Process reliability and significance of reclaimed water quality parameters

R. Mujeriego and K. Peters

ABSTRACT

Microbial quality of reclaimed water can be adequately interpreted by graphical adjustment of experimental results to a lognormal probability distribution; the method serves to verify the degree of conformity to the proposed distribution and to visualize the time variability of the results. A conventional physico-chemical treatment can inactivate up to 1.0 μlog of common bacterial indicators, while maintaining time variability below 1.0 (μlog/100 ml). Dissolved organic matter in the influent will significantly alter the performance and the reliability of disinfection process, highlighting the need for improved adjustments of disinfectant dose to actual quality of treated water. Although those process may reach inactivation rates as high as 6.0 μlog for common bacterial indicators, their reliability may be considerably affected, forcing standard deviations beyond 3.0 (μlog/100 ml). Natural processes can provide inactivation rates from 3.0 to 3.5 for common microbial indicators, under the weather conditions studied. Although those values are lower than those of physical and chemical disinfectants, the reliability of natural processes is more stable, with standard deviations ranging from 0.65 to 1.1 (μlog/100 ml). Natural processes are particularly sensible to external inputs of microbial indicators, due to the presence of wildlife.

Key words | inactivation efficiency, microbial indicators, process reliability, water reclamation

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INTRODUCTION

Reclaimed water quality evaluation is required to determine conformity with applicable criteria and standards. Statistical methods for data analysis have become common tools (Benjamin & Cornell 1970; Mujeriego 2006; Asano *et al.* 2007) for assessing process efficiency and performance reliability. Both factors are taken into account by regulations expressed by percentile conformity. Both types of information are also important for developing simulation studies on the public health risks involved in beneficial uses of reclaimed water (Tanaka *et al.* 1998).

Reclaimed water quality regulations have followed two main approaches over the last decades: those adopting strict quality requirements (California State 2001; USEPA 2004), particularly for microbial parameters, and those with less restrictive microbial limits that emphasize the socio-economic and epidemiological conditions of the nearby populations (WHO 2006). In addition, water reuse has to conform to public health requirements relative to the way in which reclaimed water is applied (user requirements). While strict microbial limits expands the possibilities for unrestricted use of reclaimed water and reduces the number and specificity of the quality indicators normally established by public health agencies, the adoption of less restrictive quality limits normally results in more diverse and specific microbial quality parameters to be controlled. As a result, states with strict reclaimed water quality limits have adopted basic microbial indicators like total coliforms as the main control parameter (California State 2001), while those with less restrictive quality limits normally recommend varying levels of more specific microbes like

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helminth eggs and *E. coli*. Such analytical requirements have considerable technical and economic implications for the routine operation of water reclamation projects, aside from the scientific interest that a detailed knowledge of the microbial content of reclaimed water may have in developing new quality criteria and standards for different beneficial uses.

OBJECTIVES

The main objective of the study was to statistically quantify the microbial inactivation efficiency and the performance reliability of different water reclamation processes. Large groups of bacterial indicator results, obtained over a 2-year follow-up study, were evaluated to determine their conformity to normal probability distribution. The significance and the extent of the microbial inactivation taking place during each treatment step, and the changes in process reliability were determined and interpreted in terms of the physical and chemical characteristics of influent water.

METHODS

A 2-year field study was conducted, from 2003 to 2005, in three water reclamation plants in Catalonia, Northeast Spain to determine, under normal operating conditions, the variability of different physico-chemical and microbiological parameters at the inlet and outlet of the facilities, and also at the intermediate points of different treatment steps. Eight sampling periods were used, evenly distributed along the 4 seasons of the 2 years of the study. Each sampling period covered a systematic sampling at the three WRP, during the five working days of a week, with one week recess between two consecutive WRP samplings. Sampling started in March 2003 and ended in January 2005. Forty experimental values were obtained in each sampling point, with the exception of sampling points 5 and 7 in Mataró WRP, where 20 and 15 results were available, respectively.

The water reclamation plants (WRP) surveyed were the WRP of Castell Platja d'Aro, the WRP of Empuriabrava, and a demonstration WRP in Mataró. The WRP of Castell Platja d'Aro (*http://www.ccbgi.org/sanejament_fitxa.php?id_municipi = 7*) has a capacity of 35,000 m^3/day, and receives a secondary effluent (sampling point CPA1) from a conventional activated sludge plant. The reclamation process includes:

1. Physico-chemical treatment: coagulation (optional) using PAX in a 57 m^3 completely mixed reactor. Coagulant is added when influent suspended solids are higher than 20 mg/l.
2. Rapid sand filtration with 4 Hydroclear filters, at 7.8 m/h filtration rate. Sampling point CPA2.
3. Disinfection with UV light, using two closed reactors in line, each provided with four lamps of medium intensity placed perpendicular to flow direction. The UV light dose applied varied from 25 to 35 mJ/cm^2, based on the reading of water transmittance. Sampling point CPA3.
4. Final disinfection with sodium hypochlorite, using a maximum dose of 5 mg Cl_2/l, and a Cxt value (total residual chlorine) varying from 80 to 135 mg Cl_2.min/l. Sampling point CPA4.

The WRP of Empuriabrava (http://www.ccbgi.org/sanejament_fitxa.php?id_municipi = 31) has a capacity of 8,750 m^3/day, with daily flows ranging from 1,800 m^3/day to 5,600 m^3/day, depending on seasonal population changes of nearby coastal resort towns. It receives the effluent from a two-line extended aeration treatment plant; effluent from each secondary sedimentation (EPB1) tank flows through a stabilization pond (3,600 m^3) (EPB2) and a polishing pond (6,000 m^3) (EPB3), before entering the water reclamation process. The reclamation process includes a 7 ha constructed wetland system, with 3 parallel vegetated cells of 160 m × 50 m (8,000 m^2) and a depth of 0.5 m. Water is distributed among the three cells, flows by gravity to an outlet channel (EPB4) and enters a shallow lagoon of 4.5 ha and 0.20 m of depth. Water flows from the lagoon center towards a peripheral weir (EPB5). Nitrified effluent from the wetland system (ammonia < 5 mg NH_4—N/l) is pumped to the nearby natural wetland system at the Natural Park of Aiguamolls de l'Empordà. The two natural steps of the wastewater treatment plant and the two natural steps of the constructed wetland system have been considered in the microbial inactivation evaluation study.

The WRP of Mataró is a demonstration facility with a capacity of 20 m^3/day. It receives effluent (MAT1) from a

large conventional activated sludge treatment plant (57,000 m^3/day), which includes a significant fraction of textile industrial effluents. The reclamation process includes:

1. Physico-chemical treatment with initial mixing (PAX, 12 min), coagulation-flocculation (polymer, 19 min), lamelar settling (2 m/h), pressurized multilayer rapid sand filtration (9.5 m/h). Sampling point MAT2.
2. Disinfection with sodium hypochlorite, with doses ranging from 3 to 6 mg Cl_2/l, using a 200 mm tubular reactor with 90 min theoretical contact time. Sampling point MAT4.
3. Disinfection with UV light, with 2 Berson in Line 20 reactors in line, each with one perpendicular Berson 400 Multiband lamp. The theoretical UV light dose applied varied from 35 to 45 mJ/cm^2. Sampling points MAT3.

The plant has several storage tanks to balance water flows of different treatment steps. The plant has a large flexibility for disinfection alternatives: water can be disinfected either by chlorine alone (MAT4) or by a combination of UV light reactors (MAT3) followed by chlorination (MAT5).

Microbiological methods

Water quality was evaluated using common bacterial indicators. Bacterial concentrations were determined by membrane filtration using 47-mm cellulose acetate filters with a nominal pore size of 0.45 μm (Millipore EZ-Pack™ membrane filters, Cat. No. EZHAWG474). Total coliform bacteria were cultured on mEndo LES agar (Difco, Sparks, MD) at 37°C for 24 h, faecal coliform bacteria were cultured on mFC agar (Difco, Sparks, MD) at 44.5°C for 22–24 hours, *E. coli* were cultured on Chromocult® Coliform Agar (Merck KGaA, Germany) for 24 h at 37°C, enterococci were cultured in Enterococcus agar (Difco, Sparks, MD) for 48 h at 37°C and suspected colonies confirmed on Bile esculine agar (Difco, Sparks, MD) for a minimum of 3 h at 44°C. Detection limit was 1 CFU/100 ml in secondary effluents, and 1 CFU/l in disinfected effluents.

Statistical analysis

Experimental results were evaluated using graphical adjustment to a lognormal probability distribution, using the statistic $F(x_i) = i/(n+1)$ and regression lines on scattered plots with probability distribution scales (Benjamin and Cornell 1970) using Sigmaplot software; the *Kolmogorov-Smirnov* goodness-of-fit test was used to verify adjustment between experimental values and proposed distributions. Microbial values "less than 1 CFU/100 ml" were taken into account to calculate the $F(x_i)$ statistic, but were ignored when drawing the regression lines. To have groups with similar number of experimental values when conducting the ANOVA test, "negative" values were replaced by their estimates from the regression line and the original $F(x_i)$ values. Mean bacterial concentrations are expressed by the back transformed of decimal logarithmic values (CFU/100 ml), while standard deviations are expressed by the percentile difference of decimal logarithm transformed values. Standard deviations in this paper have been estimated using the expression $s = \log X84 - \log X50 = \log X50 - \log X16$ and the numerical values derived from the graphical distributions. Values of "*s*" in natural logarithms can be calculated using the expression $s\,(ln) = 2.30\,s\,(log)$. The SPSS 14.0 for Windows (Inc 2007) was used for statistical analysis. The F test has been used to compare multiple means of one variable as a function of different treatments. The *Student-t* test was used to compare two different data groups. The Levene test was used to determine equality of variances for different treatments of a variable. The Student-Newman-Keuls test was used to compare the means of different treatments of the variable when variances were equal. The Tamhane test was used when variances were not equal. A level of significance (p-value) of 0.05 was adopted.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate total and faecal coliforms concentrations at the four sampling points of the WRP of Castell Platja d'Aro; similar graphs were obtained for *E. coli* and enterococci. The figures show that: (1) the four groups of experimental values conform to a lognormal probability distribution (regression lines), and (2) the standard deviations (slope of regression lines) of the four lognormal distributions are not equal, thus preventing a direct application of the ANOVA test. The Kolmogorov-Smirnov

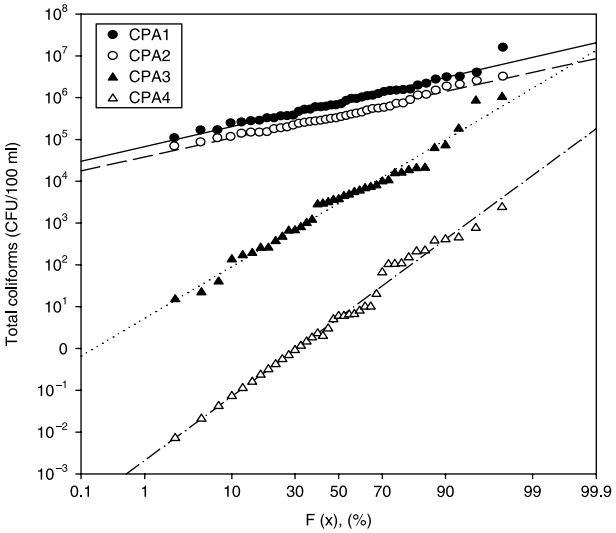

Figure 1 | Probability distribution of total coliforms at the WRP of Castell Platja d'Aro.

test confirmed the normality of the four variable groups, while the Levene test revealed a statistically significant difference between the standard deviations of two successive treatment steps. Application of the Student-Newman-Keuls and the Tamhane test to the lognormal distributions of the four microbial indicators indicates that: (1) microbial inactivation taking place at the physico-chemical treatment step is insignificant, while the time variability of the results is stable (similar standard deviations), (2) microbial inactivation takes mainly place during the UV light and chlorine disinfection processes, with the time variability of the results remaining stable (similar standards deviations), (3) UV light disinfection (constant application dose) is likely affected by parameters such as water transmittance at 254 nm, resulting in a time variability larger than in the influent, and (4) subsequent application of a constant chlorine dose further improves microbial inactivation, but does not recover the lower time variability observed after UV light disinfection.

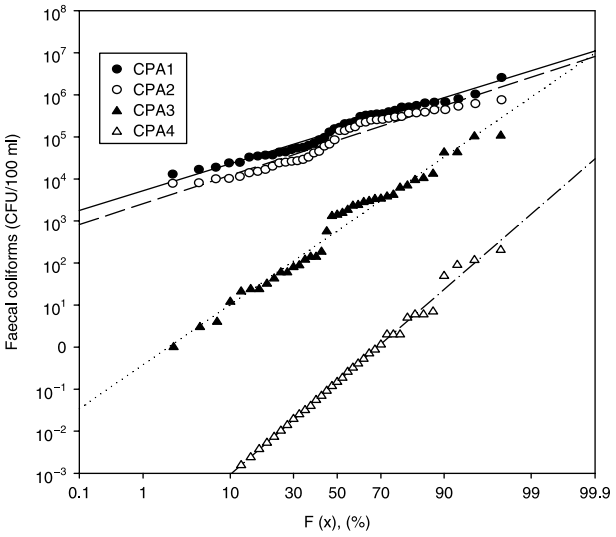

Figure 2 | Probability distribution of faecal coliforms at the WRP of Castell Platja d'Aro.

Table 1 summarizes the average microbial inactivation of the treatment steps applied at the WRP of Castell Platja d'Aro, estimated by difference of the mean values in their influents and effluents. Only UV light and chlorine disinfection processes have been included, as the physico-chemical treatment had an insignificant inactivation effect. Table 1 also includes the standard deviation of the microbial concentrations at the three sampling points. Overall average inactivation rates (μlog) range from 4.4 for enterococci to 5.7 for faecal coliforms, with values close to 4.8 for total coliforms and *E. coli*. Chlorine disinfection had higher inactivation efficiency than UV light disinfection, as they were applied at this WRP plant. Time variability of microbial concentrations shows a significant increase (higher standard deviation) as water goes through the first disinfection step, with standard deviations ranging from 1.1 to 1.3, and then through the second disinfection step, with standard deviations ranging from 1.1 to 1.6. Disinfection processes improve microbial quality, but increase microbial time variability.

Figures 3 and 4 illustrate total and faecal coliforms concentrations, at the four sampling points of the WRP of Empuriabrava; similar graphs were obtained for *E. coli* and enterococci. The figures show that: (1) the four groups of experimental values conform to a lognormal probability distribution (regression lines) and (2) the standard deviations (slope of regression lines) of the four lognormal distributions are similar in all cases, allowing a direct application of the ANOVA test to the four different treatment steps. The Kolmogorov-Smirnov test confirms the normality of the four variable groups, and the Levene test indicates a statistically significant difference between the standard deviation of some successive treatment steps. Application of the Student-Newman-Keuls and the Tamhane test to the lognormal distributions of the four microbial indicators indicates that: (1) microbial

Table 1 | Microbiological inactivation at the WRP of Castell Platja d'Aro achieved by UV light and chlorine disinfection steps, during 8 sampling seasons in 2003–2005

Microorganism	Number of values	Inactivation (μlog/100 ml)	Standard deviation (μlog/100 ml)
TC	118	2.115 + 2.745 = 4.860	CPA2: 0.409; CPA3: 1.113; CPA4: 1.374
FC	118	2.149 + 3.594 = 5.743	CPA2: 0.621; CPA3: 1.289; CPA4: 1.602
EC	118	2.141 + 2.674 = 4.825	CPA2: 0.652; CPA3: 1.216; CPA4: 1.099
FE	117	1.926 + 2.518 = 4.444	CPA2: 0.515; CPA3: 1.228; CPA4: 1.316

inactivation advances steadily as water flows through the stabilization pond, the polishing pond and the constructed wetland (three similar natural processes), (2) microbial quality deteriorates after water goes through the storage lagoon, most likely due to microbial contributions from natural fauna and (3) time variability of the microbial quality remains stable (similar standards deviations) as water flows from one treatment step to the other, including the storage lagoon.

Table 2 summarizes the average microbial inactivation of the three treatment steps applied in the WRP of Empuriabrava, estimated by the difference of the mean values recorded at the influent and the effluent of each step. Only the stabilization pond, the polishing pond and the constructed wetland system have been included, as the storage lagoon slightly increases microbial concentrations. Table 2 also includes the standard deviation of the microbial concentrations at the four sampling points considered. Overall average inactivation rates (μlog) range from 2.2 for enterococci to 3.6 for faecal coliforms, with values of 3.4 for total coliforms and 3.0 for *E. coli*. No single process has similar inactivation efficiency for the four microorganisms. The time variability of microbial concentrations shows a remarkable stability (similar standard deviation) as water flows through the three natural treatment processes, with individual standard deviations ranging from 0.65 to 1.1. In addition to an increase of its microbial content, water flowing through the storage lagoon results in a slight increase on time variability of concentrations of the four types of indicators.

A comparison between inactivation rates achieved by physical and chemical disinfection processes and those achieved by natural processes clearly indicates that the former can normally achieve inactivation rates ranging from 4.4 to 5.7 μlog (could be presumably higher under more controlled conditions), while natural processes achieve an inactivation rate from 2.2 to 3.6 μlog, under the weather conditions of the area. Furthermore, physico-chemical

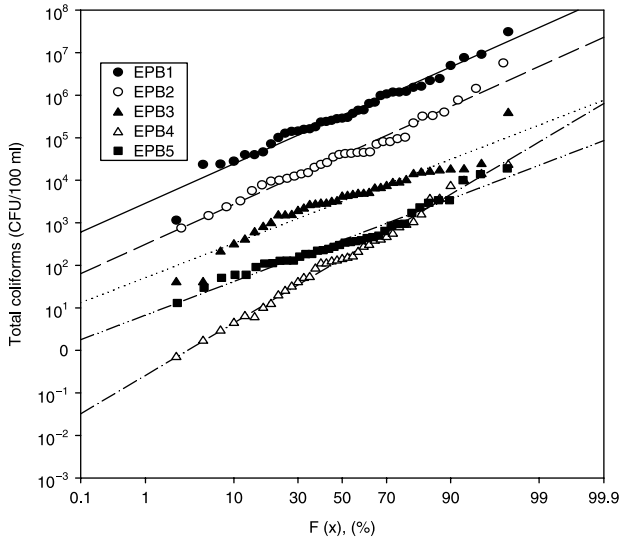

Figure 3 | Probability distribution of total coliforms at the WRP of Empuriabrava.

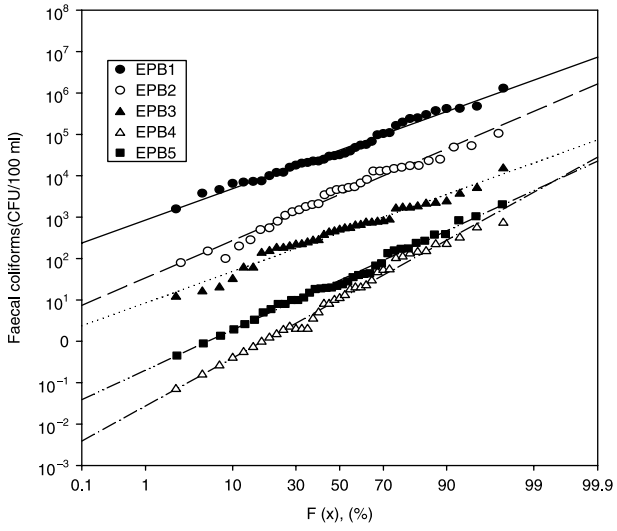

Figure 4 | Probability distributions of faecal coliforms at the WRP of Empuriabrava.

Table 2 | Microbiological inactivation at the WRP of Empuriabrava achieved by stabilization ponds, polishing ponds, and constructed wetlands, during 8 sampling seasons in 2003–2005

Microorganism	Number of values	Inactivation (μlog/100 ml)	Standard deviation (μlog/100 ml)
TC	151	0.941 + 1.080 + 1.348 = 3.369	EPB1: 0.841; EBP2: 0.841
			EPB3: 0.747; EPB4: 1.103
FC	151	1.077 + 0.923 + 1.602 = 3.602	EPB1: 0.682; EBP2: 0.809
			EPB3: 0.687; EPB4: 1.040
EC	151	0.943 + 1.141 + 0.974 = 3.058	EPB1: 0.719; EBP2: 0.750
			EPB3: 0.732; EPB4: 0.792
FE	151	0.701 + 0.859 + 0.665 = 2.225	EPB1: 0.590; EBP2: 0.716
			EPB3: 0.636; EPB4: 1.045

treatment results in the lowest time variability, with standard deviations from 0.40 to 0.65, while natural processes normally provide an effluent with slightly higher time variability, ranging from 0.65 to 1.1.

Figures 5 and 6 illustrate the total and faecal coliforms concentrations at the five sampling points of the WRP of Castell Platja d'Aro; similar graphs were obtained for *E. coli* and enterococci. The figures show that: (1) the five groups of experimental results conform to a lognormal probability distribution and (2) standard deviations (slope of regression lines) of the five lognormal distributions are not equal, thus preventing a direct application of the ANOVA test. Application of the Kolmogorov-Smirnov test to the regression lines confirmed the normality of the four variable groups, while the Levene test indicates a statistically significant difference between standard deviations. Application of the Student-Newman-Keuls and the Tamhane test to the lognormal distributions of the four microbial indicators indicates that: (1) microbial inactivation taking place at the physico-chemical treatment step is significant (higher than 1.0 μlog), with the time variability of the results remaining stable (similar standard deviations), (2) the main microbial inactivation takes place during the UV light and the chlorine disinfection process, although time variability of the results increases considerably (larger standard deviations), (3) UV light disinfection (constant application dose) is likely affected by parameters such as water transmittance at 254 nm, increasing the time

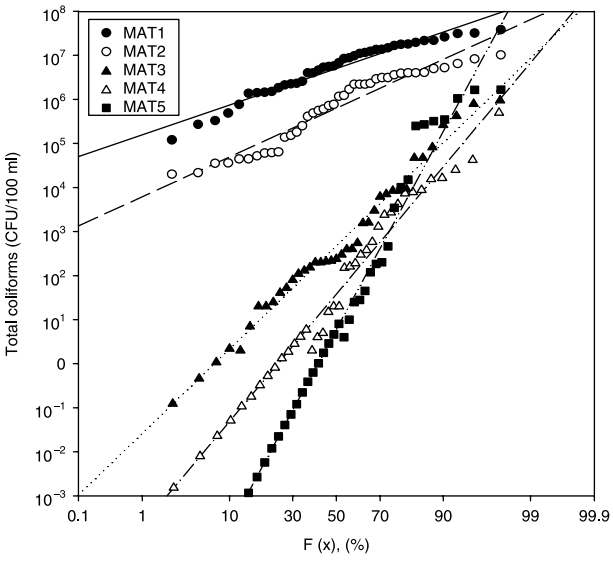

Figure 5 | Probability distributions of total coliforms at the WRP of Mataró.

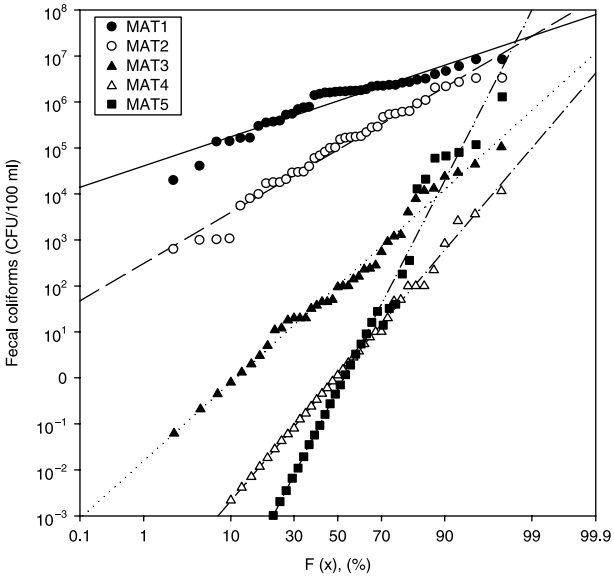

Figure 6 | Probability distributions of faecal coliforms at the WRP of Mataró.

Table 3 | Microbiological inactivation at the WRP of Mataró achieved by physico-chemical treatment, UV light disinfection and chlorine disinfection, during 8 sampling seasons in 2003–2005

Microorganism	Number of values	Inactivation (μlog/100 ml)	Standard deviation (μlog/100 ml)
TC	159	0.900 + 3.123 + 1.899 = 5.922	MAT1: 0.619; MAT2: 0.835
			MAT3: 1.717; MAT5: 3.300
FC	159	1.061 + 2.953 + 2.258 = 6.272	MAT1: 0.586; MAT2: 1.005
			MAT3: 1.535; MAT5: 3.324
EC	159	0.945 + 3.106 + 2.756 = 6.807	MAT1: 0.662; MAT2: 0.957
			MAT3: 1.903; MAT5: 3.832
FE	160	1.573 + 2.246 + 2.435 = 6.254	MAT1: 0.550; MAT2: 1.194
			MAT3: 1.592; MAT5: 2.949

variability of effluent microbial quality, and (4) subsequent application of a constant chlorine dose further improves microbial inactivation, but slightly increases time variability of microbial quality. The significant presence of textile effluents dyes, evident in that secondary effluent, could be responsible for the unreliability of the two disinfection processes, due to light absorption during UV light exposure and oxidation of organic matter during chlorine disinfection. Those conditions highlight the critical need for closer control of disinfection process, by adjusting disinfectant doses to actual light transmittance and dissolved organic matter concentration in water.

Table 3 summarizes the average microbial inactivation of the treatment steps applied in the WRP of Mataró, following the pathway MAT1-MAT2-MAT3-MAT5 that includes physico-chemical treatment, UV light disinfection and chlorine disinfection. Table 3 also includes the standard deviation of the microbial concentrations at the four sampling points considered. Overall average inactivation rates (μlog) range from 5.9 for total coliforms to 6.8 for *E. coli*, with values close to 6.3 for faecal coliforms and 6.8 for enterococci. UV light disinfection has higher inactivation efficiency than chlorine disinfection, as the processes were applied at this WRP plant. Time variability of microbial concentrations significantly increases (higher standard deviation) as water flows through disinfection processes, with standard deviations ranging from 1.6 to 1.9 in the effluent of the UV light process, and from 2.9 to 3.8 in the chlorine disinfection effluent. Disinfection processes improve average microbial quality, but considerably increase microbial time variability.

Finally, a comparative analysis was conducted between total coliforms, faecal coliforms and *E. coli* concentrations in the effluents of the disinfection processes studied at the three WRP. Only 4 out of 40 samples at the WRPs of Castell Platja d'Aro and Empuriabrava, and 3 out of 20 at the WRP of Mataró had *E.coli* values higher than total coliforms, within the 0-100 CFU/100 ml. Considering the relative simplicity of total coliforms analysis, those results raise the question of weather total coliforms could be the preferred indicator for evaluating reclamation process, when water quality limits for bacterial indicators require their absence or values lower than 10 CFU/ 100 ml.

CONCLUSIONS

Evaluation of microbial quality of reclaimed water by a lognormal probability distribution serves to verify the degree of conformity to the proposed distribution and visualize the time variability of the results. Estimated values for the mean and the standard deviation serve to evaluate process performance and reliability of different treatment steps. The method provides basic references for establishing consistent regulatory water quality limits, determining regulatory compliance, controlling water reclamations processes and facilities, evaluating new processes performance and reliability, and elaborating risk simulation models for using reclaimed water.

A conventional physico-chemical treatment can inactivate up to 1.0 μlog of common indicators, while maintaining a time variability (standard deviation) lower

than 1.0 (μlog/100 ml). That removal performance becomes statistically insignificant when only filtration is applied with no coagulant addition. Sand filtration followed by disinfection and a 3-step natural process were effective in inactivating fecal coliforms, followed by total coliforms, *E. coli* and enterococci. Although conventional physico-chemical treatment can decrease the variability of influent water quality, both suspended solids and dissolved organic matter in the influent may considerably modify effluent microbial quality. Dissolved organic matter (i.e. dyes) in the influent to reclamation plants can significantly alter the performance and the reliability (time variation) of disinfection process, highlighting the need for improved adjustments of disinfectant dose to actual quality (dissolved organics) of treated water. Constant doses of chlorine and particularly constant doses of UV light appear especially sensible to variations in water quality (transmittance $<60\%$ and dissolved organics). Although those processes, alone and in combination, may reach inactivation rates as high as 6.0 μlog for common bacterial indicators, the reliability of the process may be considerably affected, increasing standard deviations beyond 3.0 (μlog/100 ml).

Natural processes with residence times of 6 days in summer, and 17 days in winter, can provide a microbial inactivation between 3.0 and 3.5 for common indicators, under the weather conditions of the study area. Although those values are lower than those achieved by physical and chemical disinfectants, the reliability of natural processes remains quite constant when water flows from one step to another, with standard deviations ranging from 0.65 to 1.1 (μlog/100 ml). Natural processes are particularly sensible to external inputs of microbial indicators, due to the presence of wildlife.

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