Biofilm in combined sewers: wet weather pollution source and/or dry weather pollution indicator?

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Abstract In a sewer trunk, three kinds of deposit, acting as potential wet weather sources, can be found: the biofilm, the organic layer and the gross bed sediment. This research program, on the “Le Marais” catchment (Paris, France), focused on the biofilm. The objectives were to describe, using a Transmission Electronic Microscope, the architecture of the sewer biofilm and to investigate the contents and the distributions of aliphatic and aromatic hydrocarbons in biofilm. The electron micrographs illustrated a uniform film of bacteria totally covering the surface of a thick organic matrix. A large cohesion of the cell layer and organic matrix complex, due to exopolysaccharides, was noticed. Hence, the hydrocarbon contents were measured not only in the biofilm itself, but in this complex. Our results showed that almost all hydrocarbons were stored in the gross bed sediment and the organic layer and, consequently, the biofilm was not an important potential source of wet weather pollution. Comparison between the hydrocarbon distributions in the biofilm and in the other deposits indicated that the biofilm could be used as an indicator of the aliphatic hydrocarbon pollution in the organic layer.

Keywords Biofilm; n-alkanes; PAHs; pollution; sewer trunk

Introduction
The importance of urban wet weather pollution loads going through combined sewer networks and their acute impact on receiving waters (river, lake and sea) have been largely demonstrated (Saget, 1994; Schulz et al., 1994; Marsalek et al., 1999). Previous studies carried out on the “Le Marais” catchment (Paris, France) (research program entitled: “Production and transport of wet weather pollution in combined sewers”) have shown that a great part of the suspended solids at the outlet of the catchment area originated from in-sewer sources. In a sewer trunk, the three kinds of deposit, acting as potential wet weather pollutant sources, are: the biofilm, the organic layer (OL) and the gross bed sediment (GBS) (Ahyerre, 1999). This research, focused on the biofilm, had three main objectives: (i) the observation of the architecture of the biofilm attached to the trunk wall, (ii) the assessment of the biofilm contribution to the wet weather hydrocarbon pollution, (iii) the comparison of the aliphatic and aromatic hydrocarbon distributions in the biofilm with its distributions in the other deposits (OL, GBS), in order to determine whether the biofilm could be used as an indicator of the hydrocarbon pollution within the sewer trunk or not. This research should provide new data on hydrocarbon storage in sewer deposits, which can be used to target treatment solutions for urban wet weather pollution. Moreover, the use of the biofilm as an indicator of hydrocarbon pollution within the sewer system might make future projects, running at greater scales, easier, since biofilm sampling does not require specific devices.
Material and methods

Sampling site
The “Le Marais” catchment covers an area of 42 ha in an old residential district, with small business and almost no industrial activities. It is located in central Paris (France). It is densely populated (295 inhabitant ha\(^{-1}\)) and is impervious at 90%. The catchment area can be divided into 3 kinds of urban surfaces, leading to 3 types of runoff: roofs (54.5%), streets (23%), and surfaces such as courtyards, gardens and public areas (22.5%) (Gonzalez et al., 2000). The sewer network is combined, ramified and completely man-entry. It includes three ovoid trunks (“Vieille du Temple”, “St Gilles” and “Rivoli”) and around 50 egg-shaped collectors. “Vieille du Temple” and “St Gilles” trunks flow into “Rivoli”. Experiments were carried out in the “St Gilles” trunk which is 798 m long (Figure 1b).

Deposit sampling procedures
According to Ahyerre (1999), sewer deposits were divided into 3 categories: the GBS (coarse, granular and inorganic), the OL (immobile layer of organic matter) and the biofilm (organic slime on pipe wall) (Figure 1a). The GBS and the OL were sampled at the top of the “St Gilles” trunk (600 m upstream of “Rivoli”). The biofilm was sampled every 25–50 m from the St Gilles–Rivoli junction up to 600 m upstream (Figure 1b).

The GBS was sampled with the assistance of an adapted shovel that isolates the sediment during sampling and traps the fine particles of this sediment (Ahyerre et al., 2000). The system used to sample the OL was composed of a PVC box (85 × 30 × 50 cm) opened on two sides so that water can flow through it (Ahyerre et al., 2000). Two panels closed the box. The unit was inserted into the GBS in the direction of the flow. The samples were taken after each 5–10 days dry weather period. For sampling, the two panels were lowered and the water in the box was pumped out with a peristaltic pump. When all the wastewater was pumped out, the OL was scraped until reaching the GBS. The GBS and the OL were sampled simultaneously during 2 months (from January to February 2001) and 5 samples were collected. The biofilm, attached to the pipe wall, was scraped off using a little metallic scraper; 14 samples were collected in December 2000.

Experimental procedures
Observation of the biofilm architecture. The biofilm architecture was observed under a transmission electron microscope (JEOL JSM-6301-F) linked with a EDS detector (ISIS 300). To avoid alteration of the biofilm architecture during the drying step, the biofilm was slowly desiccated at 20°C during 3 weeks before observation.

Sample drying and grain size partitioning. Before extraction, samples were dried until a constant weight at 40°C. It appeared that the OL and biofilm textures were both homogeneous while the GBS was heterogeneous. The latter was mainly composed of gravel and
various size stones. Hence, dry GBS samples were sieved and divided in two fractions: smaller and larger than 400 µm (Ahyerre, 1999).

**Extraction and separation of hydrocarbons.** All solvents were purified by distillation before use. To avoid contamination, the glassware used for sampling and analysis was cleaned with 5% Decon detergent (Prolabo), rinsed with de-ionised water (Milli-Ro 5 Plus, Millipore) and heated at 450°C for 2 h to eliminate any trace of organic matter. About 1 to 2 g.dw (dry weight) was Soxwave® (Prolabo) extracted for 10 minutes with a CH$_2$Cl$_2$-MeOH mixture (35 mL/5 mL) after addition of perdeuterated internal standards (dodecane D$_{26}$, tetracosane D$_{50}$ and triacontane D$_{62}$, naphthalene D$_{8}$, phenanthrene D$_{10}$, pyrene D$_{10}$ and benzo[a]pyrene D$_{12}$). The extract was eluted on a chromatographic column (5.5 mm ID x 30 cm in length) containing 2 g of silica gel. The total aliphatic hydrocarbons (TAHs) were eluted with hexane and the polycyclic aromatic hydrocarbons (PAHs) were eluted with a hexane–CH$_2$Cl$_2$ mixture (80/20, v/v).

**Chromatographic analysis.** Hydrocarbons were determined by GC-MS (GCD 1800 A, Hewlett Packard) using a PONA fused silica capillary column, 50 m × 0.20 mm ID × 0.5 µm film thickness (Hewlett Packard). The carrier gas was helium (1.0 mL.min$^{-1}$). The injector temperature was set to 300°C and 1 µL was injected. The column temperature was programmed from 70–300°C, at 5°C.min$^{-1}$, and held for 24 min at 300°C. System control and data acquisition were monitored with a HP ChemStation software. The GCD apparatus was operating in the scan mode. The whole analytical procedure was validated using certified sample (marine sediment SRM1941a (NIST)) which is certified for PAHs, whereas aliphatic concentrations are given as non-certified, i.e. indicative (Gonzalez et al., 1999).

**Results and discussion**

**Biofilm architecture in sewer system**

**General characteristics.** Biofilm can be found on almost any surface exposed to polluted waters. It represents a microbial community with various inhabitants such as sessile bacteria, protozoa, fungi and algae (Fuchs et al., 1996). In a sewer system, the cell morphology of the dominant filament is a rod shaped cell of 1.2–2 µm diameter and 2–5 µm long. The dominant filament is identified as most probably Sphaerotilus natans, a bacterium also called “sewage fungus” (Cao and Alaerts, 1995). Two main stages may be considered in the biofilm development. Firstly, microorganisms multiply and colonise the support (e.g. riverbank, trunk wall). Once the bacteria layer is established, they secrete a matrix of mucilaginous extracellular polymers: the exopolysaccharides (EPSs) (Scott et al., 1995). The EPSs consist mainly of polysaccharides, proteins, uronic acids, humic acids, DNA and cell fragments (Späth et al., 1998). Cells within the matrix of EPSs are collectively called a “microbial biofilm” (Fuchs et al., 1996; Decho, 2000). The EPS matrix forms a stabilising and protective microenvironment and may serve a variety of specific functions to cells. At first, EPSs possess very cohesive physical properties. Therefore, sediment or particles, which are embedded in this matrix, are stabilised against resuspension. Secondly, the highly hydrated EPSs may also be considered as a protective adaptation to prevent cell desiccation due to prolonged tidal exposures. (Scott et al., 1995; Decho, 2000).

Biofilm attached to the “St Gilles” trunk walls. A superposition of an organic matrix and a cell layer was observed. Figure 2a illustrates that an organic matrix was present between the cell layer and the trunk wall. This matrix, attached to the trunk wall, had a fibrous structure and might be mainly composed of vegetal fibers and organic matter. Its thickness was important since it reached several millimetres. A uniform film of bacteria totally covered the organic matrix surface. This cell coat was thin (estimated at 5–10 µm thick) and seemed
to be composed of a monolayer of bacteria (Figures 2a, 2c). The cell coat consisted of numerous bacteria compacted together (Figure 2d) and might be bound by a polysaccharide coat. Moreover, mineral fragments of various sizes (such as quartz) were embedded in the organic matrix (Figure 2b).

The organic matrix, including abiotic materials, and the cell coat were strongly linked together. Experiments performed on the biofilm collected in the “St Gilles” trunk have even shown that the bacteria layer and the organic matrix were inextricable. The large cohesion of this complex was due to EPSs, produced by bacteria, which acted as a genuine “glue”. Cohesive properties enabled the complex to be strongly fixed to the trunk wall and prevented it from any erosion due to the dry weather wastewater flow.

Therefore, the architecture of the biofilm attached to this sewer trunk was not greatly different from the microbial biofilm found in other ecosystems (e.g. river bank). Nevertheless, in the case of this sewer system, the biofilm was not directly attached to the trunk wall because of its inorganic nature, but to a thick organic matrix.

Hydrocarbon contents in sewer biofilm

Hydrocarbon stock. As previously shown, the complex formed by the cell layer and the organic matrix was inextricable. Therefore, the hydrocarbon contents were measured not only in the biofilm itself but in the complex. TAHs and PAHs were also measured in the OL and the GBS. In the latter, two fractions, i.e. below and above 400 µm, were considered, GBSi and GBSs, respectively.

The mean PAH content in the biofilm was 3.7 ± 6.1 µg.g⁻¹dw. This important content variation, illustrated by the high Relative Standard Deviation (RSD = 160%), may be linked to the biofilm architecture. Indeed, the heterogeneous distribution of mineral fragments, embedded in the organic matrix (Figure 2b), induced a variability of the biofilm density and, consequently, a variation of the content values. But, despite the observed heterogeneity along the “St Gilles” trunk, PAH amounts were always of the same order of magnitude. Our results were in good agreement with a previous study carried out in the combined sewer of Bad Mergentheim (Germany) by Michelbach and Wöhrle (1994). Indeed, they reported that the mean PAH content in the biofilm was 6.4 µg.g⁻¹dw. When compared to the other deposits, the biofilm PAH content was 4, 8 and 6 times smaller than...
the OL, GBSi and GBSs ones, respectively (Table 1). Our results were in accordance with previous works carried out on this catchment (Gonzalez, 2001). She reported that the biofilm PAH contents were 6 times lower than the other deposits. These low values suggested that a PAH biodegradation might occur in biofilm. Biodegradation of aromatic pollutants by fixed bacteria is a well-known phenomenon (Galil, 1994; Arcangeli and Arvin, 1995; Zhang et al., 1995). Therefore, we supposed that the bacteria forming the biofilm degrade PAHs sorbed onto the organic matrix.

An opposite trend was observed for TAHs. The average content in the biofilm was nearly 200 µg·g⁻¹dw which was 3, 5 and 3 times greater than the OL, GBSi and GBSs ones, respectively (Table 1). These high TAH amounts in the biofilm were probably linked to the biological nature of the organic matrix. Indeed, this matrix, which was mainly composed of faecal matter and vegetal fibres, was rich in aliphatic hydrocarbons (Rocher, 2000).

Studies carried out in the “St Gilles” trunk allowed the evaluation of the total mass of OL, GBS and biofilm. OL, GBSi, GBSs and biofilm mass were estimated at 1,200, 2,800, 13,700 and 22 kg, respectively. (Ahyerre, 1999; Oms et al., 2002). Hence, TAHs were estimated at 4.4, 81, 117 and 935 g in biofilm, OL, GBSi, GBSs, respectively. PAHs were estimated at 0.1, 16, 86 and 307 g in biofilm, OL, GBSi, GBSs, respectively. Therefore, hydrocarbons were mainly stored in the GBS. Percentages of TAHs and PAHs, stored in this deposit (GBSi+GBSs), reached 93% and 96% of the total stock, respectively (Figures 3a, 3b).

Table 1 Aliphatic and aromatic hydrocarbon contents in deposits and calculated ratios

<table>
<thead>
<tr>
<th></th>
<th>Biofilm (n=14)</th>
<th>OL (n=4)</th>
<th>GBSi (n=5)</th>
<th>GBSs (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH (µg·g⁻¹dw ± SD)</td>
<td>3.7 ± 6.1</td>
<td>13.6 ± 19.4</td>
<td>30.5 ± 39.1</td>
<td>22.3 ± 18.1</td>
</tr>
<tr>
<td>Major PAH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Major PAH</td>
<td>42</td>
<td>45</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>LMW/HMW</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Alk/Nalk</td>
<td>0</td>
<td>0</td>
<td>0.07</td>
<td>0.08</td>
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<tr>
<td>TAH (µg·g⁻¹dw ± SD)</td>
<td>196 ± 59</td>
<td>68 ± 18</td>
<td>42 ± 21</td>
<td>68 ± 80</td>
</tr>
<tr>
<td>Major aliphatic hydrocarbon</td>
<td>C29</td>
<td>C29</td>
<td>C18</td>
<td>C18</td>
</tr>
<tr>
<td>% Major aliphatic hydrocarbon</td>
<td>10</td>
<td>16</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>UCM (µg·g⁻¹dw ± SD)</td>
<td>1,886 ± 1,139</td>
<td>838 ± 1,080</td>
<td>585 ± 456</td>
<td>260 ± 102</td>
</tr>
<tr>
<td>UCM/R</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>LMW/HMW</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Prist/phyt</td>
<td>1.3</td>
<td>1.4</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>C17/pr</td>
<td>1.9</td>
<td>2.0</td>
<td>1.8</td>
<td>1.7</td>
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<tr>
<td>C18/ph</td>
<td>3.7</td>
<td>4.0</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Σn-alkanes/C16</td>
<td>25</td>
<td>33</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>C17/C29</td>
<td>0.4</td>
<td>0.2</td>
<td>2.4</td>
<td>5.0</td>
</tr>
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</table>

For PAHs: LMW = Light Molecular Weight (ring number ≤ 3 cycles), HMW = High Molecular Weight (ring number > 3 cycles), Alk = alkylated PAHs and NAlk = non alkylated PAHs, Pyr = pyrene and Fluo = fluoranthene. For aliphatic hydrocarbons: UCM = Unresolved Complex Mixture, R = Resolved alkanes, LMW = Light Molecular Weight (carbon number ≤20), HMW = High Molecular Weight (carbon number > 20), Prist = pristane and Phyt = phytane.

Figure 3 Percentages of TAHs (a) and PAHs (b) in the 3 kinds of deposits and percentages of TAHs (c) and PAHs (d) in the biofilm and the OL without taking into account the GBS.
Remaining hydrocarbons were mainly stocked in the OL. TAHs and PAHs contained in this layer represented 7 and 4% of the total stock, respectively (Figures 3a, 3b). Hence, hydrocarbon stock in the biofilm was a lot smaller than the OL and GBS ones. Portions of TAHs and PAHs stored in this deposit were lower than 0.5% and 0.1%, respectively.

Therefore, the hydrocarbon contents underlined that the sewer biofilm has not accumulated hydrocarbon pollutants. It was even likely that PAHs sorbed on the organic matrix were partially degraded by microorganisms from the biofilm. Moreover, the total mass of biofilm lining the “St Gilles” trunk wall was found to be a lot smaller than the other deposit ones. This characteristic combined with the low hydrocarbon contents in the biofilm led to the conclusion that the hydrocarbon quantity stored in this deposit was insignificant.

**Biofilm as a wet weather pollution source.** Sewer deposits play a significant role as a source of pollution in the combined sewer overflow. Experiments carried out on the “Le Marais” catchment have shown that 30–80% of the suspended solid mass at the outlet of the catchment area originated from in-sewer sources (Gromaire, 1998). Indeed, during storm events, deposits were resuspended and the biofilm was eroded from the trunk wall. The main involved mechanism is the shear stress resulting from the increased velocities under storm flow conditions (Crabtree et al., 1995; Michelbach, 1995; Ahyerre et al., 2000). Recent studies have even established that the OL was the main source of eroded solids (Ristenpart et al., 1995; Ahyerre et al., 2000). To approximately assess the biofilm contribution to the wet weather pollution, we assumed that (i) the GBS was not resuspended, (ii) the OL was entirely resuspended and (iii) the biofilm was entirely eroded. These hypotheses were accepted even if, in reality, the GBS might be partially eroded, a small part of the OL might resist resuspension and a small part of the biofilm might resist erosion (Arthur et al., 1996; Ahyerre, 1999). Admitting these hypotheses, the OL contribution to the TAH and PAH pollutants reached 95 and 99% of the total in-sewer pollution respectively, whereas the biofilm contribution was only 5 and 1% respectively (Figures 3c, 3d). These low values emphasised that, at the “Le Marais” catchment scale, the biofilm was not an important source of wet weather pollution and could be disregarded.

**Hydrocarbon distribution in sewer biofilm**

The comparison between the TAH and PAH distributions in the different deposits was carried out to establish whether similarities between the distribution in the biofilm and the distributions in the other deposits occurred. Our investigations were carried out in order to determine whether or not the biofilm could be used as an indicator of the hydrocarbon pollution within the sewer. The use of biofilm as a pollution indicator may make the study of hydrocarbon pollution in combined sewers easier. Indeed, biofilm sampling is very convenient compared to the sampling of OL and GBS that requires specific devices. This would greatly simplify further research run at greater scales.

**TAH distributions.** The biofilm showed a very similar aliphatic distribution pattern to the one of the OL (Figure 4). Their distribution patterns were bimodal: peaked at around C18 and C29. Heavy compounds (carbon number > 20) were predominant since most of the \(n\)-alkanes had 24 to 31 carbon atoms.

The LMW/HMW ratios also underlined the abundance of high molecular weight \(n\)-alkanes since values were estimated at 0.4 in both deposits (Table 1). For the biofilm and the OL, the values of Pr/Ph, C17/Pr, C18/Ph, \(n\)-alkanes/C16 and C17/C29 ratios were 1.3/1.4, 1.9/2.0, 3.7/4.0, 25/33 and 0.4/0.2, respectively. These very close values confirmed that both deposits had the same aliphatic distribution patterns. In addition, the magnitude of the unresolved complex mixture (UCM) (i.e., the “hump” under the \(n\)-alkanes on a gas chro-
matographic trace) compared to the TAHs was nearly the same in the biofilm and in the OL, since the UCM/R values were estimated at 10 and 12 for the biofilm and the OL, respectively (Table 1). The two fractions of GBS (GBSi and GBSs) exhibited almost the same aliphatic distribution patterns, with the exception of GBSi which showed more high weight compounds than GBSs (Figure 4). These two aliphatic distributions contrasted significantly with the biofilm and OL distributions. They were centred on C18 and most of the n-alkanes were light compounds (carbon number ≤20). LMW/HMW values, estimated at 1.5 and 4.9 for the GBSi and the GBSs respectively, underlined the predominance of light compounds (Table 1).

**PAH distributions.** Aromatic distribution patterns were similar for all the deposits. The same PAHs were observed, i.e. a group containing phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene and chrysenene (Figure 5). Phenanthrene, fluoranthene and pyrene were the most abundant PAHs. Indeed, their sum accounted for 96, 98, 89 and 88% of the total PAHs for biofilm, OL, GBSi and BSs, respectively.

Relative parts of the 3 main PAHs were almost similar in OL, GBSi and GBSs, whereas the biofilm was composed of a greater percentage (38.5%) of phenanthrene and a lower percentage of fluoranthene (17.4%). The greater relative part of phenanthrene led to the most important LMW/HMW value for the biofilm since these ratios were estimated at 0.4, 0.1, 0.2 and 0.3 for biofilm, OL, GBSi and GBSs, respectively. The Alk/NAlk ratios, depicted in Table 1, indicated that no alkylated PAHs were detected in the biofilm and the OL whereas alkylated PAHs accounted for 6.5 and 7.4% of the total PAHs in the GBSi and the GBSs, respectively.

Therefore, TAH distributions in the biofilm and the OL being similar, the biofilm could be used as an indicator of the aliphatic hydrocarbon pollution of the OL. The PAH distribution in the biofilm was found to be slightly different than the other deposit distributions.
Nevertheless, for future research, the PAH analysis in the biofilm could be carried out in order to estimate the main PAHs present in the OL and the GBS. Indeed, the 6 main PAHs detected in all the deposits were the same.

Conclusions
The observation of the biofilm architecture under a transmission electron microscope has shown that a uniform film of bacteria, forming the biofilm, totally covered a thick organic matrix. This bacteria layer/organic matrix complex had a large cohesion due to EPSs, secreted by bacteria, which acted as a genuine glue. Therefore, the hydrocarbon contents were measured not only in the biofilm itself, but in this complex.

The results from the “Le Marais” catchment, in central Paris, underlined the fact that the hydrocarbon stock in biofilm is not important. Indeed, the parts of TAHs and PAHs stored in the biofilm were only estimated at 0.4 and 0.02% of the total in-sewer hydrocarbon stock, respectively. TAHs and PAHs were mainly stored in the GBS (93 and 96%, respectively) and, to a lesser extent, in the OL (7 and 4%, respectively). Therefore, we concluded that, in the sewer system of this catchment, the biofilm was not an important source of the wet weather pollution. During a rain event, the biofilm contribution to the hydrocarbon pollution only reached 5 and 1% of the total in-sewer pollution.

Comparison between the hydrocarbon distributions in the three kinds of deposits gave us information about the reliability of the biofilm as a hydrocarbon pollution indicator. Our results showed that TAH distributions in the biofilm and the OL were similar but PAH distribution in the biofilm was slightly different than the other deposit distributions. Thus, in our case, the biofilm could be used as an indicator of the aliphatic pollution in the OL.

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