

# 124 - EVALUATION OF THE MICROBIOLOGICAL INOCULATION PROCESS WITH BIOFILM USE ON GRANULAR ACTIVATED CARBON

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Highlights:

- $\cdot$  Granular activated carbon removes organic matter and micropollutants by adsorption and biodegradation.
- · Inoculation time combined with the supply of dissolved oxygen and nutrients generated conditions for microbial growth.
- SEM analysis showed the presence of microorganisms on the surface and in the pores of the CAG.
- Microbiome, flow cytometry and ATP are viable techniques for microbiological assessment of biofilms and BACs.

Keywords: BAC; biofiltration; biofim; microorganisms; microbiome;

#### **INTRODUCTION**

Biological treatment of drinking water aims to reduce water instability by oxidizing biodegradable organic matter and inorganic compounds in reduced form. Granular activated carbon (GAC) columns remove organic matter both by adsorption and biodegradation, and microbiological characterization of the biofilm and GAC using modern techniques is necessary. With the advent of molecular biology, research into the composition of biofilms associated with CAG has made great progress, and it is now possible to identify bacterial genera involved in the dynamics of organic matter biodegradation. Techniques such as fluorescence in situ hybridization (FISH), ATP concentration determination, flow cytometry (FCM), polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and metagenomics are currently essential to advance biofilm studies. It is therefore necessary to study ways and processes of inoculating CAG for use in treatment and to improve the biostability of drinking water in water treatment plants (WTPs). The aim of this work is to evaluate the process of inoculating microorganisms into fixed bed columns of CAG, considering: a) characterizing the biofilm and granular activated carbon for use in fixed bed columns; b) carrying out a literature review on the types of technology for detecting bacteria that colonize the BAC, and; 3) evaluating the BAC reactor in terms of the microbiological activity resulting from inoculation by biofilms.

#### **METHODOLOGY**

The biofilm chosen to inoculate the activated carbon was WTP supply water together with the biofilm adhered to the adduction chamber to enhance the concentration of microorganisms and consequently the inoculation of the charcoal. The physicochemical and microbiological characteristics of the biofilm followed APHA, 2017 and are shown in Table 1. The granular activated carbon (GAC) used was Synth













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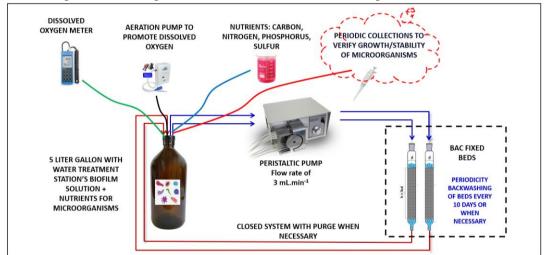
brand with a grain diameter of between 1 and 2 millimeters. The characterization of the GAC used BET, BJH, Raman, SEM, EDS and FT-IR methods.

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PARAMETERS	UNITS	VALUES	PARAMETERS	UNITS	VALUES
Turbidity	NTU	5,65	TOC	mg.L <sup>-1</sup>	4,08
Color	uC	35,00	Total solids (TS)	mg.L <sup>-1</sup>	194,00
pН	-	6,86	Total dissolved solids (TSD)	mg.L <sup>-1</sup>	48,00
Conductivity	µS/cm (25°C)	63,88	Total suspended solids (TSS)	mg.L <sup>-1</sup>	146,00
COD	mg O <sub>2</sub> .L <sup>-1</sup>	47,05	Total coliforms	cfu/100 mL	148,30
BOD <sub>5</sub>	mg O <sub>2</sub> .L <sup>-1</sup>	9,50	E. coli	cfu/100 mL	2,00

Table 1: Physicochemical and microbiological characteristics of the raw water supply sample (biofilm).

The inoculation and biological activity of the GAC filter was guaranteed by promoting dissolved oxygen (DO) from an air pump with a flow rate of 4 L.min-1 by means of a silica wafer-type diffuser placed inside the biofilm reservoir with a volume of 5 liters and the introduction of nutrients such as nitrogen, phosphorus and sulfur (figure 1).

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Samples for PCR tests to verify DNA amplification followed the standard methodology according to Fish (2017). The microbiome tests followed the Caporaso et al. 2010 method's, to identifying the bases of the 16S rRNA gene (bacterial and archaeal communities) and the Bradley, et al. 2016, method's to identifying the 18S rRNA bases (eukaryotes).

## **RESULTS AND CONCLUSIONS**

The characterization of CAG using the BET and BJH methodologies showed a coal with a surface area of 486 m<sup>2</sup>.g<sup>-1</sup>, a pore volume of 0.2753 c<sup>3</sup>.g<sup>-1</sup>, an average pore diameter of 11.34 Å and a pH of 8.85, classified as a micropore type coal (< 20 Å). The Raman and EDS tests showed it to be a coal with a high aromaticity O/C = 0.15 (when the oxygen/carbon O/C ratio is lower) which generates a better affinity for treating organic substances and those with aromatic rings. After 4, 7 and 10 months of inoculation of the biofilm on the activated carbon sample (with control of DO and nutrients), samples of the carbon were taken for SEM tests to check for the presence of microorganisms, which found









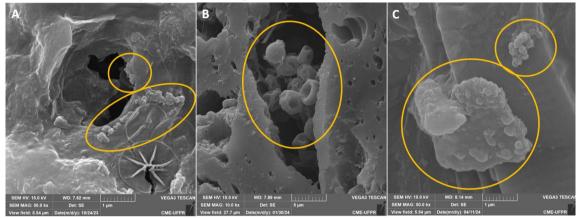




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cylindrical and pennate diatoms and circular clusters resembling a colony of bacteria (figure 2). DNA was also extracted from the biofilm and GAC samples for PCR testing. Both showed relevant DNA concentrations of around 6.7 ng. $\mu$ l<sup>-1</sup> in the preliminary NanoDrop test.

Figure 2: Result of SEM images – Scanning Electron Microscopy showing microorganisms on the surface of the inoculated granular coal sample after four (A), seven (B) and ten months (C).



(A) 50,000x magnification; (B) 10,000x magnification; (C) 50,000x magnification.

After this stage, microbiome tests were carried out on the biofilm and granular activated carbon samples inoculated with the biofilm, which showed positive results in comparison with the 16S rRNA and 18S rRNA bases for the biofilm and 16S rRNA for the inoculated granular carbon, which proved that the inoculation technique employed achieved its objective.

## ACKNOWLEDGMENTS

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