

THE INACTIVATION OF *ESCHERICHIA COLI* BY MICROALLOYED ALUMINIUM BASED COMPOSITE

UDC 628.161.3 + 546.62

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Abstract. *The inactivation of Escherichia coli by microalloyed aluminium based composite was studied. The composite is material in form of steel wire, plated by microalloyed aluminium. Its effects are based on spontaneous dissolution in contact with water, with generation of Al(III) and OH⁻ ions, and finally voluminous insoluble Al(OH)₃. Bearing in mind its great efficacy in purification of water from many chemical pollutants, efficient inactivation of microbiological pollutant of water – E. coli, was expected, too. The experiments were carried out with the water model-solutions (WMS) based on adapted natural surface water, inoculated by the E. coli. Treatments were performed in the original semi-flow system (SFS). The results show that approximately at every 10 min, the number of E. coli bacteria was reduced for about one log₁₀ count, with complete inactivation as the outcome of the treatment. At the end of the treatment, the Al(OH)₃ precipitate still contained the low amount of coprecipitated E. coli bacteria, which died in relatively short period.*

Key words: *microalloyed aluminium, composite, water model-solution, Escherichia coli, semi-flow system, disinfection of water.*

INTRODUCTION

The quantity of naturally occurring, unpolluted high-quality water sources are less than ever. Due to this fact, there is a permanent need to use all available water resources, which are, as a rule, hygienically unsuitable [1]. Chlorination is the major method of water disinfection used worldwide [2, 3, 4], and still the cheapest way and often the best mean to disinfect potable water supplies and control bacterial growth in the distribution system. However, in the last time much concern has been raised about the potential formation of undesirable disinfection by-products (DBPs) of chlorination that could be possibly deleterious to health [3, 4]. During the chlorination of drinking water, a complex mixture of DBPs forms from chlorine and the organic and inorganic compounds present

in raw water [2, 5]. The chlorination DBPs are important mainly when surface water is used for drinking water as more organic compounds are present in it, than in ground water. Because of current concerns over such chlorination DBPs, many water utilities in the industrialized world are changing chlorination practices to minimize potential risks associated with DBPs formation, and to reduce public objections to chlorine-related tastes and odors in treated waters [2, 5].

The present study was conducted to assess capability of microalloyed aluminium based composite for inactivation of *E. coli*, used, until now, in many treatments for degradation and removal of chemical pollutants from water. The mechanism of its influence on dozen inorganic cations, anions and molecules, and organic compounds (mainly based on reduction and hydrolysis) are closely investigated [6, 7, 8, 9]. Obtained results show that its efficacy in purification of water is very high, considering that the rest of the pollutant concentrations are usually under maximal allowed concentration (MAC).

Providing its action on simple, frequently very stable, chemical compounds, the assumption came that the effects of composite may be toxic and destructive for *E. coli* in water, too, because it is very complex and sensitive system. Therefore, Bojić *et al.* [10] applied the MABC for inactivation of *E. coli*, in simple, stationary conditions, for the first time. That work showed high efficacy of composite with reduction of *E. coli* for more than five \log_{10} counts, and the possibility of complete disinfection of water, in relatively short period.

EXPERIMENTAL

Microalloyed aluminum based composite

Microalloyed aluminium (MA) was prepared by microalloying aluminium of 99.8% purity with: Sn, Ga and Bi [11]. After microalloying, exact consistency was determined by flame atomic absorption spectrophotometry on Varian AAS Spectra A-20 (Mulgrave, Victoria, Australia). Obtained MA had exactly: 0.105% Sn, 0.098% Ga, 0.040% Bi. By microalloying, the oxide layer, which otherwise protects metal from corrosion [12, 13], became porous and permeable for ions, because of many defects in crystal lattice [11]. The microalloyed components formed many defects in structure of metal, as well, such as interstitials and vacancies, and irregularities in charge distribution with origination of many microgalvanic cells. These defects provoke decreasing of energy barrier for transport of Al^{3+} and O^{2-} ions. Because of that, stationary potential of MA decrease to about -1.5 V/SCE (saturated calomel electrode) [11].

The composite was formed from a steel wire (mesh 1.2×1.2 mm) plated by MA, by liquid metal metallization [11]. Its surface is amorphous, in the form of fish scales, with very high real area according to geometric. Structure of the MABC in aqueous medium can be represented by the following scheme: $[\text{Fe} - \text{Al}_{\text{Micalloy}} - \text{Al}_{\text{Micalloy}2}\text{O}_3 \times n\text{H}_2\text{O} - \text{Me}] | \text{H}_2\text{O}$ (K^+ , A^- , M, colloids...) (Fig. 1). As can be seen, beside local microgalvanic cells in MA, there is also a macro galvanic cell $-\text{Fe} | \text{Al}_{\text{Micalloy}}$, which leads to the increase of anode activity, and rate of dissolution [11].

The effects of the composite are based on its spontaneous reaction with water. The main process on microanodes is dissolution of aluminium and generation of Al(III) ions, and on microcathodes reduction of water to H_2 , with releasing of OH^- ions. Every parti-

cle, beside water molecules, which arrives to the surface of composite, may become participant of many physicochemical processes. Al(III) and OH⁻ ions form indissoluble Al(OH)₃, which coagulate to gelatinous, voluminous precipitate of very developed surface [16], which coprecipitate part of pollutants from water, or the products of their chemical degradation [6].

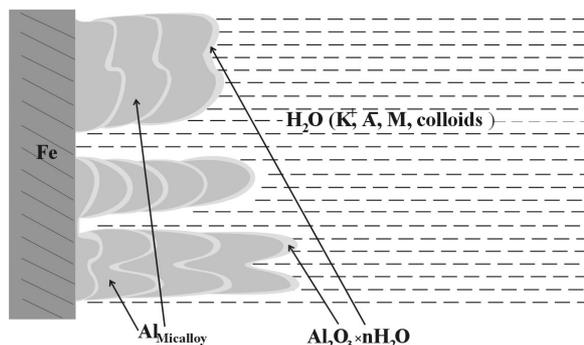


Fig. 1. A model of structure of microalloyed aluminium based composite in aqueous media.

Bacterial strain and culture conditions

It is known that the most frequently present microbiological contaminant of water is coli bacteria, and first of all *Escherichia coli*, which is possibly the best indicator of fecal contamination in water because it accounts for more than 95% of the coliform genera in human feces [17, 18]. Another reason for application of *E. coli* in this study is its high resistance to many physical and chemical disinfection agents, and ability to survive in very deficient living conditions [19, 20].

The *Escherichia coli* KF-4 strain was maintained and grown in the medium containing: peptone 10.0 g, glucose 5.0 g, NaCl 3.0 g, NH₄Cl 1.0 g and CaCl₂ 0.010 g per dm³ of deionized distilled water [21]. The exponential phase starter culture (age from 100 to 400 min) [21] was used for inoculation in all experiments. The density of starter cultures was checked by HACH 2100 AN turbidimeter (Loveland, Colorado), for turbidity value 20.0 nephelometric turbidity units (NTU). This density was equivalent to 10⁸ *E. coli* per cm³. The culture was grown at 37±0.5°C in a water bath.

Sample handling

The experiments were carried out with the water model-solutions based on natural surface water from the River Nišava. Water was taken as a grab samples, allowed to settle and filtered through the fast filter paper Schleicher & Schuell 589 (Dassel, Germany). After that, water samples were sterilized by autoclaving (200 kPa, 30 min, 120°C), and pH was adjusted to 7.90±0.10 (mean pH of the river). Finally, they were inoculated with the exponential phase starter culture of *E. coli*, in the following procedure: bacteria from defined volume of culture were collected by centrifugation, washed with 0.9% saline, and transferred to 8.0 dm³ of adapted natural surface water. The initial bacterial population (IBP) in WMS, 2500, 25000, and 250000 CFU cm⁻³, were chosen according to changeable range of fecal coliform pollution of the River Nišava.

Water model-solutions treatment

The treatments were performed in original semi-flow system (Fig. 2), consisted of: the main bath (MB) 10 dm³ volume; the peristaltic pump, with flow rate at 90 dm³ h⁻¹; and chamber for composite (CC), with 16 wires, dimension 10×10 cm. Water from CC was repeatedly returned to the MB by overflow pipe, which enabled work in a closed loop. The temperature was kept by flow thermostat. The system was acid washed and sterilized before use.

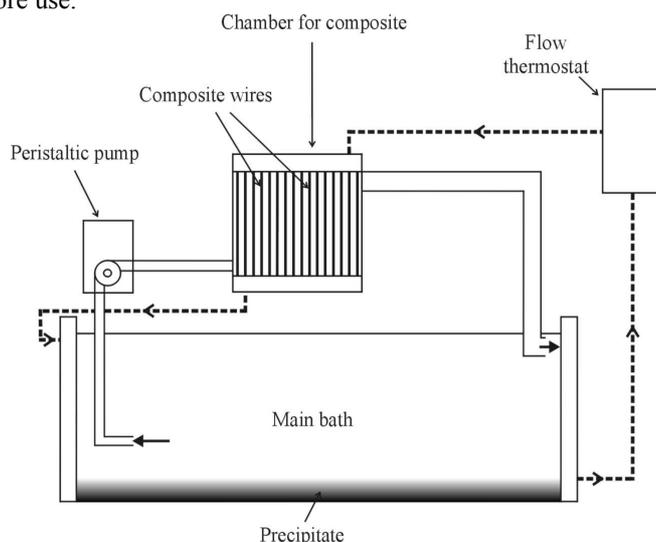


Fig. 2. Schematic diagram of the original semi-flow system.

The prepared WMS were equilibrated at 20°C (mean summer temperature of the river), and transferred to the MB. After taking the zero probes, the peristaltic pump and pump in thermostat were run. Samples were withdrawn at timed intervals from the MB, by pipette. The control experiments were done in identical conditions, without composite wires. All experiments typically were performed in duplicate. At the end of treatment of every WMS, the number of viable bacteria was determined at the same time in all samples, by standard membrane filtration procedure for fecal coliform [1], in triplicate.

In all WMS was monitored pH by pH-meter Corning Checkmate Modular Testing System (Corning, USA), with a combined electrode. Concentration of soluble part of aluminium was monitored too, by inductively coupled plasma atomic emission spectrometry, on ARL ICP model 3410 (Valencia, CA., USA).

At the end of the treatments, *E. coli* bacteria were determined in the formed Al(OH)₃ precipitates, after total sedimentation. Sampling of the precipitates was done for: 0, 20, 40, 60, 80 and 100 min, after finishing of the treatment and decantation of water phase. Plate counts were performed by plating 1.0 cm³ of homogenized samples of precipitate on Endo agar, in triplicate. Separated precipitates were also observed by microscope Nikon Microphot – FXA (Nikon, Tokyo, Japan), with the possibility of measuring dimensions of particles.

After treatment and neutralization, in water phase were determined Al and Ga by ICP AES method, and Sn and Bi by AAS with vapor generator accessory VGA-76 (Varian).

RESULTS

Fig. 3 shows rapid decrease of *E. coli* bacteria during the treatment of all three WMS, with complete inactivation in relatively short period. The patterns of all curves were similar, as well as inactivation rate constants. The constants were calculated based on Chick's law in which $\ln[(N, \text{CFU cm}^{-3} \text{ at a time } t)/(N_0 \text{ CFU cm}^{-3} \text{ at } t = 0)]$ was plotted as a function of time. The slope of the lines was k , and in the case of WMS with IBP of 250000 CFU cm^{-3} it was 0.189 min^{-1} with $R^2 = 0.94$. For WMS with IBP of 25000 CFU cm^{-3} k was 0.185 min^{-1} ($R^2 = 0.92$), and for WMS with IBP of 2500 CFU cm^{-3} , k was 0.177 min^{-1} ($R^2 = 0.92$).

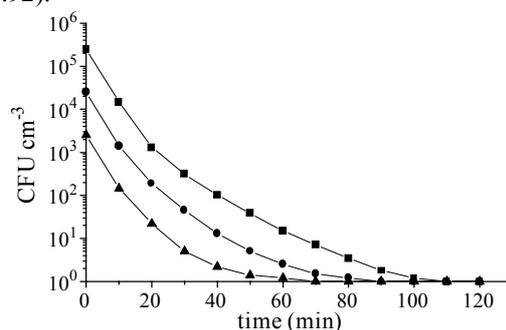


Fig. 3. Inactivation of *E. coli* in water model-solutions with initial bacterial population of 2500 (▲), 25000 (●), and 250000 (■) CFU cm^{-3} , during the treatment by the MABC, at 20°C in semi-flow system.

In the first half of the treatments, reduction of *E. coli* was about one \log_{10} count at every 10 min, and it was slower in the next stadium. Thence, 99% of *E. coli* reduction in all WMS was achieved for about 20 min. Opposite, the period of complete inactivation quite depends of initial bacterial population. In WMS with IBP of 250000 CFU cm^{-3} the complete inactivation happens after about 100 – 110 min, in WMS with IBP of 25000 CFU cm^{-3} for 80 – 90 min, and in WMS at 2500 CFU cm^{-3} for 60 – 70 min. Control experiments show no measurable change in number of *E. coli* bacteria in all three WMS, which indicates that SFS do not provide any deleterious effect on *E. coli* in water.

The changes in pH during the treatment of WMS by the MABC are shown in Fig. 4. It is notable that the pH of the WMS at the beginning of treatment regularly increases, and after approximately 30 – 40 min achieves constant value of about 9.

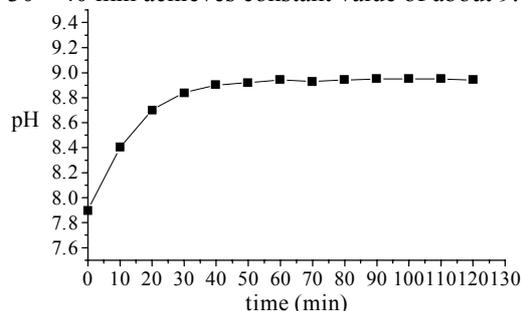


Fig. 4. Changes in pH of WMS during the treatment by the MABC.

The change in concentration of the total soluble aluminium during the treatment of WMS is shown in Fig. 5.

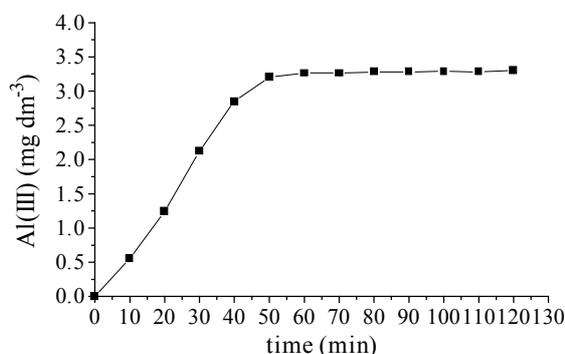


Fig. 5. Changes in concentration of the total soluble aluminium during the treatment of WMS by the MABC.

Like in the previous case, at the beginning of the treatment concentration of Al increase, and constant value of about 3.3 mg dm^{-3} , is achieved after 40 – 50 min.

In precipitate formed in WMS with IBP of $250000 \text{ CFU cm}^{-3}$ it is found about 690 CFU cm^{-3} , 45 CFU cm^{-3} were in Al(OH)_3 from WMS with IBP of $25000 \text{ CFU cm}^{-3}$, and precipitate of WMS with IBP of 2500 CFU cm^{-3} contained $4 - 5 \text{ CFU cm}^{-3}$. However, number of *E. coli* bacteria in precipitates decrease in time relatively quickly, and after between 60 – 90, 30 – 40, and 15 – 20 min, respectively, they totally disappear (Fig. 6).

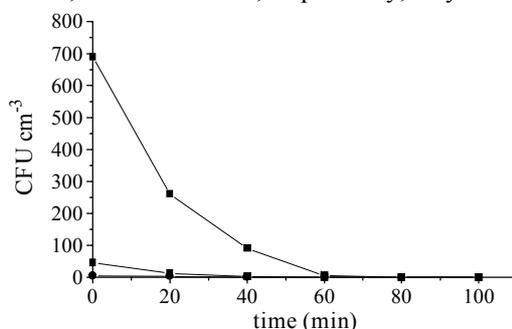


Fig. 6. Survival of coprecipitated *E. coli* in Al(OH)_3 , after the MABC treatments of WMS at: 2500 (▲), 25000 (●), and 250000 (■) CFU cm^{-3} .

It was observed by microscope that precipitates contained split microalloyed aluminium particles. The diameters of these particles vary from about $25 - 150 \mu\text{m}$. Looking at them with more attention, evolution of some gas bubbles was marked. The supposition is that it is hydrogen generated by reaction of MA particles with water.

Concentration of Al in water phase finally decline to $0.005 - 0.01 \text{ mg dm}^{-3}$, quite below MAC value 0.2 mg dm^{-3} [1]. Concentrations of microalloyed elements Sn, Ga and Bi, were below detection limits of applied methods ($0.0002 \text{ mg dm}^{-3}$ for Sn and Bi, and 0.001 mg dm^{-3} for Ga).

DISCUSSION

The results of the presented study show that the composite, applied in semi-flow conditions, prove as a powerful device for inactivation of *E. coli*. The SFS had no, or only a minor influence, so that the reduction of *E. coli* is definitively the result of the MABC effects. A large decrease of several \log_{10} counts of bacteria was observed, with complete inactivation at the end of the treatment. In the WMS with IBP of $250000 \text{ CFU cm}^{-3}$, which represented extremely polluted natural surface water, *E. coli* was completely inactivated after less than 110 min, whereby after 65 – 70 min number of *E. coli* was below 10 CFU cm^{-3} . In all three WMS the rates of *E. coli* inactivation decrease in time. This phenomenon likely issues from the fact that in conditions of extremely low number of bacteria, especially in heterogeneous system as this, there is a less probability of their contact with the composite.

Comparing of inactivation curves in Fig. 3, and their inactivation rate constants, great similarity between function which represent change of CFU number in time, can be notice. It indicate that IBP, from 2500 to $250000 \text{ CFU cm}^{-3}$, do not affect the way in which the composite acts to *E. coli*, and that process of inactivation, in kinetical meaning, follows the same principles in wide spread of *E. coli* population. As can be seen from Fig. 3, only period of complete inactivation vary in depending of IBP. This period regularly increase, for about 20 min, with increasing of IBP for one order of magnitude.

The supposition is that effect of the MABC to *E. coli* is based on processes, which are result of its spontaneous reaction with water. One of these is generation of Al(III) ions. Considering that pH during the MABC treatment is in range between 8 and 9, aluminium is mainly in form of aluminate Al(OH)_4^- [13, 22, 23]. Pettersson et al. [24] and Kinrade [22] investigated toxicity of this species in biological systems. Aluminate is less toxic ion than, for example Al^{3+} or Al(OH)^{2+} [22, 23], but it is still important factor of composite toxic activity. In thin layer of solution, near the MABC surface, the local increasing of Al concentration is expected [6, 11], which may be manifested as a higher toxicity for *E. coli* in this area.

The pH of solution during the treatment quickly increases, and soon achieves constant value (Fig. 4). The toxicity of OH^- ion for bacteria was quite investigated [19, 25], and some results show that already at pH 9 number of viable bacteria decrease to about 40 – 45%, after 3 h [25]. Studies of Blackburn *et al.* [26] and Duffy *et al.* [20] show higher survival of *E. coli* at pH between 5 and 6, than 7 – 8, which indicate that the toxicity of OH^- ion is higher than H^+ . Considering that OH^- ion is generated in solution beside the surface of composite, we expect an increase of local pH and its effects for *E. coli* in this area.

The coprecipitation of *E. coli* on Al(OH)_3 permanently happens during the treatment, and this phenomenon is one of the well-known methods for inactivation of bacteria, with well efficacy [19, 27]. Coprecipitated *E. coli* bacteria died in precipitate in relatively short period. Effects of precipitate are, probably, based on adsorption of Al(OH)_3 particles to components of cell membranes with inhibition of their function, influence of MA particles, split from composite wires, which evidently behave in precipitate similarly as composite itself, as well as toxicity of Al(III) and OH^- ions, arise by Al(OH)_3 dissociation.

Another effect of the composite, probably the most important, is its very negative stationary potential, which is a reason to composite behaves as powerful reducing agent. The electrons, released by generation of Al(III) ions, mainly were utilized to reduction of water, but they also affect *E. coli* bacteria when they enough approach to composite surface. In

this work, contact between bacteria and composite surface is significantly supported by convection which exist in semi-flow system. The MABC reduction activity may be based on presence of very unstable Al^+ ion, known intermediate species in process of Al oxidation [11, 13]. On the other side, there is a nascent hydrogen $H\cdot$ [13], usually generated as the first stage in anodic formation of gaseous hydrogen, during the water reduction [11, 15]. Mentioned reduction phenomena, probably, happen at the surface of composite, or in thin layer of solution where mentioned particles can come by diffusion.

The presented semi-flow system enables the whole volume of WMS to continuously go through chamber for composite, which is the approximate simulation of flow system. In comparison with work in stationary conditions [6, 7, 8, 10], where pollutants may come to the composite surface only by diffusion, in SFR water is "filtering" through composite wires, which increase probability of contact between bacteria and the composite. The convection in semi-flow system accelerates diffusion of $Al(III)$ and OH^- ions, and accordingly dissolution of MA. Result of that is generally increasing of composite activity.

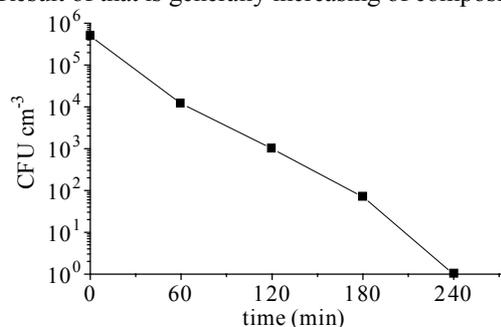


Fig. 7. Inactivation of *E. coli* in water model-solution with IBP of 500000 CFU cm^{-3} , during the treatment by the MABC in stationary conditions [10].

Bojić *et al.* [10] were applied the MABC in WMS of *E. coli*, based on 0.1% NaCl, in stationary conditions (Fig. 7). 1.0 dm^3 of WMS with IBP of 500000 CFU cm^{-3} , was treated by 4 composite wires, whereby absolute initial loading of composite was equal as in the case of WMS with IBP of 250000 CFU cm^{-3} in this paper: 1.25×10^7 CFU cm^{-2} . The comparison of semi-flow and stationary conditions, at absolute initial loading of composite, show that the semi-flow system is several times effective, considering that in stationary conditions *E. coli* reduction of one \log_{10} count was achieved for 50 – 60 min (Figs. 3 and 7). Convection in SFS was reflected to coprecipitation of *E. coli* at $Al(OH)_3$, too, considering that Bojić *et al.* [10] found only about 250 to 300 *E. coli* per cm^3 of precipitate after treatment in stationary conditions, and in the precipitate of WMS with IBP of 250000 CFU cm^{-3} , in the semi-flow conditions, was found 690 CFU cm^{-3} .

CONCLUSION

- The inactivation of *E. coli* by microalloyed aluminium based composite has been investigated in conditions adequate to natural surface waters (where *E. coli* can be found as fecal pollutant), in the original semi-flow system which represents the simulation of very efficient flow conditions, in laboratory terms.

- It is shown, that at approximately every 10 min, the number of *E. coli* bacteria was reduced for about one log₁₀ count, with complete inactivation as the final outcome of the treatment.
- At the end of the treatment, the Al(OH)₃ precipitate still contained the low amount of coprecipitated *E. coli* bacteria, which was perished in relatively short period.
- The effects of the composite are based, mainly on its reduction activity, as well as toxicity of Al(III) and OH⁻ ions, and coprecipitation on Al(OH)₃.
- The way on which the composite acts to *E. coli* do not depend of initial bacterial population, and only the period of complete disinfection depends of IBP.
- It is notable that application of the composite does not produce any chemical contamination of water phase.
- Providing until now applications of the composite, this study expands the investigation of the material, which may present a base for a new conjoint treatment for purification and disinfection of the big volumes of high chemically, and microbiologically polluted waters from different origins in flow conditions.

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INAKTIVACIJA *ESCHERICHIA COLI* KOMPOZITOM NA BAZI MIKROLEGIRANOG ALUMINIJUMA

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U ovom radu je ispitivana inaktivacija bakterije Escherichia coli, kompozitom na bazi mikrolegiranog aluminijuma. Kompozit je materijal u vidu čelične mrežice, prevučene mikrolegiranim aluminijumom. Princip njegovog delovanja je baziran na spontanom rastvaranju u kontaktu sa vodom, uz generisanje Al(III) i OH⁻ jona, iz kojih konačno nastajaje voluminozni slabo rastvorni Al(OH)₃. Imajući u vidu uspešnu primenu kompozita u prečišćavanju voda od velikog broja hemijskih zagađivača, pretpostavljeno je će biti efikasan i u inaktivaciji mikrobiološkog zagađivača vode – E. coli. Eksperimenti su rađeni sa vodenim model rastvorima na bazi prerađene prirodne površinske vode, inokulisane sa E. coli. Tretmani su vršeni u originalnom poluprotočnom sistemu. Rezultati pokazuju da približno na svakih 10 minuta broj E. coli bakterija opadne za jedan red veličine, uz postizanje potpune inaktivacije na kraju tretmana. Zapaženo je da talog Al(OH)₃ po završetku tretmana još uvek sadrži malu količinu živih bakterija, koje umiru u relativno kratkom periodu.