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Studies of BDOC and bacterial dynamics in the drinking water distribution system of the Northern Parisian suburbs

Etude de la dynamique du CODB et des bactéries dans le réseau de distribution de la banlieue nord de Paris.

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Résumé de l'article

La dégradation de la qualité de l'eau dans les réseaux de distribution, due à la reviviscence bactérienne, est, à l'heure actuelle, un souci majeur pour les producteurs d'eau potable. Dans ce contexte, une bonne connaissance des facteurs de contrôle du développement bactérien dans ce type de milieu s'avère nécessaire. Le but de la présente étude est de comprendre le rôle du carbone organique dissous biodégradable (CODB) dans la dynamique bactérienne en réseau de distribution. Cet article présente les résultats d'une étude en cours, lancée à l'initiative du Syndicat des Eaux de l'Ile de France, sur le réseau de distribution de la banlieue nord de Paris alimenté par l'usine de production de Méry-sur-Oise.

Le CODB a été déterminé par la méthode de bioessai proposée par SERVAIS et al. (1987, 1989). La biomasse bactérienne libre a été estimée par microscopie à épifluorescence après coloration des bactéries à l'acridine orange, et la méthode d'incorporation de thymidine tritiée utilisée en écologie bactérienne a été adaptée, afin d'estimer la production bactérienne des bactéries présentes dans l'eau du réseau. De plus, la biomasse et l'activité des bactéries fixées ont été étudiées. Une méthode d'estimation de la biomasse, basée sur la mesure de l'activité exoprotéolytique potentielle des bactéries, a été développée. Pour l'estimation de la production bactérienne, la méthode d'incorporation de thymidine tritiée a été adaptée pour être utilisée pour les bactéries fixées.

Les résultats obtenus mettent clairement en évidence une décroissance significative de la teneur en CODB dans les canalisations de faible diamètre dans la plupart des situations. Lorsque l'on porte la décroissance du CODB entre l'eau refoulée et l'eau présente dans les canalisations de faible diamètre en fonction du CODB dans l'eau refoulée, une corrélation significative est observée (*fig. 1*); l'intersection de la droite de corrélation avec l'abscisse indique la présence d'un seuil (environ 0,16 $mgC.L^{-1}$) en-dessous duquel aucune décroissance de CODB n'est observée. Ce résultat, qui doit encore être confirmé, est important en vue de définir un objectif à atteindre en fin de filière, en terme de teneur en CODB.

Dans l'eau refoulée, l'abondance bactérienne est proche de 1 x 104 cellules par mL. Dans le réseau de distribution, elle est toujours supérieure avec des valeurs observées allant jusqu'à 7 x 10^5 $bact.mL^{-1}$; elle semble surtout liée à l'absence d'un résiduel de chlore libre (*fig. 2*). La température et la concentration en CODB dans l'eau refoulée sont aussi déterminantes comme le montre la figure 3 où l'abondance bactérienne dans les canalisations de faibles dia-mètres a été portée en fonction de la température pour deux gammes de concentration en CODB dans l'eau refoulée. Les taux de croissance des bactéries (calculés à .partir des estimations de production bactérienne et de biomasse) sont dans la gamme 0,005 à 0,1 h^{-1} (*fig. 4*), en l'absence de chlore libre ce qui correspond à des temps de génération compris entre 7 et 140 heures. La température semble fixer la valeur maximale du taux de croissance, sous ce maximum une large gamme de valeurs est observée traduisant la variabilité des conditions nutritionnelles. Les plus hauts taux de croissance observés dans le réseau sont proches des taux de croissance de bactéries mesurés dans les milieux aquatiques naturels.

Les résultats obtenus sur les bactéries fixées montrent une biomasse bactérienne fixée dans la gamme 0,25 à 0,65 $\mu gC.cm^{-2}$, ce qui correspond à une abondance de 1 x 10^7 à 2,6 x 10^7 $bact.cm^{-2}$ (*tableau 1*). Ainsi donc, dans une canalisation de 100mm de diamètre, on peut dire que la biomasse fixée est, en moyenne, approximativement de 50 à 75 fois plus élevée que la biomasse moyenne des bactéiesJibres (2 x 10^5 $bact.mL^{-1}$) (*tableau 2*). Le taux de croissance des bactéries fixées apparaît du même ordre de grandeur que celui des bactéries en suspension. Ceci signifie que dans un réseau de distribution, l'essentiel de la production de biomasse bactérienne s'effectue sur les parois des canalisations, les bactéries en suspension résultant principalement d'un décrochage de bactéries.

Un modèle mathématique de la dynamique du CODB et des bactéries dans les réseaux de distribution, incluant les connaissances acquises concernant le contrôle de l'activité bactérienne par la matière organique dissoute, est actuellement développé (*fig. 5*). Il inclut une représentation mathématique des cinétiques des processus d'adsorption-désorption des bactéries (*tableau 4*), de fixation des bactéries, d'utilisation de la matière organique biodégradable et de la croissance bactérienne (*tableau 3*), ainsi que de l'impact du chlore libre sur les bactéries libres et fixées (*fig. 6*). Bien que préliminaire, il permet de simuler l'évolution longitudinale de la biomasse bactérienne libre et fixée, du CODB et du taux de chlore libre dans le cas simplifié d'une canalisation de diamètre fixé parcourue par un flux d'eau à vitesse constante (*fig. 7*). Une de ses applications permet de simuler l'impact de la teneur en CODB de l'eau injectée dans le réseau sur les biomasses libres et fixées (*fig. 8*).

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Studies of BDOC and bacterial dynamics in the drinking water distribution system of the Northern Parisian suburbs

Etude de la dynamique du CODB et des bactéries dans le réseau de distribution de la banlieue nord de Paris

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SUMMARY

The deterioration of water quality in distribution systems due to bacterial regrowth is, at the present time, a major concern of drinking water producers. In this context, a good knowledge of the factors controlling bacterial development is required ; the aim of the present study is to understand the role of biodegradable dissolved organic carbon (BDOC) in the bacterial dynamics of the distribution system.

This paper discusses the results obtained in a study carried out in order to assess the dynamics of biodegradable dissolved organic carbon and suspended bacteria in the water distribution system of the Northern Parisian suburbs fed by the Méry-sur-Oise treatment plant.

The results show clearly that a significant decrease in BDOC occurs within the smallest pipes, when the BDOC level in the finished water is higher than about 0.20 mgC.L^{-1}. However, no decrease in BDOC is observed when the BDOC in the finished water is lower than 0.16 mgC.L^{-1}. The bacterial abundance in the distribution system is primarily linked to the absence of free chlorine. Temperature and BDOC concentration in the finished water are also major controlling factors of bacterial numbers. Bacterial growth rates are in the range 0.005 to 0.1 h^{-1} in the absence of free chlorine, the highest of these values are in the same range as the growth rates measured for bacteria in natural aquatic ecosystems. Fixed biomass to the inner pipes surface are in the range 0.25 to 0.65 μgC.cm^{-2} and the average growth rate of fixed bacteria seems to be roughly in the same order of magnitude as the average growth rate of the suspended bacteria.

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A model of the dynamics of BDOC and bacteria in distribution network, incorporating the knowledge gained from this and previous studies concerning the control of bacterial activity by dissolved organic matter, is presented. It involves a mathematical representation of the kinetics of bacterial adsorption-desorption processes, bacterial attachment, bacterial utilization of biodegradable dissolved organic matter and impact of chlorine on free and fixed bacteria. It allows simulation of the impact of reducing the BDOC in the finished water on processes associated with bacterial regrowth in the distribution network.

Key-words : *biodegradable dissoled organic carbon (BDOC), bacteria, drinking water, distribution system.*

RÉSUMÉ

La dégradation de la qualité de l'eau dans les réseaux de distribution, due à la reviviscence bactérienne, est, à l'heure actuelle, un souci majeur pour les producteurs d'eau potable. Dans ce contexte, une bonne connaissance des facteurs de contrôle du développement bactérien dans ce type de milieu s'avère nécessaire. Le but de la présente étude est de comprendre le rôle du carbone organique dissous biodégradable (CODB) dans la dynamique bactérienne en réseau de distribution. Cet article présente les résultats d'une étude en cours, lancée à l'initiative du Syndicat des Eaux de l'Ile de France, sur le réseau de distribution de la banlieue nord de Paris alimenté par l'usine de production de Méry-sur-Oise.

Le CODB a été déterminé par la méthode de bioessai proposée par SERVAIS *et al.* (1987, 1989). La biomasse bactérienne libre a été estimée par microscopie à épifluorescence après coloration des bactéries à l'acridine orange, et la méthode d'incorporation de thymidine tritiée utilisée en écologie bactérienne a été adaptée, afin d'estimer la production bactérienne des bactéries présentes dans l'eau du réseau. De plus, la biomasse et l'activité des bactéries fixées ont été étudiées. Une méthode d'estimation de la biomasse, basée sur la mesure de l'activité exoprotéolytique potentielle des bactéries, a été développée. Pour l'estimation de la production bactérienne, la méthode d'incorporation de thymidine tritiée a été adaptée pour être utilisée pour les bactéries fixées.

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Dans l'eau refoulée, l'abondance bactérienne est proche de 1 x 10^4 cellules par mL. Dans le réseau de distribution, elle est toujours supérieure avec des valeurs observées allant jusqu'à 7 x 10^5 bact.mL^{-1} ; elle semble surtout liée à l'absence d'un résiduel de chlore libre *(fig. 2)*. La température et la concentration en CODB dans l'eau refoulée sont aussi déterminantes comme le montre la figure 3 où l'abondance bactérienne dans les canalisations de faibles diamètres a été portée en fonction de la température pour deux gammes de concentration en CODB dans l'eau refoulée. Les taux de croissance des bactéries (calculés à partir des estimations de production bactérienne et de biomasse) sont dans la gamme 0,005 à 0,1 h^{-1} *(fig. 4)*, en l'absence de chlore libre ce qui correspond à des temps de génération compris entre 7 et

140 heures. La température semble fixer la valeur maximale du taux de croissance, sous ce maximum une large gamme de valeurs est observée traduisant la variabilité des conditions nutritionnelles. Les plus hauts taux de croissance observés dans le réseau sont proches des taux de croissance de bactéries mesurés dans les milieux aquatiques naturels.

Les résultats obtenus sur les bactéries fixées montrent une biomasse bactérienne fixée dans la gamme 0,25 à 0,65 $\mu gC.cm^{-2}$, ce qui correspond à une abondance de 1 x 10^7 à 2,6 x 10^7 $bact.cm^{-2}$ *(tableau 1).* Ainsi donc, dans une canalisation de 100mm de diamètre, on peut dire que la biomasse fixée est, en moyenne, approximativement de 50 à 75 fois plus élevée que la biomasse moyenne des bactéries libres (2 x 10^5 $bact.mL^{-1}$) *(tableau 2).* Le taux de croissance des bactéries fixées apparaît du même ordre de grandeur que celui des bactéries en suspension. Ceci signifie que dans un réseau de distribution, l'essentiel de la production de biomasse bactérienne s'effectue sur les parois des canalisations, les bactéries en suspension résultant principalement d'un décrochage de bactéries.

Un modèle mathématique de la dynamique du CODB et des bactéries dans les réseaux de distribution, incluant les connaissances acquises concernant le contrôle de l'activité bactérienne par la matière organique dissoute, est actuellement développé *(fig. 5).* Il inclut une représentation mathématique des cinétiques des processus d'adsorption-désorption des bactéries *(tableau 4),* de fixation des bactéries, d'utilisation de la matière organique biodégradable et de la croissance bactérienne *(tableau 3),* ainsi que de l'impact du chlore libre sur les bactéries libres et fixées *(fig. 6).* Bien que préliminaire, il permet de simuler l'évolution longitudinale de la biomasse bactérienne libre et fixée, du CODB et du taux de chlore libre dans le cas simplifié d'une canalisation de diamètre fixé parcourue par un flux d'eau à vitesse constante *(fig. 7).* Une de ses applications permet de simuler l'impact de la teneur en CODB de l'eau injectée dans le réseau sur les biomasses libres et fixées *(fig. 8).*

Mots clés : *carbone organique dissous biodégradable (CODB), bactéries, eau potable, réseaux de distribution.*

1 - INTRODUCTION

The deterioration of water quality due to bacterial growth in the distribution systems is, at the present time, a major concern for drinking water producers. This originates, on the one hand, from increasingly frequent use as of low quality raw surface water containing high levels of dissolved organic matter and, on the other hand, from the increase of the size and complexity of distribution systems, resulting in longer residence times of the water delivered. Under these conditions, bacterial growth can occur and be the starting point of a trophic food web leading to the development of undesirable higher organisms like Asellus, Nais, etc.

At the present time, chlorination of treated water with, in some cases, postchlorination in the network is often used to limit bacterial growth in the distribution system. This solution, however, presents some disadvantages, the major one being the formation of unpleasant and potentially carcinogenic

organochlorine compounds responsible for tastes and odours of treated water (JESTIN *et al.*, 1987 ; KRASNER *et al.*, 1989).

An alternative strategy consists of developing treatment lines in which biodegradable dissolved organic carbon is removed by biological filtration (BABLON *et al.*, 1987 ; SERVAIS *et al.*, 1991). This reduces the amount of chlorine to be applied, thus limiting the formation of undesirable organochlorine compounds. The low chlorine demand of the water produced also guarantees a greater stability of its chlorine residual allowing avoidance of rechlorination within the distribution system. In the long term, an objective could be to completely éliminate bacterial growth solely by reducing the level of biodegradable organic matter, without any chlorination of the water, as it is sometimes practiced in Europe (for example, in the Netherlands and in some French cities), in the cases where high quality raw water is available.

In this context, a good knowledge of the factors controlling bacterial development is required and special attention has to be devoted to understand the role of biodegradable dissolved organic carbon (BDOC) in the bacterial dynamics within the distribution system. This is the aim of a present study launched since 1989 by the SEDIF (Syndicat des Eaux d'Ile de France). This paper presents a synthesis of the results obtained to date.

The présentation is divided in two parts. In the first one, the observations made in a full scale distribution system (the Parisian suburbs network) on the dynamics of BDOC, free and attached bacteria are summarized. For the investigations on the dynamics of bacterial population in the distribution system, we have used methods evolved from the recent progress in the field of bacterial ecology, in contrast with numerous studies in which classical bacteriological methods such as plate counts are used (GOSHKO *et al.*, 1983 ; LECHEVALLIER & MC FETERS, 1985 ; LECHEVALLIER *et al.*, 1987). In the second part, we present a model of the dynamics of BDOC and bacteria in a distribution network which is being developed on the basis of the knowledge gained during this and previous studies.

2 - MATERIALS AND METHODS

2.1 Sampling

During nine sampling campains, performed at different periods of the year, about 25 water samples were collected in the distribution network of the Northern Parisian suburbs, fed by the Méry-sur-Oise treatment plant, through fire hydrants and the vents located on various types of pipes (diameters from 800 to 80 mm). Some of the data presented in this paper were obtained during a previous study performed in November 1987 and February 1988 in the distribution network of the Eastern Parisian suburbs fed by the Neuilly-sur-Marne treatment plant. In order to study parameters concerning fixed biomass in pipes, sixteen chambers in which direct access to the pipes (mainly 100 mm diameter) is possible, were built. In each of these chambers, there were four sampling devices which allow the insertion of a piece of cast-iron

(surface area around 1.5 cm^2) just at the inner surface of the pipe in contact with the flowing water and its collection after colonization in the distribution system. Three sampling campains in 1990-1991 were devoted to the study of fixed bacterial biomass and activity.

2.2 Dissolved organic carbon (DOC) and biodegradable dissolved organic carbon (BDOC)

The dissolved organic carbon (DOC) was measured with a Dohrmann DC-180 Total Carbon Analyser using ultra-violet promoted persulfate oxidation of organic carbon, followed by infra-red and spectrophotometric detection of the CO_2 produced by the oxidation. The biodegradable fraction of DOC was determined according to the bioassay procedure developed by SERVAIS *et al.* (1987, 1989). It consists in following the DOC decrease in the water sample inoculated with bacteria (1 % of 2 µm pore size filtered water from river Marne), during a 30 days incubation period in the dark at 20 °C. BDOC is calculated as the difference between DOC at the beginning and at the end of the incubation. The accuracy of the method has been estimated as ± 0.05 $mgC.L^{-1}$ (SERVAIS *et al.*, 1989).

2.3 Biomass and activity of suspended bacteria

Bacterial numbers were determined by epifluorescence microscopy after acridine orange staining following the procedure of HOBBIE *et al. (1977).* Cell size was estimated on enlargements of photographs and biomass were calculated from abundance and biovolume distribution using the biovolume dependent conversion factor proposed by SIMON and AZAM (1989).

The tritiated thymidine incorporation method (FUHRMAN and AZAM, 1980, 1982) is now widely used in order to estimate bacterial production in aquatic environments. We have adapted the procedure to allow measurement in water of low bacterial levels (SERVAIS *et al.*, 1992). A 100 mL sample of water was incubated at *in situ* temperature in the presence of 2 nanomoles of tritiated thymidine (methyl ^{3}H-thymidine from Amersham – Specific activity : 40 $Ci.mmol^{-1}$) during a period of 20 hours. At the end of incucation, 5 mL of NaOH 5N was added ; after 10 minutes, 28 mL of cold trichloroacetic acid (TCA) were added ; after 10 minutes at 0 °C, the sample was filtered through a 0.2 µm pore size nitrate cellulose membrane (Sartorius) and rinsed with 5 mL of a phenol-chloroform (50% w/v) mixture and then with 5 mL of ice-cold 80 % (v/v) ethanol. This biochemical procedure has been proposed by WICKS and ROBARTS (1987) in order to eliminate radioactivity incorporated in macromolecules other than DNA. The value of incorporation in a blank sample has been substracted ; it consists in a sample initially treated by 1% (final concentration) of a saturated solution of $HgCl_2$ in order to stop biological processes.

The values of thymidine incorporation in bacterial DNA have been converted in cells production using a conversion factor of 1.5×10^{18} bacteria produced per mole of thymidine incorporated into bacterial DNA (SERVAIS, 1986). Growth rates of bacteria were calculated as the ratio of bacterial production (expressed in cell production) to cell number.

2.4 Biomass and activity of fixed biomass

Direct epifluorescence microscopic observations of bacteria fixed on cast-iron is impossible. The classical approach to estimate abundance of fixed bacteria is to enumerate them after their detachment from the support usually by sonication or sometimes using a homogenizer (CAMPER, *et al.*, 1985). Experiment carried out in our laboratory have shown however that it is quite impossible to obtain a total detachment of the fixed bacteria by sonication without breaking some of them. Moreover, sonication of cast-iron coupons results in the production of a lot of small metallic particles which cause difficulties for microscopic enumeration of bacteria. Therefore, to avoid detachment of bacteria from the support, we developed a method based on measurement of potential exoproteolytic activity (SOMVILLE and BILLEN, 1983), which has been shown to be proportional to bacterial biomass (BILLEN, 1991). Measurements are based on the detection of a fluorescent compounds (ß-Naphthylamine) (ßN) which is produced after hydrolysis a non-fluorescent substrate (L-Leucyl-ß-Naphthylamide) (LLßN) by the bacterial exoproteases. A conversion factor of 6.58 µgC of bacterial biomass per nmole of ßN per minute has been established by comparing the potential exoproteolytic activity of bacterial suspensions with the biomass estimated by epifluorescence microscopy as described in the previous section. A special incubation device has been built to incubate in presence of LLßN only the surface of the cast-iron coupon which has been in contact with the flowing water.

The method for estimating activity of fixed bacteria involved measurement of ^{3}H-thymidine incorporation as for free bacteria. The experimental procedure was as follows : the cast-iron coupons were incubated with 2 mL of distribution system water filtered on a 0.2 µm pore sise membrane and 50 µL of ^{3}H-thymidine (200 nM solution of methyl ^{3}H-thymidine from Amersham – specific activity 40 Ci. mmole^{-1}). Incubation at *in-situ* temperature in the dark lasted around 20 hours. Then the supernatant was discharged and 2 mL of NaOH 5N added in contact with the coupon. The device is heated 1 hour at 60 °C in order to make the bacterial DNA soluble. After cooling, 0.5 mL of 100 % TCA was added. After 15 minutes at 0 °C, the liquid was filtered through a 0.2 µm pore size nitrate cellulose membrane (Sartorius), then rinsed as described in the previous section. The bacterial production was calculated using the same conversion factor as for suspended bacteria.

2.5 Determination of adsorption/desorption parameters

Simple experiments allowed to experimentally determine the values of the three parameters involved in the description of the adsorption/desorption kinetics (see section 4.2.2). These were based on the use of a culture of radioactively labelled bacteria obtained by enriching a tap water sample with 500 mg. L^{-1} of yeast extract, incubating it at 20 °C for 24 h in the presence of 20 nM ^{3}H-thymidine (40-50 Ci. mmol^{-1}) and dyalizing it to remove the excess of nutrients and radioactivity. Small volumes of a dilution of this cultures were put in contact under agitation with cast-iron coupons. Aliquots from the liquid phase were collected at intervals for a few hours. The initial rate of bacterial adsorption, and the equilibrium-adsorption value were determined from the experimental results using the equation mentionned in the Section 4.2.2.

3 - FIELD RESULTS

3.1 Biodegradable dissolved organic carbon

The data collected in the Parisian suburbs distribution system covered a large range of situations ; BDOC values in the finished water at the coming out of the plant ranged between 0.20 and 0.61 mgC.L^{-1}. BDOC measurements carried out in the network show that a significant decrease in BDOC often occurred within the small pipes (100 mm diameter). In figure 1, the difference in BDOC between the finished water at the treatment plant and the water collected in the small pipes (Δ BDOC) has been plotted against the BDOC of the finished water just at the outlet of the plant. A significant correlation ($r = 0.88$, $n = 10$) is observed ; the intercept of the linear correlation seems to indicate a threshold of BDOC in the finished water (around 0.16 mgC.L^{-1}) under which there was no consumption of BDOC in the distribution system. When BDOC in the finished water was higher than this threshold, the decrease in the distribution system ranged between 0.03 and 0.22 mgC.L^{-1}. These results which have to be confirmed are very important in order to define an objective to reach in the treatment plants in terms of BDOC levels in the finished water.

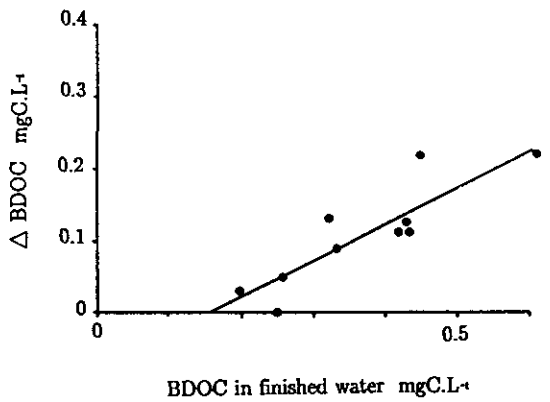

Figure 1 Difference between BDOC the finished water and the average BDOC in water colleced in the small pipes (100 mm diameter) for each sampling campains (Δ BDOC) plotted against BDOC in the finished water. Linear correlation straight line :

$$\Delta \text{ BDOC} = 0.527 \text{ . BDOC finished water} - 0.085$$
$$(r = 0.88,\ n = 10)$$

Différence entre le CODB dans l'eau refoulée et la moyenne pour chaque campagne de mesures des CODB mesurés sur les échantillons prélevés dans les petites canalisations (diamètre 100 m) (Δ CODB) porté en fonction du CODB dans l'eau refoulée. L'équation de la droite de corrélation est la suivante :

$$\Delta \text{ CODB} = 0{,}527 \text{ . CODB eau refoulée} - 0{,}085$$
$$(r = 0{,}88,\ n = 10)$$

3.2 Free bacterial biomass and activity

In the finished water, the average bacterial abundance was close to 1 x 10^4 bacteria per mL. In the distribution system, it was always higher with values up to 7 x 10^5 bacteria per mL. Even if the higher bacterial abundances were most often observed in the small pipes (100 mm diameter), the level of bacterial numbers seems to be primarily linked to the absence of free chlorine as shown in figure 2. However, in the small pipes in the absence of free chlorine, the range of bacterial abundance was very broad.

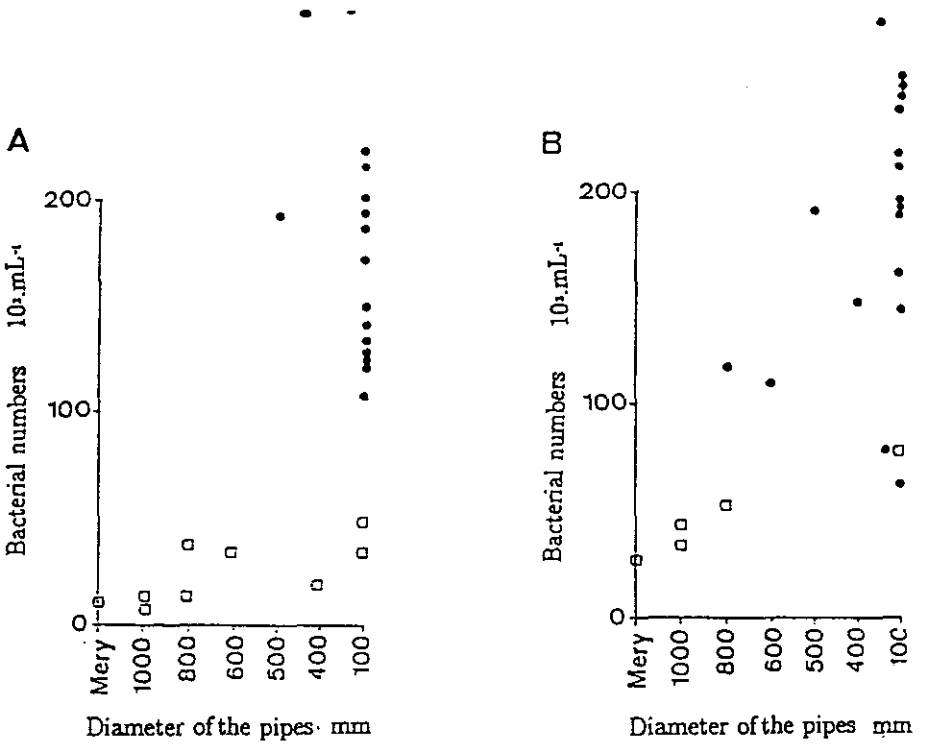

Figure 2 Bacterial abundance in the distribution system of the Parisian suburbs in February 1989 (A) an September 1989 (B) plotted against the diameter of the pipes where the sample was collected.

• Absence of free chlorine □ Presence of free chlorine

Abondance bactérienne dans les échantillons collectés sur le réseau de la banlieue parisienne en février 1989(A) et septembre 189 (B) porté en fonction du diamètre de la canalisation où l'échantillon est prélevé :

• *Absence de résiduel de chlore libre* □ *Présence d'un résiduel de chlore libre*

Temperature and the concentration of BDOC are also major control factors of bacterial abundance. In figure 3, average bacterial abundance in the small pipes in the absence of free chlorine has been plotted against temperature for various situations ; moreover, data were distinguished for two ranges of

BDOC in the finished water (0.2 to 0.4 mgC.L^{-1} and 0.4 to 0.6 mgC.L^{-1}). In each range of BDOC level in the finished water, average bacterial abundance clearly increases with increasing temperature.

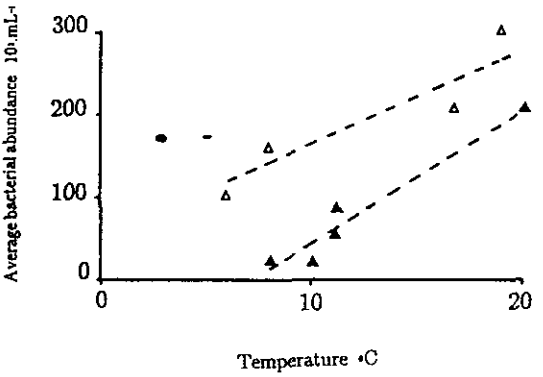

Figure 3 Average bacterial abundance for each sampling campains plotted against temperature for situations with a BDOC level in finished water in the range 0.2 – 0.4 mgC.L^{-1} (▲) and in the range 0.40 – 0.60 mgC.L^{-1} (Δ).

Abondance bactérienne moyenne pour chaque campagne de mesures portée en fonction de la température pour des situations avec un CODB dans l'eau refoulée compris entre 0,2 et 0,4 mgC.L^{-1} (▲) et entre 0,4 et 0,6 mgC.L^{-1} (Δ).

Growth rates of free bacteria (calculated as the ratio between bacterial production and free bacterial biomass estimations) are very low in the finished water (0.0005 to 0.002 h^{-1}) because of the low bacterial abundance and the presence of free chlorine (0.3 to 0.5 mg Cl_2. L^{-1}). In the distribution system, they are in the range 0.005 to 0.1 h^{-1} in the absence of free chlorine *(fig. 4)*. These growth rates correspond to generation times between 7 and 140 hours. Temperature determines a value of maximum growth rate ; below this maximum a large range of values is observed at each temperature, due to the variability of nutritional conditions for bacteria. The highest of the observed values of growth rates are in the same range as the growth rates measured for bacteria in natural aquatic ecosystems (BILLEN *et al.*, 1990)

3.3 Fixed bacterial biomass and activity

Three series of measurements have been recently carried out in order to estimate fixed bacterial biomass on thirty pieces of cast-iron collected after a period of two to three months of colonization in the Northern Parisian suburbs distribution system. Results are presented in table 1. Average fixed bacterial biomass were in the range 0.25 to 0.65 µgC.cm^{-2}. These values correspond to a fixed bacterial abundance in the range 1 x 10^7 to 2.6 x 10^7 bacteria per cm^2. These values are in the same range as the bacterial numbers (average 1 x 10^7 bacteria. cm^{-2}) observed on PVC in an experimental pilot distribution system in Nancy (HAUDIDIER *et al.*, 1988).

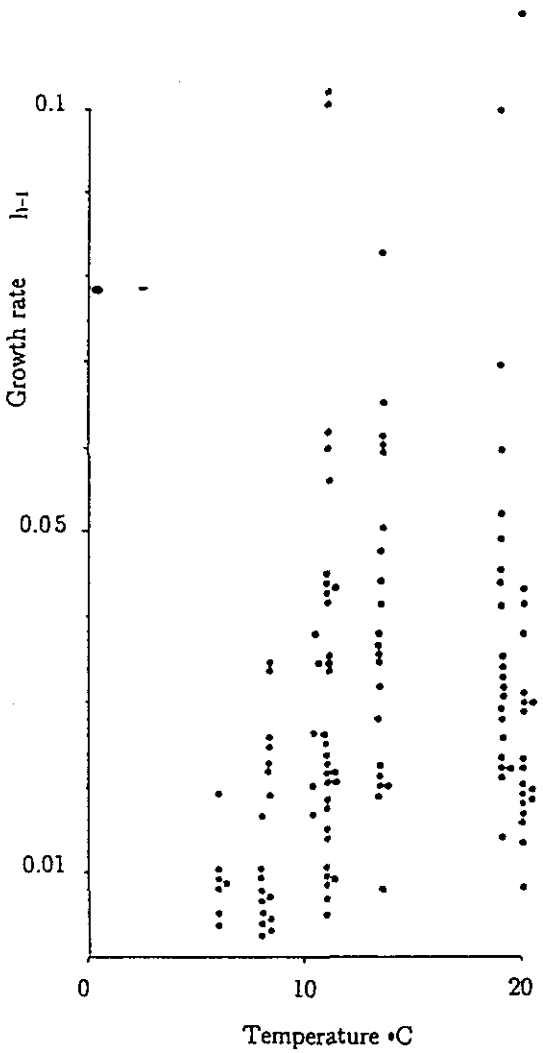

Figure 4 Bacterial growth rates in the distribution system, in the absence o free chlorine, plotted against temperature.

Taux de croissance des bactéries dans le réseau de distribution en l'absence de résiduel de chlore libre portés en fonction de la température.

With these estimations of fixed bacterial numbers, it is possible to directly compare fixed and free biomass in a distribution system. We have calculated, for the three situations, the ratio between average fixed and free bacterial numbers in a 100 mm diameter pipe where the ratio of volume to inner pipe surface is 25 liters per m^2 *(table 2)* The data show that in a small pipe fixed bacteria numbers was 53 to 77 times higher than the free bacterial numbers.

Table 1 Average fixed bacterial biomass and numbers, and production and growth rate of fixed bacteria.

Tableau 1 *Moyennes de la biomasse bactérienne fixée, de l'abondance bactérienne fixée, de la production des bactéries fixées et de leur taux de croissance.*

	Biomass ($\mu gC.cm^{-2}$)	Numbers (10^7 bact.cm^{-2})	Production ($\mu gC.cm^{-2}h^{-1}$)	Growth rate (h^{-1})
Sepember 90	0.65	2.6	–	–
December 90	0.48	2	0.0045	0.017
April 91	0.28	1.1	0.0041	0.026

Table 2 Comparison of the average abundance of free and fixed bacteria in a pipe of 100 mm diameter.

Tableau 2 *Comparaison des abondances moyennes des bactéries libres et fixées dans une canalisation de 100 mm de diamètre.*

	Fixed bacterial numbers ($10^{11}.m^{-2}$)	Free bacterial numbers ($10^6.L^{-1}$)	*Fixed bacteria* Free bacteria
Sepember 90	2.6	196	53
December 90	2	104	77
April 91	1.1	78	56

In December 1990 and April 1991, bacterial production of the fixed bacteria were also measured by tritiated thymidine incorporation. The bacterial production ranged between 0.001 to 0.008 $\mu gC.cm^{-2}.h^{-1}$ with average values of 0.0045 and 0.0041 $\mu gC.cm^{-2}.h^{-1}$ respectively in December 1990 and April 1991. The average growth rates deduced from production and biomass estimations were respectively 0.017 and 0.026 h^{-1}. The estimated growth rate of fixed bacteria seems to be roughly in the same order of magnitude as the average growth rates of the suspended bacteria which were 0.009 h^{-1} and 0.012 h^{-1} respectively in December 1990 and April 1991. In these conditions, the production of bacterial biomass in a distribution system takes primarily places at the inner surface of the pipes. The high abundance of suspended bacteria results from a detachment of bacteria from the pipes surface. This is confirmed by the fact that the production of the bacteria in the water phase is unable to explain the increase of abundance observed in the distribution system. This conclusion obtained in a full scale study is in accordance with the results of two recent studies, one performed in a laboratory reactor (VAN DER WENDE *et al.*, 1989), the other in an industrial pilot (HAUDIDIER *et al.*, 1988).

4 - MODELLING BACTERIAL DYNAMICS IN A DISTRIBUTION SYSTEM

The complexcity of the bacterial dynamics in a distribution system is such that the utilization of a mathematical model is required in order to investigate the impact of modifications of various controlling factors (BDOC level, free chlorine concentration, etc.) on the bacterial growth in a distribution network. At the present time, we are developing a model of the dynamics of BDOC and bacteria in a distribution network, incorporating the knowledge gained from this and previous studies concerning the control of bacterial activity by dissolved organic matter. In the future, this *a priori* model will be validated on the basis of experimental data.

4.1 Principles of the model

The developed model, called SANCHO, has been adapted from the model of biological filtration on GAC (CHABROL model) recently developed by BILLEN *et al.* (1992). An analogy indeed exists between the processes occurring during progression of the water within a long pipe with attached bacteria on its wall, and those occurring during filtration through a solid support. The processes taken into account in the SANCHO Model are the following :

(i) The exoenzymatic hydrolysis of dissolved organic matter by bacteria and the growth of free and fixed bacteria on the hydrolysis products ; bacterial mortality which releases organic matter is also considered.

(ii) The reversible adsorption and biological attachment of bacteria to the inner pipe surface.

(iii) Chemical consumption of free chlorine and impact of free chlorine on free and fixed bacterial activity.

The variables of the model are the following :

– Biomass of free bacteria (B_3), adsorbed bacteria (B_2), biologically fixed bacteria (B_1) expressed in $\mu gC.L^{-1}$ for B_3 and in $\mu gC.cm^{-2}$ for B_2 and B_1.

– Biodegradable dissolved organic matter (BDOC) is divided into three pools : H_1 and H_2, are polymeric organic matter which can be rapidly (H_1) or slowly (H_2) hydrolyzed by bacterial exoenzymes. S represents the monomeric substrates resulting from the hydrolysis of H_1 and H_2, which can be taken up by bacteria. All these pools of organic matter are expressed in $mgC.L^{-1}$.

– Free chlorine concentration.

Figure 5 diagrammatically represents the interaction between the variables taken into account in the SANCHO model.

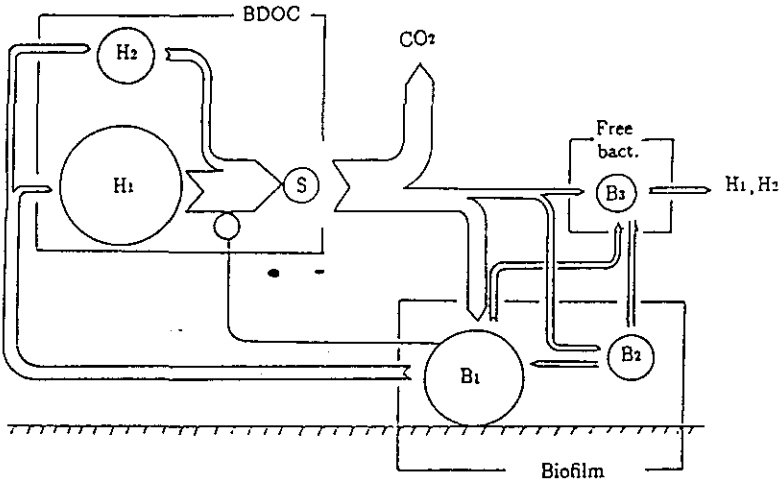

Figure 5 Schematic representation of the processes taken into the account in the model of bacterial dynamics in the distribution system (SANCHO Model).

H_1 : rapidly hydrolysable polymeric BDOC ;
H_2 : slowly hydrolysale polymeric BDOC ;
S : direct sustrate ; B_2 : adsorbed bacteria ;
B_1 : biogicaly fixed bacteria ; B_3 : free bacteria.

Représentation schématique des processus pris en compte dans le modèle de la dynamique des bactéries dans le réseau (modèle SANCHO)

H_1 : matière organique polymérique rapidement hydrolysable ;
H_2 : matière organique polymérique lentement hydrolysable ;
S : substrat direct ; B_2 : bactéries adsorbées ;
B_1 : bactéries fixées biologiquement ; B_3 : bactéries en suspension.

4.2 Kinetics of the processes involved in the model

4.2.1 *Dissolved organic matter-bacteria interaction*

The HSB model, developed in our laboratory for modelling the bacterial degradation of organic matter in natural aquatic ecosystem (SERVAIS, 1986 ; BILLEN and SERVAIS, 1988) is the basis of the SANCHO model. It can be summarized as follows :

The polymeric biodegradable dissolved organic matter (H_1 and H_2) is hydrolyzed according to a Michaelis-Menten kinetics (characterized by the parameters e_{max} and KH) by the bacterial exoenzymes which are in concentration directly proportional to bacterial biomass. Exoenzymatic hydrolysis of macromolecules yields monomeric substrates (S), like amino-acids and monosaccharides, which are rapidly taken up and utilized by bacteria (B) according to a Michaelis-Menten kinetics (characterized by the parameters $Y.b_{max}$, Ks). Bacterial mortality is supposed to obey a first order kinetic with regard to bacterial biomass. The dependency of bacterial activity

on temperature is characterized by a sigmoid function taking into account the adaptation of bacterial communities to seasonal fluctuations of temperature :

$$Act\ (T) = Act\ (T_{opt})\ exp - [\ (T_{opt}-T)^2 / (T_{opt}-T_i)^2]$$

with

T : *in situ* temperature ;
T_{opt} : temperature for which bacterial activity is maximum ;
T_i : width of the sigmoid relationship.

The values of the parameters used in the SANCHO model are listed in table 3 ; they result from previous determination summarized in BILLEN and SERVAIS (1988) and BILLEN *et al.* (1991).

Table 3 Values of the parameters of the bacterial utilization of BDOC taken into account in the SANCHO Model.

Tableau 3 *Valeurs des paramètres de l'utilisation bactérienne du CODB pris en compte dans le modèle SANCHO.*

Parameters	Units	Values
e_{1max}	h^{-1}	0.75 (at 20 °C)
KH_1	$mgC.L^{-1}$	0.25
e_{2max}	h^{-1}	0.25 (at 20 °C)
KH_2	$mgC.L^{-1}$	2.5
b_{max}	h^{-1}	0.6
Ks	$mgC.L^{-1}$	0.05
Y		0.2
T_{opt}	°C	40 – 0.5 (20-T)
T_i	°C	18 – 0.5 (20-T)

4.2.2 *Bacterial attachment*

In the model, bacterial attachment on the inner pipe surface is considered as resulting from two basically different processes (FLETCHER, 1980), adsorption and biological attachment.

1) The rapid and reversible physico-chemical adsorption process can be described by LANGMUIR theory. It assumes that the rate of bacterial adsorption onto the support is proportional to the concentration of the bacteria (B_3) in the liquid phase and to the concentration of free adsorption sites on the support. The latter is obviously equal to the difference between the maximum adsorption capacity (SB) of the support and the concentration of adsorbed bacteria (B_2) :

$$\text{adsorption rate} = \text{kads}\ B_3\ (SB - B2)$$

where kads is the adsorption rate constant for bacteria onto the support.

On the other hand, desorption rate is assumed first order with respect to the concentration of occupied adsorption sites :

$$\text{desorption rate} = \text{kdes}\ B_2$$

where kdes is desorption rate constant of bacteria from the support.

Equilibrium is reached when adsorption and desorption rates match, i.e. when

$$B_2 = SB \frac{B_3}{\frac{kdes}{kads} + B_3}$$

Direct measurements of initial adsorption rate and adsorption / desorption equilibrium conditions of labelled bacteria on cast-iron pieces allowed us to experimentally determine the value of the three parameters involved in the description of the adsorption/desorption kinetics *(table 4)*.

Tableau 4 Parameter values of the adsorption and desorption characteristics of bacteria on cast-iron.

Table 4 *Valeurs des paramètres caractéristiques de l'adsorption et de la désorption des bactéries sur la fonte.*

SB	0.3 – 0.5 ($\mu gC.cm^{-2}$)
kads	0.5 – 1 ($\mu gC.cm^{-2})^{-1}\ h^{-1}$
kdes	2 – 1 (h^{-1})

2) A slow irreversible biological attachment process involving active bacterial secretion of polysaccharides is also considered. Biological fixation results from the growth of previously adsorbed or fixed bacteria at sites where attachment is possible. As in the distribution system, fixed bacterial biomass never exceeds 2 $\mu gC.cm^{-2}$, it seems that there is never constitution of a true biofilm covering all the inner pipe surface. In fact, a 2 $\mu gC.cm^{-2}$ biomass roughly represents a 10 % coverage of the inner pipe surface. This is confirmed by microscopic observations, which shows only sparce areas of bacterial colonization. Everything happens as if a maximum capacity of bacterial biomass attachment (SP) exists for a given support, possibly corresponding to the existence of sites protected from the shear of the traversing flow. Therefore it was assumed that the rate of increase of attached bacterial biomass (B_1) depends on the growth rate of adsorbed and previously attached bacteria, in proportion to the room remaining available on the *"protected sites"*. The fraction of B_1 and B_2 production, which cannot find places at these sites, yields free bacteria in the interstitial water (B_3). In the model, we have used a value of 2 $\mu gC.cm^{-2}$ for SP.

4.2.3 *Chlorine dynamics*

As observed in the field results, the presence of chlorine in the finished water is an important controlling factor of the bacterial dynamics in the network. The SANCHO model takes into account both the processes responsible for chlorine consumption in the distribution system and the impact of free chlorine of bacterial activity.

Works by JADAS-EECART (1989) have shown that the rate of chlorine degradation, after an initial phase of rapid demand, which is satisfied during the initial storage of water after chlorination and before dechlorination and

injection in the distribution system, follows first order kinetics with regards to chlorine concentration (Cl_2) and to total demand (b) :

$$\frac{dCl_2}{dt} = - k.Cl_2.b$$

Values of the kinetic constant are around 0.1 $L.mole^{-1}.\ sec^{-1}$, or $5\ 10^{-3}\ (mgCl_2.\ L^{-1})^{-1}.h^{-1}$.

The total demand is generally attributable to DOC with a ratio b/DOC = 1 $mgCl_2/mgDOC$. It seems that BDOC is responsible for the major part of chlorine demand with a ratio b/BDOC up to 3 $mgCl_2/mgDOC$, while the ratio b/NBDOC (non biodegradable DOC) is around 0.25 $mgCl_2/mgDOC$ (VENTRESQUE, pers. comm.).

In the model, chlorine demand due to biomass fixed to the pipes is taken into account in addition to chlorine consumption by DOC. The impact of chlorine on the activity of heterotrophic bacteria has been determined by the tritiated thymidine incorporation after addition of various chlorine concentration to a natural assemblage of free bacteria *(fig. 6)*. The impact can be represented by the relationship :

$$Act\ (Cl_2) = Act(0)\ \exp\left[-\left(\frac{Cl_2 - Clm}{dCl}\right)\right]$$

with

Clm : threshold above which an impact of chlorine on bacterial activity is observed (0.03 $mgCl_2.\ L^{-1}$).

dCl : characteristic chlorine concentration (0.2 $mgCl_2.\ L^{-1}$).

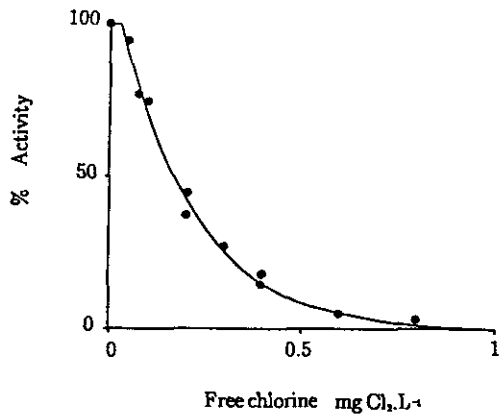

Figure 6 Impact of free chlorine on the activity of free bacteria (bacterial activity of a natural freshwater samples measured by ^3H-thymidine incorporation after addition of various quantity of hypochlorite).
• Experimental data ;
— Simulation by the relationship mentionned in the text.

Impact du chlore libre sur l'ativité bactérienne (activité bactérienne d'un échantillon d'eau douce naturelle mesurée par incorporation de ^3H-thymidine après addition de quantité croissante d'hypochlorite).
• Résultats expérimentaux ;
— Simulation par la relation mentionnée dans le texte.

However, VAN DER WENDE *et al.* (1989) suggested that fixed bacteria are more resistant to chlorine toxicity, due to the presence of an extracellular polymer i.e. mucus envelope subsiances. In the absence of experimental results, we have considered a relationship similar to that for free bacteria, but with a lower impact of chlorine on fixed bacterial activity (Clm = 0.1 $mgCl_2$. L^{-1} and dCl = 0.1 $mgCl_2$. L^{-1}).

4.3 Results of simulations

The SANCHO model calculates the spatial fluctuations, at steady state, of BDOC and chlorine concentrations as well as free and fixed bacterial biomass. In its present form, it considers the simplified case of a single pipe run by a water flux at constant speed.

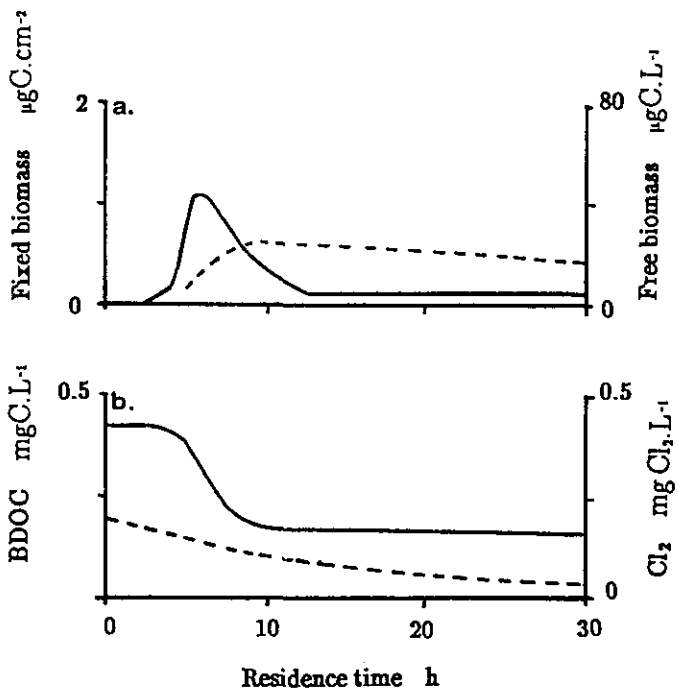

Figure 7 Longitudinal variations of (a) free (– – –) and fixed biomass (——), (b) BDOC (——), free chlorine concentration (– – –) in a 100 mm diameter pipe calculated by the SANCHO model at 20 °C (BDOC and free chlorine concenrations in inflowing water are respectively 0.4 $mgC.L^{-1}$ and 0.2 $mgCl_2.L^{-1}$).

Variation longitudinale à 20 °C de la biomasse bactérienne en suspension (– – –) et fixée (——) (a) ainsi que la concentration en CODB (——) et du résiduel de chlore libre (– – –) (b)dans une canalisation de 100 mm calculées par le modèle SANCHO (concentration en CODB et en chlore libre dans l'eau refoulée respectivement de 0,4 $mgC.L^{-1}$ et 0,2 $mgCl_2.L^{-1}$).

Figure 7 presents the simulation of the longitudinal variation of the biofilm developed in a 100 mm diameter pipe, fed by a water at 20 °C containing 0.4 $mgC.L^{-1}$ of BDOC and a free chlorine concentration of 0.2 $mgCl_2.\ L^{-1}$. Three successive areas can be distinguished. In the first one (0 to 5 h), development of biofilm is very limited because the level of chlorine keeps the bacterial growth rate very low. In the second one (5 to 10 h), an important biofilm develops. The exoenzymatic activity which is associated to bacteria allows an increase of the direct substrates concentration ; it increases the bacterial growth rate. In this area, BDOC is consumed and reaches a level down to 0.2 $mgC.L^{-1}$. In the third one (>10 h), no further decrease of BDOC is apparent and the growth rate rapidly decreases. However, free bacteria remain high and support biofilm mainly formed of adsorbed bacteria with only limited activity. The length of these three areas and the level of free and fixed bacteria reached depends on the values of the parameters chosen. Up to now, as the impact of chlorine on fixed bacteria has been arbitrarily fixed, absolute values yielded by the model have to be considered with caution. However, the trends are probably meaningfull.

As an example of a first application of this idealized model, we have tested the impact of various levels of BDOC in the finished water on the maximum level of free and fixed bacterial biomass reached in a 100 mm diameter pipe at 20 °C *(fig. 8)*. The importance of BDOC on bacterial dynamics is clearly shown by the calculations with the SANCHO model. The impact of BDOC is slightly different on free and fixed bacteria ; at BDOC values higher than 0.5 $mgC.L^{-1}$, the fixed biomass reaches a plateau while this is not the case for free bacteria which continues to increase with increasing BDOC.

5 - CONCLUSIONS

This work shows the results of applying recently developed methods for estimating BDOC, free and fixed bacterial biomass and activity in the study of drinking water microbiology. The experimental results gained in the Parisian suburbs distribution network allow us to quantify the relative contribution of the various processes involved in the bacterial dynamics. In particular two points have to be kept in mind :

– BDOC is a major controlling factor of bacterial growth. For the Parisian suburbs distribution network, we can define a threshold in terms of BDOC in the finished water below which the water seems to be biologically stable in the distribution system. Even if this point needs confirmation, it is of special importance in order to define objectives for the functioning of treatment plants.

– The importance of the role played by fixed bacteria in controlling the dynamics of the free microbial population has been demonstrated ; when a significant increase in cells number is observed, it is mainly due to detachment from the biofilm.

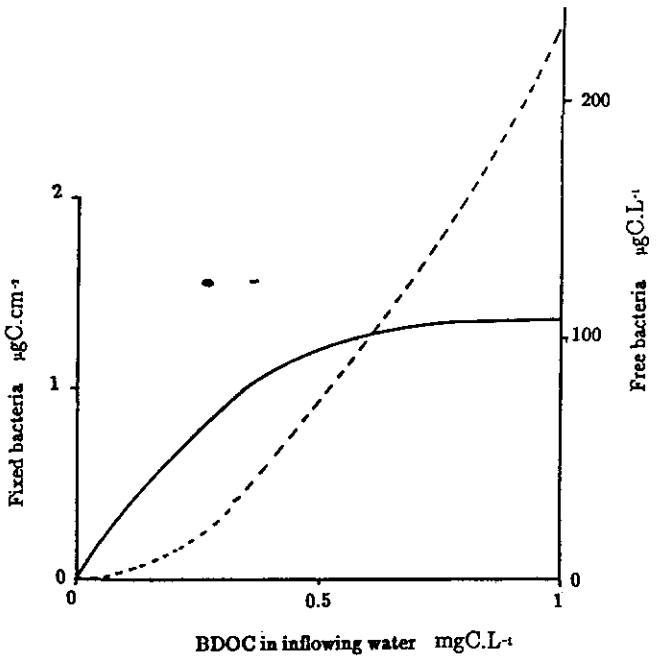

Figure 8 Maximum fixed (——) and free (– – –) bacterial biomass reached (in a 100 mm diameter pipe at 20 °C with a free chlorine residual of 0.2 $mgCl_2/L$ in the inflowing water) calculated by the SANCHO model for various BDOC concentrations in the inflowing water.

Maxima de biomasse bactérienne fixée (——) et en suspension (– – –) atteints dans une canalisation de 100 mm à 20 °C avec un résiduel de chlore libre de 0,2 $mgCl_2.L^{-1}$ dans l'eau refoulée calculés par le modèle SANCHO pour différentes concentrations en CODB de l'eau refoulée.

A model of the dynamics of BDOC and bacteria in distribution network, which incorporate the knowledge of bacterial dynamics gained in this study, has been developed. Even if experimental determination of various parameters used in the model are still required, it already offers the opportunity to show the influence of various factors on bacterial dynamics, especially BDOC concentration in the inflowing water, in a simplified distribution system. In the future this model will be a tool for a rational management of drinking water networks and treatment lines.

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REFERENCES

BABLON G., VENTRESQUE C., ROY F. (1987). Evolution of organics in a potable water treatment system. *Aqua, 2*, 110-113.

BILLEN G., SERVAIS P. (1988). Modélisation des processus de dégradation bactérienne de la matière organique en milieu aquatique. *In* : Micro-organismes dans les écosystèmes océaniques. Masson, Bianchi, M., ed., pp. 219-245.

BILLEN G., SERVAIS P., BECQUEVORT S. (1990). Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments : Bottom-up or top-down control ? *Hydrobiologia, 207*, 37-42.

BILLEN G. (1991). Protein degradation in aquatic environments. *In* : Microbial enzymes in aquatic environments. Chrost, R, ed., Springer-Verlag, pp 348-389.

BILLEN G., SERVAIS P., VENTRESQUE C., BOUILLOT P. (1992). Functioning of biological filters used in drinking water treatment : the CHABROL Model. *Aqua*, 41, 4, 231-241.

CAMPER A.K., LECHEVALLIER M.W., BROADAWAY S.C., Mc FETERS G.A. (1985). Evaluation of Procedures to Desorb Bacteria from Granular Activated Carbon. J. Microbiol. Methods, *3*, 187-198.

FLETCHER M. (1980). The question of passive versus active attachment mechanisms in non specific bacterial adhesion. *In* : Microbial adhesion to surfaces. Berkeley *et al.*, ed., Ellis Horwood, USA, pp. 197-210.

FUHRMAN J.A., AZAM F. (1980). Bacterioplankton secondary production estimates for coastal waters of British Columbia Antarctica and California. *Appl. Environm. Microbiol., 39*, 1085-1095.

FUHRMAN J.A., AZAM F. (1982). Thymidine incorporation area measure of heterotrophic bacterioplankton evaluation in marine surface waters : Evaluation and field results. *Mar. Biol., 66*, 109-120.

GOSHKO M.A., MINNIGE H.A., PIPES W.O., CHRISTIAN R.R. (1983). Relationships between standard plate counts and other parameters in water distribution systems. *JAWWA, 75*, 568-571.

HAUDIDIER K., PAQUIN J.L., FRANCAIS T., HARTEMANN P., GRAPIN G., COLLIN F., JOURDAIN M.J., BLOCK J.C., CHERON J., PASCAL 0., LEVI Y., MIAZGA J. (1988). Biofilm growth in drinking water network : A preliminary industrial pilot plant experiment. *Wat. Sci. Techn., 20*, 109-115.

HOBBIE J.E., DALEY R.J., JASPERS S. (1977). Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environm. Microbiol., 33*, 1225-1228.

JADAS-HECART A. (1989). Contribution à l'étude de la demande en chlore à long terme d'une eau potable. Modélisation et identification des précurseurs organiques. Université de Poitiers, Ph. D.Thesis.

JESTIN J.M., LEVI Y., HOTELIER J. (1987). Les odeurs générées par la chloration. *Tribune du Cedebeau, 40*,17-26.

KRASNER S.W., BARRETT S.E., DALE S., IIWANG C.J. (1989). Free chlorine versus monochloramine for controlling off-tastes and off-odors. *JAWWA, 81*, 2 : 86-93.

LECHEVALLIER M.W., Mc FETERS G.A. (1985). Interactions between heterotrophic plate count bacteria and coliforms organisms. *Appl. Environm. Microbiol., 49*, 1338-1341.

LECHEVALLIER M.W., BABCOCK T.M., LEE R.G. (1987). Examinations and characterization of distribution system biofilms. *Appl. Environm. Microbiol., 53*, 2714-2724.

MORGAN P., DOW 5. (1985). Environmental control of cell-type expression in prosthecate bacteria. *In* : Bacteria in their natural environments. Fletcher, M. & Floogate, ed., Academic Press, p. 131-170.

SERVAIS P. (1986). Etude de la dégradation de la matière organique par les bactéries hétérotrophes en rivière. Développement d'une démarche méthodologique et application à la Meuse belge. Université Libre de Bruxelles, Thèse, 271 p.

SERVAIS P. (1989). Bacterioplankton biomass and production in the river Meuse (Belgium), *Hydrobiologia, 174*, 899-110.

SERVAIS P., BILLEN G., HASCOET M.C. (1987). Determination of the biodegradable fraction of dissolved organic matter in waters. *Wat. Res, 21*, 445-50.

SERVAIS P., ANZIL A., VENTRESQUE C. (1989). A simple method for the determination of biodegradable dissolved organic carbon in waters. *Appl. Environ. Microbiol., 55*, 2732-2734.

SERVAIS P., BILLEN G., VENTRESQUE C., BABLON G. (1991). Microbial activity in granular activated carbon filters at the Choisy-le-Roi drinking water treatment plant. *JAWWA, 82*, 62-68.

SERVAIS P., LAURENT P., RANDON G. (1992). Mesure de la biomasse et de l'activité bactérienne dans l'eau de distribution. *Rev. Sc. de l'Eau*, in press.

SIMON M., AZAM F. (1989). Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Progr. Ser., 51*, 201-213.

SOMVILLE M., BILLEN G. (1983). A method for determining exoproteolytic activity in natural waters. *Limnol. Oceanogr., 28*, 190-193.

VAN DER WENDE R., CHARACKLIS W.G., SMITH D.B. (1989). Biofilms and bacterial drinking water quality. *Water Res., 23*, 1313-1322.

WICKS R.J., ROBARTS, R.D. (1987). The extraction and purification of DNA labelled with (methyl-3H) thymidine in aquatic bacterial production studies. *J. Plankton Res., 9*, 1159-1166.