Technical Note

Silver-embedded granular activated carbon as an antibacterial medium for water purification

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Abstract: Silver (Ag) particle embedded granular activated carbon (GAC) was made for the first time to assess its ability in inhibiting the growth of *Escherichia coli* (*E. coli*), a water-borne bacterial pathogen. Ag-GAC was made by impregnating GAC with $AgNO_3$ and then reducing it to metallic Ag. Plate assay showed slight inhibition of *E. coli*, even with Ag-GAC prepared from 0.005 mol L^{-1} $AgNO_3$, but this and shake flask tests showed a conspicuous effect only for higher concentrations of 0.1–1 mol L^{-1} $AgNO_3$. Flow tests further indicated that Ag-GAC made from 1.0 mol L^{-1} $AgNO_3$ caused a desirable three orders of reduction in *E. coli* number concentration in less than 30 s. An optimum of 9–10.5 wt% of embedded Ag in the final Ag-GAC product was necessary for the requisite complete inhibition of *E. coli*, killing bacteria in the contact-mode for up to 350 L of flowing water. These results prove that Ag-GAC possesses antibacterial properties and can be used for disinfection to produce potable quality water.

Keywords: silver; granular activated carbon, *Escherichia coli*; antibacterial

INTRODUCTION

Granular activated carbon (GAC), because of its porous nature and large surface area, shows high adsorption capacity for various organic contaminants. GAC is, therefore, one of the most important adsorbents used in the field of water purification for the production of potable water.[1] If one can take further advantage of its large surface area, by impregnating its pores with metallic silver (Ag) particles to make Ag-GAC composites, one can inhibit bacterial growth, or even completely remove it. The aim here was to make Ag-GAC solid composite for the first time and test it for water disinfection, taking advantage of the roles of GAC as adsorbent and Ag as biocide.[2] Although the exact mechanism of the biocidal action of Ag is not fully understood,[3] one of the accepted explanations is that Ag, by the oligodynamic effect, interferes with the functioning of transport proteins, thereby preventing the uptake of nutrients into the cell, causing its starvation and death. Germ death on exposure to Ag took some time, which has also been analyzed here.

Previous work reported in the literature has focused primarily on the role of colloidal solutions of metallic Ag particles,[2,4] or Ag ions in some cases,[3] in mitigating bacterial growth. In contrast, very few studies have used Ag in a solid matrix, that too being restricted to either a polymeric foam[5] or a polymeric hydrogel.[6] This note describes the preparation, microstructure and antibacterial efficacy of the Ag-GAC composite, starting from pre-synthesized GAC powder.

EXPERIMENTAL

Granular activated carbon (GAC, 200/325 to 20/50 mesh size particles, Filtrex Technologies Pvt. Ltd, India), $AgNO_3$ (99%, Qualigens, India), sodium borohydride ($NaBH_4$, Loba Chemie, India) and Millipore water (of resistivity 18.2 MΩ) were used. All experiments were performed with fresh $AgNO_3$ solution and in a dark place to prevent decomposition of $AgNO_3$ in light. Around 1 g of measured GAC powder was soaked in different beakers containing 20 mL $AgNO_3$ solutions of various concentrations (0.001–1.5 mol L^{-1}). After 24 h of impregnation in the dark, the powder samples were washed with water to remove $AgNO_3$ loosely adsorbed, until no $AgNO_3$ was observed in the filtrate. This ensured that only strongly adsorbed $AgNO_3$ was retained within GAC. The powder samples collected in filter papers were dried in a vacuum desiccator. By adding 10 mL of 0.2 mol L^{-1} $NaBH_4$, impregnated $AgNO_3$ was chemically reduced (over 24 h) to form Ag particles. Washing with water to remove excess $NaBH_4$ was followed again by drying.

To assess antibacterial activity of Ag-GAC, three tests using *Escherichia coli* (*E. coli*) were done: (a) plate assay; (b) shake flask test; and (c) flow test. For all

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water disinfection studies, *E. coli* is used as a test organism, as it is of fecal origin. If this organism is killed, as a standard protocol, all other water-borne disease-causing organisms are assumed killed. For plate assay, melted M-Endo Agar (Himedia) was prepared and to it 1 mL of diluted (overnight grown) *E. coli* culture was added so as to have 105 colony forming units in 1 mL of agar. About 20 mL of seeded agar was then added to pre-sterilized petriplates and solidified by refrigerating for 4 to 6 h. Four 7 mm diameter holes were made in the seeded agar using a sterilized borer. About 20 to 50 mg of Ag-GAC was added in these holes, with 1 to 2 drops of sterilized water. The plates were incubated at 37 °C for 20 h and inhibition zone diameter was measured.

For the shake flask test, 50 mL of sterile was taken, and inoculated with *E. coli*, so as to have 3 log cells in the shake flask. 0.5 g of Ag-GAC was added to it, and the contents were stirred on a rotary shaker at ambient temperature. Samples were drawn periodically from the flask and tested for the number of surviving *E. coli* by plate count method and M-Endo agar, using standard procedures. The aim was for 3 log reduction in *E. coli* concentration. This implies reduction by a factor of 1000; that is, when water containing *E. coli* in the range of 1000 to 9900 per mL was passed through the Ag-GAC sample, the output water will have no *E. coli* count. Finally, for the flow test, 5 g Ag-GAC was taken in a small burette. Tap water spiked with 3 log *E. coli* was fed through it by gravity, at a flow rate of 50 mL min^{-1}. Water samples were collected, at the end of every 50 L being fed, and tested for the number of *E. coli* survivors.

RESULTS AND DISCUSSION

Powder X-Ray diffraction (ARL 'X' TRA, Thermo Electron Corporation, Switzerland) of Ag-GAC product granules showed exactly same diffraction peaks as that of pure crystalline Ag, whereas pure GAC had no peaks, confirming the presence of metallic Ag in the product. Surface morphology of GAC by scanning electron microscopy (Quanta 200 with EDAX facility at 20 kV, FEI) showed both smooth areas and rough surfaces (with pores and edges). The pores were irregular in shape and of size range 2–10 μm. Ag particles formed both on the outer surface and inside the pores of Ag-GAC granules, appearing as white spots (Fig. 1(a)). Their morphology varied from single particles to aggregates of particles. Energy dispersive X-Ray (EDAX) of the white spots gave a high intensity Ag peak, with very low intensity peaks of C, N and O. First, this verified that the white spots were indeed Ag particles. Secondly, it showed the presence of a very small amount of unconverted $AgNO_3$ in Ag-GAC, with the rest being metallic Ag. In fact, the relative atomic weight percentages of the three elements, Ag, N and O (obtained from EDAX), were consistent with relative weight percentages estimated from gravimetry of the overall product.

Table 1 shows weight percentages of $AgNO_3$ and metallic Ag, after impregnation and chemical reduction, respectively. On increasing the concentration of $AgNO_3$ in the impregnating solution, there was a gradual increase and finally saturation in the amount of $AgNO_3$ impregnated. Hence, simultaneously, there was saturation of the final amount of metallic Ag embedded in Ag-GAC too. This is consistent with the mechanism of adsorption in the impregnation technique.

Figure 1(b) shows inhibitory zones formed in the plate test, results of which in Table 2 show that GAC

Table 1. $AgNO_3$ impregnation and its reduction to metallic Ag in Ag-GAC (from gravimetry)

$AgNO_3$ molar conc. (mol L^{-1})	wt% of $AgNO_3$ impregnated	wt% of Ag embedded
0.1	21.62	8.86
1	24.60	10.37
1.5	27.49	11.36

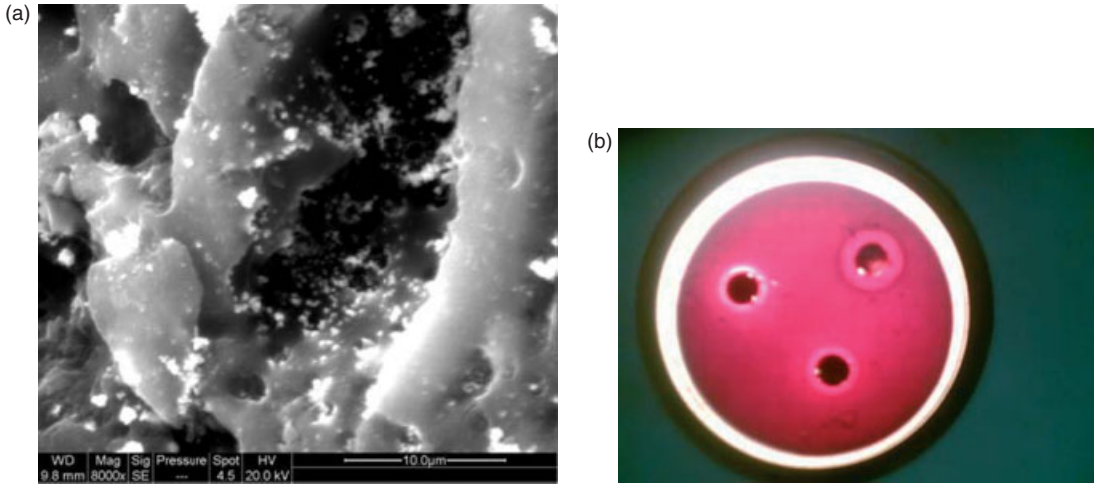

Figure 1. (a) SEM image of Ag (white spots)-embedded GAC, made from 1.0 mol L^{-1} $AgNO_3$. (b) Inhibitory zones formed by this Ag-GAC against *E. coli* in the plate test. This figure is available in color online at www.interscience.wiley.com/jctb.

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impregnated with as low as 0.005 mol L^{-1} $AgNO_3$ possessed slight biocidal activity. However, it was significant only for samples made from 0.1 mol L^{-1} $AgNO_3$, and effectively reached maximum inhibition at 1 mol L^{-1} $AgNO_3$. This proved 0.1 to 1 mol L^{-1} $AgNO_3$ concentration to be optimum in antibacterial activity, which corresponds to 8.86 to 10.37 wt% of Ag in Ag-GAC samples (Table 1). Shake flask results (Table 3) showed that Ag-GAC samples, after a contact period of 5–15 min, brought about 3 log reduction in *E. coli* number concentration. The time needed depends on the Ag concentration; higher concentration, as expected, increased the rate of kill of *E. coli*. Finally, flow test results in Table 4 demonstrated that the optimum (based on previous tests) Ag-GAC sample brought about 3 log reduction in *E. coli*, for up to 350 L of water flown by gravity. Thereafter, the exit *E. coli* concentration started to build up as Ag-GAC would gradually exhaust. So the test was discontinued after 450 L of water had been passed.

As suggested both by the SEM image in Fig. 1(a), and from flow tests showing sustained antibacterial effects at least up to 350 L of water, one concludes that the Ag particles were strongly adhered to the GAC. This also implies that the *E. coli* were subjected to contact-kill mode by Ag particles, and were not killed by any Ag leached-out from the GAC. Such a desirable, strong adhesion of Ag to GAC was possible due to the slow (over a time period of 24 h) and sustained impregnation of $AgNO_3$ from its aqueous solution to GAC. As a result, subsequent chemical reduction of $AgNO_3$ to Ag caused the Ag particles to strongly adhere to GAC. Alternative impregnation attempts by spraying $AgNO_3$ onto GAC therefore were not successful.

Table 2. Inhibitory zones exhibited by Ag-GAC samples (made from solutions of different $AgNO_3$ concentrations) in plate test

Type of Ag-GAC sample ($AgNO_3$ concentration used for impregnation) (mol L^{-1})	Inhibitory zone (diameter)
1.5	11 mm
1.0	11 mm
0.5	10 mm
0.1	9 mm
0.05	slight inhibition
0.005	slight inhibition
0.001	no inhibition

Table 3. Reduction of *E. coli* count (in log units) with time, due to biocidal activity of Ag-GAC samples (made from solutions of different $AgNO_3$ concentrations) in the shake flask test

	$AgNO_3$ concentration used in Ag-GAC synthesis			
Contact time (min)	1.5 mol L^{-1}	1.0 mol L^{-1}	0.5 mol L^{-1}	0.1 mol L^{-1}
0	3.2 log	3.1 log	3.1 log	3.0 log
2	1.2 log	1.3 log	2.1 log	2.5 log
5	0.2 log	0.4 log	1.1 log	1.4 log
15	0	0	0	0
30	0	0	0	0

Table 4. Survivor count of *E. coli* (in log units) in water flown (gravity-driven) through Ag-GAC samples (made from 1.0 mol L^{-1} $AgNO_3$) in flow test

Days	Water passed (L)	Cumulative amount of water passed (L)	Input *E. coli* count	Output *E. coli* count
1	50	50	3.2 log	0
2	50	100	3.15 log	0
3	50	150	3.3 log	0
4	50	200	3.1 log	0
5	50	250	3.3 log	0
6	50	300	3.25 log	0
7	50	350	3.2 log	0
8	50	400	3.1 log	1.5 log
9	50	450	3.0 log	2.7 log

CONCLUSIONS

Ag-GAC composites were made by impregnating GAC with aqueous $AgNO_3$ solution, followed by chemical reduction to metallic Ag particles within GAC. XRD confirmed that crystalline Ag formed, while SEM images showed the Ag particles to be either as individual particles or as aggregates. EDAX and gravimetric estimates of relative atomic weight percentages of Ag, N and O were consistent, which, coupled with plate assay, shake flask test and flow tests showed that the optimum composition with respect to antibacterial properties was about 9–10.5 wt% of Ag in Ag-GAC. With this new product, it was possible to remove a number concentration of 3 log *E. coli* for up to a maximum of 350 L of flowing water (in gravity-driven input water), in less than 30 s of contact time. These results show the potential of Ag-GAC for use in water purification to produce potable quality water.

Presently, world over, safe drinking water is made by disinfecting either chemically or by UV treatment and occasionally by heating/boiling. Except for chemical treatment, other methods require electricity, and therefore these methods cannot provide people with safe water in areas where there is no electricity/fuel. Chemical treatments are easy to perform and cheaper. Unfortunately, all chemicals produce byproducts, which can prove harmful to the consumer in the long run. Therefore there is an urgent need for alternatives. Silver impregnation is very effective in overcoming the problems of the above routes. It has been in use since ancient times, and has no known side effects. Therefore, silver disinfection is a relevant potential alternative.

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