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# BIOFILMS AND BACTERIAL DRINKING WATER QUALITY

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**Abstract**—Bacterial growth in potable water distribution systems was studied in a pilot reactor system designed to model the plug flow characteristics in a water main. Experiments were performed to measure specific cellular growth rates and numbers of cells growing on the pipe walls (biofilm cells) and cells suspended in the water phase (planktonic cells) to determine the relative contribution of biofilm accumulation to the bacterial populations in distribution mains. The experiments were performed with chlorine-free water at a water treatment plant as well as with disinfectant (chlorine) added to the reactor influent to examine the effect of chlorine on biofilm accumulation and planktonic cell numbers. The results indicate that biofilm growth and detachment accounted for most, if not all, the planktonic cells present in the bulk water of a chlorine free system. Chlorine affects the accumulation and spatial distribution of the biofilm.

*Key words*—biofilm, planktonic cells, cell growth rate, plug flow, chlorine

## NOMENCLATURE

RT = RotoTorque reactor
RTS = RotoTorque reactor system consisting of four (4) RT in series
HPC = heterotrophic plate count
EPS = extracellular polymeric substances
$X_1$ = planktonic cell concentration in RT1 (No. $L^{-3}$)
$X_0$ = planktonic cell concentration entering RT1 (No. $L^{-3}$)
$X_b$ = biofilm cell density (No. $L^{-2}$)
$F$ = volumetric flow rate through RT1 ($L^3$ $t^{-1}$)
$V$ = liquid volume in RT1 ($L^3$)
$F/V$ = dilution rate ($t^{-1}$)
$V/F$ = hydraulic residence time (t)
$A$ = surface area for biofilm accumulation in a RT ($L^2$)
$\mu$ = specific growth rate of planktonic cells ($t^{-1}$)
$\mu_b$ = specific growth rate of biofilm cells ($t^{-1}$)
$r_d$ = specific biofilm detachment rate ($t^{-1}$).

## INTRODUCTION

Occurrences of excessive bacterial populations in distribution systems, sometimes referred to as events of blooms in the literature, have troubled utilities because of their possible implications for the hygienic safety of their product. The water utility industry uses "regrowth" and "aftergrowth" synonymously to describe the processes contributing to the increase in number of cells with travel time away from the treatment plant.

Beginning in the spring of 1984, a water utility in the northeast U.S. recovered excessively high numbers of coliforms from the water distribution system. Coliforms were recovered from sampling locations throughout the system. The coliforms present were identified mainly as species of *Klebsiella* (Martin *et al.*, 1982) and *Enterobacter*. There was no evidence of *Escherichia coli*, the coliform most frequently associated with fecal contamination. Statistical analysis of the data suggested that the blooms might be a result of bacterial growth in the distribution system in addition to breakthrough in the treatment facilities (Characklis *et al.*, 1988). It has often been suggested that cells growing on the inside walls of water mains, as biofilms (Characklis, 1973; Donlan and Pipes, 1986), play an important role in the growth phenomenon. Because the immobilized cells do not wash out as fast as their suspended counterparts, relatively large biofilm cell populations can accumulate in the system. Biofilm environments also offer ecological advantages like partial protection against disinfectants (LeChevallier *et al.*, 1984; McFeters *et al.*, 1985; Nagy and Olson, 1985). The concentration of planktonic cells in the distribution system may increase as a result of planktonic growth and contributions from biofilm erosion and biofilm sloughing. Experiments were done using a pilot reactor, the RotoTorque System, that simulated the plug flow characteristics of the water distribution system.

The objectives of this study were as follows: (1) quantify the relative contribution of detached biofilm cells and replication of planktonic cells to the total planktonic cell concentration in a drinking water distribution system and (2) determine the effect of

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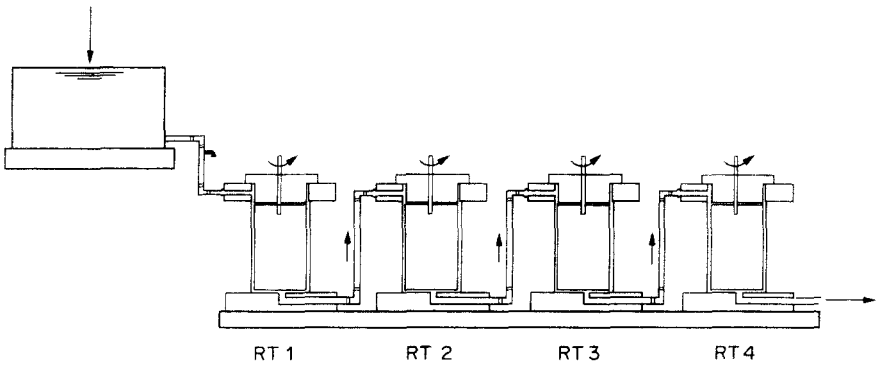


Fig. 1. Schematic illustration of the RotoTorque System consisting of 4 RotoTorques in series. A tank with overflow receives the effluent from a pilot filter and is used as the influent for the RotoTorque System.

chlorine on biofilm accumulation and bacteriological water quality.

## MATERIALS AND METHODS

### *RotoTorque System*

The RotoTorque System (RTS; Fig. 1) consists of four (4) RotoTorque reactors (RT; Fig. 2) in series. Each RT is a continuous flow stirred tank reactor (CFSTR) with a 15 cm (6 in.) i.d. water pipe section forming the outer wall of the reactor. A solid PVC drum spins inside the pipe section. The reactor can be easily dismantled so that pipe sections of different material or varying age can be conveniently substituted in the reactor. The liquid volume of each RT is 1.575 liters and the wetted surface area is 0.207 $m^2$.

The RTS simulates the plug flow nature of a water main in that concentration gradients exist in the direction of flow. Mains of variable length carrying water at variable flow rates are conveniently modeled with this system. The volumetric flow rate determines the hydraulic residence time of the RTS. (Results from tracer studies in the RTS matched the predictions of a mathematical model that describes fluid transport in the RTS under conditions of ideal mixing.) The rotational speed of the drum creates the shear stress on the inside pipe wall surface of a water main. Therefore, combinations of main length and flow velocity are modeled by selecting the appropriate combination of rotational speed and volumetric flow rate. Since the system allows independent control of hydraulic residence time and shear stress, their effects on biofilm processes can be studied independently. The 4 RT serve as "windows" at fixed positions along the modeled main. Water and biofilm samples can be collected from these "windows". Two (2) parallel RTS were used in the experimental work, each RTS containing 4 RT in series.

The RTS were located at a water treatment plant that purifies lake water using the "direct filtration" process. Water quality parameters and other physical and chemical properties of the treatment plant effluent are presented in Table 1.

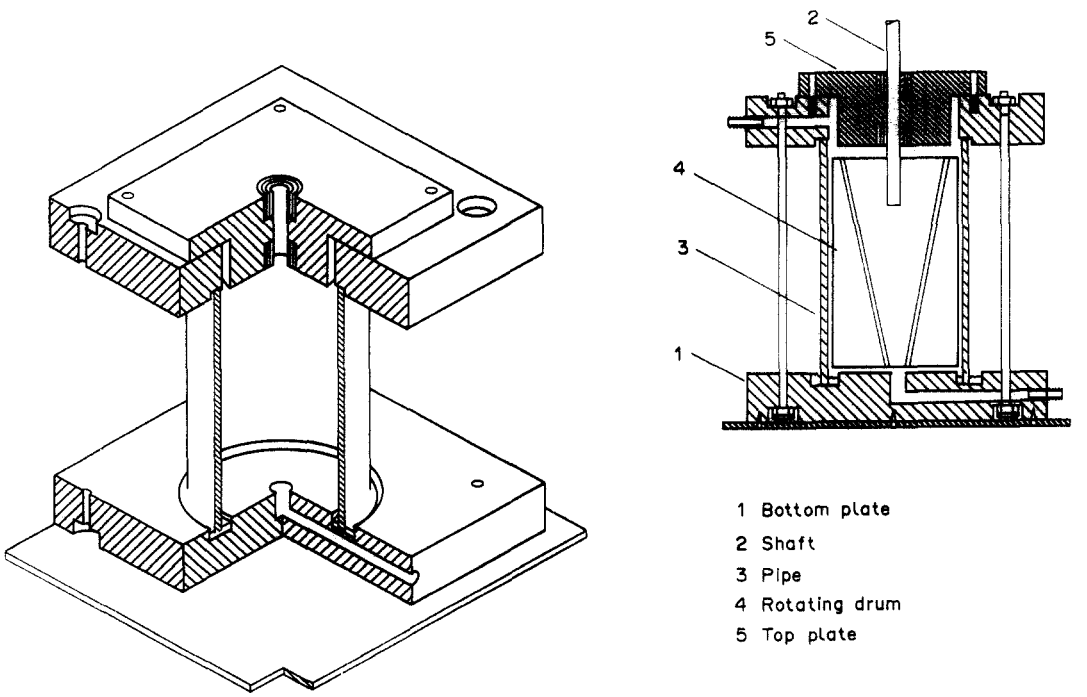


Fig. 2. Schematic illustration of a RotoTorque reactor.

Table 1. Physical and chemical properties of the water treatment plant effluent (RTS influent) during the period of biofilm accumulation (average values)

| | |
|---|---|
| pH | 7.2 |
| Alkalinity | 15 mg/l (as $CaCO_3$) |
| TOC | 2 mg/l (as carbon) |
| Color | 1 Color Unit |
| Turbidity | 0.3 NTU |
| Ammonia | 0.1 mg/l (as nitrogen) |
| Nitrate | 0.2 mg/l (as nitrogen) |
| Temperature | 21°C |

*Procedures and analytical methods*

*Biofilm sampling and analysis.* The rotation of the PVC drums was stopped and the drums were lifted from the RT to obtain biofilm samples. Duplicate biofilm samples were collected by means of a vacuum technique (Fig. 3). A 50 ml plastic sterilized Erlenmeyer was supplied with a bent, glass suction tube and connected to a vacuum pump through a second tube. Biofilm from 12.6 $cm^2$ (2 $in^2$) surface was vacuumed off for each sample using a sterile phosphate buffer (20–40 ml). Buffer was dripped on the remaining biofilm during the last phase of sampling and was also sampled by the vacuum. A new Erlenmeyer was used for each sample and the samples were stored on ice and analyzed the next day.

The number of viable biofilm cells in a biofilm sample was determined by means of a spread plate technique [heterotrophic plate count (HPC); Means, 1981]. The biofilm samples were homogenized and dilution series were made in phosphate buffer. Each dilution (0.1 ml) was plated out on R2A agar in triplicate and the plates were incubated at room temperature for 7 days. (R2A agar is routinely used by the water authority to monitor the bacterial water quality in the distribution system, enabling use of a data bank for reference.) After this incubation time, the colonies were counted with a Quebec colony counter.

*Water sampling and analysis.* The number of viable planktonic cells was determined in water samples obtained form the taps on each RT. Dilutions of these samples in sterilized tap water were made as necessary. Plating and counting of the water samples was done in a similar manner as indicated for biofilm samples.

Chlorine concentration was determined as free chlorine (HOCl and $OCl^-$) using a colorimetric method with a sensitivity of 0.1 mg/l. (Hach Co.; test kit model CN-66). The sensitivity was higher than 0.1 mg/l for low chlorine concentrations.

## GROWTH RATES OF BIOFILM CELLS AND PLANKTONIC CELLS

The purpose of the experiments was to quantify the relative contribution of detached biofilm cells and replication of planktonic cells to the total planktonic cell concentration in a drinking water distribution system. In this way, the relative contribution of biofilm processes to the regrowth problem could be determined.

*Experimental design*

When the hydraulic residence time in the RTS is significantly shorter than the generation time of the planktonic cells, replication of planktonic cells in the RTS is negligible. As a consequence, the measurement of biofilm ($X_b$) and planktonic cell numbers ($X_l$) in the RTS for two suitable hydraulic residence

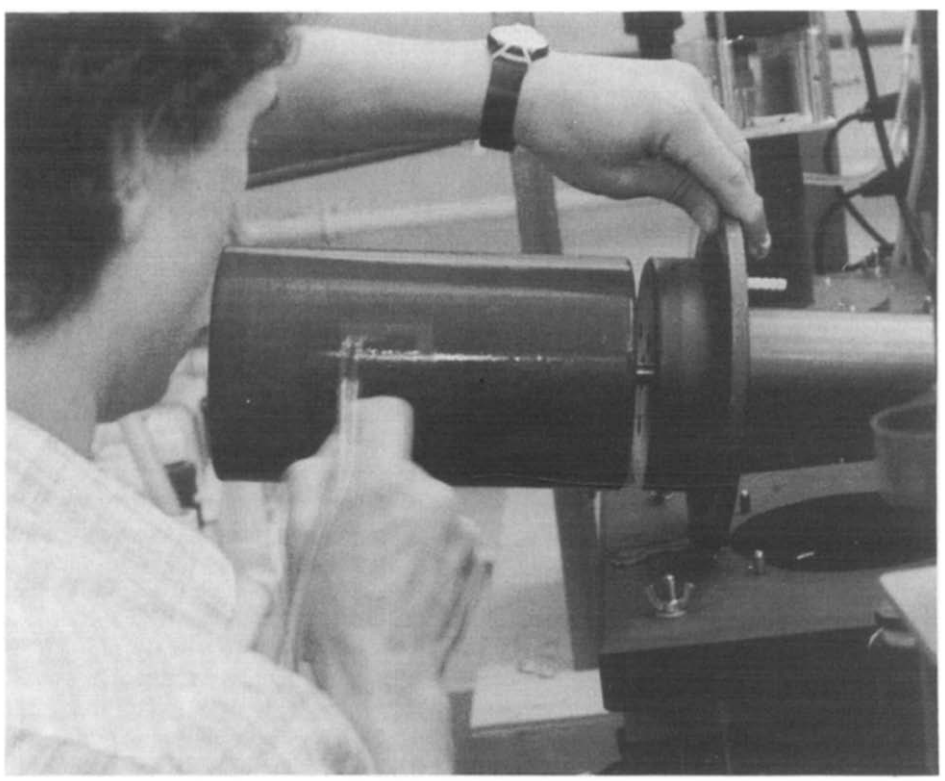

Fig. 3. The biofilm sampling process from a RotoTorque (RT 2 is pictured) uses a vacuum technique to remove biofilm from a defined or measurable wetted surface area.

times ($V/F$), one short and one long, permits the calculation of the specific growth rate in the water ($\mu$) and in the biofilm ($\mu_b$) by means of material balances. Consider the following mathematical expressions which describe the balance for cells in the RTS:

*Planktonic cell balance in RT 1*

$$V\frac{dX_1}{dt} = F[X_0 - X_1] + \mu X_1 V + r_d X_{b1} A \quad (1)$$

rate of accumulation; net rate of transport out of reactor; net rate of growth in the bulk liquid; net rate of detachment from the biofilm

*Biofilm cell balance in RT 1*

$$A\frac{dX_{b1}}{dt} = \mu_b X_{b1} A - r_d X_{b1} A. \quad (2)$$

rate of accumulation; net rate of growth in the biofilm; net rate of detachment from the biofilm

At steady state, equations (1) and (2) simplify to:

$$\frac{F}{V}[X_1 - X_0] = \mu X_1 + r_d X_{b1}\frac{A}{V} \quad (3)$$

$$\mu_b = r_d \quad (4)$$

The equations contain three unknowns; $\mu$, $\mu_b$ and $r_d$. However, at high flow rate (short hydraulic residence time), the dilution rate will be much larger than the specific planktonic growth rate ($\mu$) and planktonic growth rate ($\mu X_1$) will be negligible. Thus, the set of equations is determinant.

$$\frac{F}{V}[X_1 - X_0] = r_d X_{b1}\frac{A}{V} \quad (5)$$

$r_d$ can be calculated from equation (5) and $\mu_b$ can be determined from equation (4). The values for $\mu_b$ and $r_d$ determined for the short hydraulic residence time can be used to determine $\mu$ in the long hydraulic residence experiments [equation (3)].

The RTS hydraulic residence times used for the experiments were 7.3 h ($F = 0.87$ l/h; this is also the original flow rate under which the biofilm accumulated) and 0.22 h ($F = 28.2$ l/h). Dilution rate ($D$) is the reciprocal of hydraulic residence time ($V/F$). Thus, the corresponding dilution rates are 0.14 $h^{-1}$ and 4.5 $h^{-1}$, respectively. The dilution rate of 0.14 $h^{-1}$ is low enough to expect cellular reproduction in suspension in addition to growth in the biofilm. The dilution rate of 4.5 $h^{-1}$ is too high for significant microbial cell replication in suspension (Van der Kooij and Hijnen, 1982; Van der Kooij *et al.*, 1982). In order to improve the accuracy of the experiment, the RTS influent was filtered during the conditions of the high dilution rate so that the planktonic cell concentration in the influent ($X_0$) was zero, thus eliminating "background noise" in the cell number measurement. An additional experiment at an intermediate flow rate of 2.7 l/h (hydraulic residence time = 2.3 h and dilution rate = 0.43 $h^{-1}$) was conducted for comparison. The rotational speed in all of the experiments provided a shear stress at the reactor surface simulating 0.92 m/s (3 ft/s) flow velocity in a pipe of 0.15 m (6 in.) i.d. The pipe sections in the RT were made of new cement-lined iron pipe with a bitumastic coating.

*Results*

Most microbial activity occurred near the inlet to the system as indicated by the relative high numbers of biofilm cells and the large increase in planktonic cell numbers (Fig. 4). Thus, cell growth rate calculations were made for RT 1 using data from Tables 2 and 3 and equations (3)–(5). The calculated specific growth rate of *biofilm* cells under the low flow rate conditions was 0.00025 $h^{-1}$. This means that the cell turnover time ($\theta$) or cell generation time in the biofilm (the reciprocal of the specific growth rater) was approx. 17 days. The calculated specific growth rate of the *planktonic* cells under the low flow rate conditions was $-0.12$ $h^{-1}$. The negative value may be explained by analytical error (a small error in the count of detached biofilm cells at the high flow rate results in a large error in the calculation of the specific planktonic cell growth rate) or by decreasing cell viability or cell dieoff in RT 1A water. The latter is quite possible as suggested by the declining viable bacterial numbers in the consecutive RT operating at the low flow rate (Fig. 4). The specific biofilm cell growth rate for the intermediate flow rate (2.7 l/h) is 0.006 $h^{-1}$, thus considerably higher than for the low flow rate.

## THE INFLUENCE OF CHLORINE ON BIOFILM ACCUMULATION

The purpose of a second set of experiments was to determine the effect of chlorine on biofilm accumulation and bacteriological water quality.

*Experimental design*

Two RTSs were used in parallel for these experiments. The RT used PVC pipe sections as the outer cylinder to improve the sampling of biofilm. The RTS were cleaned with a hot alkaline solution (10 g/l Alconox powder detergent), rinsed with hot water and disinfected with 95% ethanol. The RTS influents were prepared by mixing chlorine-free filter effluent with chlorinated treatment plant effluent in 2 feed tanks equipped with overflows. One RTS received influent with a measured free chlorine concentration of 0.8 mg/l free chlorine while the other RTS received influent with a measured free chlorine concentration of 0.2 mg/l. Chlorine has been shown to react with various components of the system such as construction materials, biomass (Characklis *et al.*, 1980), and other particulate or dissolved components (Haas and Karra, 1984) which results in declining chlorine concentrations through the RTS. The flow rate through both systems was 1.8 l/h and the rotational speed of the drums was 150 rpm simulating a flow velocity of

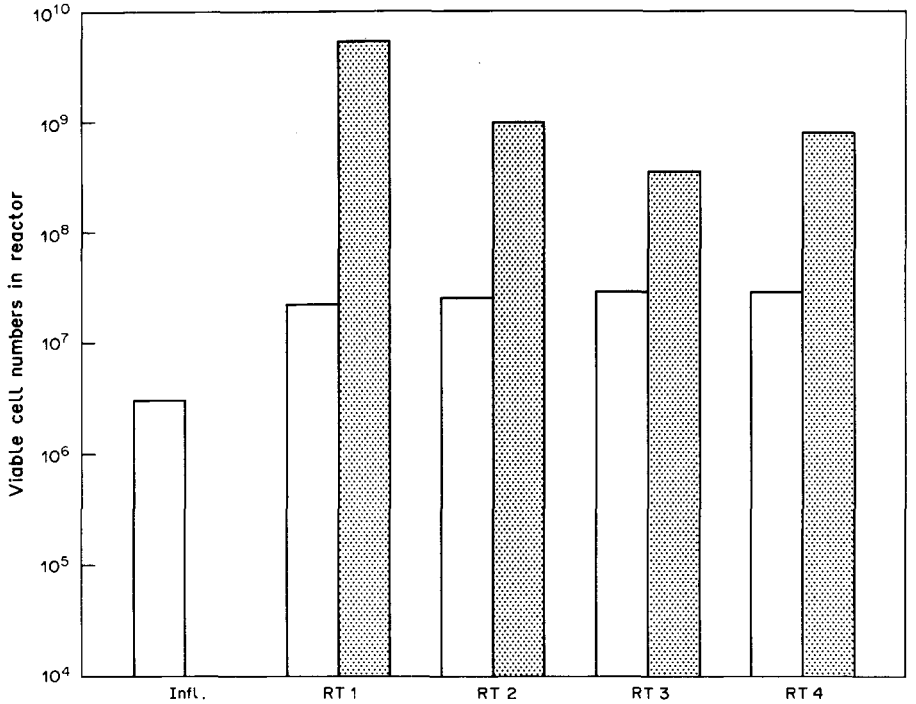


Fig. 4. Number of viable cells (heterotrophic plate count) in the bulk water (= planktonic cells) and biofilm in a RotoTorque System after an exposure time of 85 days to chlorine-free water. The overall hydraulic residence time is 7.3 h. The data represent cell numbers per RotoTorque or per 1.575 liters (= RT volume) influent. □, planktonic cells; ▩, biofilm cells.

0.92 m/s (3 ft/s). Every 2 weeks, water from all RT and both feed tanks was analyzed for chlorine concentration and planktonic cell concentration and the biofilm in each RT was analyzed for biofilm cell numbers.

*Results*

Biomass accumulation at the low chlorine concentration (0.2 mg/l) was determined for exposure times of 17 and 45 days. Biofilm cell numbers increased through the RTS [Fig. 5(a) and (b)]. The accumulation of planktonic cells was correlated with biofilm accumulation, i.e. as biofilm accumulation increased, planktonic cell numbers increased. The maximum number of planktonic cells observed at low chlorine concentration was approximately the same as for the chlorine-free system. Yet, the measured free chlorine residual in the bulk water never decreased below 0.05 mg/l at any point in the system.

Table 2. Viable planktonic cells (mean and SD of heterotrophic plane count) in a disinfectant free RotoTorque System after an exposure time of 85 days

| | Flow rates in the RTS (cfu/ml) | | |
|---|---|---|---|
| Sample | 0.87 l/h | 28.2 l/h | 2.7 l/h |
| Infl. | 2300 ± 200 | 0 ± 0 | 1900 ± 140 |
| RT 1 | 14,600 ± 740 | 470 ± 134 | 13,900 ± 1000 |
| RT 2 | 13,300 ± 1070 | 70 ± 8 | 16,100 ± 2600 |
| RT 3 | 11,800 ± 1850 | 1250 ± 316 | 17,700 ± 1400 |
| RT 4 | 4500 ± 2400 | 380 ± 22 | 17,700 ± 400 |

Biomass accumulation at the high chlorine concentration (0.8 mg/l) was determined for exposure times of 17 and 38 days [Fig. 6(a) and (b)]. Not surprisingly, viable cell numbers were lower than in the other experiments due to higher chlorine levels. For the first 17 days, the RTS remained essentially free of cells. However after approx. 38 days, a "patchy" biofilm had accumulated throughout most of the RTS. At the same time, low numbers of planktonic cells were found throughout the RTS. The measured free chlorine residual never decreased below 0.5 mg/l in the bulk water at any point of the system.

## DISCUSSION

*Growth rates of planktonic cells and biofilm cells*

Relative high bacterial numbers were observed in the simulated distribution system even in the presence

Table 3. Viable biofilm cells (mean and SD of heterotrophic plate count) in a disinfectant free RotoTorque System after a exposure time of 85 days and a hydraulic residence time of 7.3 h (= low flow rate)

| | Biofilm cells (cfu/ml) |
|---|---|
| RT 1 | $2.6 (\pm 0.7) \times 10^{10}$ |
| RT 2 | $4.7 (\pm 1.8) \times 10^{9}$ |
| RT 3 | $1.8 (\pm 0.2) \times 10^{8}$ |
| RT 4 | $3.8 (\pm 0.2) \times 10^{9}$ |

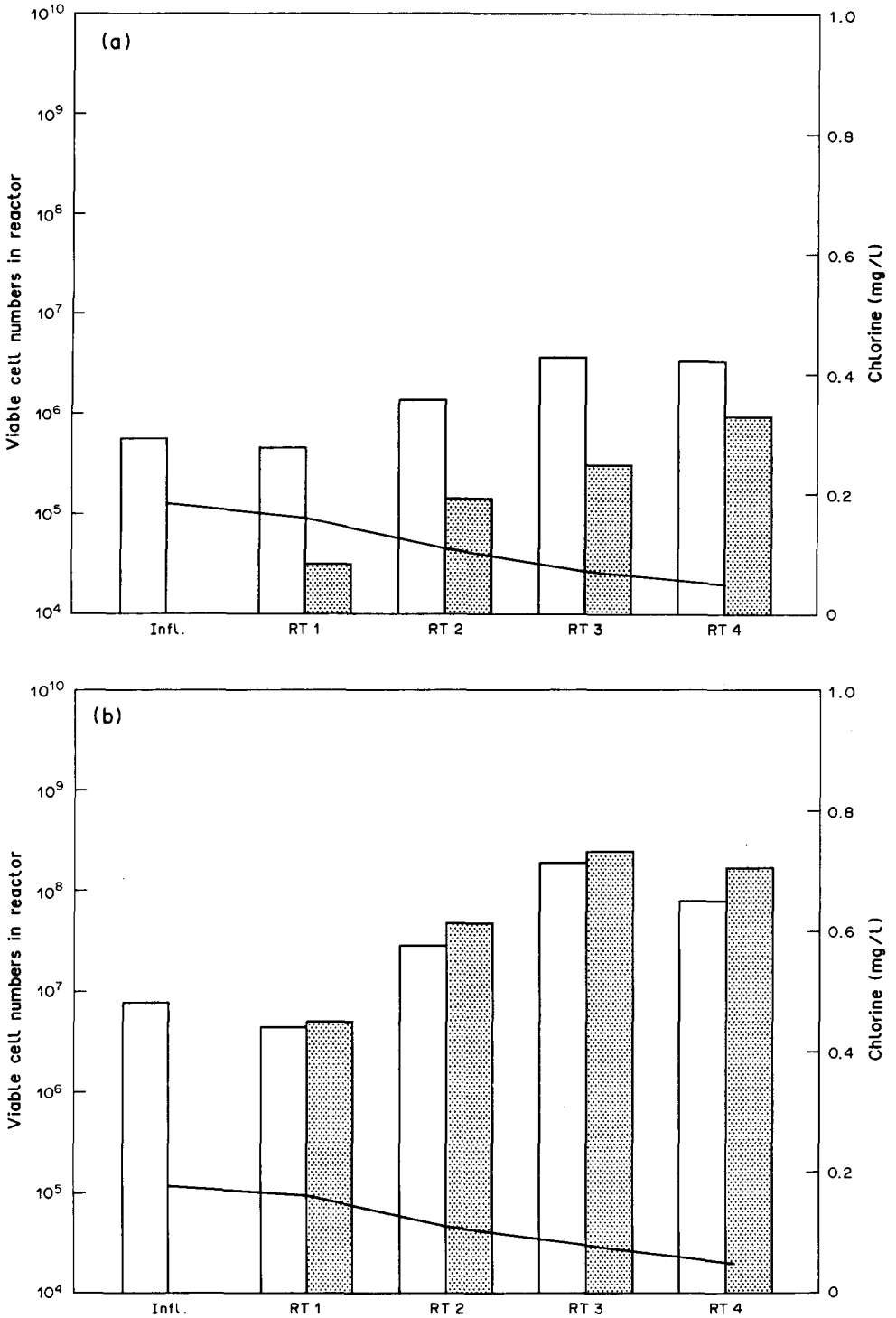


Fig. 5. (a) Numbers of viable cells (heterotrophic plate count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 17 days to water with a low chlorine concentration. The overall hydraulic residence time is 3.5 h. The data represent cell numbers per RotoTorque or per 1.575 liters (= RT volume) influent. □, planktonic cells; ■, biofilm cells; ——, chlorine. (b) Numbers of viable cells (heterotrophic plate count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 45 days to water with a low chlorine concentration. The overall hydraulic residence time is 3.5 h. The data represent cell numbers per RotoTorque or per 1.575 litres (= RT volume) influent. □, planktonic cells; ■, biofilm cells; ——, chlorine.

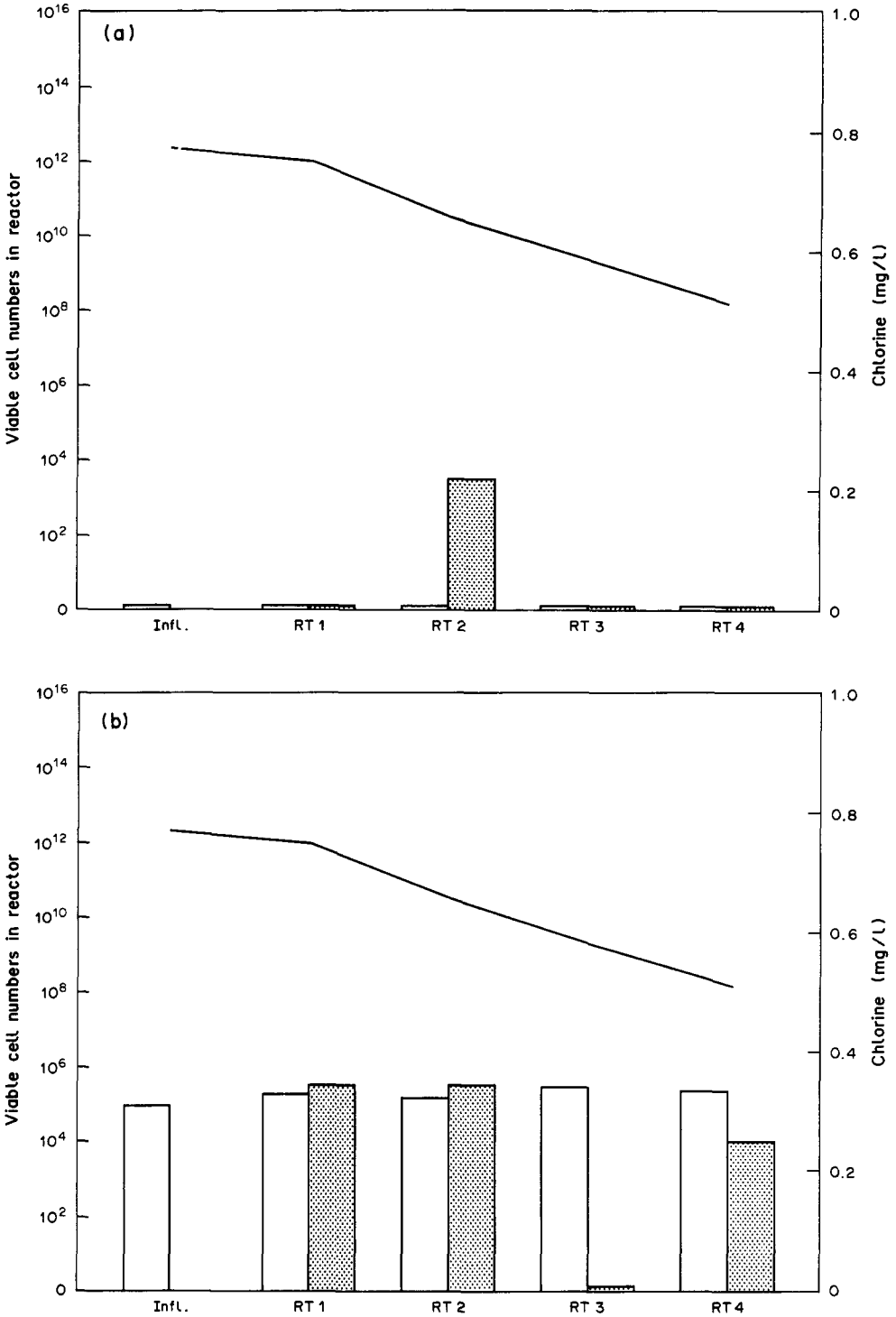


Fig. 6. (a) Numbers of viable cells (heterotrophic plate count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 17 days to water with a high chlorine concentration. The overall hydraulic residence time is 3.5 h. The data represent cell numbers per RotoTorque or per 1.575 liters (= RT volume) influent. □, planktonic cells, ▩, biofilm cells; ——, chlorine. (b) Numbers of viable cells (heterotrophic plate count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 38 days to water with a high chlorine concentration. The overall hydraulic residence time, is 3.5 h. The data represent cell numbers per RotoTorque or per 1.575 liters (= RT volume) influent. □, planktonic cells; ▩, biofilm cells; ——, chlorine.

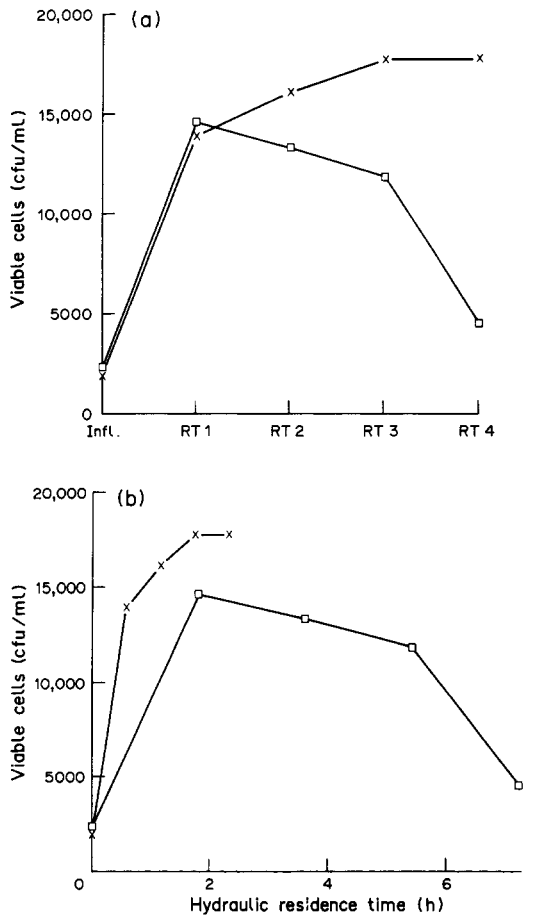


Fig. 7. (a) Numbers of viable planktonic cells (heterotrophic plate count) in the RotoTorque System at steady state as a function of distance through the system (no disinfectant). Overall hydraulic residence time is 7.3 h (□) and 2.3 h (×). (b) Numbers of viable planktonic cells (heterotrophic plate count) in the RotoTorque System at steady state as a function of hydraulic residence time through the system (no disinfectant). Overall hydraulic residence time is 7.3 h (□) and 2.3 h (×).

of a measurable chlorine residual. The specific growth rate of planktonic cells in the finished water is very low. Thus, significant increase of cell numbers in the bulk water is primarily due to detachment of biofilm cells. Net cell production rate is the product of specific growth rate and population cell numbers. In the biofilm, the low specific cell growth rate is countered by a high density of cells resulting in a net biofilm cell production rate that is relatively high compared to the planktonic cell production rate and the input rate of cells at the influent (Fig. 4). At increased flow rate (2.7 l/h), a significantly higher specific growth rate (0.006 $h^{-1}$) was determined for the biofilm cells. Nutrient loading rate, the product of nutrient concentration and flow rate, increases with increasing flow rate which may have contributed to a greater biofilm activity but was ignored in our analysis. Therefore, higher biofilm detachment rates may have occurred during the high flow rate experiment. Accordingly, calculated planktonic growth rate at the low flow rate may be underestimated since it was calculated by subtracting the biofilm growth rate of the high flow rate from the total growth rate of the low flow rate. However, another determination suggests that planktonic growth can be neglected as compared to cells detaching from the biofilm. If all microbial growth was occurring in suspension, $\mu$ would have to be approx. 0.5 $h^{-1}$ to account for growth in RT 1A for the low flow rate. Such a high growth rate may be possible for an organism in rather ideal growth conditions but not for organisms growing at low temperatures in unsupplemented tap water (Van der Kooij and Hijnen, 1982; Van der Kooij *et al.*, 1982).

The planktonic cell concentration at low flow rate increases through the first RT and then decreases through the remainder of the RTS [Fig. 7(a)]. The decrease in cell numbers with distance (time) through the reactor may reflect a nutrient limitation. At the intermediate flow rate, the limitation apparently occurs after the water has passed three RTs. However, if the cell numbers are related to residence time, nutrient limitation occurs at approximately the same residence for both flow rates [Fig. 7(b)].

The results from the RTS can be extrapolated to a water distribution pipe segment of 15 cm (6 in.) i.d. by estimating the corresponding hydraulic residence time in the pipeline. The hydraulic residence time in the RTS has to be corrected for the different substrate loading rate on the biofilm resulting from a reduced volume and an increase in surface area. The correction factor was calculated as 4.9. Thus, a residence time of 5 h in the RTS is approx. 25 h in the 15 cm (6 in.) i.d. pipe line. The extrapolation is simple because biofilm growth is largely dominant over planktonic growth and, hence, the effect of nutrient consumption in the water phase can be neglected.

*Effect of chlorine on biofilm accumulation*

Chlorine reduces accumulation of biofilm and influences its distribution in the RTS. For the chlorine-free system, most biofilm accumulated at the entrance to the reactor system probably through the rapid consumption of nutrients. Less biofilm accumulation was observed in the remaining RTs (Fig. 4). A low chlorine concentration (0.2 mg/l) in the RTS influent reduced the biofilm in the first reactor, thus leaving a larger potential for biofilm accumulation in the subsequent reactors where the free chlorine residual was lower [Fig. 5(a) and (b); Maul *et al.*, 1985]. Planktonic cell numbers increased 1–2 orders of magnitude in conjunction with increased biofilm accumulation between the exposure times of 17 and 45 days.

At the higher chlorine concentration (0.8 mg/l), planktonic cells were observed only in the presence of biofilm [Fig. 6(a), (b)]. Planktonic cells appeared only after an initial biofilm had been established. These

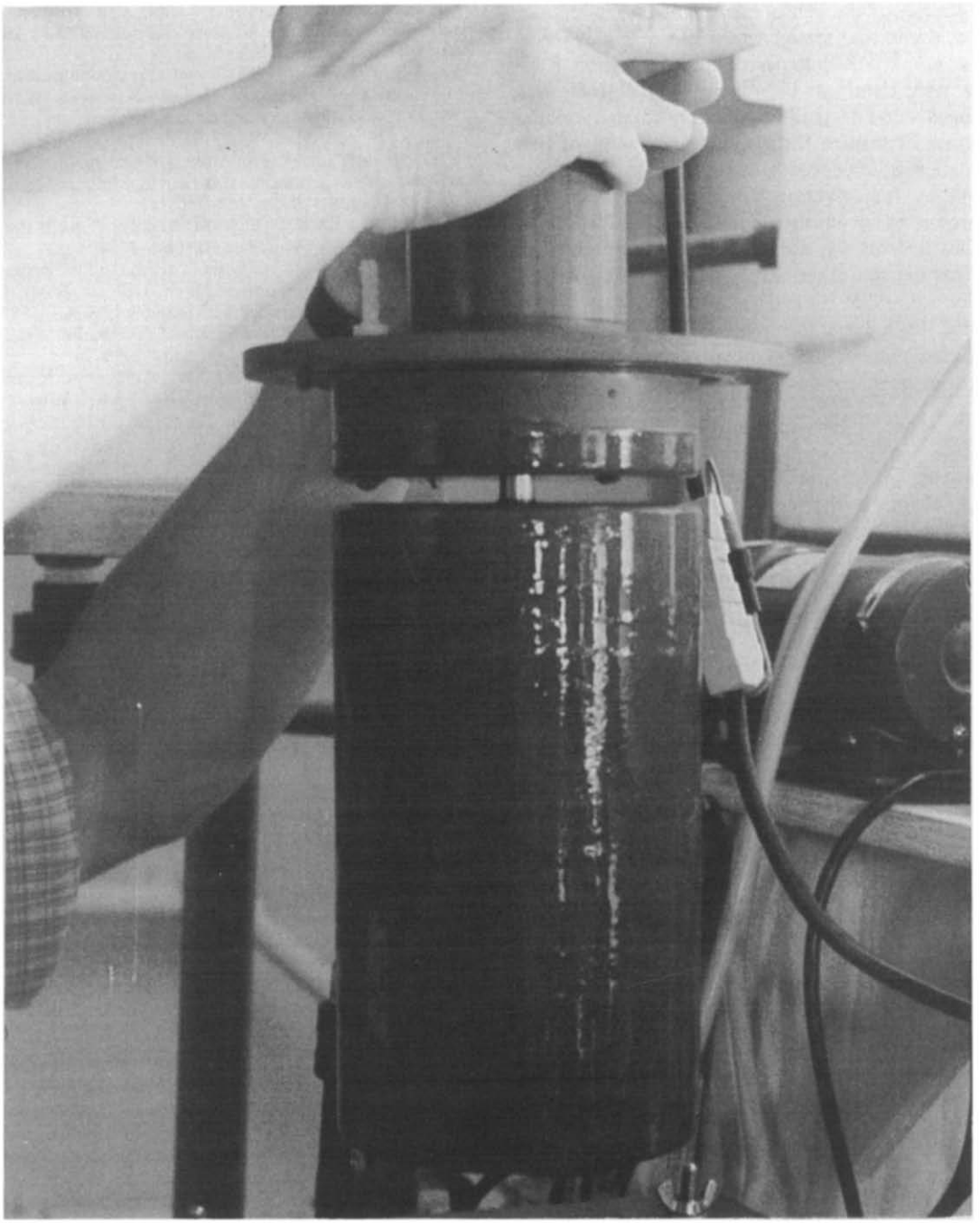

Fig. 8. The biofilm that had accumulated in RotoTorque 1 after approx. 3 months under chlorine-free conditions was considerably thicker than the biofilm in RotoTorque 2 (Fig. 4) or the biofilms observed in the chlorinated systems.

results are consistent with the results from the growth rate experiment which showed the importance of biofilm cell growth to the increase of planktonic cell numbers.

Biofilm cell growth and detachment may be even more dominant in water distribution systems with (low) chlorine concentrations than in chlorine-free systems. The biofilm environment is believed to protect cells against the activity of chlorine by diffusional resistance and neutralization of chlorine through the reaction with biofilm and pipe wall materials while planktonic cells do not find such protection in their environment. Thus, microbial growth in the biofilm will be even larger than the growth of planktonic cells which experience a higher chlorine concentration.

Bacterial species less susceptible to chlorine may accumulate selectively in the distribution system. Biofilm and planktonic cells isolated from the RTS at high chlorine concentration after 38 days represented only a few species. An estimated 75–90% belonged to

one species of the genus *Pseudomonas*. Wolfe *et al.* (1985) found that these bacteria can be highly chlorine tolerant. The colonies of this species, on $R_2A$-agar, were clearly of the "smooth type", indicating the production of significant amounts of extracellular polymer substances (EPS). Rate and extent of EPS formation is influenced by many environmental conditions but it seems clear that this species was capable of producing significant amounts of EPS. The lack of species diversity was not observed in the chlorine-free system or by other experimenters (Brazos and O'Connor, 1985; Maki *et al.*, 1986; Ridgeway and Olson, 1982). Thus, chlorine may lead to selection of a limited number of microbial species in the early stages of biofilm accumulation.

## CONCLUSIONS

Biofilm growth and detachment accounted for most, if not all, the planktonic cells present in the bulk water. Planktonic growth was negligible.

Chlorine affects the accumulation and spatial distribution of biofilm in a plug flow reactor system carrying treated drinking water.

When influent with a free chlorine concentration of 0.8 mg/l was used, bacteria grew only in the protective biofilm environment and not in the water phase. At low free chlorine residuals (<0.2 mg/l), biofilm accumulation in the RTS was substantial.

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