weight (Mw) constituting 100–300 kDa) and low molecular weight CTN_2 (with Mw constituting 50-60 kDa) with a degree of deacetylation constituting, 95 and 75-85%,

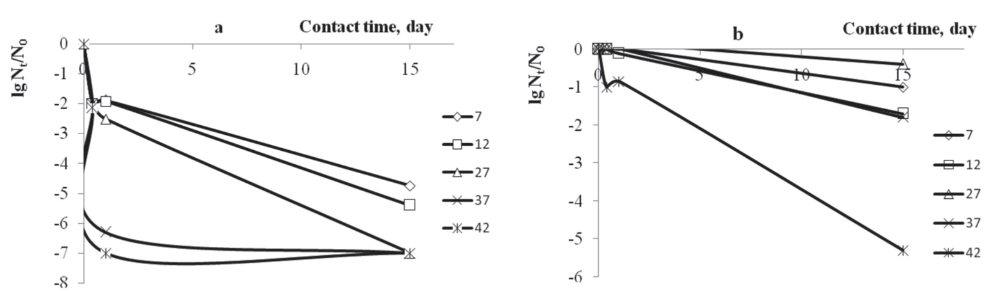

Fig. 4. The kinetics of disinfection of tap water (pH = 7.2) from *E.coli* (a) and *C. albicans* (b) using CTN_1 (0,1 mg/dm^3) at different temperature (figures indicated on the curves, ^{o}C).

respectively), whereas, in the case of *C. albicans*, a high molecular weight CTN with a degree of deacetylation amounting to 95% is a more effective disinfecting agent.

The highest degree of inactivation of the culture of *C. albicans* with CTN_1 is achieved in a weakly acidic medium (pH 5.0), whereas at pH 8.5 the disinfecting effect is insignificant.

For the first time a significant contribution of the flocculation of microorganisms with chitosan to the total effect of water disinfection is shown. This factor is essential when choosing a rational scheme of disinfection of water with chitosan, because it indicates the possibility of reducing the dose of chitosan required for disinfection in the case of applying the water filtration after treatment with chitosan.

It is shown that the presence of organic and inorganic impurities in water reduces both the disinfecting and flocculating effect of the polysaccharide on the microbiological object, which is obviously connected with the competing influence of these impurities on the interaction of CTN molecules with microorganisms.

It is shown that the antimicrobial effect of chitosan increases with increasing temperature from 7 to 42 °C. Achieving a high degree of disinfection of water at low temperatures requires evidentially increasing the dose of chitosan.

REFERENCES

Abebe, L., Chen, X. and Sobsey, M. 2016. Chitosan Coagulation to Improve Microbial and Turbidity Removal by Ceramic Water Filtration for Household Drinking Water Treatment. *Int. J. Environ. Res. Public Health.* 13 (3) : 269

Bolgova, E.S., Saprykina, M.N. and Goncharuk, V.V. 2016. New test object for assessing the quality of disinfected water. *Water & Water Pur. Tech. Sc. Tech. News.* 20 (3): 3-7

Chung, Y. C. and Chen, C. Y. 2008. Antibacterial characteristics and activity of acid-soluble chitosan. *Biores. Tech.* 99 (8) : 2806-2814

Eaton, P., Fernandes, J. C., Pereira, E., Pintado, M. E. and Xavier Malcata, F. 2008. Atomic force microscopy study of the antibacterial effects of chitosans on *Escherichia coli* and *Staphylococcus aureus. Ultramicroscopy.* 108(10) : 1128-1134

Fabris. R., Chow, C. W. K. and Drikas, M. 2010 Evaluation of chitosan as a natural coagulant for drinking water treatment. *Water Sci. Tech.* 61 (8): 2119-2128.

Goncharuk, V.V., Rudenko, A.V., Koval, E.Z. and Savluk, O.S. 2004. The problem of water infection with pathogens of mycoses and prospects of resolving this problem. *J. Water Chem. Tech.* 26 (2) : 1-18.

Goncharuk, V.V., Potapchenko, N.G., Vakulenko, V.F., Savluk, O. S., Kosinova, V. N. and Sova, A.N. 2008. Water disinfection by joint effect of ozone and UV-radiation in a flow-through mode. *J. Water Chem. Tech.* 30 (1) : 51-58.

Goy, R.C., Britto, D. de and Assis, O.B.G. 2009. A review of the antimicrobial activity of chitosan. *Polímeros.* 19(3) : 241-247.

Grigorieva, L.V. 1981. Handbook on sanitary microbiology. *Chisinau: Cartea Moldoveneasca.* 206.

Kangama, A., Zeng, D., Tian, X. and Fang, J. 2018. Application of Chitosan Composite Flocculant in Tap Water Treatment. *J. Chem.* 2018: 1-9.

Kong, M., Chen, X. G., Liu, C. S., Liu, C. G., Meng, X. H. and Yu, L. J. 2008. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli. Colloids and Surf. B: Biointerfaces.* 65(2): 197-202.

Kong, M., Chen, X. G., Xing, K. and Park, H. J. 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int. J. Food Microbiol.* 144 (1) : 51-63.

Li, J., Jiao, S., Zhong, L., Pan, J. and Ma, Q. 2013. Optimizing coagulation and flocculation process for kaolinite suspension with chitosan. *Colloids and Surfaces A: Physicochem. Engin. Aspects*. 428 : 100-110.

Li, Q., Mahendra S., Lyon D. Y., Brunet L., Liga M. V., Li D. and Alvarez P. J. J. 2008. Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Res.* 42 (18) : 4591-4602.

Naresh, M. and Olsen, M. 2004. *Mycotoxins in Food: Detection and Control*. England. Woodhead Pablishing Ltd.: 473.

Pontius, F. W. 2016. Chitosan as a Drinking Water Treatment Coagulant. *Am. J. Civil Engin.* 4 (5) : 205-215.

Potapchenko, N.G., Goncharuk, V.V., Kosinova, V.N. and Sova, A.N. 2001. Disinfection of water by ozone. 1. Interrelationship between kinetics of the dying-of bacterian in bidistilled water and ozonization parameters. *J. Water Chem. Tech.* 23 (4) : 57-65.

Raafat, D., von Bargen, K., Haas, A. and Sahl, H.-G. 2008. Insights into the Mode of Action of Chitosan as an Antibacterial Compound. *Appl. Envir. Microbiol.* 74 (12) : 3764-3773.

Tymchuk, A.F. and Grubnyak, A.E. 2017. Influence of natural and synthetic flocculants on the sedimentation stability of suspensions. *Visn. Odes. Nac. Univ., Him*. 22 (2) : 71-81

Unuabonah, E. I., Adewuyi, A., Kolawole, M. O., Omorogie, M. O., Olatunde, O. C., Fayemi, S. O. and Taubert, A. 2017. Disinfection of water with new chitosan-modified hybrid clay composite adsorbent. *Heliyon.* 3 (8) : 1-29

Yang R., Li, H., Huang, M., Yang, H. and Li, A. 2016. A review on chitosan-based flocculants and their applications in water treatment. *Water Res.* 95 : 59-89.

Zabolotnyi, D. I., Rudenko, A. V., Goncharuk, V. V., Saprykina, M. M. and Bolgova, O. S. 2019. Drinking water. Microscopic fungi. Mycotoxins (literature review). *J. National Acad. Med. Sci. Ukraine*. 25 (3) : 253-259

Zeng, D., Wu, J. and Kennedy, J. F. 2008. Application of a chitosan flocculant to water treatment. *Carbohydrate Polym.* 71 (1): 135-139