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## WASTEWATER AS A SOURCE OF NITRIFYING BACTERIA IN RIVER SYSTEMS: THE CASE OF THE RIVER SEINE DOWNSTREAM FROM PARIS

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**Abstract**—The River Seine downstream from Paris receives large amounts of ammonium (about 200  $\mu\text{mol/l}$ ) from treated and untreated wastewater effluents. In such large river systems, due to the slow growth of nitrifying bacteria, the small size of the nitrifying population present in the water column often represents the limiting factor for nitrification of the contaminating ammonium. In this work we demonstrate that discharge of urban effluents can represent an important seeding of nitrifying bacteria, influencing the dynamics of nitrification in the river downstream. A nitrifying bacteria biomass in wastewater was deduced from  $\text{H}^{14}\text{CO}_3^-$  potential nitrifying activity measurements. These were found to be higher in untreated wastewater (1–200  $\mu\text{gC/l}$ ) and in treated effluents (0.8–30  $\mu\text{gC/l}$ ) than in the receiving river water (0.5–5  $\mu\text{gC/l}$ ). A retrospective analysis of the nitrification process in the River Seine downstream from Paris suggests that the overall ammonium oxidation rate has been continuously reduced over the past 20 years (from 1.5 to 1.0  $\mu\text{mol/l/h}$ ), as a result of the improvement of the treatment of Paris wastewater and the reduction of the discharge of untreated wastewater (from 14% to 0.2% of the total wastewater discharge). © 2000 Elsevier Science Ltd. All rights reserved

**Key words**—nitrifying bacteria, wastewater, potential nitrifying activity,  $\text{H}^{14}\text{CO}_3^-$  incorporation method, River Seine, wastewater treatment management

### INTRODUCTION

Direct or indirect (through organic N mineralisation) ammonium contamination of river water due to wastewater discharge is of widespread occurrence in densely populated watersheds. Nitrification is the main process by which ammonium can be eliminated from polluted river waters. However, because of the low growth rates of nitrifying bacteria, the high dilution and flushing rates characterising river systems are the major factors controlling the development of their planktonic nitrifier populations. Accordingly, in small streams, nitrification of contaminating ammonium mostly occurs in the benthos (Cooper, 1984; Schwert and White, 1974). In estuaries, on the other hand, high rates of planktonic nitrification are maintained because nitrifying bacteria are associated with suspended matter in a way which has been compared to a fluidised bed reactor (Owens, 1986). In the case of large rivers, the greater depth of the water column which reduces the significance of benthic activity, and the absence

of hydrological mechanisms allowing particulate material to be maintained in suspension, are both factors which limit nitrification. Indeed, the development of planktonic populations of nitrifiers after a point ammonium contamination in large rivers is known to be relatively slow even in favourable summer conditions (Chestérikoff *et al.*, 1992) so that large residence times are needed before a significant nitrification can occur. The seeding of river water with nitrifying bacteria might therefore be crucial to the dynamics of nitrification in large rivers. Here we demonstrate that considerable amounts of nitrifying bacteria are brought into rivers through the discharge of treated and untreated urban wastewater, and that this leads to a significant seeding of the water column.

The observations reported in this paper concern the River Seine downstream from Paris (Fig. 1) which is a large 7th order river. Sixty kilometres downstream from Paris, it receives the River Oise and becomes of 8th order. After the Oise confluence, the River Seine runs over 100 km before reaching the navigation dam of Poses, the start of the estuarine zone. Since 1966, the river discharge was gradually regulated by the successive construction of three storage-reservoirs on the major upper

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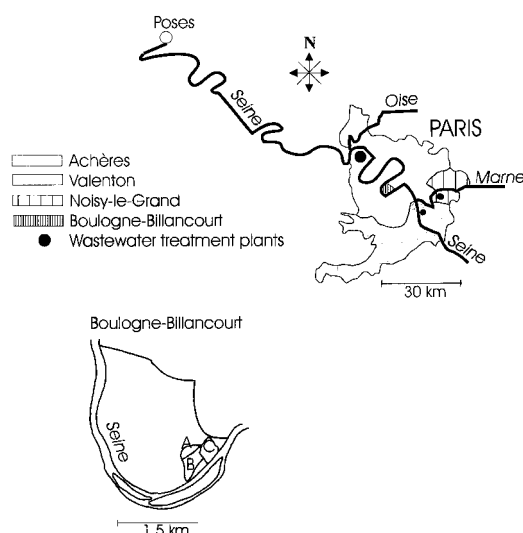


Fig. 1. The River Seine within the Paris agglomeration. The delimited surfaces represent the different catchment area of the purification plants of Achères (107,750 ha), Valenton (68,800 ha), and Noisy-le-Grand (12,850 ha). The lower part of the figure represents an enlargement of the Boulogne-Billancourt sewer catchment area (544 ha), with three sub-areas of 4.7 ha (A), 28 ha (upstream from A, B and C) and 47.5 ha (A+B+C+upstream). (A: Catchment area of one street, B: of three streets, C: of a cemetery.)

branches of the stream: the Seine Reservoir (1966, volume of  $205 \times 10^6 \text{ m}^3$ ), the Marne Reservoir (1974, volume of  $350 \times 10^6 \text{ m}^3$ ) and the Aube Reservoir (1990, volume of  $170 \times 10^6 \text{ m}^3$ ). They allowed the maintenance of a minimum discharge in summer for navigation and production of drinking water for the Parisian agglomeration. Actually, the characteristic discharge in summer is of  $200 \text{ m}^3/\text{s}$  with a temperature of  $20^\circ\text{C}$  (Fig. 2). Around the agglomeration of Paris, wastewater discharge of the 10 million inhabitants represents a severe disturbance in the ecological functioning of the river. Before 1954, all wastewater from the agglomeration was directly discharged in the River Seine without any treatment. Since 1954, and the construction of the first wastewater treatment plant of Achères

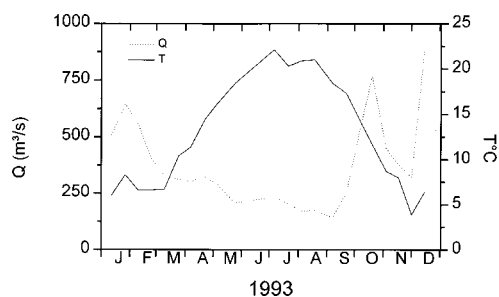


Fig. 2. A characteristic annual cycle of discharge (Q in  $\text{m}^3/\text{s}$ ) and water temperature ( $T$  in  $^\circ\text{C}$ ) of the Seine river measured at Poses. Year 1993.

(about 20 km downstream from Paris), Paris effluents were gradually treated before being discharged in the river. Indeed in 1966, 1971 and 1978, the second, third and fourth wastewater treatment blocks of Achères were started-up. In 1974, a second purification plant was started-up at Noisy-le-Grand (on the Marne River, just before the confluence with the Seine) and in 1987, a third one started-up in Valenton (10 km upstream from Paris) (see Fig. 1). Still, this treatment capacity was insufficient during the daily peaks of wastewater production, and a fraction of wastewater was still discharged without any treatment. Only since 1992, with the construction of the large wastewater storage basin of Sèvre-Achères, could this problem be solved. Thus, at the present time, Paris wastewater is treated by three purification plants. The plant of Achères is the most important, treating daily about  $2,000,000 \text{ m}^3$  of sewage (this capacity makes it the world's second most important treatment plant) with a classical activated sludge process. The discharge of the effluents of this plant results in a spectacular increase of the ammonium concentration in the river, reaching  $500 \mu\text{mol/l}$  during summer months which is far above the threshold value of  $150 \mu\text{mol/l}$  considered as a major pollution by the Agence de l'Eau Seine-Normandie (A.E.S.N.).

At the present time, the ammonium brought by Paris wastewater is only slowly nitrified, even under favourable summer conditions, because of the slow growth of the nitrifying population and the too short residence times (around 10 days for a mean discharge of  $250 \text{ m}^3/\text{s}$ , Chestérikoff *et al.*, 1992). Only 100 km downstream, in the estuarine zone, the nitrifying population has increased enough to cause significant ammonium depletion. In complete contrast with this, past records of ammonium concentration in the River Seine downstream from Paris, taken before the installation of the full capacity of the present wastewater purification plants, show that rapid nitrification did occur in the river itself a few kilometres downstream from the point of wastewater discharge while the nitrogen inputs were practically the same as today (Chestérikoff *et al.*, 1992). Why was nitrification in the summer more rapid in the past than at the present time while the ecological conditions in the River Seine did not significantly change in terms of temperature, oxygenation and ammonium concentration? We suggest in this paper that the biomass of nitrifying bacteria present in untreated wastewater represents another important factor that could influence the nitrification rate in the river downstream from Paris. The objectives of our work are to determine the nitrifying biomass associated with Parisian wastewater, to determine the role of wastewater discharge as a seeding mechanism of nitrifying bacteria for the river and to assess the possible implications of wastewater discharge in the nitrification rates in the receiving Seine river.

## METHODS

*Site description and sampling*

All three wastewater purification plants of the Parisian agglomeration were considered (Fig. 1) in this study because they are characterised by contrasting treatment schemes.

The *Achères* plant, a classical high load activated sludge plant, receives wastewater from about 6.5 million inhabitant-equivalents. It is the most important of the three. It consists of five complete treatment lines receiving wastewater from five different sewers and functioning in parallel (Fig. 3A) (*Achères* 1, 2, 3a, 3b and 4). Each line involves primary treatment and decantation, activated sludge process and secondary decantation. Total untreated wastewater discharges measured on the days of sampling were 2,125,440 m<sup>3</sup>/day (June 1994) and 2,372,000 m<sup>3</sup>/day (March 1994). Seven percent of this discharge underwent primary treatment only. Alongside these five classical treatment lines, a sixth pilot plant follows the same treatment process, but is completed by nitrification on the biological filter (Fig. 3A) (BIOSTYR<sup>®</sup>, OTV<sup>®</sup>). This experimental pilot treats 30,000 m<sup>3</sup>/day.

The purification plant of *Valenton* (Fig. 3B) includes four treatment lines receiving wastewater from one main sewer and functioning in parallel (*Valenton* 1, 2, 3 and 4). Each line involves primary treatment and decantation, anaerobic activated sludge with denitrification, aerobic activated sludge with nitrification and secondary decantation. A fraction of the nitrate-rich nitrified effluent is re-circulated into the denitrifying plant. The total discharge of wastewater measured on the sampling day (April 1994) was of 253,400 m<sup>3</sup>/day.

The purification plant of *Noisy-le-Grand* (Fig. 3C) has a maximum treatment capacity of 2800 m<sup>3</sup>/day (June 1994) and has only one treatment line that follows the same pathway as in the *Achères* purification plant, but is completed by nitrification on a fixed bed reactor (SESSIL<sup>®</sup>) where wastewater trickles along long narrow suspended plastic strips colonised by nitrifying bacteria. A tertiary decantation normally follows biological treatment but was not in use on the day of sampling.

The three purification plants were sampled on dry days to avoid dilution of wastewater with collected rainwater.

In all three purification plants, and for each independent treatment line, samples of untreated wastewater and treated wastewater were collected using refrigerated automatic samplers. To obtain a daily mean sample, sampling occurred every 30 min during 24 h, mixing all aliquots together. Several samples of untreated wastewater were also directly collected in the sewers network of Boulogne-Billancourt in September 1994 at three stations collecting wastewater from a catchment area of 4.7 ha, 28 ha and 47.5 ha, respectively (see Fig. 1). Samples from the River Seine were collected in June 1992 and March 1994 with a bucket from bridges located a few kilometres upstream and downstream from the wastewater discharge of *Achères*.

*Chemical and biological measurements*

Measurements of inorganic nitrogen concentrations and of nitrifying biomass were carried out on each waste- or river water sample taken.

*Dissolved inorganic nitrogen.* All chemical analysis were made on 0.45- $\mu$ m filtered water. Ammonium was measured with the indophenol blue method according to Slawyc and McIsaac (1972). Nitrate was measured after cadmium reduction into nitrite, and nitrite was measured with the sulfanilamide method according to Jones (1984).

*Nitrifying biomass.* Nitrifying biomass was estimated by measuring potential nitrifying activities with the nitrapyrine-chlorate sensitive H<sup>14</sup>CO<sub>3</sub><sup>-</sup> incorporation method modified from Somville (1978) as described by Brion and Billen (1998). Samples are incubated in the dark with and without nitrapyrine and chlorate (specific inhibitors of nitrification) with H<sup>14</sup>CO<sub>3</sub><sup>-</sup> during 20 h. They are filtered in five replicated on 0.2  $\mu$ m membranes, placed in scintillation vials and the radioactivity is counted by liquid scintillation. The C incorporation rate by nitrifiers is calculated by difference and converted to N oxidation rate by using the growth yield of nitrifiers. Potential nitrifying activity measurements (measured at saturating ammonium and oxygen concentrations and at a constant temperature of 20°C) can be directly correlated with the nitrifying biomass (Belser and Mays, 1982) with 1  $\mu$ gC of nitrifying bacteria oxidising 0.04  $\mu$ mol NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>/h (Bion and Billen, 1998).

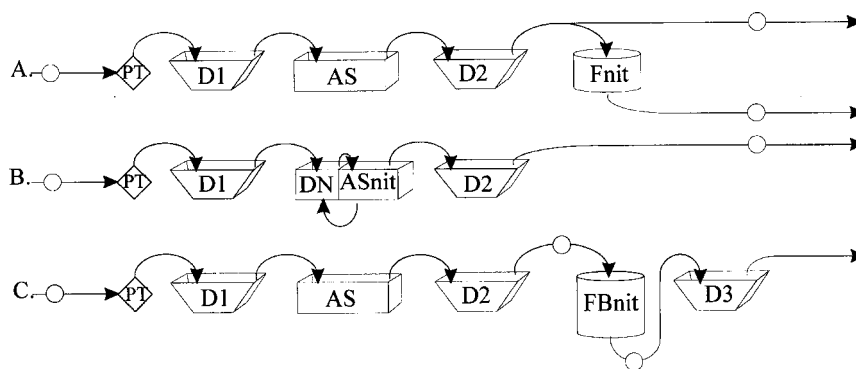


Fig. 3. Schematic representation of one of the treatment lines of the purification plants of *Achères* (A), *Valenton* (B) and *Noisy-le-Grand* (C). PT: primary treatment. D1,2,3: Primary, secondary and tertiary decanter. AS: Activated sludge reactor. DN: Denitrification plant. AS NIT: Activated sludge with nitrification reactor. F NIT: Biofilter with nitrification (BIOSTYR<sup>®</sup>). FB NIT: Fixed bed reactor with nitrification (SESSIL<sup>®</sup>). Arrows represent the circulating wastewater, and circles represent the automatic sampler positions. *Achères* has five treatment lines like the one presented, each receiving wastewater from a different sewer; *Valenton* has three, receiving wastewater from the same sewer; and *Noisy* has one.

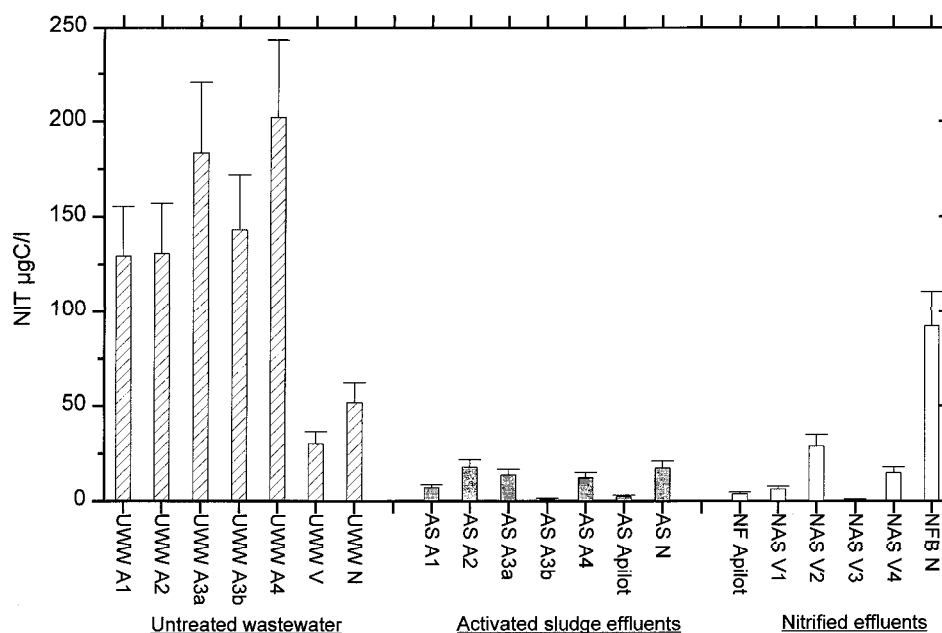


Fig. 4. Nitrifying biomass (NIT in  $\mu\text{gC/l}$ ) calculated from potential nitrifying activity measurements, with error bars corresponding to the variability of five filtration replicates, in the different kinds of wastewater. UWW A1, A2, A3a, A3b, A4, N and V: untreated wastewater arriving at the plants of Achères (A), Noisy (N) and Valenton (V) before primary treatment. AS A1, A2, A3a, A3b, A4 Apilot, and N: activated sludge effluents after secondary decantation of the plants of Achères (A) and Noisy (N). NF Apilot: Nitrified effluent from the BIOSTYR<sup>®</sup> pilot of Achères. NAS V1 to 4: Nitrified effluent from the nitrifying activated sludge of Valenton after secondary decantation. NFB N: Nitrified effluent from the fixed bed reactor of Noisy, without tertiary decantation (out of use at the time of sampling).

## RESULTS

### *Nitrifying biomass and dissolved inorganic N concentrations in wastewater*

In untreated wastewater nitrifying biomass collected either at the entrance of the purification plants or within the Boulogne sewer system varied from 1 to 200  $\mu\text{gC/l}$  (Figs 4 and 5). Classically in

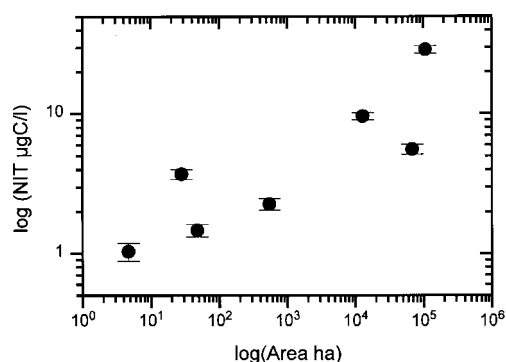


Fig. 5. Nitrifying biomass measured in untreated wastewater (NIT in  $\mu\text{gC/l}$ ) in function of the urban catchment area (area in ha) with error bars corresponding to the variability of five filtration replicates. Takes into account the catchment areas of the plants of Achères, Noisy and Valenton and the sewer network of Boulogne-Billancourt.

these samples, ammonium concentrations were high (2100–2760  $\mu\text{mol/l}$ ), and nitrite and nitrate concentrations were low (1–30  $\mu\text{mol/l}$  for both) (Table 1).

In non-nitrified activated sludge effluents, nitrifying biomass varies from 1 to 18  $\mu\text{gC/l}$  (Fig. 4) and ammonium, nitrite and nitrate concentrations (Table 1) are of the same order as in untreated effluents except for the Noisy activated sludge plant where the higher nitrate and nitrite concentrations of the effluents (360 and 87  $\mu\text{mol/l}$ , respectively) show that there is probably some nitrification occurring in the activated sludge reactor itself.

Nitrifying biomass in the effluents of the three nitrification systems studied differs considerably (Fig. 4). Effluents from the experimental nitrifying biofilter of Achères have a nitrifying biomass of 4  $\mu\text{gC/l}$ . Those from the nitrifying–denitrifying activated sludge systems of Valenton vary from 0.8 to 30  $\mu\text{gC/l}$  and those from the fixed bed reactor at Noisy-le-Grand are 90  $\mu\text{gC/l}$ .

Ammonium concentrations in the three nitrifying systems were from 5 (Noisy) to 500 (Valenton) times lower than in the untreated effluents, depending on the efficiency of each system. Accordingly, nitrate was high (1570 to 1910  $\mu\text{mol/l}$ ), even in the denitrifying plant of Valenton showing a malfunctioning on the day of sampling. Nitrite concentrations stay low (1  $\mu\text{M}$ ) except in Noisy (197  $\mu\text{M}$ ).

Table 1. Daily means of ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>) concentrations in different kinds of wastewaters<sup>a</sup>

	NH <sub>4</sub> (μM)	NO <sub>2</sub> (μM)	NO <sub>3</sub> (μM)
Untreated wastewater <sup>b</sup>			
UWW A (five sewers)	2092	28	30
UWW V (one sewer)	2443	1	28
UWW N (one sewer)	2714	2.9	0.7
Activated sludge effluents <sup>c</sup>			
AS A (five activated sludge lines)	2756	4.9	19
AS Apilot (one activated sludge line)	1900	0	48
AS N (one activated sludge line)	2136	87	363
Nitrified effluents <sup>d</sup>			
NF Apilot (one nitrification line)	262	11	1570
NAS V (four nitrification lines)	4.3	1.1	1570
NFB N (one nitrification line)	471	197	1914

<sup>a</sup>For the treatment plants receiving wastewater from distinct sewers and/or having several treatment lines functioning in parallel as Achères and Valenton, the concentration values given for the corresponding untreated and treated wastewater are averages balanced for discharge.

<sup>b</sup>UWW A, V and N: untreated wastewater arriving to the plants (before primary treatment) or Achères (A), Valenton (V) and Noisy (N).

<sup>c</sup>AS A, Apilot, and N: activated sludge effluents after secondary decantation of Achères (A), Achères'pilot (Apilot), and Noisy (N).

<sup>d</sup>NF Apilot: nitrified effluent from the BIOSTYR<sup>®</sup> pilot of Achères. NAS V: nitrified effluent from the nitrifying activated sludge of Valenton after secondary decantation. NFB N: Nitrified effluent from the fixed bed reactor of Noisy, without tertiary decantation (out of use at the time of sampling).

#### Variations in nitrifying bacteria biomass and dissolved inorganic N in the river just below the wastewater plant of Achères

The situations observed in the Seine downstream from Achères in June 1992 and March 1994 show an increase of nitrifying biomass by a factor of 2 to 4, in good agreement with what is expected from the dilution of Achères effluents (Table 2). Downstream the wastewater discharge, ammonium, increased in the river by a factor of 7 while nitrite and nitrate were not significantly affected which is in good agreement with what is expected from the dilution of Achères effluents (Table 2).

## DISCUSSION

We report in this paper the first data on nitrifiers' biomass in wastewater deduced from the measurements of potential activity. The data available in the current literature are scarce and most of them use the Most Probable Number (MPN) counting technique. These numbers converted to biomass show values of about 2–3 orders of magnitude lower than our findings (Table 3). This considerable difference is easily explained by the fact that the MPN technique, widely used in the last decades to enumerate nitrifying bacteria, is presently known to greatly underestimate the real size of the nitrifying populations. Belser and Mays (1982) showed that MPN counts in soils and sediment samples were only 0.1–5% of the estimated populations that would be required to produce the observed nitrifying activity. The fluorescent antibody technique also reveals lower values. This technique also underestimates nitrifiers' counts because not all of the sample's serotypes can be detected. Montuelle *et al.* (1996) showed that in spite of the isolation of 10 different serotypes of *Nitrobacter* sp., the fluorescent antibody counts were still lower than the MPN counts. The method we used is closer to the nitrification inhibitor sensitive BOD<sub>5</sub>, which can be considered as a nitrifying activity measurement in terms of oxygen consumption rate. The data given by Koopman *et al.* (1989), converted to nitrifiers' biomass are quite similar to our values in decanted activated sludge effluents. The modelling approach used by Henze (1992) gave higher values for nitrifiers' biomass in untreated wastewater and similar values for treated effluents.

Comparing the different biomass levels in the different kinds of untreated and treated wastewaters, we see that nitrifying biomass in untreated wastewater generally exceeds that found in treated wastewater, including nitrified effluents (Fig. 4). This is rather surprising, considering that neither domestic nor industrial wastewater originally carries nitrifying bacteria, and that anoxic conditions thought to be associated with wastewater should

Table 2. Concentration in ammonium, nitrate and nitrite, and nitrifying bacteria upstream and downstream from the purification plant of Achères, measured in June 1992 and March 1994<sup>a</sup>

	June 1992		March 1994				
	Q (m <sup>3</sup> /s)	NIT (μgC/l)	Q (m <sup>3</sup> /s)	NIT (μgC/l)	NH <sub>4</sub> (μM)	NO <sub>2</sub> (μM)	NO <sub>3</sub> (μM)
River upstream	150	5.23	350	0.45	30	2	250
Treated wastewater	20.6	–	25.5	10.61	2756	5	19
Untreated wastewater	4	–	1.9	165.17	2092	28	30
Mixed effluents	24.6	46.69 <sup>b</sup>	27.4	21.65	2709	7	20
River downstream	174.6	9.98	377.4	2.01	220	2	241
Calculated <sup>c</sup>		11.03		2.01	225	2	233

<sup>a</sup>Q: discharge, NIT: nitrifiers biomass, NH<sub>4</sub>: ammonium concentration, NO<sub>2</sub>: nitrite concentration and NO<sub>3</sub>: nitrate concentration. As wastewater arrives from five different collectors and is treated in five separate treatment lines in parallel, the concentration and biomass values given for the untreated and treated wastewater are averages balanced for discharge.

<sup>b</sup>In June 1992, only the final mixed effluent was analysed for nitrifying bacteria abundance.

<sup>c</sup>Calculated from the given concentrations and discharges considering the simple mixing of effluents in river water.

not be suitable for the growth of nitrifying bacteria in the sewer network. Although leaching of agricultural soils could represent a source of nitrifying organisms, this does probably not happen in the concreted and asphalted coverings dominating the highly urbanised areas of Paris. We also observe that nitrifying biomass concentrations in untreated wastewater increase with increasing drainage area of the sewer system (Fig. 5). This means that there must be, somewhere within the sewer system, places where the growth of nitrifying bacteria is still possible. Indeed, if there was no growth, concentration should be constant or even decrease. Where this growth is occurring is still an open question that cannot be answered without further investigations. One hypothesis is that nitrifying organisms develop where oxygenated conditions are generated (like in the heads of the network and beyond small waterfalls widely dispersed over the network). The bacteria are released continuously at these places and are dragged to the bigger collectors, their concentration increasing until the purification plant.

Secondary treatment of wastewater by an activated sludge process followed by a secondary decantation always resulted in a reduction of the nitrifying biomass (Fig. 4). This is probably related to the fact that nitrifying bacteria are associated to particulate material that is trapped in the decanter. Tertiary treatment with different nitrification systems surprisingly did not necessarily result in an increase of the nitrifying biomass in treated water (Fig. 4). Indeed, the nitrifying biofilter effluents of Achères show a reduction of the biomass, probably because of the effective mechanical filtering effect and the active bacterial grazing occurring in these kind of filters. The effluents of the nitrifying–denitrifying activated sludge system of Valenton have about the same nitrifying biomass as non-nitrifying activated sludge effluents. Apparently, most of the

nitrifying biomass produced in the process is retained by final decantation. Finally, only the effluents of the fixed bed reactor of Noisy-le-Grand show higher biomasses than in the incoming untreated wastewater, probably because of the failure of the tertiary decantation treatment at the time of sampling.

According to Chestérikoff *et al.* (1992) and Brion (1997) nitrifying bacteria concentration in the River Seine upstream from Paris ranges from 0.48  $\mu\text{gC/l}$  during winter floods to 5.23  $\mu\text{gC/l}$  during summer low waters. This is at least two time lower for most cases than in untreated and treated wastewater effluents. It follows that a discharge of wastewater can represent a significant seeding of nitrifying organisms for the river. This is well illustrated by our measurements that showed that the effluents of Achères were responsible for a significant increase of the nitrifying biomass in the river, especially during summer low-water conditions (Table 2). Bonnet *et al.* (1997) also showed that wastewater from a classical activated sludge treatment plant played a role in seeding the sediments of the receiving small river with *Nitrobacter* cells.

The impact of wastewater discharge on river water quality has generally long been studied from a strictly chemical point of view, considering only the effect of the associated input of organic or inorganic matter on the ecosystem. Recently however, it has been stressed that wastewater discharge has also significant direct biological effects on river microbial dynamics. Purification plants and untreated wastewater discharges have been shown to release large sized heterotrophic bacteria which actively take part in organic matter degradation in river water and compete with autochthonous bacteria (Garnier *et al.*, 1992a,b). Similarly, the release of protozoans by purification plants has been shown to influence the disappearance rate of faecal bacteria in rivers and

Table 3. Biomass of nitrifying bacteria in various treated wastewater; a review of literature<sup>a</sup>

	NIT ( $\mu\text{gC/l}$ )	Technique used	Authors
Untreated wastewater	1940	Model	Henze (1992)
	1.02–200.29	PNA	This work
Primary decanted effluents	0.08–0.97 <sup>b</sup>	FA	Abeliovitch (1987)
	0.008–0.32 <sup>b</sup>	MPN	Strom <i>et al.</i> (1976)
Decanted activated sludge effluents	0.002–0.62 <sup>b</sup>	MPN	Strom <i>et al.</i> (1976)
	0.0161–0.105 <sup>b</sup>	MPN	Koopman <i>et al.</i> (1989)
	16.1 <sup>c</sup>	BOD <sub>5</sub>	Koopman <i>et al.</i> (1989)
	19.32 <sup>d</sup>	Model	Henze (1992)
	1.61–18.51	PNA	This work
Decanted, nitrified activated sludge effluents	0.43 <sup>b</sup>	MPN	Strom <i>et al.</i> (1976)
	0.08–29.38	PNA	This work

<sup>a</sup>NIT: nitrifiers biomass; PNA: potential nitrifying activity measurements; MPN: Most Probable Number counts; FA: fluorescent antibody technique; BOD<sub>5</sub>: biochemical oxygen demand with the inhibition of nitrification; Model: activated sludge modelling approach.

<sup>b</sup>Calculated from the given abundance using a mean biomass/cell of  $7.33 \times 10^{-8} \mu\text{gC/cell}$  (Brion and Billen, 1998).

<sup>c</sup>Calculated from the given values of BOD<sub>5</sub>, assuming a stoichiometry of 1.9 O<sub>2</sub> consumed per oxidised NH<sub>4</sub><sup>+</sup>, and using the maximum specific activity of Brion and Billen (1998).

<sup>d</sup>Calculated from the given chemical oxygen demand (COD) values, considering that 1  $\mu\text{g}$  of microbial C corresponds to 2.84  $\mu\text{g}$  COD (Gaudy and Gaudy, 1981).

coastal seawaters (Menon, 1993). Similarly, we show here that the seeding of nitrifying bacteria accompanies ammonium input through wastewater discharge and affects the dynamics of nitrification in the river. In favourable temperature conditions, the amount of nitrifying bacteria brought by the wastewater discharge, taking advantage of the large ammonium concentration, will directly influence the nitrification rate of this ammonium.

Paradoxically, seeding of nitrifying bacteria is more pronounced with untreated sewage (particularly from large sewer networks) than with treated effluents (even in the case of nitrifying treatments), as the latter contains lower nitrifying biomass than the former (Fig. 4). This would imply that the progressive improvement of wastewater treatment on a river initially receiving untreated wastewater should result in a reduction of the seeding of this river with nitrifying organisms. Depending on the kind of improvement, the result on the river will be different. If the improvement consists in the installation of nitrification plants, the reduced seeding will be accompanied with a reduced ammonium release and, logically, this will no longer cause an ecological problem, but may even be a benefit for the river by diminishing the biological oxygen demand related to high ammonium concentrations. On the contrary, if the improvement only consists of the establishment of classical activated sludge treatment

plants, ammonium release to the river will still be high while the seeding of nitrifying bacteria is reduced. This will result in a reduction of the nitrification rates in the river downstream of the plant so that high levels of ammonium are maintained for a longer time in the river.

A retrospective analysis over the last 20 years of the evolution of the treatment capacity of the Achères plant and of the ammonium profiles in the River Seine below Paris illustrates this quite well (data are from the Syndicat Interdépartemental de l'Assainissement de l'Agglomération Parisienne S.I.A.A.P. and from the A.E.S.N.).

Ammonium profiles measured in the River Seine downstream from Paris under low-water summer conditions (Fig. 6) show apparent nitrification rates decreasing significantly (from 1.53 to 1.26  $\mu\text{mol/l/h}$ ) between 1976 and 1989 on the one hand and between 1991 and 1993 on the other hand (from 1.17 to 1.01  $\mu\text{mol/l/h}$ ). These decreases correspond, respectively, to two major modifications in the treatment of Paris wastewater at Achères, each resulting in lower amounts of untreated wastewater discharge (14% of total wastewater discharge in 1976, 2% in 1989–1992 and 0.2% in 1993) in the river (Table 4). Indeed, in 1978, a fourth treatment line was set up at Achères and in 1992, the construction of a new collector and a buffer basin (storage basin of Sèvre–Achères) allowed a better

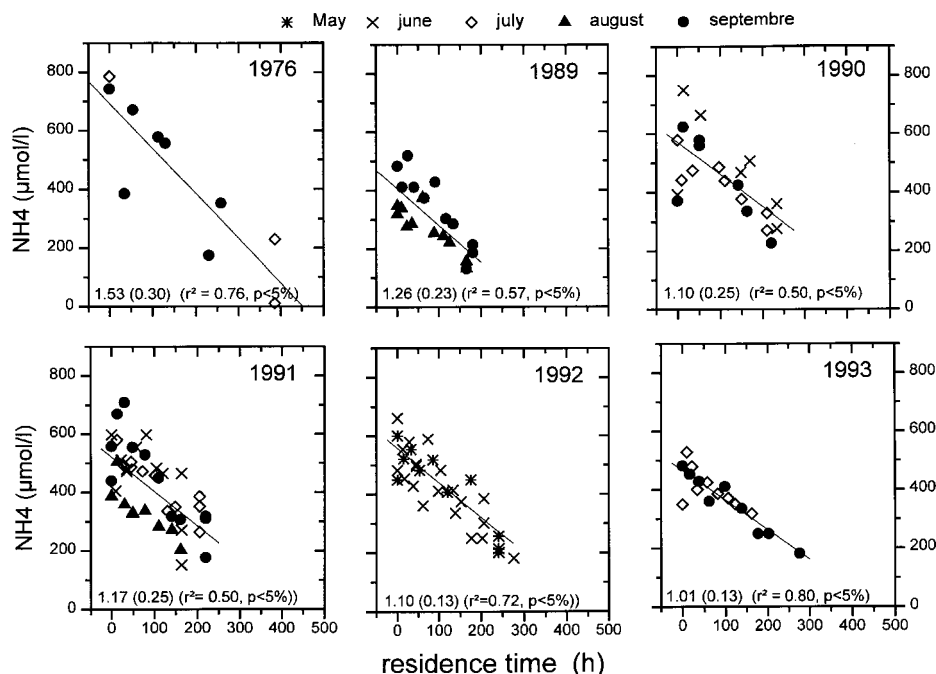


Fig. 6. Ammonium concentration ( $\text{NH}_4$  in  $\mu\text{mol/l}$ ) profiles in the River Seine downstream of the Oise confluence at different stages in the history of Paris wastewater treatment. (A) 1976: Data from the A.E.S.N. (Agence de l'Eau Seine–Normandie). Very dry summer and about 1/4 of Paris effluents are still discharged without treatment. (B–F) 1989–1993: data from the A.E.S.N. Discharge of raw sewage is much reduced. Residence time of a water mass was calculated using discharge, wet sections and stream segment lengths values. It is set to 0 at the Oise confluence.

Table 4. Influence of the treatment efficiency of the Parisian wastewater from 1976 to 1993 on the release of nitrifying bacteria to the River Seine and on the apparent nitrification rate<sup>a</sup>

Year	Q UWW (1000 m <sup>3</sup> /day)	Q AS (1000 m <sup>3</sup> /day)	Q Seine(1000 m <sup>3</sup> /day)	ΔNIT (μgC/l)	dNH <sub>4</sub> /dt (μmolN/l/h)
1976	248	1500	11,250	6.33 ± 1.3	1.54 ± 0.3
1989–1992	45.1	2230	15,660	2.56 ± 2	1.24 ± 0.27
1993	4.6	1975	15,250	1.78 ± 0.38	1.01 ± 0.13

<sup>a</sup>Q: Discharge of untreated wastewater (UWW), activated sludge effluents (AS) and Seine water (Seine). They are mean summer values for Achères (Gousaille, pers. comm.) and for the Seine river (AESN) for the periods corresponding to the longitudinal profiles given in Fig. 6. ΔNIT: increase of the nitrifying biomass in the River Seine due to wastewater discharge. This is calculated from the mean biomass measured in untreated wastewater and activated sludge effluents of Achères (165.17 μgC/l in untreated wastewater and 10.61 μgC/l in treated effluents) and taking into account the dilution of wastewater in river water. Errors are calculated from the standard deviation on the mean summer discharge of wastewater. dNH<sub>4</sub>/dt: apparent nitrification rate calculated as the slopes of the profiles represented in Fig. 6. As the slopes for the years 1989–1992 were not significantly different, data for this period were pooled to determine one apparent nitrification rate.

management of the peak wastewater flows. In this example, the significant improvement of wastewater treatment at Achères thus resulted in the decrease of the seeding of nitrifying bacteria to the river, while the ammonium input stays important. Smaller nitrifying activity caused by this decrease and the low growth rate of nitrifying organisms, result in a high level of ammonium over longer stretches in the River Seine. This ammonium is only nitrified downstream, in the estuary, where the nitrifying population becomes large enough to display high nitrification rates (Brion *et al.* (2000), in press).

#### CONCLUSIONS

This paper presents nitrifying biomass data deduced from potential nitrifying activity measurements in different kinds of treated and untreated wastewaters from the Parisian agglomeration. Major conclusions of our results are:

- Highest nitrifying biomasses are found in untreated wastewater, especially from collectors with large catchment areas.
- The treatment of the wastewater by any kind of process including a particle retention step (decantation or filter) results in a decrease of the nitrifying biomass in the effluents.
- Nitrifying biomasses in treated or untreated wastewaters are higher than in the receiving river and the present discharge of the Parisian effluents represents a significant seeding in nitrifiers.
- The significant improvement of wastewater treatment in Paris over the last 20 years resulted in the decrease of the seeding of nitrifying bacteria to the river, while the ammonium input stayed important. As a consequence, overall ammonium oxidation rates in the river downstream from Paris decreased.

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