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Bacteria supported on carbon films for water denitrification

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HIGHLIGHTS

- Mesoporous carbon-xerogels films have been obtained.
- The mesoporosity is independent of the reached carbonization degree using microwaves.
- Carbon xerogel films are very interesting materials as supports of bacteria *E. coli* to remove nitrates from water solution.
- Low carbonization degree and/or more acid PZC favor the Gram negative bacteria growth on carbon films.

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ABSTRACT

This work shows the behavior of new carbon xerogel films as bacteria supports to remove nitrates from water solution. The preparation method of carbon-xerogel films with well developed mesoporosity obtained by microwave assisted carbonization, as well as the particular effects that the microwaves produce during the carbonization process on the final chemical characteristic of these materials, have been described. Bacteria *Escherichia coli* were supported on the films working well in the denitrification process of water solutions. Low carbonization degree and/or more acid pH of point of zero charge favor the Gram negative bacteria growth on these types of films.

1. Introduction

Different types of carbon films have been used as a coating of structural materials [1]. On the other hand, the preparation of ceramic structures coated with well adhered carbon-xerogel films has also been studied [2]. Thus, the development of new types of carbon xerogels for different applications has grown considerably during the last years [3–8].

Actually, the development of carbon-based coatings to be applied on different structural materials as concretes [9], honeycomb monoliths [2] or foams [10], is reaching high interest due to their potential environmental applications: biological treatments, adsorption, or catalysis. Carbon-xerogels are very good candidates for this type of coatings and they are typically microporous materials with low mesopore volumes [11], being the presence of mesopores specially poor when carbon xerogels are formed in film shape [2,4]. Recently, several works in which microwave radiation is used during the synthesis process of resorcinol–formaldehyde organic gels have been published [11–13], and they show that this type of treatments make possible to obtain carbon xerogels in short periods of time, being this aspect very interesting from an economical point of view. On the other hand, the use of microwave radiation as a heating source for pyrolysis has already been shown as a very rapid method when microwave receptors are present in contact with the sample [14], nevertheless as far as we know, the microwave carbonization of resorcinol–formaldehyde organic gels has not been described yet, which can be due to the fact that these organic polymers hardly absorb the microwaves produced by standard ovens which normally operate at a frequency of 2.45 GHz [15].

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The nitrate toxicity from water consume is low, however the secondary effects from nitrates reduction to nitrites, and the later nitrosamide formation, are very dangerous for human and animal health [16].

On the other hand, high nitrate concentration in water favors the overpopulation of some aquatic plants, because they have a lot of available nitrogen for their proteins synthesis. This plant overpopulation in waters produce the eutrophication in rivers and lakes, where they also proliferate harmful microalgae [17,18].

Some heterotroph bacteria use the oxygen from nitrates and nitrites to breathe in water anaerobic conditions, being N_2 the final product of this process [19]. There are several bacteria genus which are able to carry out the water denitrification by means of the above mentioned mechanism, as *Pseudomonas* [20] or *Halomonas* [9]. Previous works showed that *Escherichia coli* (*E. coli*) supported on activated carbons can also be used in water denitrification processes [21–23]. The effect of the chemical characteristics of commercial and lab prepared activated carbons on the bacteria adsorption was studied, finding that the higher PZC and mineral matter were positive for the adsorption and for the denitrification process. On the other hand, bacteria adsorption of carbon also produced an enlargement of the surface negative charge which increased the adsorption capacity of metallic species in water [22].

This work collects the preparation method of carbon-xerogel films with well developed mesoporosity obtained by microwave assisted carbonization and also by conventional carbonization. Thus, the particular effects that the microwaves produce during the carbonization process on the final chemical characteristic of these materials, are shown; both, mesopore volumes and final oxygen contents of the carbon films do not depend on the carbonization degree developed by using microwaves. Bacteria *E. coli* were supported on the films, and the behavior of these in the denitrification of water solutions was evaluated. Bacteria supported on the films obtained by microwave assisted carbonization, showed the best denitrification behavior which would be related to a more favorable surface chemical properties of the films for the bacteria growing. The objective of this work is centered in two aspects: (i) the development of original carbon-based films from a new microwave-assisted carbonization process, as a new approach for ceramic coatings. (ii) studying the performance of the carbon films as bacteria supports for water denitrification.

2. Experimental

Six carbon-xerogel films with three different carbonization degrees were prepared from an original organic xerogel film (XO) using two different carbonization techniques: microwave assisted carbonization (series XMW) and conventional carbonization in thermal oven (series XF). The organic xerogel film was prepared by dissolving resorcinol (R) and formaldehyde (F) in water and using Cs_2CO_3 as polymerization catalyst (C). The stoichiometric R/F and R/C molar ratios were 0.50 and 2300 respectively. The mixture was stirred to obtain homogeneous solutions that were cast into flat glass molds. The molds were closed in order to avoid an evaporation processes during the later curing period which included 1 day at room temperature, 1 day at 50 °C and 5 days at 80 °C. After the curing, the films were thermally dried at 110 °C and atmospheric pressure. The thickness of the dry organic films was around 0.1 cm approx.

The microwave assisted carbonization process was carried out using an oven model Savoid MSG-20810-S, which was adapted with an entry of gases, heating with 800 W of power in radiation periods of ten cumulative minutes, always in a stream of argon. After each period there was a pause in the radiation also under an argon stream. For the first period of treatment the organic xerogel films were situated between two sheets of graphite as a microwave receptor in order to induce a greater warming on the surface of the organic film. During the second and subsequent periods the top graphite sheet was removed. The cooling of the carbonized material was also held in a stream of argon. In these conditions, carbon xerogel films with a percentage of weight loss of 13, 17 and 28 wt.% were obtained using 3, 4 and 7 treatment periods, respectively. Carbon xerogel films are referred as XMW13, XMW17 and XMW28 indicating the digits the weight loss percentage after carbonization in each case.

Other three carbon films, with the same carbonization degree developed in the XMW series, were obtained from the same original organic xerogel film, by conventional carbonization in a thermal oven. In these last cases the carbonization was carried out in an argon flow at 340, 400 and 520 °C during 30 min using a heating rate of 10 °C/min and therefore obtaining the corresponding 13, 17 and 28 wt.% of weight loss, respectively. These carbonization temperatures were chosen after a thermogravimetric study in argon of the organic xerogel film. The carbon films obtained by conventional carbonization are referred as XF13, XF17 and XF28 indicating the digits the weight loss after carbonization in each case.

The porous characteristics were studied by N_2 and CO_2 adsorption at 77 and 273 K, respectively, using an equipment Autosorb-1C Quantachrome, and applying BET, Dubinin–Radushkevich (DR) and BJH methods to the adequate adsorption or desorption data. The samples were also characterized by scanning electron microscopy (SEM) using a ZEISS DSM 950 (30 kV) scanning electron microscope, Infrared spectroscopy (IR) using a Nicolet 20SXB FTIR spectrometer, magnetic nuclear resonance (RMN) using a Bruker Advance 500 instrument equipped with a standard-bore 11.74 T superconducting magnet operating at 125.76 MHz for 13C, Raman, using a Micro-Raman JASCO NRS-5100 dispersive spectrometer with a 532 nm laser line, and elemental analysis using a Thermo Scientific – Flash 2000 elemental analyzer. Finally, pH of point of zero charge (PZC) measurements were obtained suspending 250 mg of each sample on 4 mL of degasified and distilled water. Suspensions were stirred and thermostated at 25 °C measuring the pH periodically until the reading was constant. The obtained final pH was considered as the PZC for each sample [24].

The bacterium used in the experiments was *E. coli*, ATCC 25922 strain, supplied by the Microbiology Department of the Granada University. To immobilise this bacterium on the different supports it was first incubated at 310 K for 2 days in 50 ml of Tryptic Soy Broth (TSB) media buffered at pH 7 in sterile conditions and aerobic medium. Then, 1 ml of this suspension was added to another that contained 0.4 g of the sterilised carbon film in 50 ml of TSB shaking this new suspension at 310 K for 3 days. After this time, the film with the immobilised bacteria was separated and washed many times with sterilised distilled water. Carbon films with the immobilised *E. coli* are hereafter referred to in the text with the name of the film followed by the letter B.

Bacteria supported on films so obtained (the above 0.4 g) were used to study the denitrification process of water solutions, which contained 100 ml of a sterilised nitrate solution of 40 mg l^{-1} (from $NaNO_3$) where a carbon source of 2.6 ml of ethanol was also added. The water solutions were buffered at pH 7 with an appropriate phosphate solution. Denitrification processes were carried out in batch mode, at 298 K under soft agitation, using special glass flasks as bio-reactor where the anaerobic conditions were always kept flowing with argon.

The denitrification process was monitored analyzing the nitrate and nitrite concentrations each day several times. The concentration of nitrate was measured directly into the bio-reactor with a selective electrode. For the analysis of nitrite concentration aliquots from the reaction media were taken. Nitrite concentration was determined at 543 nm after coupling diazotized sulfanilamide