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Aquatic Natural Organic Matter (NOM) Removal in Biological Filters

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The aim of this work was to study the biodegradability of aquatic natural organic matter (NOM) through measurements of the biodegradable dissolved organic carbon (BDOC) in several water samples. Two different bioassays were used to determine BDOC: the Billen-Servais method (inoculum: raw water) and the Joret-Lévi method (inoculum: acclimated sand) and three different types of water were studied in this work: a natural water from the Úzquiza Reservoir (Burgos, Spain), a synthetic water prepared using natural fulvic acids extracted from the Úzquiza Reservoir and a synthetic water prepared using a commercially suppliedhumic acid. The natural water from the Úzquiza Reservoir (Burgos, Spain) showed the most biodegradable NOM whereas the solutions of pure humic substances had a low biodegradability, showing values slightly higher the fulvic acids. The Billen-Servais method (inoculum: raw water) gave substantially lower BDOC values than the Joret-Lévi method (inoculum: acclimated sand) for the three types of NOM studied. Additionally, several filtration tests were performed using columns filled with either sand (a non-adsorbing media) or granular activated carbon: GAC (an adsorbing media), including two operating conditions: sterile filtration and biologically active filtration. GAC filters showed a substantially greater TOC removal than sand filters, due to the high adsorptive capacity of GAC for humic substances; biologically active filters showed a slightly greater efficiency (about 3%) than sterile filters, which was similar regardless of the type of filter used (GAC or sand).

Key words: Biodegradation assays, Biodegradable dissolved organic carbon (BDOC), Granular activated carbon (GAC) filters, Sand filters,humic substances, Natural organic matter (NOM).

Several studies have shown the existence of microbial regrowth in drinking water distribution systems despite the correct quality of the drinking water coming from the drinking water treatment plant. This regrowth potential is usually related to a relatively high amount of biodegradable dissolved organic carbon (BDOC) present in water[1,2,3,4]; for instance, it has been reported thatthe use of ozone as a final disinfectant results in an increase in biodegradability of aquatic natural organic matter (NOM)[5,6,7] and therefore, it can lead to extensive bacterial regrowth in the distribution system, an obvious disadvantage of terminal ozonation[8,9,10,11,12]. Several reports of the presence of coliforms in chlorinated drinking water indicate that chlorine is not always effective for controlling regrowth[2,13]; furthermore, increased dosages of chlorine to control additional bacterial regrowth would lead to an increase in disinfection by-products (DBP) formation. Therefore, reducing the concentration of biodegradable compounds in water to sufficiently low levels in important in controlling regrowth.

Microscopic observation and cultivation techniques have shown that these bacteria are mostly present on the walls of distribution pipes, that is to say, in the form of pipeline biofilms. Bacteria growing in these biofilms are chemoheterotrophic and most of these

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microorganisms use oxygen as a hydrogen acceptor; besides coliform bacteria, representatives of the genera *Flavobacterium*, *Cytophaga*, *Xanthomonas*, *Arthrobacter*and*Corynebacterium*have been identified frequently in drinking water[8]. The major problem with regrowth is the multiplication of opportunistic potentially pathogenic bacteria, e.g., *Legionella pneumophila*(in hot-water systems), *Mycobacterium kansasii*, *Pseudomonasaeruginosa*and*Klebsiellapneumonia* [13]. Emtiazireported that biofilms of different drinking water conditioning sampling sites were made up by different microbial populations and even within one compartment the biofilms were not unique[14]. Most of the bacteria identified by Emtiazi in his study were beta-*Proteobacteria*; saprophytic mycobacteria and legionellae were also detected in all biofilms. *Pseudomonas aeruginosa* and facultatively pathogenic mycobacteria were detected sporadically (even directly after UV disinfection) whereas *Legionella pneumophila* and the indicators of faecal contamination, *Enterococusfaecium* and *Enterococusfaecalis* were not found in any biofilm.

DOC cannot be used to predict the level of bacterial regrowth in distribution systems, since in most environments only a small fraction of DOC is actually susceptible to microbial attack, the rest being made of refractory organic compounds (mainly humic substances) not available for bacterial growth[15,16]. The traditional biochemical oxygen demand (BOD) test is not usually sensitive enough for the low carbon concentrations of drinking waters[17]. Therefore, BDOC is the most suitable parameter to which growth and activity of heterotrophic microorganisms respond in natural waters[18,19,20].

One of the most useful methods to eliminate BDOC in drinking water treatment plants is the use of biofilters (biologically active filters), consisting of porous media with high specific surface area on which a large amount of aerobic biomass grows naturally when waters containing biodegradable organics are treated; there are two main types of porous media: sand (a non-adsorbing medium) and granular activated carbon (GAC, an adsorbing medium). The combination of the adsorption and biodegradation mechanisms in GAC is usually called biological activated carbon (BAC)[12,21,22,23,24,25,26,27,28]; the biodegradation is a result of the presence of microorganisms on the external surface and in micropores of the GAC[29]. Some authors have reported that GAC filters present a higher efficiency than non-adsorbing filters, not only due to the contribution of the adsorption mechanism to NOM removal but also due to an observed increase in biodegradation rates for GAC filters relative to non-adsorbing media. According to some authors[2], this behaviour may be due to a better utilization of sorbed substrate (the ability of GAC to better adsorb and retain organic matter would increase the chance of biodegradation) [25], a more favourable acclimation environment and/or a higher surface area of GAC relative to sand, which means that GAC filters can support a larger bacterial population; for instance, Krasner[30] reported a number of about 150 million bacteria/cm^{3}GAC whereas only approximately 2-5 million bacteria/cm^3 sand. Other investigators even reported a greater amount of biomass attached to GAC than the previously mentioned values, such as Velten[31] who reported 5 x 10^9 cells/cm^3 GAC and Magic-Knezev[32] who reported 0.1-4 x 10^{10} cells/cm^3 GAC; this latter author also reported a lower amount of bacterial population for sand filters in comparison with GAC filters (3 x 10^7 - 2 x 10^8 cells/cm^3 sand for slow sand filters and 3 x 10^7 - 2 x 10^{10} cells/cm^3 sand for rapid sand filters). However, other authors indicated that the rate and efficiency of biodegradation in GAC filters is approximately similar to that observed in non-adsorbing filters and that GAC and sand filters contain similar number of bacteria[33]. An exhaustive review of biofilm processes in BAC filters was reported by Simpson[34].

The combination of pre-ozonation and biofiltration has been found to be very effective for NOM removal, since ozonation increases BDOC levels in water [19,25,35,36,37,38,39,40]. Some authors[41] have reported that most of BDOC is likely to be biodegraded on the GAC surface before adsorption, therefore, the production of BDOC by ozonation prior to GAC treatment would favour the extension of the GAC service life and the reduction of DOC loading to GAC[34]. However, other authors have reported that in the case of waters having low SUVA (UV_{254}/TOC) values (which indicates a high biodegradability of the NOM present in the water), ozone application does not improve DOC biodegradation efficiencies of the biofilters[25]. Finally, another advantage of BAC filters is that they have also been shown to be useful for the removal of bromate, a typical ozonation by-product[42].

In this study, BDOC was determined for several types of water (natural and synthetic) using two different biodegradation assays; additionally, a synthetic water was treated by filtration using sterile and biological activated GAC and sand filters in order to study the efficiency of both types of filters.

MATERIALS AND METHODS

Types of water

Three different types of water have been used in this study: a natural water from the Úzquiza Reservoir (Burgos, Spain), a synthetic water prepared using natural fulvic acids extracted from the Úzquiza Reservoir and a synthetic water prepared using a commercially supplied humic acid (Aldrich Co. UK).

Extraction of fulvic acids

The extraction of fulvic acids from the reservoir water is based on the resin adsorption procedure described by Thurman [43]. In this procedure Amberlite XAD-7 resin is used to isolate the humic substances, pumping initially the natural water through the column at pH 2.0; humic substances adsorbed on the resin are eluted with 0.1 N NaOH. Fulvic and humic acids are then separated by precipitation at pH 1.0; after precipitation for 24 h, the sample is centrifuged (8000 rpm – 20 min): humic acids precipitate whereas fulvic acids remain in solution [44].

TOC Analysis

TOC (Total Organic Carbon) was measured with a carbon analyzer (Shimadzu TOC-5050), based on the combustion-infrarred method.

Biodegradation assays

Two different biodegradation assays were performed, both based on the measurement of the biodegradable dissolved organic carbon (BDOC). BDOC is the fraction of the dissolved organic carbon (DOC) which can be metabolized by bacteria within a period of a few days to a few months; in drinking water treatment, the removal of this form of carbon is of particular importance because BDOC reaching the distribution system can be responsible for bacterial regrowth and associated problems [15,45].

In the two BDOC methods used in this study, an inoculum of autochthonous bacteria (usually water from the same environment as the sample) is added to the samples. After incubation for several days, BDOC is calculated as the difference between the initial and the final DOC (DOC remaining at the end of the incubation period); this minimum value of DOC observed after incubation is called refractory dissolved organic carbon (RDOC), which can be defined as the fraction of DOC not biodegradable by the bacterial inoculum [46].

The two bioassays performed are the following:

Method of Billen-Servais

In this method [15]the water sample (filtrated through a 0.2-mm filter) is inoculated with a small amount (usually 1% by volume) of raw water (or collected at some point in the process, usually after sand filtration), which has been passed through a 2-mm filter in order to eliminate protozoa. After the incubation period (usually in darkness at 20ºC for 28 days), BDOC is obtained from the difference between the initial and the final DOC.

The Billen-Servais method was used in this study with slight modifications [7]: 150-ml water samples were inoculated with 5 ml of raw water from the Úzquiza Reservoir. Based on preliminary tests the incubation period was reduced to 7 days, since DOC levels were suitable for accurate analyses. Incubation temperature was set at 25ºC.

Method of Joret-Lévi

This method [19,47]provides a more rapid assay for BDOC than the previous one, using prewashed, biologically active sand as inoculum. This sand is normally taken from a drinking water treatment plant that does not use prechlorination and it is washed with distilled water until there is no detectable release of DOC; then, the water sample is inoculated with the sand (usually 100 g of sand per 300 ml of water). Incubation takes place in darkness for 8 days (in some cases for 3-5 days). BDOC is taken as the decrease in DOC during the test[48,49].

The Joret-Lévi method was used in this study with slight modifications[7]: the sand inoculum was not taken from a treatment plant but a bioacclimated medium was produced in the laboratory: an acclimated biofilm was established on 2-3 mm diameter sand media by seeding it with raw water from the Úzquiza Reservoir and continously feeding it with the raw water over a period of three months. 150-ml water samples were inoculated with 50-ml of bioacclimated sand. Incubation took place in darkness at 25ºC for 7 days to coincide with the previous test. An additional sand media was acclimated with synthetic solutions of the pure humic substances inoculated with natural water in order to check the relative efficiency of both acclimated filters.

For the two methods, samples were filtered through a porous membrane (0.45 mm) previously washed with distilled water before analysis of DOC[50] (APHA 2005),in order to avoid organic carbon contamination.

Filtration column tests

Filtration tests were performed on GAC and sand column filters using two different operating conditions: sterile filters (using $HgCl_2$ to avoid microorganism growth on the filter) and biologically active filters (seeded with natural water from the reservoir). The columns (4-cm-diameter and 1-m-height glass cylinders) were fixed bed operated in up-flow mode and the operating parameters were the following: the depths of filter media (sand or GAC) were 50 cm, the empty bed contact time (EBCT) in the columns was 17 min and influent flow rate was 1.2 m/h. According to some reports [51,52], biofilters usually reach steady state (the biofilter has developed a mature biofilm) after 40-50 days from the start-up process, corresponding to roughly 3800 bed volumes of operation; in this study, the biofilters were operated during 45 days (3800 bed volumes) before data collection for the experiments.

RESULTS

The results for BDOC measurements are shown in Figure 1. Due to the fact that for the three types of NOM studied different initial TOC concentrations were used, the absolute BDOC values obtained cannot be compared directly; this comparison can be made by analyzing the BDOC-to-TOC ratio (that is, the percentage of BDOC in each case), as shown in Figure 1. It can be seen that the NOM from the natural reservoir water was the most biodegradable; the pure humic substances (fulvic and humic acids) had a low biodegradability, showing values slightly higher the fulvic acids, which consist of smaller macromolecules than the humic acids; this result was similar for the two biodegradation assays used in this study. The Billen-Servais method (inoculum: raw water) gave substantially lower BDOC values than the Joret-Lévi method (inoculum: acclimated sand) for the three types of NOM studied.

Table 1. Efficiency percentages (%) of filters for NOM removal

Type of filter	Percentage of toc removal (%) Sterile	Biological	Difference(%)
Sand	3.3	6.3	3.0
GAC	82.4	85.3	2.9

NOM: commercial humic acids.

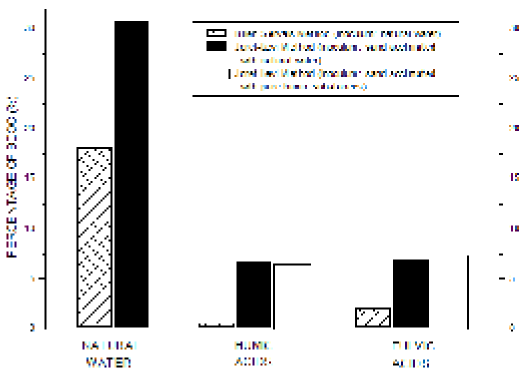

Fig. 1. BDOC measurements (% BDOC: BDOC/TOC ratio) for several types of water

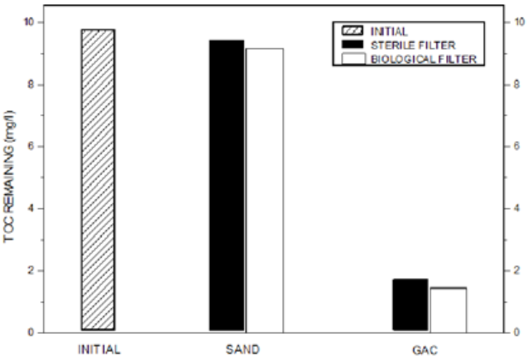

Fig. 2. Filtration tests: sand and GAC filters operating on either sterile or biologically active conditions. NOM: commercial humic acids, initial TOC = 9.78 mg/l.

Figure 2 shows the results of the filtration tests for both types of filters, sand (non-adsorbing) and GAC (adsorbing) filters, each one of them studied using two operating conditions: sterile and bioacclimated media; the water used in these tests was a synthetic solution of commercial humic acids. Sterile filters only remove NOM from water via adsorption whereas in biologically active filters adsorption and biodegradation occur together; the difference between them represents the TOC removed via biodegradation. It can be observed in Figure 2 that sand filters (both, sterile and biologically active) only achieved a slight TOC removal whereas GAC filters removed NOM with much greater efficiency (percentages of TOC removal around 82-85%); for both cases, biologically active filters achieved a slightly higher TOC removal (about 3%) than sterile filters.

DISCUSSION

As was reported earlier, Figure 1 shows that the pure humic substances were less biodegradable than the natural water from the reservoir. Humic substances consist of large organic macromolecules that can be considered as refractory to biodegradation [16,53]; these substances only account for about 50% of TOC for the natural water from the Úzquiza Reservoir[44], the rest of the organic matter being comprised of smaller and more readily biodegradable compounds (low molecular weight hydrophilic acids, amino acids, etc), which explains the higher BDOC percentage value found for the natural water. The BDOC value measured in the present study for the Úzquiza reservoir water (0.45 mg C/l for the Billen-Servais method) is within the range of values reported in the literature (0.4 – 0.9 mg C/l) for natural waters with low TOC content[15]; concerning the Joret-Lévi method, the BDOC/TOC ratio measured for the Úzquiza reservoir water (about 30 %) is also in accordance with the literature, where BDOC percentages about 10 – 50 % DOC [19] and 20 – 65 % DOC[54]are reported.

Generally, organic compounds with high values of SUVA (specific ultraviolet absorbance: UV-to-TOC ratio) have a low biodegradability, due to the greater presence of aromatic and other unsaturated configurations[5,55]. According to the data reported by Yapsakli[25] and based on the classification proposed by Edzwald&Tobiason[56], waters with SUVA values > 4 consist mainly of high molecular weight substances (e.g., humic acids) showing a low degree of biodegradability whereas SUVA values < 2 indicate waters with a high content of low molecular weight and highly biodegradable compounds; SUVA values between 2 and 4 correspond to intermediate situations. This is in accordance with the SUVA values measured for the waters studied in this work[44]: commercial humic acids (5.0 L/mg × m) > natural fulvic acids (2.9L/mg × m) > natural water (2.5L/mg × m).

Another observation that can be deduced from Figure 1 is that the Billen-Servais method (inoculum: raw water) gave substantially lower BDOC values than the Joret-Lévi method (inoculum: acclimated sand) for the three types of NOM studied; this result indicates that the biodegradation carried out by biofilm bacteria (bacteria attached to a support medium) was more efficient than that performed by bacteria suspended in water, due mainly to the greater amount of biomass present in the biofilm (although it also raises the possibility of release of organic matter from this biomass or adsorption of organic matter from solution by the biomass)[57]. Some authors have found similar results in analogous bioassays [11,58]and some of them have proposed that the BDOC bioassays based on acclimated biofilms are more useful than those based on suspended bacteria, since acclimated bacteria attached to surfaces mimic the bacterial regrowth process in the distribution system (which also consists of bacteria attached to a surface: the walls of distribution pipes) more completely than the use of unattached species of bacteria [11,57].

Figure 1 also shows that there was no substantial difference between using either sand acclimated with natural water or with pure humic substances (Joret-Lévi method) for the solutions of humic and fulvic acids. This result was expected for the fulvic acids since they represent the majority organic fraction (45% of the whole NOM) of the Úzquiza Reservoir water[44]; however, even though a higher efficiency of sand acclimated with humic acids was initially expected for the biodegradation of the commercial humic acids (since these compounds are not present in the natural water), the results showed no net appreciable effect.

Figure 2 shows NOM removal achieved in the filtration tests. The slight TOC removal achieved by the sterile sand filter is attributed to a minimum adsorption of the humic acids onto the sand surface; as was expected, the sterile GAC filter showed a greater TOC removal than the sterile sand filter, due to the high adsorptive capacity of GAC for humic substances. Concerning the comparison between sterile and biologically active filters, it can be observed in Figure 2 that biologically active filters showed a slightly greater efficiency than sterile filters, which was similar regardless of the type of filter used (GAC or sand).

The percentages of TOC removal achieved in the filtration tests are shown in Table 1, where it can be seen that the contribution of the biodegradation mechanism to NOM removal was about 3% (see column "difference" in Table 1). This result is in agreement with some studies from the literature, such as those by Yapsakli[25] and Velten[27], who reported 2-5% and 3% of biological removal of DOC respectively. However, the literature is not consistent with regard to this issue, since other authors have reported greater DOC biological removals, such as 8% [59] and as high as 15% [38]. Velten also suggested that low yield in biofilters is beneficial for operation, since by-product formation (in this case bacterial cells) is low in comparison with target-compound removal (in this case NOM).

The biological removal of TOC achieved in this study (3%) was similar for both types of filters (GAC or sand), therefore our results agree with the hypothesis that biofilms (bacterial biomass) grown on either, adsorbing (GAC) or non-adsorbing (sand) media, show similar efficiencies for biological removal of NOM[21,33].

CONCLUSIONS

The biodegradability of three different types of NOM has been studied in this work using several biodegradation assays and filtration tests.

The natural water from the Úzquiza Reservoir (Burgos, Spain) showed the most biodegradable NOM; the solutions of pure humic substances (natural fulvic acids extracted from the mentioned reservoir and commercial humic acids) had a low biodegradability, showing values slightly higher the fulvic acids, which consist of smaller macromolecules than the humic acids.

The Billen-Servais method (inoculum: raw water) gave substantially lower BDOC values than the Joret-Lévi method (inoculum: acclimated sand) for the three types of NOM studied. There was no appreciable effect between using either sand acclimated with natural water or with pure humic substances for the biodegradation of the commercial humic acids and the natural fulvic acids using the Joret-Lévi method.

GAC filters showed a substantially greater TOC removal than sand filters, due to the high adsorptive capacity of GAC for humic substances; biologically active filters showed a slightly greater efficiency (about 3%) than sterile filters, which was similar regardless of the type of filter used (GAC or sand).

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