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Highly efficient Ag/C catalyst prepared by electro-chemical deposition method in controlling microorganisms in water

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Abstract

The present work highlights the high efficiency of silver nano-particles deposited over activated carbon, by electro-chemical deposition method, in controlling microorganisms in water. The anti-bacterial activity of the catalysts were determined qualitatively by testing the presence of coliforms in water after contacting with the catalyst, using a Readycult reagent. The catalytic characteristics of these materials are obtained by SEM (scanning electron microscopy), XRD (X-ray diffraction) and TPR (temperature programmed reduction). TPR results clearly indicate the presence of metallic silver in the dried catalyst prepared by electro-chemical deposition method and the presence of silver oxide and/or nitrate precursors in the catalysts prepared by impregnation method. SEM results indicate the presence of Ag particles in nanometer size. Comparison of the anti-bacterial activity of the Ag/C catalyst prepared by electro-chemical deposition method with that of the Ag/C catalyst prepared by conventional impregnation technique indicate that lower amount of former is sufficient in controlling the microorganism which is not the case with the latter. The main advantage of Ag/C catalyst prepared by electro-chemical deposition is that no pretreatment conditions like reduction are required for deactivation of microorganism in water, which is not the case with the catalysts prepared by impregnation technique.

Keywords: Ag nano-particles; Electro-chemical deposition; Microorganisms; Active carbon

1. Introduction

Water pollution is one of the main crisis faced by millions of people and majority of the water-borne diseases are spreading because of the poor quality of water, particularly due to the presence of bacteria and viruses in the water. There are several methods of water purification like chlorination, iodination, ozonation, UV-purification, reverse osmosis and using silver catalysts. Chemical purification like adding chlorine or iodine or applying ozone to kill the bacteria has several disadvantages, for example excess chlorination could lead to cancer. Other methods like UV-purification and reverse osmosis are not cost affective. Ever since silver has been recognized as an anti-bactericide, its application in purification of water is increasing. Heinig [1] developed a catalytic cartridge-containing layer of silver micro-crystals deposited on α-alumina and showed that lightly bound nascent oxygen on micro-crystals of silver readily oxidizes bacteria or viruses and completely disintegrates them. The anti-bacterial and antibiotic action of silver compounds are reported in the literature [2–4]. The catalytic oxidation by the metallic silver in the walls of the container as well as reaction with the dissolved monovalent silver ion probably contributes to the bactericidal effect of silver vessels [5]. Supported silver catalysts are reported to be effective in deactivating microorganism [6,7]. Antelman [8] in his research indicated that in an aqueous medium the peroxide Ag(III) works about 240 times as fast as Ag(I) and is upto 200 times more effective a disinfectant than Ag(I) compounds or metallic silver. Electrically

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driven silver ions are reported to overcome a critical problem in the treatment of serious bone infections [9] and severely burned hands and figures [10]. Further, silver is used in the silver–copper ion treatment in the sanitation of swimming pools with minimal or no chlorine usage [11].

Generation of silver nano-particles in water by silver ion generators is recently a popular technique. The present work aims in the generation of silver nano-particles on active carbon by electro-chemical deposition method where in these nano-particles are utilized in controlling the microorganisms in water. Also, the advantages of these catalysts over the Ag/C catalysts prepared by conventional impregnation technique have been emphasized in the study of the biological activity of these catalysts in controlling the microorganisms. Further, the differences in the activities of the two catalysts has been explained by the characteristic techniques used viz., XRD, SEM and TPR.

2. Experimental

2.1. Preparation of catalysts

2.1.1. Preparation of Ag/C catalyst by conventional impregnation method

Ag/C catalyst with silver loading of 2 wt.% was prepared by impregnating aqueous solution of requisite amount of silver nitrate on to dried and purified active carbon. The excess amount of water was then evaporated on a hot plate followed by drying in an oven at 110 °C for about 12 h. The dried sample thus obtained was calcined in air at 400 °C for 4 h followed by reduction in H_2 flow for a period of 2 h at 250 °C. The resultant catalyst has been designated as AgC-IMP. The active carbon (M/S. Norit, with surface area $\sim$1000 $m^2\,g^{-1}$) support used, has been purified by a sequential treatment with hot conc. HNO_3, hot distilled water, hot conc. NH_3 solution and hot distilled water for at least three times and was then dried in an oven at 110 °C prior to the impregnation of silver over it.

2.1.2. Preparation of Ag/C by electro-chemical deposition

The silver solution was firstly generated by passing low but constant dc voltage electricity (40 V) through the silver electrodes (0.4 mm thickness and 10 mm width and 100 mm long plates) immersed in distilled water. This solution was then used to test the presence of microorganism activity in the water samples. The Ag/C catalyst by electro-chemical deposition method designated as AgC-EC was prepared by taking requisite amount of purified active carbon in distilled water and silver particles were generated by the above method while maintaining a constant rapid stirring for a calculated amount of time so as to obtain a 2 wt.% of Ag in the finished catalyst. The excess water was then evaporated over a hot plate followed by drying in an oven at 110 °C for 12 h.

2.2. Characterization of catalysts

2.2.1. Scanning electron microscopy (SEM)

This technique has been used to find out the range of Ag particle sizes obtained when run time of passage of low voltage current passed through the silver electrodes immersed in water was varied. The aqueous samples containing silver particles were mounted on aluminium stubs coated with gold in Hitachi-5GB vacuum evaporator and micrographs were recorded on a Hitachi S-520 SEM instrument.

2.2.2. Powder X-ray diffraction

The XRD patterns of reduced sample of AgC-IMP and dried sample of AgC-EC were recorded on a Siemens D 5000 X-ray diffractometer using Ni filtered Cu Kα radiation.

2.2.3. Temperature programmed reduction (TPR)

TPR patterns of dried samples of AgC-IMP and AgC-EC were obtained using a home made on-line quartz microreactor interfaced to a thermal conductivity detector (TCD) which in turn is connected to a data station (comprising of a standard GC-software supplied by Hindetron, India) for recording the profiles. H_2/Ar (6 vol.% of H_2 and balance Ar) mixture was used as the reducing gas at a heating ramp of 5 K/min from 303 to 973 K and kept at the final temperature isothermally for 30 min. The experimental details of the TPR run are discussed elsewhere [12].

2.3. Activity test

Ready cult® Coliforms 50 supplied M/S. E. Merck; Germany was used for the detection of total coliforms in the water samples. The composition of this reagent is as follows.

2.3.1. Composition in g/blister

Tryptose-0.25, NaCl-0.25, sorbitol-0.05, tryptophan-0.05, di-potassium hydrogen phosphate-0.135, potassium dihydrogen phosphate-0.1, lauryl sulfate sodium salt-0.005, X-GAL-0.004, MUG-0.0025 and IPTG-0.005.

2.3.2. Principle

The high nutritional quality of the peptones and the incorporated phosphate buffer guarantee rapid growth of coliforms where as lauryl sulfate largely inhibits the accompanying flora, especially the gram positive. By adding the chromogenic substrate X-GAL, which is cleaved by coliforms and the fluorogenic substrate MUG that is highly specific for *E. coli*, the simultaneous detection of total coliforms and *E. coli* is possible. The presence of total coliforms is indicated by a blue-green color of the brath and *E. coli* by a blue fluorescence under UV-light.

2.3.3. Procedure

To a 50 ml of water (to be tested for coliforms) sample taken in a sterile, transparent 100 ml vessel with screw cap, the granules of Ready cult coliforms reagent was added by

Table 1
Effect of run time on the particle size of silver

S. no.	Voltage (V)	Run time (min)	Range of Ag particle size (nm)
1	40	5	50–100
2	40	10	100–120
3	40	20	250–1000
4	40	30	600–1500

breaking the snap pack. The vessel was sealed, shaken completely to dissolve the granules and incubated for 24 h at 310 K. Any color change of the brath to blue-green confirms the presence of coliforms. If the brath remains slightly yellow (no color change), it is an indication of absence of total coliforms. This is a blank test, i.e., without adding any Ag catalyst. The same procedure was adapted for testing the activity of the catalysts by adding required amount of the catalyst to the water sample and stirred well for ∼1 h, followed by filtration to remove the catalyst particles, before the ready cult was added to it.

3. Results and discussion

3.1. SEM results

The Ag nano-particles are generated electro-chemically by passing a dc current (40 V) through silver electrodes dipped in distilled water (50 ml). The effect of run time (duration of passage of the current) on the size of the particles generated is examined by carrying out SEM analysis of the Ag solutions thus obtained. Table 1 presents the Ag particle size obtained from SEM analysis at different run times. It can be seen that just a 5 min time is sufficient enough to generate the Ag particles in the nano-range in 50 ml water when a 40 V dc current is applied and beyond this time agglomeration of particles seem to take place producing Ag particles of bigger size. Fig. 1 represents the SEM micrograph of nano-size Ag particles in water generated by electro-chemical method after a run time of 5 min. The figure clearly shows the formation of nano-particles of Ag in the range of 50–200 nm.

During the electrolysis run, as the size of the particle (Ag) increases, the distance between the particles decreases which results in the increase in the current (mA) (initial current of 5 mA has been increased up to 8 mA). It is reported that the aqueous solution containing the silver particles (nano-range) by electro-chemical method to be in the range of 10–20 ppm of Ag [13]. For supporting Ag particles on activated carbon by this method, it is thus assumed that the concentration of Ag is ∼10 ppm. Based on this assumption, a 2 wt.% of Ag deposited on activated carbon (AgC-EC) has been prepared by suspending ∼5 g of carbon in the distilled water with vigorous stirring and maintaining 40 V dc current through the silver electrodes, for sufficient amount of time.

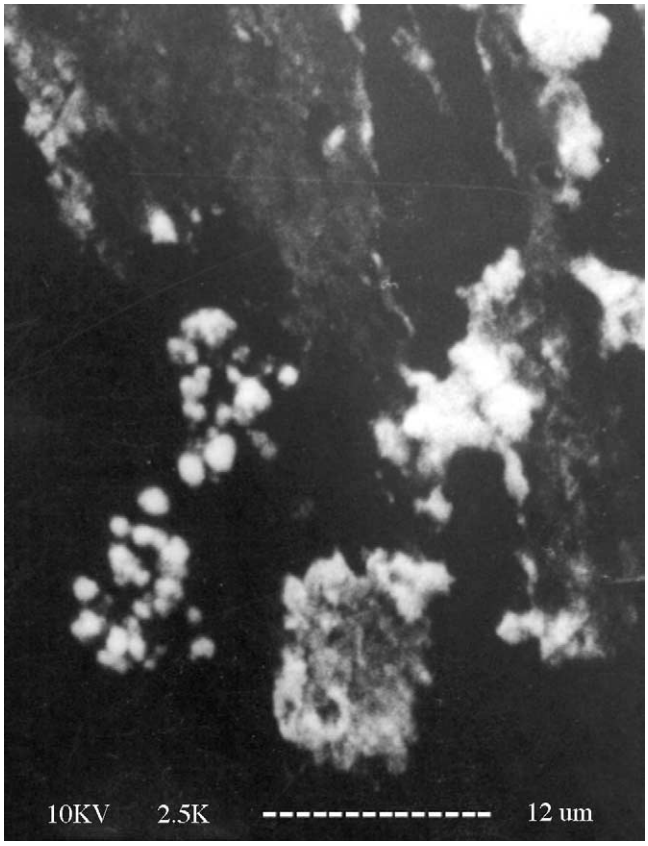

Fig. 1. Scanning electron micrograph of silver nano-particles in water generated by electro-chemical method.

3.2. X-ray diffraction

Fig. 2 depicts the XRD patterns of AgC catalysts, i.e., AgC-EC (dried) and AgC-IMP (reduced). The XRD pattern of impregnated Ag over active carbon is recorded for the

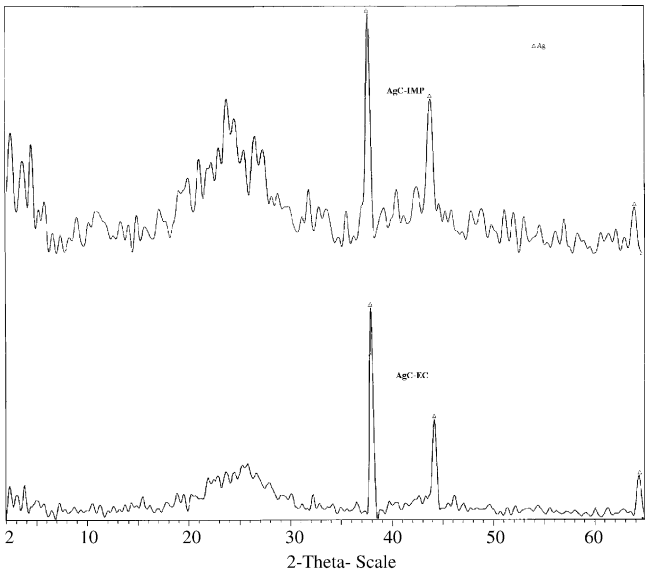

Fig. 2. X-ray diffraction patterns of AgC catalysts.

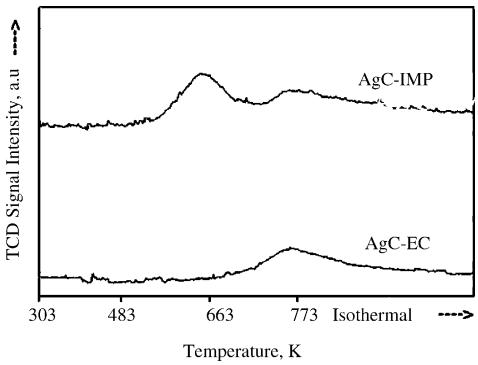

Fig. 3. Temperature programmed reduction profiles of AgC Catalysts.

reduced form of the catalysts where as the pattern of Ag/C catalyst prepared by electro-chemical deposition method is of the dried form. The two catalysts showed amorphous carbon phase and silver in metallic phase [Ag with d values 2.36_x, 2.04_4, 1.25_3 – ASTM card no. 4–783]. The pattern of AgC-EC clearly shows that the dried form of the catalyst itself is in metallic form and thus need not be reduced any more. On the other-hand, the impregnated catalyst has to be reduced in H_2 flow for at least 2 h at 250 °C to get the metallic phase of Ag.

3.3. TPR results

The TPR patterns of dried Ag catalysts viz., AgC-EC and AgC-IMP are presented in Fig. 3. The TPR pattern of AgC-EC catalyst exhibit only a single reduction peak centered at T_{max} of 740 K that which is also observed in TPR pattern of the other catalyst, AgC-IMP. This signal may be attributed to the gasification of the support, active carbon. It is reported to be due to the reaction of carbon with hydrogen resulting in the formation of methane confirmed by the coupled FID analysis technique [14] and is represented as follows:

$$C + H_2 \rightarrow CH_4$$

No other peaks corresponding to reduction of oxidic species of Ag are observed in the TPR pattern of AgC-EC catalyst. This clearly suggests that silver is already in the metallic form in this catalyst. It can be further confirmed from the TPR pattern of conventionally prepared AgC-IMP catalyst which showed a low temperature reduction peaks centered at T_{max} of ∼600 K. This reduction signal is originated due to the reduction of Ag precursors, possibly either the reduction of $AgNO_3$ precursor or the reduction of Ag_2O. It is reported by Gang et al. [15] in the H_2-TPR carried out on silver catalysts pretreated in oxygen below 100 °C after reduction at 500 °C for 2 h to have observed the existence of three peaks at 80, 140 and 460 °C. They attributed it due to molecularly adsorbed oxygen, to surface adsorbed atomic oxygen and the high temperature one assigned to the bulk-dissolved oxygen, which is the most difficult one to be reduced, respectively. Boccuzzi et al. [16] have shown in the TPR patterns of Ag/Ti fresh samples, that a broad and asymmetric peak at T_{max} = 383 K is ascribed to the reduction of oxygen species on finely dispersed silver and to the reduction of Ag_2O. Also, Paryjczak et al. [17] have ascribed a double peak at 370–500 K observed in the TPR study carried out by them over 4 wt.% Ag on γ-$Al_2O_3$to be due to different oxide species of Ag. Based on these reports it seems that the reduction signal observed in the TPR pattern of AgC-IMP sample in this study correspond to the reduction of $AgNO_3$ precursor and/or the reduction of silver oxide. Thus it clearly indicates that silver is in reduced form in AgC-EC where as in oxidic form or in unreduced state in the impregnated catalyst.

For controlling the microorganisms in raw water, it is assumed that silver should be in metallic form so that it can take dissolved oxygen from water. Thus for the catalyst AgC-EC, no pretreatment is needed for controlling the microorganisms in water. Whereas the silver catalyst prepared by conventional impregnation technique requires reduction prior to use in controlling microorganisms in water because silver is in oxidic form and also $AgNO_3$ precursor in the catalyst may leach into the water there by causing depletion of concentration of silver in the catalyst.

3.4. Activity test

The *E. coli* lacZ gene encoding ß-galactosidase (ß-gal) is the classical histochemical reporter gene [18]. It can be detected using a variety of substrates, all of which have galactose linked through a ß-D-glycosidic linkage to a moiety whose properties change upon liberation from galactose [19]. Several substrates yield colored or fluorescent soluble products, which are useful when quantifying ß-gal activity [20,21] or visualizing transduced cells live in vivo [22–24]. The fluorescent products can even be used to kill cells in vivo [23]. However, for localization of cells containing transduced lacZ, chromogenic substrates that yield a precipitated product are desirable [25–27]. The most common such substrate is an indole derivative, 5-bromo-4-chloro-3-indolyl-ß-D-galactoside [28].

When ß-gal cleaves the glycosidic linkage in X-gal, a soluble, colorless indoxyl monomer is produced. Subsequently, two of the liberated indoxyl moieties form a dimer, which is non-enzymatically, oxidized (Fig. 4). The resultant halogenated indigo is a very stable and insoluble blue compound [28]. The dimerization and oxidation reactions require transfer of an electron, which is facilitated by electron acceptors of the proper redox potential [29]. The ferric and ferrous ions included in most X-gal reaction buffers provide this function [30].

The scheme presented in Fig. 4 shows the action of X-gal in detecting the presence of microorganisms, which is evidenced from the blue coloration, found after incubation. The Readycult containing X-gal is mixed in the raw water sample to be tested along with required amount of catalyst stirred well for an hour and incubated for 24 h. The resulting bluish-green coloration of the brath indicates the presence of

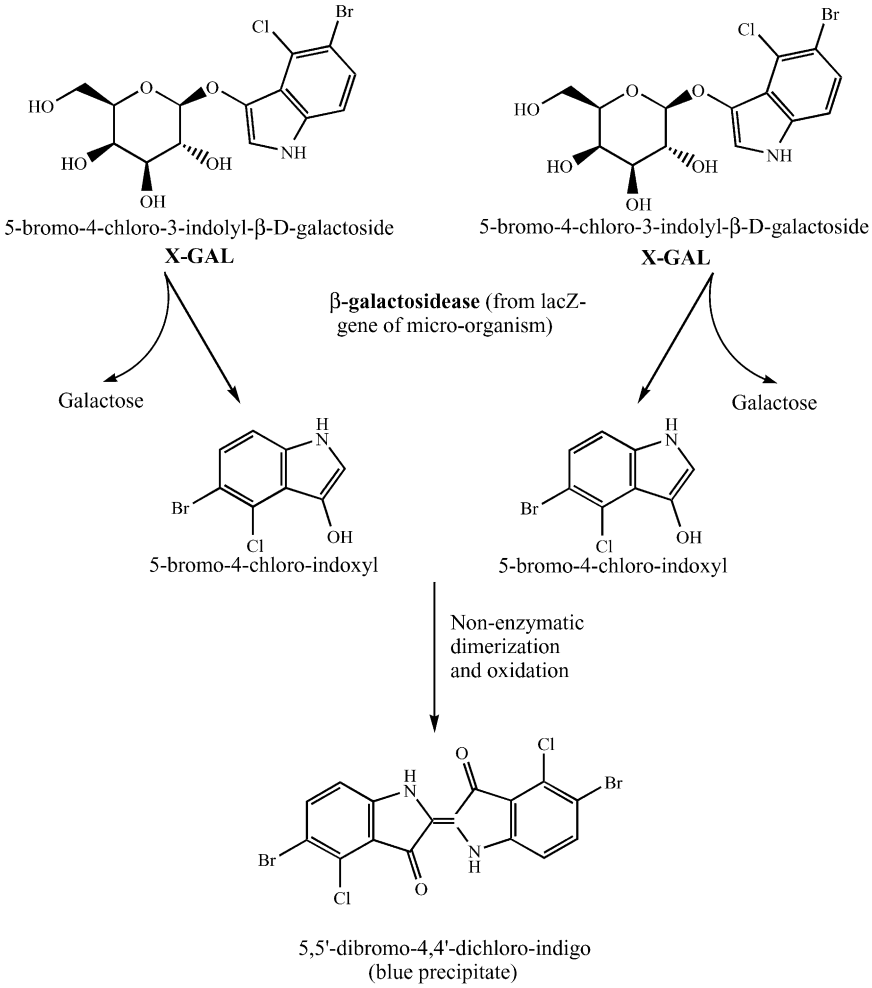

Fig. 4. Scheme showing the action of X-gal (from Readycult reagent) in detecting the microorganisms in water.

microorganisms and the absence of any coloration show the effectiveness of Ag catalyst in controlling the microorganisms. Since, if the microorganism is killed by the action of Ag, then there is further reaction of ß-galactosidase present in the microorganism with X-gal responsible for the coloration of the brath.

IPTG, isopropyl ß-D-thiogalactopyranoside is a chemical analogue of galactose, which cannot be hydrolyzed by the enzyme ß-galactosidase. Therefore, it acts as an inducer for activity of the lac operon of the microbe by binding and inhibiting the lac repressor without being degraded and is hence often used in conjunction with a ß-galactosidase substrate such as X-gal.

MUG, 4-methyl lumbelliferyl-ß-D-glucuronide trihydrate is a fluorescent substrate for ß-D-glucuronidase (GUS) encoded by the gusA gene isolated originally from *E. coli*. Cleavage of the substrate MUG by a ß-glucuronidase activity leads to the generation of the fluorigenic product 4-MU, 7-hydroxy-4-methyl coumarin, which can be visualized or detected by irradiation with UV-light.

A report [31], discuss three mechanisms of deactivation that silver utilizes to incapacitate disease-causing organisms. They are catalytic oxidation, reaction with cell membranes, and binding with the DNA of disease organisms to prevent unwinding. Among all the metals, silver is unique in its affinity towards oxygen. It is reported that atomic oxygen has an almost prefect fit in the octahedral holes of gold, silver [32] and copper. However, in gold the electron cloud of oxygen tends to be expelled by lattice oxygen of gold atoms and this block the movement through holes. Copper, on the other hand forms the oxide providing an impossible barrier. Silver offers so little repulsion to oxygen that only a small amount of thermal energy is required to readily move the atomic oxygen through the silver lattice [33].

Silver in its atomic state, has the capacity to absorb oxygen and act as a catalyst to bring about oxidation. Atomic (nascent) oxygen absorbed onto the surface of silver ions in solution will readily react with the sulfhydryl (–S–H) groups surrounding the surface of bacteria or viruses to remove the hydrogen atoms (as water), causing the sulfur atoms to form an R–S–S–R bond; blocking respiration and causing the bacteria to expire. Micro-crystals of silver have a tendency to lightly bound nascent oxygen (with a binding energy of only 40 kcal/mol) and these species readily oxidizes bacteria or

Table 2
Effect of Ag concentration in water on the destruction of coliforms

S. no.	Catalyst code	Volume of 10 ppm Ag aq. solution (ml)	Volume of raw water (ml)	Total coliforms status (A: absence, P: presence)
1	Without catalyst	–	50	P
2	Ag solution (10 ppm)	10	40	P
3	Ag solution (10 ppm)	20	30	P
4	Ag solution (10 ppm)	30	20	A
5	Ag solution (10 ppm)	40	10	A

Table 3
Comparison of activities of AgC-EC and AgC-IMP in controlling the microorganism

S. no.	Catalyst	Catalyst weight (g)	Volume of water (ml)	Total coliforms status (A: absence, P: presence)
1	AgC-EC	1	50	A
2	AgC-EC	0.5	50	A
3	AgC-EC	0.2	50	A
4	AgC-IMP	1	50	A
5	AgC-IMP	0.5	50	P
6	AgC-IMP	0.2	50	P

viruses, resulting in complete disintegration [1]. There are many forms of silver viz., silver salts, organic precursors of silver, metallic form of silver on carbon, which may exhibit microscopic particle size and show germicidal, antibiotic and other effects, but are not always found to be safe and are less effective than the silver generated by electro-deposition method. In the case of silver nano-particles in water solution generated by electrolytic method, ∼30 ml of such solution is required to deactivate bacteria in a 20 ml raw water (Table 2). Moreover, it is very difficult to separate the Ag particles in the solution and to reuse them. Thus this Ag solution deposited on active carbon seems to be more advantageous particularly in reusing the catalyst. Silver particles produced by this method are ideal sized and provide the greatest biological benefit and are proved to be non-toxic as well. The biological activities of AgC-EC and AgC-IMP, the two different types of silver catalysts used, are presented in Table 3 clearly shows the difference and superiority of the AgC-EC system in controlling the microorganisms. Although the impregnated Ag catalyst (AgC-IMP) showed anti-bacterial activity, they are needed in high concentration, i.e., only if taken ≥1 g amount (in ∼50 ml raw water) can effectively control microorganisms in water. Also, more importantly these catalysts have to be pretreated subjecting to reduction in H_2 flow at 523 K for at least 2 h to get metallic silver particles on active carbon prior to their application. Thus, again AgC-EC catalyst prepared by electro-chemical deposition method, which contains silver already in metallic form, is advantageous over the impregnated catalyst as the pretreatment is not required. Moreover, even ∼0.2 g of this catalyst is sufficient enough or effective in controlling the microorganisms in water.

4. Conclusions

1. The catalytic ability of the silver deposited on carbon by electro-deposition route is more effective than that of silver deposited on carbon by conventional impregnation technique in controlling the microorganisms in water.
2. The electro-deposition method is easier and less expensive.
3. Silver is deposited in metallic form directly in this method, which is more cost effective as the pretreatment processes viz., calcinations and/or reduction can be avoided.
4. This method yields smaller particles (nano-particles) which increases the intrinsic activity (activity per site) and hence superior activity compared to the catalysts prepared by conventional method. The high intrinsic activity of this catalyst is evidenced from its effectiveness in controlling the microorganism in water with lower weight.
5. The electro-deposition method can also be extended to make other supported catalysts like Cu, Ni to increase the catalytic activity in various other reactions too.

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