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Research Article

Fast and complete decontamination of bacteria and yeast from water by silica-supported carbon nanoparticles simple filtration

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Abstract

The present work aim to study the adsorption of different models of bacteria and yeast by silica-supported carbon nanoparticles (SCNP). SCNP was prepared at room temperature by ultrasonication of anthracene in toluene, in presence of ferrocene as catalyst and silica as nucleation sites. The strength of bacterial removal by the resultant SCNP was studied with *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (as bacterial models) and *Candida albicans* (as yeast model). The bacterial suspension was left for 10 minutes in contact with the carbon material, filtrated and we enumerated bacteria in the filtrate obtained. The bacterial adsorption results exhibited complete adsorption efficiency after short contact time with SCNP. These results demonstrate the strength of the SCNP to completely adhere the bacteria and yeast. Similar experiment was conducted on silica particles without carbon, but no bacterial adsorption was observed. This clearly indicates the effectiveness of the SCNP to easily and simply adsorb bacteria and yeast without outside interference parameters.

Keywords: carbon, nanoparticles, ultrasonication, adsorption, bacteria.

1. Introduction

Water pollution is one of the most undesirable environmental problems in the world and it constitutes the major mode of transmission of pathogenic bacteria. Worldwide, more than one million and three hundred thousand children are dead because of diarrheal illness every year [1]. One approach of monitoring the quality of water is by controlling its safety and ensuring its cleanliness against yeast such as *Candida albicans* (*C. albicans*) and bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli (E. coli)*. This last is used as fecal indicator for water safety and monitoring management [2-4].

Moreover, *E. coli*, lives normally in the intestines of healthy people and animals. One may be exposed to *E.coli* from contaminated water or food sources. Some pathovars are pathogenic [5]. *S. aureus* is a type of bacteria that about 30% of people carry in their noses. Sometimes, the bacterium causes infections. In healthcare settings, these infections can be serious or even fatal [6]. *P. aeruginosa*, bacteria are widely found in the environment. Serious infections usually occur in people in the hospital; however healthy people can also develop mild illnesses, especially after exposure to water [7]. *C. Albicans candida* yeasts normally live on the skin and mucous membranes. Overgrowth of these organisms can cause symptoms to develop [8]. They may remain in the environment and resist the common disinfection processes [9]. Thus, development of new techniques to clean water from viruses and bacteria become inherently demanded.

There are several methods available for the removal of micro-pollutants from waters and wastewaters

such as flocculation, chlorination [10,11], photocatalytic process[12], biological processes, adsorption and filtration[13-14]. Some of these techniques have been shown to be effective however they have some limitations such as excess amount of chemical usage (chemical processes) and accumulation of concentrated sludge (biological processes). The adsorption technique, which is based on the transfer of pollutants from the solution to the solid phase, is considered as one of the efficient wastewater treatment method [15]. Carbon materials have emerged as one of the best for the removal of chemical and biological contaminants from water, e.g. carbon nanotubes[16] are known to be good candidates for water decontamination. They can concentrate bacterial pathogens from water systems that are contaminated. Adsorption by carbon material is better than other conventional removal techniques in terms of initial cost, simplicity of design, ease of operation, and non-toxicity of the utilized adsorbents. It is known as a universal adsorbent due to its microporous, homogenous structure, high surface area and its ability to bind to a large variety of pollutants [17]. Indeed, various pollutants such as pesticides, odors, algae toxins and industrials micropollutans can be removed using granular carbon filters [18].

The aim of this work is to develop SCNP and evaluate its effect on a variety of bacterial strains and explore its effectiveness towards their complete adsorption and removal from water within a short period of time.

2. Materials and methods

2.1 Materials

SCNP used in this study was prepared according to the method described by H. H. Hammud elsewhere [19]. The importance of this material lies in its easiness of preparation from simple compounds by ultrasinaction of anthracene and ferrocene dissolved in toluene at room temperature, in presence of silica particles for two hours. The reaction yielded silica-supported carbon nanostructures. Other carbonaceous materials are known to be prepared at high temperatures and under specific conditions [20].

2.2 Strains used in the study

In this work, we studied the (removal) effect of SCNP on the following bacteria:

Escherichia coli (CMUL 469) (E. coli), *Pseudomonas aeruginosa* (CMUL 313) (*P. aeruginosa*) and *Staphylococcus aureus* (CMUL 381) (*S. aureus*); as well as a yeast, the *Candida albicans* (CMUL 064) (*C. albicans*). This category were provided from the microbial collection of the Lebanese University CMUL: Collection Microbiologique de l'Université Libanaise. Of each strain we performed a suspension in a physiological saline solution (PSS) (0.9% NaCl), with an adjusted turbidity to become equal to 0.5 McFerland (~ 10^8 CFU / mL) and then diluted to 1/100 in a PSS prior to use for testing.

To determine the number (N) of microorganisms in each milliliter, a series of decimal dilutions is prepared in PSS ($10^{-1} \rightarrow 10^{-6}$), and then one milliliter of each dilution is cultivated by incorporation method in a nutrient agar (Canada®, Spain) for bacteria and Sabourand agar for Candida *albican* (Canada®, Spain).

After 24h of incubation, the petri dish where the number of colonies was counted, from the dilution that allows an easy counting of the colonies (50-100 colonies).

N = total number of bacteria per milliliter

n = number of counted colonies at chosen dilution

10^{+x} = chosen dilution to count the *n* number of colonies

$N = n \times 10^{+x}$ CFU/mL

10 mL of each suspension were added in a Petri dish (diameter = 90 mm), then 1g of SCNP was introduced and the petri dishes were placed under slight agitation (70 rpm) for 10 min.

After contact times, the contents of each petri dish were filtered through a filter paper (Whatmann with a pore diameter = 11 µm), sterile (autoclaved 115ºC during 20 min). The obtained filtrate in a sterile Erlenmeyer flask (work in a classe B laminar hood) was diluted from$10^{-1} \rightarrow 10^{-4}$ in PSS.

1 mL from the filtrate and each dilution were deposited in the petri dish then 18 mL of nutrient agar (for bacteria) and Sabourand agar (for yeast) were added. The mixture was then manually mixed by rotation and left to solidify at room temperature. The petri dishes were incubated for 24h-48h at 37ºC.

A loop amount of the retained SCNP after filtration from water with *E. coli* was suspended in 1mL of PSS. The main solution was then diluted to 10^{-4} and enumerated as described above.

To control the effectiveness of SCNP material, similar work was performed on the resin and on the silica particles separately and independently.

3. Results and discussion

In order to evaluate the yeast and bacterial adsorption process on SCNP and to estimate their removal efficiency from water using SCNP, a suspension of different kinds of microorganism in presence of SCNP was placed under agitation. The obtained results showed that after 10 minutes of contact time, the observed efficiency was 100% (table 1).

Table 1: **Efficiency of SCNP towards bacteria and yeast removal after 10 minutes of contact**

strain tested	N (CFU)/mL	observed growing colonies after treatment (CFU)/mL
E. coli	$134x10^5$	0
P. aeruginosa	$33x10^5$	0
S. aureus	$87x10^4$	0
C. albicans	$31x10^4$	0

The number of *E. coli* found after filtration on a loop of SCNP removed from the Whatmann paper was $134x10^5$/mL. Similar results were obtained with *P aeruginosa*, *S. aureus* and *C. albicans* as shown in table 1. These results give evidence that all these micro-organisms were adsorbed on SCNP and demonstrate the strength of the SCNP to completely remove the bacteria and yeast from the suspension.

A similar experiment was performed on silica without carbon nanoparticles to find out the effectiveness against the silica particles for the removal of bacteria (ex. *E. coli*) from water. These results showed no retention efficiency and therefore no bacteria adhered on the surface on the silica particles (table 2).

Table 2: Control over silica particles without SCNP

Name of the bacterium	agitation time (min)	number of bacteria without silica (CFU/mL)	Number of bacteria after filtration (with silica) (CFU/mL)
E. coli	10	~10^6	~10^6

Results from Table 2 show that the silica used as support for carbon nanoparticles do not present any retention effect on the studied microorganisms.

The feasibility of the SCNP as effective bacterial adsorbent agent was evaluated using *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* as a model bacteria and yeast. It was revealed that all tested microorganisms had adsorbed on the surface of the carbon supported on silica surface particles where they were initially grown. It was clear they have the maximum adsorption affinity. The filtered water was completely clear from bacteria or yeast. This indicates the importance of the SCNP as an ideal adsorbent compared to others in the adsorption process and contact time regarding various models of gram positive, gram negative and yeast. The bacterial adsorption on the surface of the SCNP occurred spontaneously without any special treatments.

Moreover, the results of the viability of bacteria after the bacterial capturing reaction by adsorption showed no difference in bacteria viabilities when compared with the control sample that does not contain SCNP as well as that after the reaction in the absence of adsorption, indicating the negligible effect of SCNP themselves on the bacteria viability.

Due to the fact that all bacteria completely adsorbed on the surface of the SCNP, this means that the cell surface characteristics have little efficiency on the carbon nanoparticles. The primary forces of the bacterial adsorption or adhesion to the various material surfaces include dispersion van der Waals forces, electrostatic interactions, and hydrophobic attractions [21-23].

The mechanism which is mainly responsible for the nonselective attachment of bacterial cells to the SCNP is not clear. However, in view of the dominant hydrophobicity on the SCNP surfaces [24], the hydrophobic attraction between the surfaces of bacteria and SCNP could be a major driving force for the reaction. Additional studies are necessary to address the nature of the binding interactions between bacterial cells and SCNP. Nevertheless, the highly efficient and on-selective bacteria adsorption phenomenon strongly suggests the possibility of the multiwalled carbon nanotubes clusters as ‘‘universal’’ bacterial adsorbents that would be efficient for any bacterial cells [25].

Nonetheless, due to the large efficiency of SCNP as highly non-selective bacterial adsorbent, it is suggested that SCNP would be considered as universal adsorbent for bacteria that may work for any bacterial cells.

Compared to silver impregnated activated carbon and silica sand which were used to eliminate and destroy water borne *E. coli* under plate assay and shake flask technique by Karnib *et al*[26]. Even they showed high antibacterial effect against *E. coli*, however, bacterial growth was completely inhibited on using silver impregnated activated carbon at all the tested concentrations after one hour of incubation. This means that at least one hour is needed to deactivate bacteria not only just by carbon material but additionally with silver impregnated particles. Meanwhile in this study with simple room temperature prepared carbon material, only 10 min were enough to adsorb not only *E. coli* but a larger range of bacterial variety.

Additionally, Rivera-Utrilla *et al*. showed that the number of bacteria adsorbed on demineralized commercially activated carbon in a solution of pH value equal to the iso-electric point was negligible, however, in the presence of cations the proportion of adsorption increased but to a certain value without reaching the maximum level of adsorption[18]. This means that water will still contain amounts of bacteria and other microorganisms that survive throughout the adsorption process.

The performance of multi-walled carbon nanotubes clusters was evaluated and optimized by its bacterial separation process by Moon *et al*. using *E. coli* bacterium as a model[25]. Again, agitation and incubation time were conducted for one hour before magnetic separation of paramagnetic complexes. Sophisticated techniques were used to synthesize the separating materials, and others to identify their performance and presence in bulk aqueous solution after magnetic separation. When compared to this method of bacterial adsorption, just filtration paper, incubation in petri dishes and enumeration of bacterial colonies if found were the only and simple efficient and easy methods used to eliminated not only *E. coli* bacterial type from water but also other types as described above.

4. Conclusion

SCNP offered advantage for water purification-total bacterial and yeast retention while operating at room temperature during short time experiment unlike other carbon materials such as carbon nanotubes and activated carbon. The obtained results of SCNP for retaining bacteria and yeast demonstrated their highly effective property as universal spontaneous bacterial and yeast adsorbent. The ease of the preparation of the sample as well as the cost of the method constituted a main reason of its usage. Further development of SCNP is still required for design and optimization of results improvement. Moreover, the separation of the microorganisms and/or yeast should be validated with larger varieties of organisms to generalize the process. This would allow to achieve bacterial capturing and separation procedures and to extend them to future application concepts such as sampling units and bio sensing.

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