Quantifying bacterial biomass fixed onto biological activated carbon (PAC and GAC) used in drinking water treatment

Céline Stoquart, Benoit Barbeau, Pierre Servais and Gabriela A. Vázquez-Rodríguez

ABSTRACT

Hybrid processes coupling the use of powder activated carbon (PAC) with membrane filtration for drinking water production are emerging as promising alternatives to conventional technologies due to their enhanced control of dissolved contaminants. The quantification of biomass colonizing PAC is crucial for modeling, designing control strategies and improving the overall performance of these processes. The aim of this study was to examine the applicability of several common methods developed for colonized granular activated carbon (GAC) to PAC. Six analytical methods (based on the measurement of proteins, polysaccharides, heterotrophic plate counts, potential glucose respiration, bacterial ATP and a potential acetate uptake rate, which is proposed herein) were compared. The results showed that the rates of glucose respiration and of acetate consumption could be used interchangeably. Proteins were also an interesting alternative for on-site measurements. It was concluded that biological PAC-based processes sustained a level of heterotrophic activity similar to or greater than that observed in GAC biofilters.

Key words | biofilters, drinking water, extracellular polymeric substances, microbial activity, substrate uptake rates

Céline Stoquart
Benoit Barbeau
Gabriela A. Vázquez-Rodríguez (corresponding author)
NSERC Industrial Chair on Drinking Water,
Department of Civil, Mining and Geological Engineering,
École Polytechnique de Montréal,
NSERC-Industrial Chair in Drinking Water,
C.P. 6079, Succursale Centre-Ville,
Montréal, QC,
Canada H3C 3A7
E-mail: *g.a.vazquezr@gmail.com*, *gvazquez@uaeh.edu.mx*

Gabriela A. Vázquez-Rodríguez
Permanent address:
Centro de Investigaciones Químicas,
Universidad Autónoma del Estado de Hidalgo,
Carr. Pachuca-Tulancingo km. 4.5,
42184 Mineral de la Reforma, Hgo.,
Mexico

Pierre Servais
Écologie des Systèmes Aquatiques,
Université Libre de Bruxelles,
Campus de la Plaine, CP 221,
Boulevard du Triomphe,
B-1050 Brussels,
Belgium

INTRODUCTION

Biological filters have been used extensively for the production of drinking water in North America and Europe. Some of their advantages include the biological stabilization of treated water via the removal of dissolved organic matter and ammonia, a decrease in chlorine demand and the formation of fewer disinfection by-products. Currently, these processes are becoming increasingly important due to the rise of green technologies and the emergence of new contaminants that may be amenable to biodegradation, such as several pharmaceuticals, taste and odor compounds and cyanotoxins (Brown 2007). Granular activated carbon (GAC) is the preferred medium for biological filters because it offers more sites for microbial attachment than non-adsorptive supports (e.g. sand or anthracite) (Wang *et al.* 1995). Moreover, both the irregular surface and the porous structure of GAC protect the biomass against shear forces and toxic loads (Chaudhary *et al.* 2003; Herzberg *et al.* 2003).

Recent developments in membrane technologies for drinking water production include the integration of powder activated carbon (PAC) in processes referred to as hybrid membrane processes (HMPs) (Stoquart *et al.* 2012). PAC targets dissolved compounds that are not retained by low-pressure membranes. It offers faster adsorption rates than GAC (Yener *et al.* 2008) and can be injected and recovered continuously from a slurry-type suspension. This

doi: 10.2166/aqua.2013.101

configuration offers high flexibility to address sudden source water quality variations by simply adjusting the age of the PAC slurry to control adsorption performance (Stoquart *et al.* 2012).

When using activated carbon (AC), biodegradable compounds can be removed by adsorption, biodegradation or a combination thereof. Within the HMP, the relative importance of both mechanisms is intimately related to the age of the PAC slurry. Assessing the relative importance of both mechanisms is paramount for modeling the process and thus optimizing its performance. Therefore, reliable techniques for evaluating microbial colonization onto PAC are required.

Several methods have been developed to evaluate bacterial biomass fixed on GAC. As proposed by Lazarova *et al.* (1994), they can be classified as either non-destructive or destructive (i.e. techniques that disrupt the biofilm). Non-destructive methods include direct microscopy and techniques based on the measurement of specific activities, such as the potential glucose respiration (PGR) (Servais *et al.* 1991), the overall oxygen consumption (Urfer & Huck 2001) or the potential nitrifying activity developed to estimate the autotrophic nitrifying biomass (Kihn *et al.* 2000). On the other hand, destructive methods, which require detaching the biomass from the support media, include total cell counting by epifluorescence (Nishijima *et al.* 1997), the enumeration of heterotrophic plate counts (HPC) (Camper *et al.* 1985a, b) and the measurement of compounds related to the viable biomass, such as phospholipids (Wang *et al.* 1995) and ATP (Velten *et al.* 2011), and the biofilm density, such as the concentrations of polysaccharides (Quintelas *et al.* 2008) and proteins (Drogui *et al.* 2012; Papineau *et al.* 2012) in extracted extracellular polymeric substances (EPS).

All the aforementioned techniques have only been applied to GAC, not biological PAC. Non-destructive methods are the most interesting because the detachment of the biomass is undesirable due to issues such as incomplete recovery and the impact of the procedure on cellular integrity (Lazarova *et al.* 1994). Amongst the non-destructive methods, PGR allows the biomass potentially responsible for the removal of biodegradable compounds to be evaluated. However, the PGR method requires the use of a radiolabeled substrate (i.e. ^{14}C-glucose), which is expensive, not always readily available and may pose logistical challenges. One promising alternative substrate is acetate, a ubiquitous compound that can be used as a substrate by almost all eubacterial and fungal cells (Wang *et al.* 1995). For instance, the abundance of acetate-utilizing bacteria has been estimated microautoradiographically as 47–93% of the total bacteria in wastewater-activated sludge (Nielsen & Nielsen 2002). In addition, acetate is a common ozonation by-product (Urfer & Huck 2001). Hence, acetate-biodegraders are likely to be present in drinking water biofilters. Moreover, acetate is poorly adsorbable onto GAC (Moteleb *et al.* 2002) and PAC (Stoquart *et al.* 2013), which minimizes potential bias in the evaluation of the acetate uptake rate. Thus, measuring the potential acetate uptake (PAU) rate could be an interesting alternative for evaluating active bacterial biomass fixed on solid media.

To the best of our knowledge, no published studies have aimed to quantify the biomass developed on the PAC surface from drinking water treatment processes. This work was thus conducted to evaluate the applicability of several existing analytical methods (i.e. the assessment of PGR, HPC, ATP and EPS) and to propose a new method based on measuring PAU rates for characterizing heterotrophic biomass on biological PAC obtained from two hybrid pilot membrane processes. The results obtained for PAC were also compared with those measured on a biological GAC (BAC) sample collected from a full-scale biofilter. Finally, the results obtained from the six tested analytical methods were compared.

MATERIALS AND METHODS

Description of samples

AC characteristics

Three types of wood-derived AC commercialized by Pica™ (Picahydro LP 39, Picahydro L30-260 and Picabiol 2) were tested; the non-colonized media are referred to as NP25, NP200 and NGAC, respectively. NP25 and NP200 are PAC with median particle sizes of 15–35 and 210–260 μm, respectively. NGAC has a median particle size of 500–700 μm. Their iodine numbers vary between 900 and 1,000 mg/g.

AC colonization

The colonization of PAC was carried out in four parallel HMP pilots operating under two configurations. The pilots were fed with coagulated-settled river water (Des Mille-Iles River, Laval, Canada). The average main influent characteristics were 2.9 mg DOC/L, 0.23 mg BDOC/L and 154 μg N-NH_3/L. In the first configuration (PAC/UF), the PAC treatment was integrated to a submerged ultrafiltration (UF) unit described in detail by Léveillé *et al.* (2013). Two PAC/UF reactors were filled with NP25. In the first AC/UF reactor (P7-5), a PAC concentration of 5 g dry weight (dw)/L, a solids retention time (SRT) of 7 days and a hydraulic residence time (HRT) of 69 min were maintained. In the second PAC/UF pilot (P60-10), the PAC concentration, the SRT and the HRT were 10 g dw/L, 60 days and 77 min, respectively.

In the second configuration (PAC + UF), water was fed into two parallel stirred-tank reactors filled with NP200, which was maintained inside the reactors using a 55–85 μm sieve. The effluent water was then fed into the UF modules. The PAC concentrations within the reactors were 1 g dw/L (P67-1) and 5 g dw/L (P67-5). Unlike the PAC/UF configuration, both reactors were operated continuously without any PAC replacement (i.e. with variable SRT). At the sampling time, an SRT of 67 days had been reached in both PAC + UF reactors.

The BAC samples were obtained from the surface of a full-scale dual media filter (80 m^2 surface area) composed of layers of sand ($D_{10} = 0.45$ mm, $H = 15$ cm) and GAC (with NGAC-type characteristics; $H = 180$ cm). The empty bed contact time in the filter ranged from 20 to 30 min depending on the water demand.

A summary of the characteristics of the five colonized AC samples is presented in Table 1.

Biomass measurements

The biomass measurements were performed on the same day for all analytical techniques and sample types tested (i.e. the five colonized samples and the three virgin samples). The virgin samples (NP25, NP200 and NGAC) were used as controls. Before any experiment, 10 g dw of each of the virgin AC samples was humidified overnight at room temperature in 1 L of Milli-Q water. The pH of the slurry (approximately 3) was raised to 7.0 by adding 1 M NaOH. The colonized PAC slurries (P7-5, P60-10, P67-1 and P67-5) were withdrawn at the same time from the four reactors. All the PAC samples (colonized and virgin) were recovered by filtration on a Grade 41 paper filter (Whatman). Finally, the BAC sample was collected from the surface of the biofilter.

PAU rate

According to Monod kinetics, the rate of substrate depletion (dS/dt) and the specific microbial growth rate (μ) can be expressed by Equations (1) and (2), respectively:

$$\frac{dS}{dt} = -\frac{\mu X}{Y} \quad (1)$$

Table 1 | Biological AC sample characteristics

Sample ID[a]	Type of carbon	Median diameter (μm)	Pilot unit	Carbon used for starting up the pilot	SRT (days)	Concentration in the reactor (g dw/L)
P7-5	PAC	15–35	PAC/UF[b]	NP25	7	5
P60-10	PAC	15–35	PAC/UF[b]	NP25	60	10
P67-1	PAC	210–260	PAC + UF[c]	NP200	67	1
P67-5	PAC	210–260	PAC + UF[c]	NP200	67	5
BAC	GAC	500–700	Biological GAC	NGAC	N.A.[d]	350

[a]The first number refers to the age (d) of the carbon and the second number refers to the PAC concentration (g dw/L) within the contactor.
[b]Hybrid membrane process with integrated AC treatment.
[c]Hybrid membrane process with AC pretreatment.
[d]N.A.: not available.

$$\mu = \mu_{\max} \frac{S}{S + K_S} \quad (2)$$

where X is the biomass concentration, Y is the growth yield, $\mu_{\max}$ is the maximum specific growth rate and K_S is the half-saturation constant. Equation (3) is obtained by substituting Equation (2) into Equation (1) as follows:

$$\frac{dS}{dt} = -\frac{\mu_{\max} X}{Y} \cdot \frac{S}{S + K_S} \quad (3)$$

If $S >> Ks$, Equation (3) can be simplified as:

$$\frac{dS}{dt} = -\frac{\mu_{\max} X}{Y} \quad (4)$$

q_S, the specific substrate removal rate, is defined by Equation (5):

$$q_S = -\frac{dS}{X\,dt} \quad (5)$$

Thus, Equation (4) can be rewritten to give Equation (6):

$$q_S = \frac{\mu_{\max}}{Y} \quad (6)$$

Equation (6) shows that under substrate saturating conditions (i.e. $S >> Ks$), the substrate is removed following zero-order kinetics during the first few hours of incubation. Thus, q_S (hereafter referred to as the PAU rate) can be considered an estimate of the microbial biomass fixed on the media because the uptake rate and biomass are proportional (Equation (6)).

The PAU rates were estimated in 1 L beakers containing 500 mL of test medium. The latter contained a saturating initial concentration of sodium acetate (15 mg Na-acetate/L, equivalent to 4.4 mg C/L) and was supplemented with NH_4Cl (0.65 mg N/L) and phosphate buffer (0.06 mg P/L). After adding 10 g wet weight (ww) of AC, the slurries were maintained at 20 °C under agitation at approximately 150 rpm using a magnetic stirrer. Samples were withdrawn periodically by filtering 40 mL of slurry through a 0.45 μm polyethersulfone membrane previously rinsed with 1 L of Milli-Q water.

The acetate concentration was determined in duplicate samples by ion chromatography (Dionex Corporation, Sunnyvale, USA). The apparatus was equipped with an IonPac AS18 column and suppressed conductivity detection. An EluGen® EGC-KOH cartridge was used as the eluent. The limit of detection, the limit of quantification, the reproducibility and the accuracy of this method are 0.0159 mg/L, 0.0530 mg/L, 3 and 4.5%, respectively. The PAU rates were calculated from the initial slope of the acetate content obtained by linear regression, normalized per gram of AC dw in the assay. These rates are expressed as specific acetate uptake rates in mmol Na-acetate per g AC dw per hour. Regressions made to calculate PAU rates were always significant ($p < 0.05$) with $R^2 > 0.96$, except for P7-5 ($R^2 = 0.61$) and P60-10 ($R^2 = 0.72$).

PGR rate

The production of $^{14}CO_2$ due to the mineralization of ^{14}C-glucose under saturating conditions was measured according to the method of Servais *et al.* (1991). This method uses a similar fundamental approach to the PAU method, although radioactive glucose replaces acetate as a substrate and mineralization (CO_2 production) is measured rather than substrate consumption. A mixture containing ^{14}C-glucose and non-radiolabeled glucose was prepared to obtain a final glucose concentration of 1 mM and a radioactivity of 0.1–1 μCi/mL. One milliliter of this mixture was added to 1 g ww of AC in a penicillin flask closed with a rubber septum. After 3 h of incubation at 20 °C, the sample was acidified by adding 2 mL of 10% H_2SO_4 through the septum. Samples were bubbled for 10 min to extract the CO_2, which was trapped in a mixture of Carbo-SorbE (Perkin Elmer) and PermafluorE+ (Perkin Elmer, 1:4 v/v). The radioactivity was determined by liquid scintillation with a Packard Tri-Carb (TR-1600) scintillation counter. The amount of glucose mineralized was measured in triplicate and expressed as nmol glucose per g AC dw per hour.

Heterotrophic plate counts

For each sample, 1 g ww of the AC sample was homogenized in a blender at 16,000 rpm and 4 °C for 3 min with a mixture of Zwittergent (10^{-6} M), EGTA (ethylene

glycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 10^{-3} M), Tris buffer (0.01 M, pH 7.0) and 0.1% peptone (Camper *et al.* 1985a). The culturable cells were analyzed in duplicate on decimal dilutions using the membrane filtration technique (9215 D Standard Methods, (APHA 2012)). The membrane filters were incubated on R2A agar at 20 °C in the dark. After 7 days, the HPCs were determined and expressed in CFU per g AC dw.

Bacterial ATP

An ATP detection kit was used (Profile®-1 Reagent Kit, New Horizons Diagnostics, USA). The kit consisted of a microluminometer, filtration devices (0.4 μm pore diameter), a lysing reagent for non-bacterial ATP (somatic releasing agent, SRA) and a second reagent ensuring both bacterial lysis and leaching of bacterial ATP (bacterial releasing agent, BRA). Prior to the analysis, the samples were homogenized, just as for the measurement of HPC, and then 10-fold diluted in phosphate buffer to minimize ATP adsorption on AC. After the filtration of 225 μL of the suspension in the filtration device, 60 μL of SRA was added and evacuated twice to discard the non-bacterial ATP. In a second step, 60 μL of BRA was added to the sample in the Filtravette device, filtered and recovered. Finally, 25 μL of the filtrate was mixed with 225 μL of reconstituted luciferin-luciferase reagent (Molecular Probes®, USA) in a new Filtravette device and the relative luminescence units were recorded. Positive controls were obtained by adding two standards with high concentrations of ATP (ranging from 0.8 to 2.7 μg ATP/L) to the samples. The results were compared to a standard curve (0.01–3 ng ATP/L) and expressed as ng ATP per g AC dw. No replicates were made.

EPS: polysaccharides and proteins

EPS were extracted in duplicate using approximately 2 g ww of sample according to the method proposed by Liu & Fang (2002). The samples were resuspended in 10 mL of phosphate buffer supplemented with 60 μL of formaldehyde (36.5% v/v) and then incubated at 4 °C for 1 hour under agitation. After the addition of 4 mL of 1 M NaOH, the samples were incubated for 3 h at 4 °C. Next, the slurries were centrifuged twice at 12,000 g and 4 °C for 15 min. Finally, the supernatants were recovered and filtered through sterile 0.45 μm pore size membranes.

The protein content of the extracts was determined using a bicinchoninic acid method-based commercial kit (Pierce© BCA Protein Assay Kit, Thermo Scientific, USA) with a bovine serum albumin (BSA) standard. The results were expressed in mg of proteins (equivalent BSA) per g of AC dw or in mg C per g of AC dw, knowing that carbon represents 45% of the mass of BSA. The polysaccharides were analyzed by the phenol-sulfuric acid method using glucose as a standard (Dubois *et al.* 1956). The results were expressed in mg polysaccharides (equivalent glucose) per g of AC dw or mg C per g of AC dw, knowing that carbon represents 40% of the molecular weight of glucose. The total amount of EPS was obtained by summing the concentrations of proteins and polysaccharides (both expressed as mg C per g of AC dw).

Dry weight

The virgin and biological AC were dried for 24 h at 105 °C according to the standard method 2540 B (APHA 2012) to obtain the dry weights of samples. The results of all microbiological measurements were then expressed per g of AC dw.

RESULTS AND DISCUSSION

The analytical methods described previously were applied to quantify the biomass fixed onto AC samples. Concerning biological PAC samples, different levels of biomass density were expected because the colonization was carried out under variable PAC ages and concentrations within the reactors. First, P7-5 and P67-5 were two PAC samples colonized at the same concentration in the pilot reactor (i.e. 5 g dw/L). Although these two PAC had distinctive sizes, no significant differences with respect to biomass density were expected due to this factor (Markarian *et al.* 2010). However, higher ages (67 vs. 7 days) were expected to lead to higher biomass densities for AC colonized under equivalent conditions. Second, two reactors were operated with the same PAC age (67 days) but variable PAC concentrations inside the reactor (P67-1 and P67-5). In that case, equivalent colonization (expressed per g of AC dw) is not

expected; Markarian *et al.* (2010) noted that the development of biomass on biological PAC is limited by substrate availability rather than the available colonization surface. Consequently, it was hypothesized that PAC colonized at a lower concentration in the pilot reactor would support a higher biomass density on its surface. The results gathered from each analytical method are presented and discussed separately as follows.

PAU rates

No decrease in the acetate concentration was observed in the experiments conducted with virgin materials, even after 24 h of contact (data not shown). This finding confirms that any acetate removal is due to biological activity.

The initial acetate concentration applied in the assays was approximately 3-fold higher than K_s (the value embedded in Biowin™ software is 5 mg/L). This concentration led to the expected zero-order kinetics for acetate removal when the biomass growth is negligible compared to the biomass initially present on the AC. Hence, all colonized samples removed acetate at a constant rate during the first 4–6 h (Figure 1).

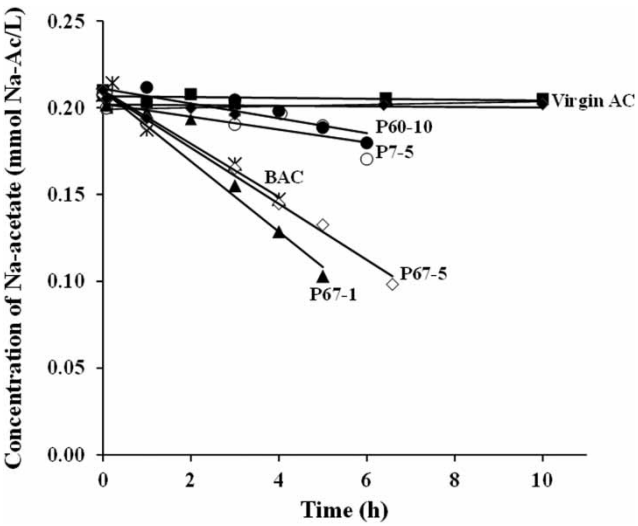

Figure 1 | Acetate uptake by virgin and colonized samples of AC. The symbols represent the experimental results, and the lines represent the data from the linear regression.

Figure 2(a) depicts the results of the PAU tests. The lowest PAU rate was found for the P7-5 sample, which was expected to have the lowest level of bacterial colonization due to the short SRT of the reactor (7 days), whilst the maximum PAU rate was measured for the P67-1 sample (colonized at the highest SRT). Actually, the PAU rate measured for the P67-1 sample was not significantly different from that measured for the biological GAC filter (i.e. the BAC sample). Sample P67-5 presented a statistically significantly higher PAU rate than sample P7-5 ($p < 0.05$) confirming that higher SRT led to a higher active biomass on the PAC surface. In addition, the PAC samples colonized at a lower PAC concentration (P67-1) had a statistically significantly higher PAU rate than its counterpart colonized at 5 g/L (P67-5) ($p < 0.05$). In this way, the positive effect of a high substrate availability on the activity of the biomass fixed onto PAC appears to be confirmed too.

Based on these preliminary results, the measurement of PAU rates is a promising method. This finding should be confirmed by validating this method with a wider set of samples, including more BAC samples. In addition,

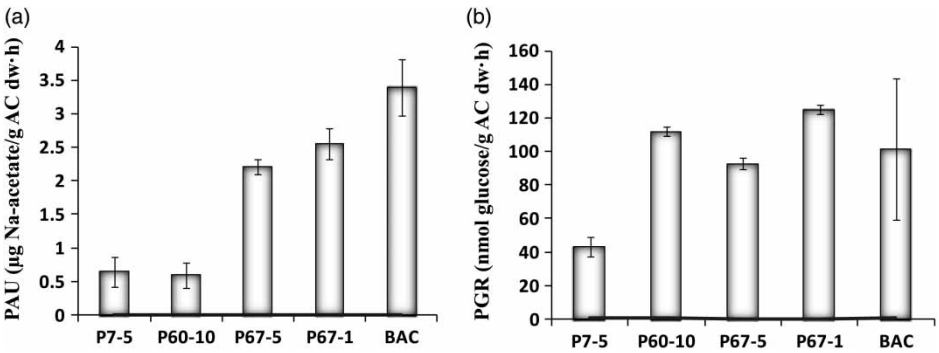

Figure 2 | (a) PAU rates of biological AC samples. Error bars represent the standard errors of the regression slopes used for calculating PAU rates. (b) PGR rates of biological AC samples. Error bars represent standard deviations. Solid lines (——) represent the background values measured on the corresponding virgin samples.

monitoring the depletion of acetate in the first hours of the assay in short time intervals is necessary for properly monitoring the zero-order kinetics.

PGR rates

Figure 2(b) presents the PGR rates measured on colonized AC. The background values obtained for the virgin PAC were between 0.07 and 0.44 nmol/g AC dw · h. These values were not significantly different from one another ($p > 0.05$) and were considerably lower (120- to 1,900-fold) than the values obtained for the colonized materials. Therefore, the PGR values shown in Figure 2(b) and elsewhere in this paper were not corrected for the background measurement.

The PGR of the sample P7-5 (43.1 nmol/g AC dw · h) was significantly different from the virgin samples ($p < 0.05$). The PGR rates obtained for the samples with higher SRT (i.e. at least 60 days) led to the same conclusions as for the PAU method, with P60-10 being more colonized than P7-5 and P67-1 being more colonized than P67-5. All the samples with a high SRT presented a PGR not significantly different from the BAC sample.

To compare our results with the values of PGR found in the literature (usually reported as μg of C-biomass/cm^3), the PGR rates were converted by considering a mean density value of 1.33 g of wet AC per cm^3 and a correspondence factor of 1.1 μg C of bacterial biomass per nmol of glucose respired per hour. This last value was originally obtained by calibrating the radiochemical method with bacteria washed off from GAC filter samples and enumerated by epifluorescence microscopy (Servais *et al.* 1991). Following this conversion, our PGR values obtained for the colonized PAC samples ranged from 17.7 to 69.4 μg C/cm^3, whereas a biomass of 38.6 μg C/cm^3 was measured at the surface of the biological filter (sample BAC). This value is higher than the PGR measured previously by Niquette *et al.* (1998) at the top of the same full-scale GAC biofilter (20 μg C/cm^3). This difference could be partly explained by the temperature in the filter (16 °C in our case vs. 11 °C measured previously). In an another study reporting the colonization of a biofilter at 16 °C, the PGRs were comprised between 2.5 and 10.9 μg C/cm^3 (Servais *et al.* 1991), while an average value of 4.7 μg C/cm^3 was proposed as representative of the fixed biomass in a GAC biofilter at steady state (Servais *et al.* 1991). The higher biomass densities observed on our PAC samples are most likely explained by the amount of support area available for the colonization and the nutrients limitation. Indeed, the PAC samples were colonized at low concentrations (i.e. 1 to 10 g/L), whereas a larger mass of GAC typically provides support for the colonization at the surface of a BAC filter. In addition, the biomass densities measured on BAC come from second-stage filters, while our PAC was colonized as a first-stage process. Niquette *et al.* (1998) observed a similar trend (i.e. higher colonization in first- as opposed to second-stage BAC filters). As described earlier, this result is explained by the higher nutrient loading offered in first-stage processes.

Heterotrophic plate counts

The results of the analysis of HPC are presented in Figure 3(a). The densities of HPC on biological PAC ranged from 4.2×10^7 to 3.0×10^8 CFU/g PAC dw, whereas the value for the BAC sample was 3.3×10^7 CFU/g GAC dw. The results obtained are of the same order of magnitude as the

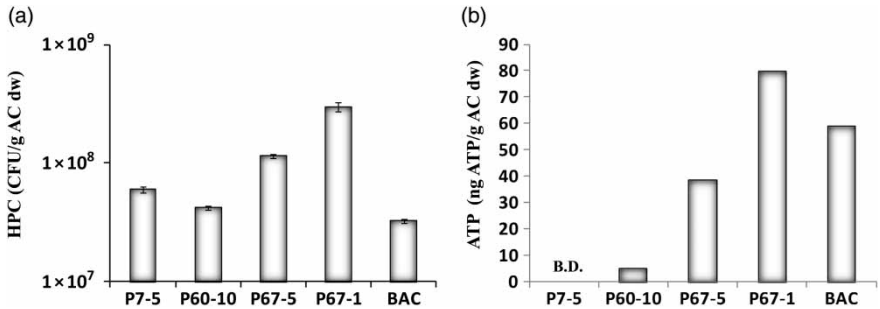

Figure 3 | (a) HPC in biological AC samples. Error bars represent standard deviations. (b) Bacterial ATP contents in biological AC samples. B.D.: Below detection limit.

literature values for BAC. However, no further comparison can be drawn. The culture conditions (i.e. culture medium and incubation temperature) applied were indeed highly variable, leading to potentially large variations in the HPC results. LeChevallier *et al.* (1984) measured 10^8 CFU/g GAC on a TLY medium, Camper *et al.* (1985b) measured 5×10^7 CFU/g GAC after incubating at 35 °C, and Niemi *et al.* (2009) measured 6×10^7 to 10^8 CFU/g GAC dw after incubating at 25 °C.

As mentioned before, the detachment of bacterial cells from the support medium is critical when using the plate count procedure to assess biomass density. In this study, the higher values of culturable cells obtained for biological PAC may be the result of the homogenization step being more efficient for smaller particles. Indeed, the shear stress is inversely proportional to the cross-sectional area of the material with area parallel to the applied force vector.

Bacterial ATP

The results obtained for the bacterial ATP are presented in Figure 3(b). The ATP concentration measured on sample P7-5 was below the detection limit, whereas sample P60-10 presented an ATP concentration of only 5 ng ATP/g PAC dw. Samples P67-5 and P67-1 presented ATP concentrations of 39 and 80 ng ATP/g PAC dw, respectively. These results may have been biased by the impact of residual adsorption. Positive controls confirmed that ATP readily adsorbed onto PAC particles. Luminescence losses ranging from 70 to 99% were detected after the addition of an ATP standard to the PAC. Thus, the residual adsorption capacity of PAC is an important concern when using ATP and future work should aim for an improved separation of the biofilm from the PAC particles before conducting the ATP analysis.

For colonized GAC, the measured value (58.9 ng ATP/g BAC dw or 14.07 ng ATP/g BAC ww) was lower than the usual ATP contents. Niemi *et al.* (2009) measured 0.5–0.7 nmol ATP/g GAC dw (equivalent to 254–355 ng/g GAC dw), while Velten *et al.* (2011) measured 1,170 ng ATP/g GAC ww at the surface of a fully colonized biofilter. In the former case (Niemi *et al.* 2009), a prior extraction of the ATP had been carried out, thereby indicating that the method used herein could most likely be improved by including an ATP extraction step before the addition of the luciferin-luciferase complex.

EPS: polysaccharides and proteins

The results of the analysis of extracellular proteins and polysaccharides are presented in Figure 4(a). The proteins ranged from 6.7 to 14.0 mg/g PAC dw and 8.1 mg/g BAC dw (or 1.9 mg/g BAC ww). The results obtained for the BAC were lower than those obtained in laboratory-scale GAC columns removing microcystin-LR from drinking water (17.2–37.2 mg/g GAC ww) (Drogui *et al.* 2012). Polysaccharides were present at lower concentrations than proteins: 1.0–3.6 mg/g PAC dw and 5.1 mg/g BAC dw. Figure 4(b) presents the total EPS measurements as well as the polysaccharide-to-protein ratio. The calculated polysaccharide-to-protein ratios were similar in the various samples of biological PAC with SRT of at least 67 days

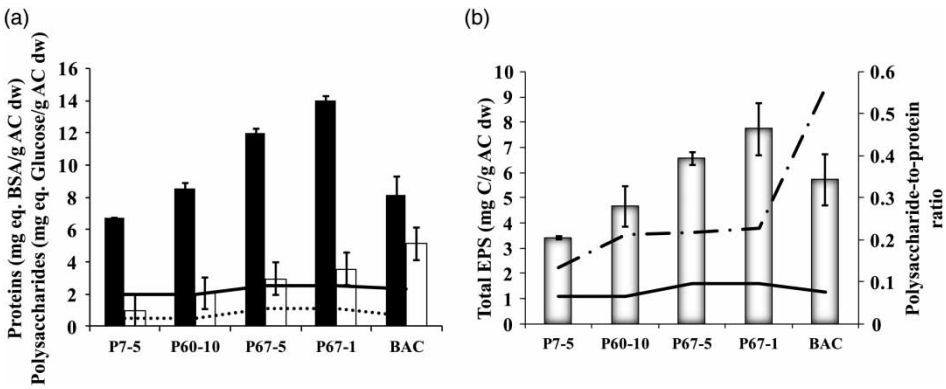

Figure 4 | (a) Measured (■) proteins, (▬) background proteins, (□) polysaccharides and (▪ ▪ ▪ ▪ ▪) background polysaccharides in biological AC samples. (b) Measured (□) total EPS, (▬) background total EPS and (▬ ▪ ▪) polysaccharide-to-protein ratio in biological AC samples. Error bars represent standard deviations.

(0.22 ± 0.04). The youngest PAC (i.e. P7-5) had a lower ratio (0.13 ± 0.002), whereas the GAC sample cumulated the greatest amount of polysaccharides (0.56 ± 0.1). As a reference, higher polysaccharide-to-protein ratios have been associated with higher SRT in activated sludge (Massé *et al.* 2006; Satyawali & Balakrishnan 2009), which is consistent with our results. In fact, the relative abundance of polysaccharides over proteins has been explained by the slower hydrolysis rate of polysaccharides (Massé *et al.* 2006). The increase in the polysaccharide-to-protein ratios at high SRT could also arise from a higher polysaccharide production, which would increase the bacterial adhesion to AC. Indeed, polysaccharides are widely recognized as key in mediating both cell-to-cell interactions and cell attachment to supports (Tay *et al.* 2001). As polysaccharides tend to accumulate (cf. low hydrolysis rate), this technique may lead to an improper assessment of AC microbial colonization for high SRT. In particular, comparing fully colonized samples with different SRT is not possible because old AC should lead to higher polysaccharides contents for the same amount of active biomass. However, by considering the total amount of EPS (i.e. the sum of proteins and polysaccharides) as presented in Figure 4(b), similar trends as the one observed for the potential activity-based methods (i.e. PAU and PGR) were obtained.

Comparison of methods

A comparison was performed by considering all the tested methods except for the ATP method, which remains to be optimized. Figure 5 compares the results obtained with our reference method (i.e. PGR) and the results of the PAU rates, log HPC and the contents of proteins, polysaccharides and total EPS. The corresponding correlation matrix is presented in Table 2. This matrix confirms that the results of the PAU tests were significantly correlated with the PGR values ($r = 0.82$), suggesting the possibility of using either technique interchangeably. In addition, there were strong correlations between methods based on potential activity and the EPS analysis ($r > 0.75$). The total EPS and proteins were better correlated with the PGR method ($r = 0.92$ and 0.89, respectively), whereas the polysaccharides results were better correlated with the PAU data ($r = 0.96$).

All of the methods investigated differentiated between the colonized AC and the corresponding virgin material ($p < 0.05$) for all of the samples tested. As confirmed by the background measurements, the EPS methods were the least sensitive (see background measurements on Figures 4(a) and (b)). In contrast, the PGR method was the most sensitive method. Indeed, by raising the ratio of the radioactive specific activity of the glucose solution added to the AC

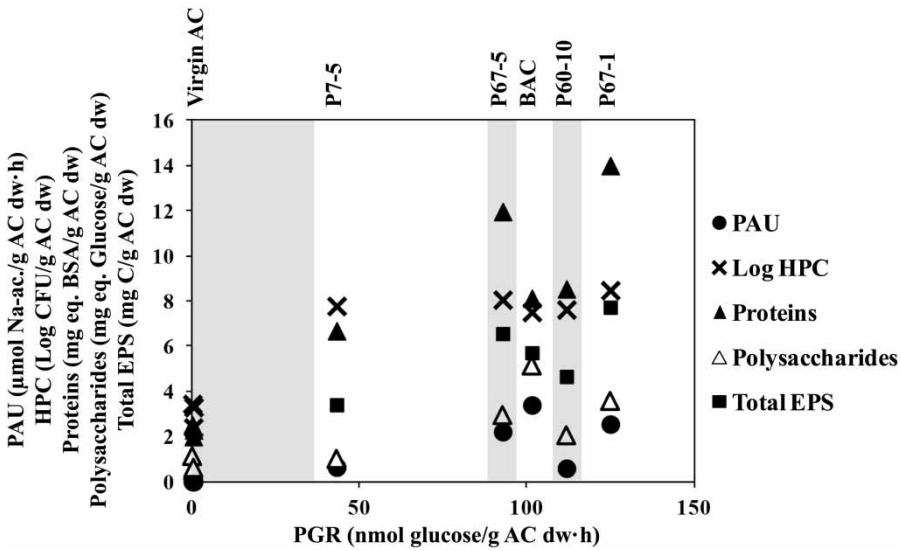

Figure 5 | PAU rate, log HPC, proteins, polysaccharides and total EPS as a function of the PGR rate measured on biological and virgin AC samples.

Table 2 | Correlation matrix (I-values, $p < 0.05$) between the results obtained with the PAU, PGR, proteins, polysaccharides, total EPS and log HPC methods

	PAU	PGR	Proteins	Polysac.	Total EPS	Log HPC
PAU	1	0.82	0.80	0.96	0.88	0.72
PGR	0.82	1	0.89	0.84	0.92	0.87
Proteins	0.80	0.89	1	0.75	0.98	0.81
Polysac.	0.96	0.84	0.75	1	0.85	0.66
Total EPS	0.88	0.92	0.98	0.85	1	0.89
Log HPC	0.72	0.87	0.81	0.66	0.89	1

*Polysac.: Polysaccharides.

(by increasing the proportion of radioactive glucose in this solution), the sensitivity can be easily improved.

Among biological PAC samples, P67-1 carbon exhibited the maximum results of all the six analytical methods, which were higher than those presented by the P67-5 sample. The enhanced microbial colonization of P67-1 carbon might be due to the higher substrate availability that prevailed in this reactor (with the lowest PAC concentration). No statistically significant difference was found between the P67-1 and BAC samples using the PGR method ($p > 0.05$), although this finding may be attributed to the considerable standard deviation of the BAC results (i.e. 41%). The total EPS method indicated that the P67-1 sample was more colonized than the BAC sample, whereas the opposite was true for the PAU method. These results support the idea that the biomass of a PAC-based process is as active as the biomass at the surface of a conventional biological GAC filter for drinking water treatment.

Both potential activity-based methods (PGR and PAU) could be used to improve the modeling of DOC and BDOC removal as well as the control strategies and the overall performance of biological processes used in drinking water production. The protein measurements were highly correlated with the potential activity methods (see Table 2). From an operational viewpoint, the protein analysis is the least cumbersome and time-consuming of all techniques tested during this project. It could be used as an easily measurable surrogate for monitoring the microbial activity on biological AC in water treatment plants.

It is worth highlighting that, amongst the methods tested, the PAU and PGR rates and HPC methods were designed to provide data on the heterotrophic biomass while the ATP and the EPS measurements allowed the evaluation of not only the heterotrophic bacteria, but the entire bacterial and non-bacterial biomass. These differences should be kept in mind and the investigation of the microbial communities established at the surface of the AC under variable SRT would provide additional insight on the results of the different methods applied in this study.

CONCLUSIONS

The results from PAU, PGR, EPS and HPC methods were strongly correlated. The PAU rate is a promising method to assess the bacterial activity on PAC and GAC. However, it should be validated for a wider set of colonized AC samples. Because protein content is well correlated with the PAU and PGR results, it would be a good surrogate for on-site biomass measurements. A high SRT increased the activity of heterotrophic biomass and the amount of EPS associated to the biofilms. Finally, it was concluded that heterotrophic activities comparable to BAC filters can be obtained in biological PAC-based drinking water systems.

ACKNOWLEDGEMENTS

The authors would like to thank the outstanding technical support of Jacinthe Mailly, Jörg Winter, Julie Philibert, Marcellin Fotsing, Mélanie Rivard, Mireille Blais and Yves Fontaine. This work was completed at the Industrial-NSERC Chair in Drinking Water of École Polytechnique de Montréal with the financial support of its partners, namely the City of Montreal, John Meunier, Inc., and the City of Laval.

REFERENCES

APHA 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association, American Waterworks Association, Water Environment Federation, Washington, DC.

Brown, J. C. 2007 Biological treatments of drinking water. *Bridge* **37**, 30–36.

Camper, A. K., LeChevallier, M. W., Broadaway, S. C. & McFeters, G. A. 1985a Evaluation of procedures to desorb

bacteria from granular activated carbon. *J. Microbiol. Methods* **3**, 187–198.

Camper, A. K., LeChevallier, M. W., Broadaway, S. C. & McFeters, G. A. 1985b Growth and persistence of pathogens on granular activated carbon filters. *Appl. Environ. Microbiol.* **50**, 1378–1382.

Chaudhary, D. S., Vigneswaran, S., Jegatheesan, V., Ngo, H. H., Moon, H., Shim, W. G. & Kim, S. H. 2003 Granular activated carbon (GAC) adsorption in tertiary wastewater treatment: experiments and models. *Water Sci. Technol.* **47** (1), 113–120.

Drogui, P., Daghrir, R., Simard, M. C., Sauvageau, C. & Blais, J. F. 2012 Removal of microcystin-LR from spiked water using either activated carbon or anthracite as filter material. *Environ. Technol.* **33**, 381–391.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. 1956 Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* **28**, 350–356.

Herzberg, M., Dosoretz, C. G., Tarre, S. & Green, M. 2003 Patchy biofilm coverage can explain the potential advantage of BGAC reactors. *Environ. Sci. Technol.* **37**, 4274–4280.

Kihn, A., Laurent, P. & Servais, P. 2000 Measurement of potential activity of fixed nitrifying bacteria in biological filters used in drinking water production. *J. Ind. Microbiol. Biotechnol.* **24**, 161–166.

Lazarova, V., Pierzo, V., Fontvielle, D. & Manem, J. 1994 Integrated approach for biofilm characterization and biomass activity control. *Water Sci. Technol.* **29** (7), 345–354.

LeChevallier, M. W., Hassenauer, T. S., Camper, A. K. & McFeters, G. A. 1984 Disinfection of bacteria attached to granular activated carbon. *Appl. Environ. Microbiol.* **48**, 918–923.

Léveillé, S., Carrière, A., Charest, S. & Barbeau, B. 2013 PAC membrane bioreactor as an alternative to biological activated carbon filters for drinking water treatment. *J. Water Suppl. Res. Technol. AQUA* **62**, 23–34.

Liu, H. & Fang, H. H. P. 2002 Extraction of extracellular polymeric substances (EPS) of sludge's. *J. Biotechnol.* **95**, 249–256.

Markarian, A., Carrière, A., Dallaire, P.-O., Servais, P. & Barbeau, B. 2010 Hybrid membrane process: performance evaluation of biological PAC. *J. Water Suppl. Res. Technol. AQUA* **59**, 209–220.

Massé, A., Spérandio, M. & Cabassud, C. 2006 Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Res.* **40**, 2405–2415.

Moteleb, M. A., Suidan, M. T., Kim, J. & Maloney, S. W. 2002 Pertubated loading of a formaldehyde waste in an anaerobic granular activated carbon fluidized bed reactor. *Water Res.* **36**, 3775–3785.

Nielsen, J. L. & Nielsen, P. H. 2002 Enumeration of acetate-consuming bacteria by microautoradiography under oxygen and nitrate respiring conditions in activated sludge. *Water Res.* **36**, 421–428.

Niemi, R. M., Heiskanen, I., Heine, R. & Rapala, J. 2009 Previously uncultured β-*Proteobacteria* dominate in biologically active granular activated carbon (BAC) filters. *Water Res.* **43**, 5075–5086.

Niquette, P., Prévost, M., MacLean, R. G., Thibault, D., Coallier, J., Desjardins, R. & Lafrance, P. 1998 Backwashing first-stage sand-BAC filters. Does it increase removal of biodegradable organic matter and ammonia? *J. Am. Water Works Assoc.* **90**, 86–97.

Nishijima, W., Shoto, E. & Okada, M. 1997 Improvement of biodegradation of organic substance by addition of phosphorus in biological activated carbon. *Water Sci. Technol.* **36** (12), 251–257.

Papineau, I., Tufenkji, N. & Barbeau, B. 2012 Impact of media aging on the removal of *Cryptosporidium* in granular media filters. *J. Environ. Eng.* **139**, 603–611.

Quintelas, C., Fernandes, B., Castro, J., Figueiredo, H. & Tavares, T. 2008 Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC). *Chem. Eng. J.* **136**, 195–203.

Satyawali, Y. & Balakrishnan, M. 2009 Effect of PAC addition on sludge properties in an MBR treating high strength wastewater. *Water Res.* **43**, 1577–1588.

Servais, P., Billen, G., Ventresque, C. & Bablon, G. P. 1991 Microbial activity in GAC filters at the Choisy-le-Roi treatment plant. *J. Am. Water Works Assoc.* **83**, 62–68.

Stoquart, C., Servais, P., Bérubé, P. & Barbeau, B. 2012 Hybrid membrane processes using activated carbon treatment for drinking water production: a review. *J. Membr. Sci.* **411–412**, 1–12.

Stoquart, C., Vázquez-Rodríguez, G. A., Servais, P. & Barbeau, B. 2013 Gamma irradiation: a method to produce an abiotic control for biological activated carbon. *Environ. Technol.* doi:10.1080/09593330.2013.803132.

Tay, J.-H., Liu, Q.-S. & Liu, Y. 2001 The role of cellular polysaccharides in the formation and stability of aerobic granules. *Lett. Appl. Microbiol.* **33**, 222–226.

Urfer, D. & Huck, P. M. 2001 Measurement of biomass activity in drinking water biofilters using a respirometric method. *Water Res.* **35**, 1469–1477.

Velten, S., Boller, M., Köster, O., Helbing, J., Weilenmann, H. U. & Hammes, F. 2011 Development of biomass in a drinking water granular active carbon (GAC) filter. *Water Res.* **45**, 6347–6354.

Wang, J. Z., Summers, R. S. & Miltner, R. J. 1995 Biofiltration performance: part 1, relationship to biomass. *J. Am. Water Works Assoc.* **87**, 55–63.

Yener, J., Kopac, T., Dogu, G. & Dogu, T. 2008 Dynamic analysis of sorption of methylene blue dye on granular and powdered activated carbon. *Chem. Eng. J.* **144**, 400–406.

First received 12 January 2013; accepted in revised form 1 August 2013. Available online 23 September 2013