Revue des sciences de l'eau Journal of Water Science



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Volume 9, Number 2, 1996

URI: https://id.erudit.org/iderudit/705248ar DOI: https://doi.org/10.7202/705248ar

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Publisher(s)

Université du Québec - INRS-Eau, Terre et Environnement (INRS-ETE)

ISSN

0992-7158 (print) 1718-8598 (digital)

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Cite this article

Kerneis, A., Déguin, A. & Feinberg, M. (1996). A colony count model for the control of drinking water distribution systems. *Revue des sciences de l'eau / Journal of Water Science*, *9*(2), 189–205. https://doi.org/10.7202/705248ar

Article abstract

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Accurate correlation measurements between colony counts and DOC consumed in the network were carried out in three distinct systems. No significant correlations were measured. For these three networks, biodegradable organic matter measurements based on DOC determinations were demonstrated to be unreliable process control parameters for monitoring bacterial regrowth.

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A colony count model for the control of drinking water distribution systems*

Un modèle du nombre de micro-organismes pour le contrôle des réseaux de distribution d'eau potable

A. KERNEIS1*, A. DEGUIN² and M. FEINBERG³

Reçu le 21 octobre 1994, accepté le 20 décembre 1995**.

RÉSUMÉ

De nombreux paramètres sont mesurés aux usines de production d'eau potable pour contrôler l'efficacité du traitement: pH, turbidité, concentration en désinfectant... Cependant, l'eau traitée n'est pas un produit fini ; sa qualité, notamment microbiologique, peut évoluer en cours de distribution. La livraison d'eau aux consommateurs peut ainsi être considérée comme l'étape finale du processus de production d'eau potable. La complexité du contrôle de la qualité de l'eau en cours de distribution repose sur l'identification des points d'échantillonnage et du nombre de prélèvements. L'objectif de cette étude est de sélectionner un paramètre de contrôle de la recroissance microbienne pour développer un contrôle de qualité plus rigoureux et précis de l'eau de distribution. Deux paramètres ont été étudiés en tant que paramètres potentiels de contrôle : le temps de séjour de l'eau dans le réseau et la matière organique biodégradable. S'il est possible de démontrer que ces paramètres ont une influence significative sur la qualité microbiologique, ils pourront être utiles à l'identification des points d'échantillonnage et à la détermination des nombres minimaux de prélèvements. Le temps de séjour a été déterminé dans un réseau en antennes, puis dans un réseau légèrement maillé. Ce paramètre a été calculé à partir de mesures de débit et de volume de réservoirs. Les conditions de mélange parfait dans les réservoirs et d'écoulement piston dans les canalisations ont été supposées. La détermination du temps de séjour a ensuite été validée par des tracages au chlorure de sodium. La mesure du carbone organique dissous (COD) naturellement consommé dans le réseau a été préférée, pour sa simplicité et son plus faible coût, aux analyses reposant sur la biodégradation

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Communication présentée au Colloque international sur la « Matière Organique Biodégradable », Ecole Polytechnique de Montréal, juin 1994.

^{**} Les commentaires seront reçus jusqu'au 13 décembre 1996.

de la matière organique in vitro. Ces déterminations reposent sur la différence entre les teneurs en COD de l'eau traitée et de l'eau en cours de distribution. Un modèle prédictif du nombre de micro-organismes déterminé sur gélose et fonction du temps de séjour a été développé. La fonction logistique, souvent appliquée à la croissance des micro-organismes dans des réacteurs de laboratoire, a été choisie comme modèle. Deux paramètres microbiologiques ont été considérés : le nombre de micro-organismes déterminé à 20 °C après trois jours (N3D) et quinze jours (N15D) d'incubation. La fonction logistique a été ajustée, à un niveau de signification inférieur à 0,05, aux données de N3D et N15D collectées en hiver, au printemps et en été dans un réseau faiblement maillé. L'ajustement du modèle à différentes saisons et différentes parties d'un réseau a permis de mettre en évidence les facteurs influençant la recroissance microbienne et par conséquence les coefficients du modèle : la saison, l'origine de l'eau (eau de surface, eau souterraine) et le type de conduite. Le modèle de N3D a permis de localiser et d'estimer la quantité d'eau de distribution dont le paramètre N3D est supérieur au niveau guide européen. Ce modèle a aussi été appliqué à la localisation de postes de rechloration sur le réseau.

L'utilisation de la mesure de la matière organique biodégradable en tant que paramètre de contrôle de process a été évaluée à partir de la mesure de la corrélation partielle entre le COD consommé dans le réseau et N3D et N15D. La corrélation partielle permet, dans ce contexte, de mesurer uniquement le lien entre le COD consommé dans le réseau et le nombre de micro-organismes en supprimant les interactions avec d'autres paramètres tels que le temps de séjour. Les résultats ont montré que pour les trois réseaux étudiés aucune corrélation partielle significative n'a été observée entre ces deux paramètres. Le COD consommé dans le réseau ne peut pas être utilisé efficacement comme paramètre de contrôle.

Mots-clés : nombre de micro-organismes, modèle, eau potable, réseau de distribution, temps de séjour.

SUMMARY

The purpose of this study is to select a process control parameter for monitoring microbial regrowth in a network and to develop a more accurate and relevant quality control of supply water. Two parameters were examined as potential process control parameters: the water residence time in the network and the concentration of biodegradable organic matter. Residence time calculations were carried out and validated by tracer studies in a branched network and then in a simply looped network. The measurement of the natural dissolved organic carbon (DOC) consumption in the network was preferred to the determination of any in vitro biodegradation. The measurement of consumption requires the determination of DOC in treated water and in supply water. It is simpler and less expensive than other biodegradable organic matter determinations. A model for colony counts as a function of the residence time was developed in order to demonstrate that this parameter can be used for process controlling. This model was very well adjusted to data collected in a network in winter, spring and summer. This process control parameter was then used in order to locate and estimate the quantity of water whose colony counts exceed the European directive guide level.

Accurate correlation measurements between colony counts and DOC consumed in the network were carried out in three distinct systems. No significant correlations were measured. For these three networks, biodegradable organic matter measurements based on DOC determinations were demonstrated to be unreliable process control parameters for monitoring bacterial regrowth.

Key words: colony counts, model, drinking water, distribution network, residence time.

1 – INTRODUCTION

Treatment of drinking water has been widely studied in order to define parameters for use in process control such as pH, turbidity, concentration of disinfectant... (ELLIS *et al.*, 1991; COLLINS *et al.*, 1991). However, treated water at the plant is not a final product. Water is supplied to customers through a network of pipes and reservoirs in which its microbial quality can change, and thus transport of water to the customer must be considered as part of the process. The complexity of water quality control in the network lies in the identification of sampling points and the number of samples:

The growth of bacteria in drinking water distribution systems is often linked to the deterioration of water quality including taste and odour (CHÉDAL *et al.*, 1990), turbidity (OLSON, 1984) and the development of potentially pathogenous bacteria (WADOWSKY *et al.*, 1982; BURKE *et al.*, 1987). Thus, European directives relating to the monitoring of drinking water recommend the determination of colony counts in drinking water at the plant and in the network. It is recommended that water suppliers produce a water whose colony counts remain lower than a given guide level in the network. No information concerning the location of sampling points in the network is given to the water supplier.

The purpose of this study was to select a process control parameter for monitoring the microbial regrowth in the network and to develop a more accurate and relevant guality control of supply water. This new guality control aims to locate and estimate, from a reduced number of samples, the quantity of supply water whose colony counts are higher than the European guidelines (Journal Officiel, 1980). Two parameters were examined as potential process control parameters: the water residence time in the network and the concentration of biodegradable organic matter. Water residence time in the network was considered since it has been widely reported to have an influence on microbial water quality (LECHEVAL-LIER, 1990; LECHEVALLIER et al., 1991). The development of a model for colony counts as a function of residence time could help the water supplier to locate sampling points in the network. It could then lead to the location and estimation of supply water whose colony counts exceed a given level. Previous studies in vitro or in a pilot scale network have demonstrated the role of the biodegradable dissolved organic carbon (BDOC) as an indicator for microbial regrowth (VOLK et al., 1992; MATHIEU, 1992). Thus, the measurement, according to a given analytical procedure, of the biodegradable organic matter in treated water at the treatment plant could be relevant for determining the number of microbial analyses to be carried out in the network. The application of this potential process control parameter to a real distribution is tested in this study.

2 - MATERIAL AND METHODS

2.1 Water sampling

Water in the network was aseptically sampled in official or domestic buildings after flushing the taps for a few minutes in order to minimize the effect of residence of water in the domestic network. The taps were heat sterilised with a flame. Microbial samples were collected in sterile flasks containing 17.5 mg/l sodium thiosulfate (final concentration) in order to reduce the remaining disinfectant. Flasks for organic carbon measurements had been heated at 500°C for 4 h. Orthophosphoric acid was added to the samples (final concentration 20 mg/l) for organic matter analysis in order to prevent any oxidation of the organic carbon. All the samples were stored at 4°C and analysed within 6 hours.

2.2 Colony counts

Colony counts were determined according to the pour plate technique using plate count agar incubated at 20°C for 3 days and for 15 days (AFNOR, 1990). The pour plate technique was preferred to the determination by epifluorescence since the process control parameters studied have to be related to the bacterial parameters listed in the European directive regulation (Journal Officiel, 1980). Results, expressed as colony forming units (CFU) per millilitre, are called N3D for 3 days of incubation and N15D for 15 days of incubation. The European directive guide level for N3D is 100 CFU/ml in supply water. The parameter N15D was considered because it allows the detection of injured and slow-growing micro-organisms (McFeters *et al.*, 1986).

2.3 Dissolved organic carbon measurement

The measurement of the natural dissolved organic carbon (DOC) consumption in the network was preferred to the determination of assimilable organic carbon or biodegradable dissolved organic carbon. The measurement of consumption requires the determination of DOC in treated water and in supply water. It is simpler and less expensive than other biodegradable organic matter determinations.

DOC determinations were carried out with a Dohrman DC 80 total organic analyzer after filtration (0.22 µm diameter *Millex* filter, Millipore, containing polyvinylidene difluoride) and acidification at pH 2 with orthophosphoric acid.

2.4 Calculation of water residence time in the network

2.2.1 Technique for calculation

Residence time in the network was calculated according to a method previously applied to the determination of the time spent by the water in the process units of treatment plants (TEEFY *et al.*, 1990). This method is based on two flow models for reservoirs and pipes (KERNEIS *et al.*, 1994).

The plug flow model applies to pipes in which fluid particles move in parallel paths. All the fluid particles leaving a pipe observe the same residence time at the same moment since no mixing in the axial direction occurs. Residence time in a pipe is equal to the pipe length divided by the water velocity in the pipe. Residence time at a node in the network corresponds to the sum of the residence times in all the pipes of the path followed by the water. Water velocity in the pipes is determined according to a classic hydraulic analysis which involves the following steps:

building of the network schematic;

- distribution of metered water demand;

- field test including pressure and flow measurements;
- calibration of the hydraulic model.

The perfect mixing model applies to reservoirs in which the flow is completely mixed and uniform. Thus, the concentration of a chemical at the effluent of the reservoir is the same as that throughout the entire reservoir. The particles leaving a reservoir do not observe the same residence time. Mean residence time for the whole quantity of water in a reservoir is then calculated. Mean residence time in reservoirs is determined from a differential equation proposed by Kennedy *et al.* (1993):

$$Q : RT_{\partial} + V = \frac{\partial (V \cdot RT)}{\partial t};$$

with Q, the influent flow rate;

V, the volume of water in the reservoir; t, the time; RT, the residence time of water at moment t;

 RT_0 , the residence time of water at moment t = 0.

2.2.2 Method of validation

Calculations of residence time were validated according to a procedure involving tracer studies (KERNEIS *et al.*, 1993). It consists of measuring the time spent by a substance to reach a node in the system. This substance, called a tracer, must obviously not alter the water quality. Its concentration must not be modified by either chemical or adsorption reactions which could interfere with the determination of residence time: the tracer must be conservative. The tracer must, moreover, be easily detectable. For example, in the USA, tracer studies have been carried out with sodium fluoride (KENNEDY *et al.*, 1991). Sodium fluoride is usually injected at the plant in order to prevent tooth decay. Injection is suspended for a few days and the decrease of tracer concentration is measured in the network. Sodium chloride has been chosen for our validation since injection of fluorides is not allowed in France. Moreover the selected tracer has, at low concentration, no impact either on health or on the organoleptic properties of water.

A solution of 200 g/l NaCl was prepared in a continuously stirred tank of 500 l. A dosing pump connected to the tank injected at a constant rate of tracer at the treatment plant outlet. The tracer concentration in supply water was 50 mg/l NaCl. The tracer was detected in the network by measuring conductivity. The conductivity meter probe (LF 192 WTW) was placed in a flask under a tap and connected to an *Iris* data logger (DTS Systems, Portsmouth, England).

The actual mean residence time was determined from conductivity measurements according to the following calculations (KERNEIS, 1994):

$$\overline{\mathsf{RT}} = \int_0^{+\infty} \frac{\mathsf{C} - \mathsf{C}_0}{\mathsf{C}_{\mathsf{m}} - \mathsf{C}_0} \cdot \mathbf{t} \cdot \mathrm{dt}$$

with \overline{RT} , the actual mean residence time;

C, the conductivity at time t;

Co, the conductivity before the injection of tracer;

C_m, the maximum conductivity after the tracer injection.

2.5 Model for colony counts in supply water

Growth of micro-organisms in pilot reactors is classically described by a logistic function of time τ , called X (τ) (Jolivet, 1982):

$$X(\tau) = \frac{a}{1 + \exp(b - c \cdot \tau)}$$
, where a, b and c are the model coefficients.

 $X(\tau)$ is a continuous function, constituting the envelope of discrete colony counts. The use of this function, generally applied to closed system reactors, has been extrapolated to a network in which water resides for a certain time. Figure 1 presents an example of the logistic function. $X(\tau)$ slowly increases for low values of τ . The slope of $X(\tau)$ keeps on increasing, up to μ_{max} , called the maximum growth rate, at the inflexion point. The expression of μ_{max} as a function of the model coefficient is: $\mu_{max} = 1/4.a.c.$ For high values of t, X tends towards an asymptote designated X_{max}.



 Figure 1
 Example of the logistic function.

 Exemple de fonction logistique.
 Exemple de fonction logistique.

This model is empirical and was selected for its simple application to process control. It is not based on the description of biological mechanisms. The coefficients are statistically determined from a sufficient number of data (bacterial counts and residence time). The model is calibrated with actual data, the effects of the biofilm and of the disinfectant on the bacterial counts are not ignored.

In order to test the influence of the pipe material, colony counts collected from different pipe materials were compared using a Mann-Whitney test.

2.6 Calculation of correlation coefficients

Colony counts and DOC have often been reported to vary as a function of residence time in the network (RIZET *et al.*, 1984). The link between these two variables does not permit the use of classic correlation coefficients such as Kendall's or Spearman's coefficients (Siegel, 1956). These parameters could vary in the same way as a function of residence time yet have absolutely no direct link between them. Thus, the influence of DOC consumption on colony counts in the network must be measured by determining the partial correlation. Partial correlation measures the direct link between DOC consumed and colony counts while residence time is kept constant. The measurements were carried out in three different distribution systems in order to obtain results in a wider context.

3 – RESULTS

3.1 Application sites

The model for colony counts was adjusted to data collected in a network located in the East of France (network *East*). Figure 2 shows the structure of the network *East* which is supplied with two treatment plants (spring and surface water) at a total rate of 3 000 m³/day. Six reservoirs are located in the network. Three looping parts in the system involve mixing of water as flow directions show in figure 2. Two sampling points used for the tracer detection are also indicated in figure 2. The pipe material in the network is mainly cement mortar lined ductile *iron*. A branch of cast iron pipes, shown in figure 2, had been scraped in 1989 and then protected by sodium carbonate injection for 3 months. This branch is called the *rehabilitated branch* in the text. Only the colony counts in the pipes supplied with plant 1 were adjusted to the model since the range of residence times is not broad enough in the part of the network connected to plant 2. During winter, spring and summer 1993, 61 samples were collected in the network at 20 different points supplied by plant 1. Colony counts determined in water from plant 2 are presented as average values for winter, spring and summer 1993.

Table 1 presents the characteristics of the two additional distribution networks, called *Centre* and *West* in the text, and considered for the measurement of total and partial correlations between colony counts and consumed DOC. The samples were obtained in spring, summer, autumn 1992 and winter 1993 for the network *Centre* and in summer 1993 for the network *West*.

Although the three systems studied are all supplied with surface water, their structures are very distinct:

- network East: looped network supplied by 2 works
- network West: long branches
- network Centre: short branches

Validation of residence time calculation with tracer studies was only carried out in two systems since the network *West* has a very simple structure.



Figure 2 Network East. Réseau Est.

Table 1 Characteristics of the studied networks.

Tableau 1 Caractéristiques des réseaux étudiés.

	West	Centre
Origin of water	surface water	surface water
Treatment	full treatment including ozonation	full treatment
Average concentration of residual disinfectant in treated water	0.3 mg Cl ₂ /1	0.2 mg Cl ₂ /I
Capacity (m ³ /day)	9 500	2 500
Number of data	26	37

3.2 Calculation of residence time

Table 2 presents the residence times calculated and determined by tracer detection in the networks *East* and *Centre*. The differences between calculated and measured data are around 1 h, even for high values, such as 91 h.

	t calculated (h)	t determined by tracer detection (h)	
Network East		, <u> </u>	
	10	11	
	17	18	
letwork Centre			
	91	93	
	10	11	
	17	18	

Table 2	Residence times calculated and determined by tracer detection.
Tableau 2	Temps de séjour calculés et déterminés par la détection du traceur.

3.3 Model for colony counts

Figure 3 presents an example of the adjustment to the logistic function of the N3D results, collected in summer in the network *East*. The coefficient of determination, R^2 , of 0.95 for 8 points demonstrates the very good adjustment of the model to N3D.



Figure 3 Example of N3D adjustment to the model. Exemple d'ajustement de N3D au modèle.

Figure 4 presents an example of the adjustment to the logistic function of N15D results, collected in winter in the network *East*. The coefficient of determination of 0.97 for 10 points also indicates the good adjustment of the model to N15D.



Figure 4 Example of N15D adjustment to the model. Exemple d'ajustement de N15D au modèle.

Prior to the model adjustments carried out at different seasons, the influence of the pipe characteristics on the model coefficients must be studied. The results of N3D and N15D in the rehabilitated branch and in the cement mortar lined pipes were compared with a Mann-Whitney test. Table 3 demonstrates the influence of the two types of pipes on N3D and N15D. It shows that the adjustments of results to the model can be identical for the cement mortar lined and the rehabilitated pipes, except for N3D in summer at a level of significance of 0.03.

- Table 3
 Comparaison of the variable N3D and N15D in the rehab ilitated branch with the cement mortar lined pipes. Levels of significance of the Mann-Whitney test.
- Tableau 3
 Comparaison des variables N3D et N15D dans la branche rénovée avec les conduites revêtues de ciment. Niveaux de signification du test de Mann-Whitney.

	Winter	Spring	Summer
N3D	0.80	0.16	0.03
N15D	0.90	0.31	1

Table 4Models X(t).

Tableau 4 Les modèles X(t).

Season	Type of pipes	Parameter	Model, X(CFU/ml) and t(h)	µ _{max} (CFU/m1/h)	Number of data	R ²
Winter	all types	N3D	$X(t) = \frac{713.4}{1 + \exp(9.7 - 0.22 t)}$	39	11	0.85
	all types	N15D	$X(t) = \frac{21\ 441.2}{1 + \exp\left(14.7 - 0.61\ t\right)}$	3 270	1 0	0.97
Spring	all types	N3D	X(t) = 5.5	0	, 7	
	all types	N15D	$X(t) = \frac{10579.7}{1 + \exp(5.5 - 0.13t)}$	344	10	0.92
Summer	cement lined	N3D	$X(t) = \frac{1 \ 107.6}{1 + \exp(6.5 - 0.34 \ t)}$	94	8	0.95
	cement lined	N15D	$X(1) = \frac{144\ 284.1}{1 + \exp(11.2 - 0.30\ t)}$	10 821	7	0.59
	rehabilitated	N3D	X(t) = 10	0	8	

.

Table 4 presents the different model adjustments carried out from the colony counts measured in winter, spring and summer. Table also shows that the logistic model fits the data very well since all the coefficients of determination are higher than 0.80, except for N15D in summer. Two types of adjustments are observed according to the maximum growth rate: μ_{max} is either nil (for some N3D adjustments) or strictly positive for N3D and all N15D adjustments.

As expected, all the maximum growth rates are higher for N15D than for N3D since injured and low growth bacteria are not counted by the N3D technique.

Table 5 shows that colony counts remain very low in water from plant 2 for every season.

Valeurs moyennes de X(t) dans l'eau de l'usine 2.

	Parameter	Mean X (CFU/ml)	Number of date
Winter	N3D	1	3
	N15D	100	3
Spring	N3D	1	4
	N15D	33	4
Summer	N3D	20	3
	N15D	1	3

Table 5 Mean values of X(t) in water of plan 2.

3.4 Correlation between colony counts and DOC consumed

Figure 5 presents the total and the partial correlation coefficients, at a level of significance of 5%, between N3D and DOC consumed in the network. No significant total or partial correlations between N3D and DOC consumed were observed. The partial correlation coefficients are lower than the total correlation coefficients for the networks *East* and *West*. On the other hand, the partial correlation was higher than the total correlation for the network *Centre*.

Figure 6 shows the total and the partial correlation coefficients, at a level of significance of 5%, between N15D and DOC consumed in the network. No significant total correlation was observed for the networks *East* and *West*. However, the partial correlation between N15D and DOC consumed was significant at a level of 5% for the network *East* in summer.

Tableau 5





4 – DISCUSSION

4.1 Selection of the process control parameter for monitoring the microbial regrowth

No total or partial correlations were observed at a level of significance of 0.05 between DOC consumed and N3D and N15D (except one). Partial correlations were lower than total correlations when colony counts are residence time dependent (for networks *East* and *West*). On the other hand, when no microbial regrowth was observed and when DOC decreased in the network, partial correlations were higher than total correlations. This explains the different results of partial correlations obtained for the network *Centre*.

The measurement of DOC consumption in the network proves to be a poor process control parameter for microbial regrowth since it is not correlated with colony counts. These results contradict previous studies conducted in pilot scale networks and in vitro. Mathieu observed, at different sampling points in a pilot scale network, a significant total correlation between BDOC and colony counts determined by epifluorescence (MATHIEU, 1992). However, no partial correlation was measured. VOLK et al. (1992) showed a linear relation between BDOC and the maximum growth of micro-organisms in vitro for waters of various origins. However, studies conducted in full size systems demonstrated that no correlation exists between BDOC and colony counts (CAPELLIER et al., 1992; NAKACHE et al., 1994; VAN DER KOOIJ, 1992). The different results obtained in full scale, pilot scale or in vitro studies can be explained by the influence of adsorption and desorption of DOC and by the level of disinfectant. In full scale networks, where residence times are higher and where pipes do not have a uniform roughness, the measurement of DOC variation in the network is probably not a good indicator of the actual DOC consumption.

The present results demonstrate that the logistic model as a function of residence time fits microbial regrowth in the network very well. Residence time appears to have a major influence on the microbial quality of water. The application of the model to a large amount of data highlighted the factors which have an influence on the model coefficients. They have to be taken into account for the location and the estimation of the quantity of water whose colony counts exceed the level proposed by the European directive. The season was first shown to have an important effect on microbial regrowth. This factor involves not only temperature but also changes of raw water quality due to the weather. The passage of water in reservoirs and pipes has often been observed to have distinct impacts on water quality (CLARK *et al.*, 1991; KERNEIS *et al.*, 1995). The origin of water was also seen to influence microbial regrowth.

4.2 Segmentation of the network

Residence time, which was selected as a process control parameter, and the additional factors having an influence on the microbial regrowth, have allowed us to accurately locate and estimate the quantity of water whose N3D is higher than 100 CFU/ml. Figure 7 presents the segmentation of the network *East* where N3D is higher than 100 CFU/ml. The use of the model for colony counts and the microbial results of the area supplied by plant 2 have demonstrated that 22% of supply water had, in summer, a N3D higher than 100 CFU/ml. Due to the very good qua-

lity of spring water, pumped in plant 2, and the water of plant 1, mixing in that looped network, the water of poorest quality was located in the centre of the network.

The segmentation of the network can be relevant for locating rechlorination points in the network. Figure 7 presents a proposal for installing a new chlorine booster at the limit where N3D is higher than the European directive guide level. This would avoid any super-chlorination at the plant 1, which could increase the production of trihalomethanes and lead to bad tastes and odours. The microbial results also showed that rechlorination does not need to be operated for every season; regular microbial analyses of supply water should determine, with accuracy, the periods of operation

In conclusion, for the three networks studied, measurement of DOC variation in the network was, demonstrated to be a poor process control parameter for monitoring microbial regrowth. It was shown that calculations of residence time are needed for the rigorous measurement of correlation between colony counts and any biological parameter. Residence time was demonstrated to be a very good process control parameter which can be used to locate and estimate the quantity of water whose N3D is higher than the European directive guide level. The model for colony counts can also be applied to the accurate location of rechlorination points in the network.





5 - ACKNOWLEDGEMENT

The authors gratefully acknowledge Olivier HEYOB for his help and support throughout the study.

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