Water treatment using activated carbon supporting silver and magnetite
Eva Valušová, Anna Vandžurová, Peter Pristaš, Marián Antalík and Peter Javorský

ABSTRACT
Recent efforts in water purification have led to the development of novel materials whose unique properties can offer effective biocidal capabilities with greater ease of use and at lower cost. In this study, we introduce a novel procedure for the preparation of activated carbon (charcoal) composite in which magnetite and silver are incorporated (MCAG); we also describe the use of this material for the disinfection of surface water. The formation process of magnetic MCAG composite was studied using ultraviolet-visible spectroscopy. The results demonstrated the high sorption efficiency of AgNO₃ to magnetic activated carbon. The antimicrobial capabilities of the prepared MCAG were examined and the results clearly demonstrate their inhibitory effect on total river water bacteria and on Pseudomonas koreensis and Bacillus mycoides cultures isolated from river water. The bacterial counts in river water samples were reduced by five orders of magnitude following 30 min of treatment using 1 g l⁻¹ of MCAG at room temperature. The removal of all bacteria from the surface water samples implies that the MCAG material would be a suitable disinfectant for such waters. In combination with its magnetic character, MCAG would be an excellent candidate for the simple ambulatory disinfection of surface water.

Key words | activated carbon, bacteria elimination, magnetic, silver, water treatment

INTRODUCTION
Microbial contamination of surface water and groundwater is a worldwide environmental problem, and inadequate disinfection can lead to the spread of waterborne diseases. Both conventional and advanced water treatment methods require considerable capital expenditure, engineering expertise and infrastructure (Shannon et al. 2008). One alternative to these limitations could be the use of point-of-use (POU) water treatment. Among the most promising candidates for this application are household water filters composed of granular carbon material. Carbon materials (namely activated carbon/charcoal) present unique advantages due to their low cost, high specific surface area, highly porous structure, chemical inertness and good physical stability (Tan et al. 2010). Due to their high rate of adsorption, activated carbon materials are excellent supporting materials for metals (Weifang et al. 2007) which could be used in the development of antibacterial composites (Tan et al. 2010). This kind of support provides high dispersion and inhibits the agglomeration of metals within the activated carbon pore structure (Tchomgui-Kamga et al. 2010).

Among antimicrobial species, silver containing activated carbon or other forms of silver nanomaterials acts as a powerful agent with a broad inhibitory biocidal capability against microbes (Marambio-Jones & Hoek 2010; Tan et al. 2010). Nevertheless, silver containing carbon particles used as water disinfectants may have some issues regarding separation and regeneration. Silver containing activated carbon is usually applied in columns or filter devices, and this may cause the blockage of filters or the loss of carbon after use in water and wastewater treatment; additionally, the reuse of these materials requires additional time-consuming procedures. The problems involved in reusing these materials usually leads to the material being discarded after use, a process which causes secondary pollution.

To overcome these disadvantages, activated carbon supporting silver may be additionally combined with a magnetic...
carrier to develop magnetic composites. Magnetic separation would be a rapid and effective method of removing these magnetic composites supporting silver as antimicrobial agents from the treated water. Using a magnet, the thus-removed magnetic composites supporting silver could therefore be reused for water disinfection avoiding any adverse environmental effects which would be involved in their disposal (Marambio-Jones & Hoek 2010).

This article presents a technique for the preparation of bifunctional silver-magnetite activated carbon composites (labelled MCAG) with both antimicrobial and magnetic properties. The magnetic property is provided by magnetite (Fe₃O₄), which has been immobilized onto porous carbon material (charcoal) using an approach modified from that which has been described previously (Koneracká et al. 1999). A permanent magnet is used in the preparation of final silver co-adsorbed active magnetic carbon particles, a process which negates the need for centrifugation or filtration procedures. The targeted antimicrobial properties of MCAG were proved against both river water bacteria and Pseudomonas koreensis and Bacillus mycoides cultures isolated from river water. The MCAG could be separated easily from those river water samples on the basis of their magnetic properties. The feasibility and applicability of using these MCAG composites as potential agents for water disinfection is also evaluated.

**METHODS**

**Preparation of magnetite-activated carbon material**

Both iron (II) chloride tetrahydrate (FeCl₂·4H₂O) – 0.054 g and iron (III) chloride hexahydrate (FeCl₃·6H₂O) – 0.0198 g (Merck, Germany) were dissolved in 10 ml distilled water and then mixed with 100 mg active carbon/charcoal (~100 mesh, produced by Imuna Pharm, Šarišské Michaľany, Slovakia under commercial name Carbosorb). The suspension was stirred on a magnetic stirrer for 30 min. After this period 1 ml NH₄OH was added to the suspension in drops and the suspension was stirred for an additional 20 min. The black activated carbon with incorporated magnetite was separated from the suspension by magnetic separation using a permanent magnet placed at the side of the vessel. The magnetite-incorporated activated carbon (henceforward MC) was then washed repeatedly with deionized water by magnetic decantation using a permanent magnet to remove impurities from the resulting MC.

**Ag⁺ sorption and release experiments**

Batch adsorption experiments were conducted to evaluate the sorption behaviour of our MC. In each adsorption experiment, various initial weighted quantities of dried MC particles were dispersed in 2 ml solutions of 12.5 mmol l⁻¹ AgNO₃. Each suspension was stirred vigorously for 20 min, and then the resulting magnetic activated carbon composites supporting silver were separated from the suspension using a permanent magnet held to the bottom of the test tube. The clear supernatants were withdrawn from the test tube using a syringe and filtered. The final non-adsorbed amount of AgNO₃ in supernatant was measured with a UV–Vis spectrophotometer JASCO V-650 (Japan) using a range from 240 to 370 nm.

The sorption percentage was calculated using the following equation:

\[ S(\%) = \frac{c_{\text{initial}} - c_{\text{non-sorbed}}}{c_{\text{initial}}} \times 100 \]

where \( c_{\text{initial}} \) refers to initial concentration (mmol l⁻¹) of AgNO₃ and \( c_{\text{non-sorbed}} \) refers to final non-sorbed concentration (mmol l⁻¹) of AgNO₃ evaluated from absorption maxima of AgNO₃ at 302 nm.

The release of AgNO₃ from magnetic activated carbon composite supporting silver was investigated as follows: 2 g of dried MC particles were dispersed in 2 ml of 12.5 mmol l⁻¹ AgNO₃. The mixture was stirred vigorously for a period of 20 min at room temperature. The formed magnetic composite was then separated from the suspension using a permanent magnet. The upper clear solution was poured off and the settled magnetic particles were rinsed three times with distilled water. After this procedure, the final release of AgNO₃ was performed by adding 1 ml of distilled water to 1.4 g of activated carbon composites supporting magnetite and silver (MCAG) and stirring the resultant mixture for 20 min. The MCAG was then settled to the bottom of the tube and the clear supernatant was filtered prior to the spectrum measurement of released AgNO₃.

Based on the above experiments, MCAG for the next biocidal study was prepared using a coprecipitation method. The activated carbon with incorporated magnetite (MC) prepared as above was re-suspended in 4 ml of deionized water. One millilitre of freshly prepared AgNO₃ (0.0042 g) (Sigma–Aldrich, USA) was added in drops to this suspension under vigorous stirring and the resulting suspension was stirred for 1 h at 250 rpm at room temperature. After this period the magnetic carbon composites with
adsorbed AgNO₃ were separated by magnetic decantation using a permanent magnet and were then washed three times with 5 ml of deionized water. In the final step, the settled magnetic activated carbon with AgNO₃ (MCAG) was dried and stored at room temperature.

**Sampling and microbiological analysis of surface water**

The experimental study was conducted from May 2011 to November 2011 using water obtained from the river Hornad which runs through the city of Kosice in East Slovakia. Immediately following sampling, the water sample was stored in cool dark conditions in a sterile tube and was transferred to the laboratory within 1 h. The cultivable bacteria counts in the water samples were obtained by plating the dilution series onto Nutrient Agar 2 (Difco, USA) plates. The plates were cultivated at 30°C for 2 days until well-defined colonies appeared. The colonies were washed with buffered saline, pooled and used in further experiments in which they are titled ‘total river bacteria’.

Random isolates from the highest dilution plates were chosen for further study based on their different colony morphologies and were identified further using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS). The raw spectra obtained for each isolate were imported into BioTyper software-version 2.0 and analysed without any user intervention (Bruker Daltonik GmbH, Leipzig, Germany). Two bacterial strains, *B. mycoides* and *P. koreensis*, were selected for subsequent analysis.

**Antimicrobial activity of MCAG**

One gram of MCAG was dispersed in 1 ml of deionized water by vortex and the antimicrobial activity of the material was then tested against total river water microbes and pure cultures of *B. mycoides* or *P. koreensis* using a single drop plate test. Fifty microlitres of dispersed MCAG was applied to a plate inoculated with 100 μl of bacterial culture. Control experiments were also conducted in which the same amount of magnetic carbon material without silver ions (MC) and active carbon only (C) were used. The plates were cultivated at room temperature overnight. The resulting zone of inhibition was then observed.

**Treatment of surface water with MCAG**

The MCAG was tested for the efficacy in removing bacteria from river water samples in both small (1 ml) and large volume (1 l) samples. MCAG (1 g per litre of sample) was added to the water samples (in Eppendorf tubes or 1.5 l flasks) and the liquid was gently shaken by a mechanical shaker or by magnetic mixer at room temperature for 30 min. After the end of the incubation period, the MCAG material was separated from the liquid using a magnetic stand (MagneSphere Technology, Promega, USA) or using a strong permanent magnet (5 × 5 × 1 cm). The cultivable bacteria counts in the supernatants were determined by plating 100 μl of supernatant onto Nutrient Agar 2 (Difco, USA).

Both before and after MCAG treatment, the river water samples were also tested for the presence of bacteria using a water disinfection test performed by the regional branch of the Public Health Authority of the Slovak Republic (SNAS Reg. No. 125/S-061). The supernatant of the MCAG treated water samples was analysed for the presence of leached out silver using atomic absorption spectroscopy (SpectrAA 220Z Varian, the Netherlands) following the method outlined by Ortiz-Ibarra *et al.* (2007). All microbial tests were performed in triplicate at minimum.

**Treatment of pure bacterial cultures with MCAG**

The same procedure was used to monitor the effect of MCAG and MC materials on pure *B. mycoides* and *P. koreensis* cultures. The liquid cultures of these bacteria were prepared in sterile flasks and incubated in an orbital shaker at 100 rpm. Cell density was monitored spectrometrically (Spekol 11, Carl Zeiss, Jena, Germany). For all treatment experiments, bacterial cell counts were adjusted to 1 × 10⁶ ml⁻¹. The cell counts of bacteria in the magnetic supernatants were determined by plating 100 μl of supernatants directly onto Nutrient Agar 2 (Difco, USA) plates. In order to analyse the presence of bacteria in magnetic sediments, the sediments were re-suspended in the original volume of water and aliquots were plated onto Nutrient Agar 2 plates.

**RESULTS AND DISCUSSION**

As has been studied previously, a major advantage of activated carbon with incorporated magnetite is its excellent adsorption capacity and its ability to be isolated magnetically (Oliveira *et al.* 2002; Yang *et al.* 2008). In order to create additional biocidal properties, we employed a technique which we have published previously (*Koneracká et al.* 1999) and produced magnetite-incorporated activated carbon (MC) with coadhered Ag⁺ cations.
**Ag⁺ sorption and release experiments**

In order to investigate the sorption process of AgNO₃ to MC particles, we prepared suspensions with different amounts of MC particles dispersed in a water solution of AgNO₃ at a constant concentration of 2.11 mg ml⁻¹. As described in Methods, we left the MC particles to uptake AgNO₃ over the relatively short period of 20 min and then allowed the magnetic activated carbon composites supporting silver to settle. The remaining non-sorbed AgNO₃ in the supernatant was detected through its characteristic spectroscopic signature using UV-Vis measurements. The spectra in Figure 1 show a gradual decrease in the intensity of well-defined absorption bands (maximum at 302 nm for non-sorbed AgNO₃) as the amount of MC particles used increased. The optical intensity of the remaining non-sorbed AgNO₃ ($A_{\text{observed}}$) from spectra b, c, d in Figure 1 was related to the reference intensity of AgNO₃ lacking magnetic activated carbon ($A_{\text{max}}$) from spectrum a. Using the equation for sorption percentage ($S\%$), each value was then plotted as a function of MC particle concentration expressed in mg ml⁻¹. The increasing profile is shown in the inset in Figure 1 and indicates progressive binding of Ag⁺ ions with MC particles. The highest amount of 50 mg MC in 1 ml volume was able to adhere $1.6 \times 10^3 \mu g$ AgNO₃, a value which corresponds to 75% sorption as a percentage of the total available AgNO₃ in the reaction suspension. Due to its observed high adsorptivity and the proven richness in micro-sized pores of activated carbon (Zhu et al. 2010), the MCAG composites may be considered to be an effective immobilization substrate.

Our MCAG composites sub-saturated with AgNO₃ were subjected to the desorption procedure described above in Methods. The slight removal of AgNO₃ from the active MCAG suspension is confirmed by the absorption band of spectrum e in Figure 1. The amount was rated from absorption intensity at 302 nm and it corresponds to 185 μg of released AgNO₃ from 1.4 g MC particles. In addition to the low levels of AgNO₃ release, the 0.1% (w/v) MCAG suspension bearing 20 mg sorbed AgNO₃ was chosen as suitable for further bactericidal study on three main grounds: firstly the excellent adsorption properties of the activated carbon, secondly the high rate of Ag⁺ ions on the surface of the MC particles, and thirdly the fact that the levels of AgNO₃ released by the MCAG particles is at a concentration which is non-toxic to human cells (Hosoba et al. 2009; Parameswari et al. 2010).

**Antibacterial activity of MCAG**

In order to analyse antibacterial activity, widely used drop plate tests were performed to evaluate inhibitory haloes surrounding MCAG samples on inoculated agar.

The bactericidal activity of MCAG was determined according to the agar diffusion method and the results are evident from Figure 2. The presence of the well dispersed colloidal MCAG particles at a concentration of 50 μg in a 50 μl drop completely prevented the growth of total river water bacteria; the clear zone of inhibition is visible in Figure 2(a) and it clearly resembles the reported Halo test (Furr et al. 1994). When a 50 μl drop of MCAG was applied to the plates of pure cultures of *P. koreensis* and *B. mycoides* isolated from the tested water, a clear zone of inhibition was observed, as is shown in Figure 2(b) and 2(c). A slightly larger zone of inhibition of Gram-negative *P. koreensis* compared with Gram-positive *B. mycoides* was observed. In the control experiments, the dispersed MC and C materials exhibited no zone of inhibition (Figure 2).

In order to prove that the microbicidal activity of the MCAG was not microorganism specific, the effect of MCAG was also tested against two separate bacterial species, Gram-positive *B. mycoides* and Gram-negative *P. koreensis* strains which were isolated from water samples. As expected, bacteria counts significantly decreased for both species. Both *P. koreensis* and *B. mycoides* revealed a
4 log units reduction of viable bacteria counts in supernatants. When the viability of bacterial cells in MCAG sediments was examined, Gram-negative \textit{P. koreensis} showed a much higher sensitivity than Gram-positive \textit{B. mycoides} cells. No viable \textit{P. koreensis} cells were detected in MCAG sediments while \textit{B. mycoides} cells were reduced by 95\% (Table 1). The higher sensitivity of Gram-negative bacteria to silver nanomaterial has been reported previously (Singh \textit{et al.} 2008).

The anti-microbial effect of MCAG could perhaps be explained as a combination of a mechanical process in which MCAG particles act as magnetic filters which retain bacteria; the bacteria are subsequently killed by Ag\textsuperscript{+} ions. The results presented in Table 1 support this idea. The MC particles were able to adsorb bacteria and remove it from water samples, but the majority of the bacteria remained viable after treatment. On the other hand, the MCAG sediment of magnetically separated water samples contained either bacteria or bacterial DNA detectable by PCR analysis (Valušová \textit{et al.}, unpublished results). These results reinforce earlier studies which have outlined the ability of carbon materials to adsorb bacteria (George \\& Davies 1998; Kim \textit{et al.} 2009).

**Table 1** | *Colony form units of \textit{Bacillus mycoides} and \textit{Pseudomonas koreensis} in untreated cultures and in magnetic supernatants and sediments after 30 min treatment with MCAG and MC

<table>
<thead>
<tr>
<th></th>
<th>\textit{Bacillus mycoides} (cfu/ml)</th>
<th>\textit{Pseudomonas koreensis} (cfu/ml)</th>
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<tbody>
<tr>
<td>Control</td>
<td>6.0 × 10^4 ± 5.0 × 10^3</td>
<td>6.7 × 10^4 ± 4.5 × 10^3</td>
</tr>
<tr>
<td>MCAG/sup.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCAG/sed.</td>
<td>2.6 × 10^3 ± 5.0 × 10^2</td>
<td>0</td>
</tr>
<tr>
<td>MC/sup.</td>
<td>5 ± bN.D.</td>
<td>7.5 × 10^2 ± 7.0 × 10^1</td>
</tr>
<tr>
<td>MC/sed.</td>
<td>3.5 × 10^4 ± 2.6 × 10^3</td>
<td>4.1 × 10^4 ± 4.5 × 10^3</td>
</tr>
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\textsuperscript{a}The bacterial counts are the mean of triplicate assays ± SD. 
\textsuperscript{b}N.D. not detected.

**Treatment of surface water with MCAG**

Water disinfection tests were also carried out on inoculated water in which MCAG had been applied for the selected periods of time. The performance of the water disinfection test was made much easier due to the magnetic properties of MCAG particles. The elimination of surface water bacteria following interaction with MCAG was studied by incubating the water samples with dispersed solutions of MCAG for 30 min at room temperature in both small and large-scale experiments. Counts of bacteria present in the supernatants were taken at the end of the incubation periods. In the small-scale experiments with river water samples containing from 1 × 10\textsuperscript{3} to 1 × 10\textsuperscript{5} cultivable bacteria per ml, no bacteria were detected in the supernatants by plate counts. Similar results were obtained for the large-scale experiments (1 l). The bacterial content of the surface river water samples before and after MCAG treatment was verified by the regional branch of the Public Health Authority of the Slovak Republic. The examined water samples contained microorganisms such as \textit{Escherichia coli}, \textit{Enterobacter agglomerans}, \textit{Pseudomonas} spp., \textit{Aeromonas} spp., \textit{Bacillus} spp. The water disinfection tests confirmed the lack of the aforementioned cultivable microorganisms in water samples after the MCAG treatment.

It can be stated that adhered cationic silver in general can be considered to have strong antibacterial properties. The external surfaces of MCAG are likely to be positively charged at circumneutral pH conditions in accordance with research regarding the attachment affinities of positive ions to activated carbon (Kim \textit{et al.} 2009). The bacterial attachment to the MCAG could be mostly restricted to the external surfaces of MCAG (including some sections of macropores) since, as estimated previously, the pore volumes inside the MC particles are smaller (Inagaki \\& Tascon 2006) than the diameter of the bacteria. According to existing studies (Fontes \textit{et al.} 1991), bacteria are negatively

**Figure 2** | Antimicrobial activity of MCAG against (a) river water bacteria, (b) \textit{Pseudomonas koreensis}, (c) \textit{Bacillus mycoides}. The concentration of tested materials (I- MCAG, II-MC and III-C) in each drop (50 \textmu l) was 1 \textmu g/\textmu l.
charged at circumneutral levels of pH; the electrostatic interaction between the external surfaces of MCAG and bacteria would therefore be attractive (Guggenbichler et al. 1999). The attachment of MCAG could be aimed at the chemically active sulphur-containing cell membrane proteins which have been recognized as preferential sites for silver binding (McDonnell & Russell 1999); the membrane proteins are inactivated during the course of attachment (Liau et al. 1997; Feng et al. 2000).

It has been suggested that the subsequent disruption of bacterial membrane may cause a significant increase in permeability, a process which then leads to uncontrolled transport. When silver ions penetrate the bacterial cell, the DNA molecule takes on a condensed form and loses its replication ability, a process which leads to cell death. It has also been reported that heavy metals react with proteins by becoming attached to the thiol group resulting in the deactivation of proteins (Liau et al. 1997; Feng et al. 2000). The second closely allied effect of the disruption is the oxidative deterioration of biomolecules associated with transition metal ions (Matsumura et al. 2003; Monteiro et al. 2009).

Whatever the mechanism involved in the bactericidal effect, it is reasonable to suggest that MCAG is a strong candidate for simple ambulatory disinfection procedures for surface water e.g. for emergency disinfection of flood water. Quantitative atomic absorption spectroscopic analysis was used to estimate the leaching of silver ions into the river water samples at the amount of 0.13 mg l$^{-1}$. Subsequent MCAG treatment (1 g l$^{-1}$, 30 min, at room temperature) resulted in decreasing silver concentrations to a level of below 0.05 mg l$^{-1}$. This finding suggests the adsorption of silver nitrate into MC is stable and indicates the general stability of the MCAG material. Our proposed procedure would be considered ‘green’ according to the World Health Organization’s Water Quality Guidelines for drinking water, which states that silver levels of less than 0.1 mg l$^{-1}$ can be tolerated without any health risk.

**CONCLUSIONS**

In this study, activated carbon was impregnated with magnetite (MC) in order to give the material magnetic properties. Using a coprecipitation method, the MC exhibited high efficiency in adsorption with Ag$^+$ ions. The adsorption capacities of MC were 32 $\mu$g AgNO$_3$ per 1 mg of MC. Moreover, 1 h was defined as adequate time for adsorption of silver ions. This MCAG composite seems to have stability against Ag$^+$ leaching and could be adopted for further studies. The positive surface charge appears to play a major role in the adsorption of bacteria. The incubation period of 30 min was sufficient for the MCAG particles (1 g l$^{-1}$ of MCAG containing 20 mg of adsorbed silver in the nitrate form) to adsorb the river water bacteria ($1 \times 10^3$ to $1 \times 10^5$ bacterial cells ml$^{-1}$) of the treated river water samples. The initial bacterial count was reduced by at least five orders of magnitude. The bactericidal activity of MCAG was confirmed using a drop plate test.

The magnetic properties of MCAG composites allow them to be removed easily from water after the bactericidal effect has occurred. These initial results indicate that MCAG may have a potential application as a surface water disinfectant.

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