Removal and relationships of microbial indicators in a water treatment and reclamation facility

Laura Alcalde, Montserrat Folch and Josefina C. Tapias

ABSTRACT

A wastewater treatment and reclamation facility in north-east Spain was monitored over 1 year to determine the occurrence and concentrations of different microbial indicators (*Escherichia coli*, fecal enterococci, somatic bacteriophages and spores of sulfite-reducing clostridia). The removal of the indicators and its relationships through the wastewater treatment and reclamation trains were evaluated. The results obtained show that the reclamation treatments evaluated present a different efficiency in indicator microorganisms' removal depending on the type of microorganism. The *E. coli* and enterococci present an average reduction slightly higher than the other indicators, followed by somatic bacteriophages and spores of sulfite-reducing clostridia. The Spearman's correlations indicate that it is not suitable to use any of the bacterial indicators evaluated to predict the content of virus or spores of sulfite-reducing clostridia. Therefore, in order to evaluate the microbiological risk of the reclaimed effluent use, it is necessary to monitor the three types of indicator microorganisms (bacteria, virus and protozoa).

Key words | microbial indicators, reclamation, reuse, treatment technology, wastewater

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INTRODUCTION

The contribution of reclaimed water to sustainable water resources is expected to increase in many areas of the world, including some parts of the USA (Asano 2002) and European areas from the northern shore of the Mediterranean Sea (Angelakis *et al*. 2003). Reclaimed water is a valuable but under-utilized resource due partly to the uncertainties about the potential health risks associated with reclaimed water exposure. When reusing, one of the greatest threats to human health is considered to be posed by pathogenic microorganisms (Toze 2006). Since a wide variety of pathogenic microorganisms can be found in wastewater, including bacteria, viruses, protozoan and parasitic worms, in order to minimize public health risks the most important goal is the reduction of pathogens during reclamation treatments. Nevertheless, due to the difficulty to determine directly all the pathogens, indicators for several of them are routinely used.

Consequently, at present, reclaimed water quality is mainly assessed through routine monitoring for the presence of bacterial indicators and nematode eggs which are included in different regulations and guidelines around the world (USEPA 2004; WHO 2006). In Spain, in December 2007, a national regulation on reclaimed water quality criteria was issued (RD 1620/2007) determining five different categories of uses: urban, agricultural, industrial, recreational and environmental. These quality criteria rely mainly on several microbiological and physicochemical parameters (*Escherichia coli*, nematode eggs, suspended solids and turbidity) although for some uses more than 70 parameters must be controlled. The maximum levels permitted for each parameter are dependent on the specific use. For the main parameters, *E. coli* maximum levels are between 0 and 10,000 colony-forming units (CFU)/100 mL, nematode eggs are between 1 egg/10 L to no limitation, suspended solids range between 5 and 35 mg/L and turbidity levels are between 1 and 15 NTU.

It should be noted that a universally accepted standard to regulate the quality of reclaimed water does not exist.

doi: 10.2166/wh.2012.213

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E. coli is one of the most used indicators in regulations and standards worldwide (Lucena *et al.* 2004; Harwood *et al.* 2005).

Bacteriophages infecting enteric bacteria and spores of sulfite-reducing clostridia have been suggested as indicators for viruses (IAWPRC 1991) and protozoa (Payment *et al.* 1985). In this sense, somatic bacteriophages are one of the groups proposed among bacteriophages (Kott *et al.* 1974).

In the Mediterranean area, as elsewhere, studies evaluating treatment technologies for reclaimed effluent production on an industrial scale monitoring different types of microbiological indicators at the same time are limited. The aim of this study is to determine and enumerate the levels of indicators in raw water, secondary and tertiary effluents in order to assess the effects of treatment trains on the removal of these microorganisms and to determine the behavior and relationships of the indicators studied.

METHODS

Water treatment and reclamation plants studied

This study was performed in a treatment facility consisting of a wastewater treatment plant (WWTP) followed by a water reclamation plant (WRP). The facility is located in Catalonia, in the north-east of Spain, which has a typical Mediterranean climate. The WWTP has a design flow of 47,500 m^3/day. The facility has a population equivalent of 210,583, a design suspended solids content of 232 mg/L and a BOD_5 of 266 mg/L. The design flow of the WRP is 16,500 m^3/day. The flow diagrams of the WWTP and the WRP trains can be found in Figures 1 and 2.

The chlorination is carried out in the WRP using sodium hypochlorite. The dosage is 8 mg/L with a contact time of 12 minutes in order to achieve residual chlorine of 1 mg/L in the reclaimed effluent.

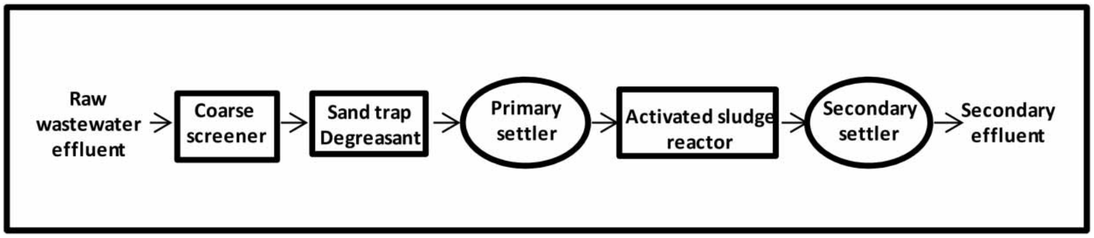

Figure 1 | Flow diagram of the WWTP.

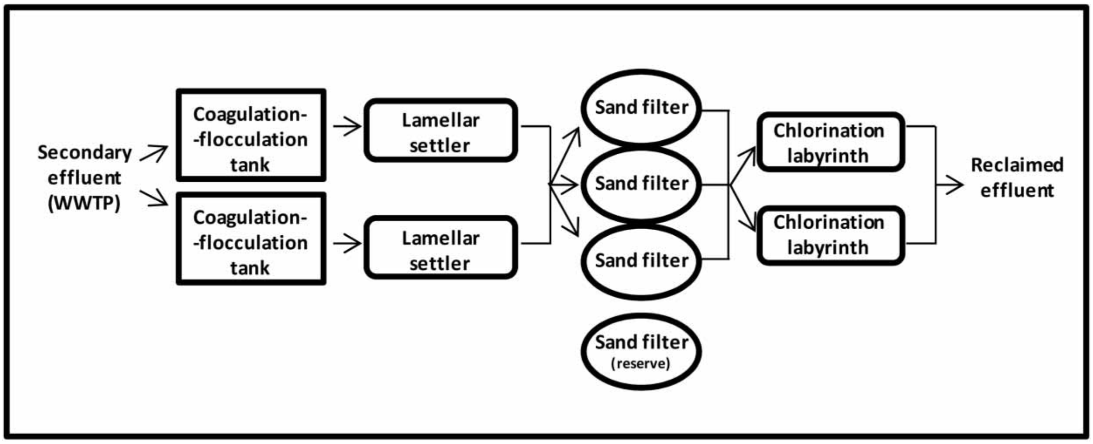

Figure 2 | Flow diagram of the WRP.

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Samples and sampling

In order to evaluate the microbiological quality of the wastewater throughout the WWTP and WRP facilities, the following sampling points were selected:

- Inlet to the primary settler (considered here the raw wastewater effluent);
- Outlet of the secondary settler (considered here the secondary effluent);
- Outlet of the chlorination labyrinth (considered here the reclaimed effluent).

Sampling was done at all sampling points weekly, on Tuesday morning, for 1 year. The number of samples analyzed for each sampling point was from 38 to 40. All samples were analyzed for the presence of microbial indicators and pathogenic microorganisms. The microbial indicators selected were *E. coli*, fecal enterococci, somatic bacteriophages and spores of sulfite-reducing clostridia. Analytical methods for microorganisms' quantification are shown in Table 1.

Water samples were collected, preserved and transported according to the procedures described in APHA (2005) on water sampling for microbiological analysis.

The SPSS software version 19.0 was used for statistical calculations. The statistics analyzed are the descriptive statitistics and Spearman's correlations. The significance level (p) used has been a bilateral p of 0.05 ($p \leq 0.05$).

Table 1 | Microbiological parameters and analytical methods utilized during this study

Parameter	Analytical method
Escherichia coli (E. coli)	APHA (2005) ref. 9222-D Membrane filtration
	Culture media: chromID^{TM} Coli (COLI ID-F) Biomérieux
Fecal enterococci	APHA (2005) ref. 9230-C Membrane filtration
	Culture media: Difco^{TM} m Enterococcus Agar
Spores of sulfite-reducing clostridia	APHA (2005) ref. 9222-D Membrane filtration
	Culture media: BBL^{TM} TSN Agar
Somatic bacteriophages	ISO 10705–2 (2000)
	Host strain: WG5-*E. coli* CN (ATCC 700078)

The water samples of the reclaimed effluent were analyzed for the somatic bacteriophage content using a concentration method, filtering the volume of water through a nitrocellulose filter of 0.22 μm, as described in Sobsey *et al.* (1982), and according to the modifications made by Méndez *et al.* (2004).

RESULTS AND DISCUSSION

Microorganism indicator densities in different water effluents

Descriptive statistical parameters of indicator microorganisms are shown in Table 2. In order to show the dispersion of the results obtained, confidence levels at 95% of the average content at the different sampling points and average removals of indicator microorganisms in secondary and tertiary treatments are shown in Figures 3 and 4, respectively.

The indicator *E. coli* in raw wastewater shows the highest concentration of all the microorganism concentrations evaluated, followed by the somatic bacteriophages, fecal enterococci and spores of sulfite-reducing clostridia (Table 1). These figures and proportions between the indicators are in agreement with those found in raw wastewaters of other Spanish WWTPs treating urban wastewater (Lucena 2008).

In the secondary effluent, the content of all the microorganisms evaluated has diminished, but the proportions are the same as in raw wastewater, *E. coli* being the most abundant, followed by somatic bacteriophages, fecal enterococci and spores of sulfite-reducing clostridia.

Tertiary effluent presents some differences regarding the proportions (relative contents) of the indicator microorganisms evaluated in comparison with the other types of samples. Somatic bacteriophages show the highest concentration of all microorganisms, followed by spores of sulfite-reducing clostridia, *E. coli* and fecal enterococci (Figure 3).

The mean content and proportions of the indicator microorganisms evaluated in the secondary and reclaimed effluents are similar to the ones described in several studies in Spain and in other areas of the world where urban waters

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Table 2 | Descriptive statistics of the concentration of indicator microorganisms at the different sampling points' effluents

	Indicator microorganisms concentration ($\log_{10}$ units/100 mL)			
	Minimum	Maximum	Average	Standard deviation
Raw wastewater effluent				
E. coli ($n = 40$)	6.30	7.61	7.02	0.29
Fecal enterococci ($n = 40$)	5.41	6.09	5.87	0.17
Somatic bacteriophages ($n = 40$)	5.74	7.46	6.69	0.32
Spores of sulfite-reducing clostridia ($n = 39$)	2.00	5.09	4.06	0.62
Secondary effluent				
E. coli ($n = 40$)	3.70	6.17	5.22	0.58
Fecal enterococci ($n = 40$)	3.15	5.10	4.12	0.56
Somatic bacteriophages ($n = 40$)	4.01	5.97	4.92	0.45
Spores of sulfite-reducing clostridia ($n = 40$)	1.18	3.42	2.46	0.47
Reclaimed effluent				
E. coli ($n = 40$)	0.00	6.01	1.81	1.99
Fecal enterococci ($n = 40$)	0.00	4.70	1.55	1.59
Somatic bacteriophages ($n = 39$)	0.30	5.78	4.07	1.06
Spores of sulfite-reducing clostridia ($n = 38$)	0.70	3.41	1.83	0.70

n: number of samples analyzed.

are treated and reclaimed (Duran *et al.* 2003; Lucena *et al.* 2004; Harwood *et al.* 2005; Mandilara *et al.* 2006; Costán-Longares *et al.* 2008).

The average reduction of the indicator microorganisms studied through the different treatments of the WWTP does not present significant differences ($p > 0.05$), having similar values for *E. coli* (1.80 $\log_{10}$ units/100 mL), fecal enterococci (1.77 $\log_{10}$ units/100 mL), somatic bacteriophages (1.75 $\log_{10}$ units/100 mL), and sulfite-reducing clostridia (1.60 $\log_{10}$ units/100 mL) (Figure 4).

WWTP treatments (pretreatment, primary settling, activated sludge process and secondary settling) have a certain disinfection capacity due to the natural die-off of the microorganisms and adsorption onto the biological flocs, and the attachment of some microorganisms to suspended particles that are removed by the sedimentation processes (Tchobanoglous *et al.* 2003).

According to several authors (Lucena *et al.* 2004; Mandilara *et al.* 2006), the most important percentage of inactivation is produced during the activated sludge biological treatment and secondary settling.

This type of wastewater treatment seems to reduce the microorganisms' concentration of the indicators in a similar manner. However, the *E. coli* and enterococci present an average reduction slightly higher than the other indicators, followed by somatic bacteriophages and spores of sulfite-reducing clostridia. These results are in agreement with those of other authors (Rose *et al.* 1996; Gantzer *et al.* 1998; Durán *et al.* 2003; Mandilara *et al.* 2006; Wen *et al.* 2009).

The average reduction of the indicator microorganisms during the reclamation treatment presents differences between the indicators evaluated. *E. coli* shows the highest removal rate (3.41 $\log_{10}$ units/100 mL), followed by fecal enterococci (2.49 $\log_{10}$ units/100 mL) somatic bacteriophages (0.90 $\log_{10}$ units/100 mL) and sulfite-reducing clostridia (0.67 $\log_{10}$ units/100 mL) (Figure 4).

There are significant differences between the removal of *E. coli* and fecal enterococci, and also between the average removal of somatic bacteriophages and sulfite-reducing clostridia (Figure 3). *E. coli* and fecal enterococci are more efficiently removed than somatic bacteriophages and sulfite-reducing clostridia.

These results can be explained by the fact that somatic bacteriophages and spores of sulfite-reducing clostridia have higher resistance to chlorine disinfection than *E. coli* and fecal enterococci (Ashbolt *et al.* 2001; Araujo *et al.* 2004).

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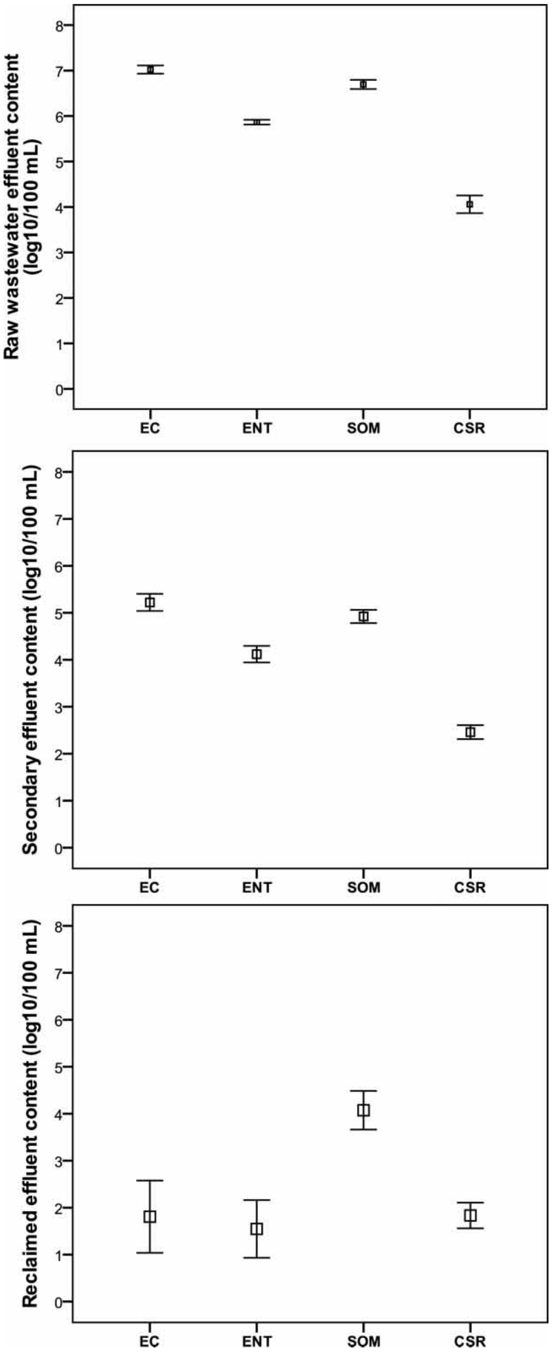

Figure 3 | Confidence levels at 95% of the average content of *E. coli* (EC), fecal enterococci (ENT), somatic bacteriophages (SOM) and spores of sulfite-reducing clostridia (CSR) in raw wastewater, secondary and reclaimed effluent.

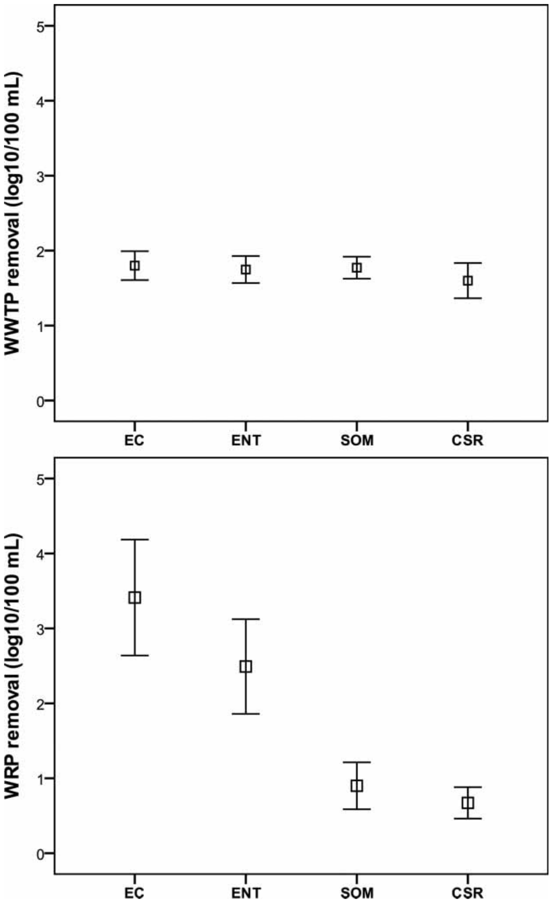

Figure 4 | Confidence levels at 95% of the average removal of *E. coli* (EC), fecal enterococci (ENT), somatic bacteriophages (SOM) and spores of sulfite-reducing clostridia (CSR) achieved by the WWTP and the WRP.

The average removal values found in this study for the microorganisms evaluated are similar to the removal values described in other studies performed in reclamation facilities consisting of a physicochemical process and a chlorine disinfection treatment (Havelaar 1987; Tartera *et al.* 1988; Mandilara *et al.* 2006).

The results obtained show that the reclamation treatments evaluated present a different efficiency in indicator removal depending on the type of microorganism. This fact has not been observed during the treatment in the WWTP, where all microorganisms determined were removed in a similar manner. This behavior is consistent with the results described in other studies (Scott *et al.* 2003; Mandilara *et al.* 2006; Costán-Longares *et al.* 2008).

The Spanish RD 1620/2007 indicates the maximum level of *E. coli* concentration allowed for irrigation of green areas (2.3 $\log_{10}$ units/100 mL). The reclaimed water obtained in the facility evaluated meets this requirement in all the samples except one which presented a concentration of 6.00 $\log_{10}$ units/100 mL. This fact was due to a failure in

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the chlorine dosage that was rapidly fixed by the plant technicians.

The differences in the proportions among the indicators evaluated can also be explained, to some extent, by the operational regime of the reclamation facility. The reclamation facility does not operate in a continuous mode, and the operation depends on the irrigation demands of the park. Therefore, the facility may not be operating for 1 to 3 days, depending on the irrigation demands. This fact can affect the efficiency and reliability of the facility.

Relationships among the indicator microorganisms evaluated

In order to determine the relationships between densities of the indicators evaluated, bilateral Spearman's correlations (p bilateral ≤ 0.05) were performed (Table 3).

In the secondary effluent, a significant correlation was found between *E. coli* and fecal enterococci ($r = 0.81$, $p = 0.00$), it being the highest correlation. Other significant correlations, but with lower correlation coefficients and weaker relationships, corresponded to the relationships between fecal enterococci and spores of sulfite-reducing clostridia ($r = 0.48$, $p = 0.00$), and between *E. coli* and sulfite-reducing clostridia ($r = 0.36$, $p = 0.02$). There were no significant correlations between somatic bacteriophages and the rest of the indicator microorganisms evaluated (Table 3).

In the reclaimed effluent, bilateral Spearman's correlation between *E. coli* and fecal enterococci ($r = 0.90$, $p = 0.00$) was found to be significant and very high. There were also significant correlations, but with a lower coefficient, between *E. coli* and somatic bacteriophages ($r = 0.65$, $p = 0.00$), *E. coli* and sulfite-reducing clostridia ($r = 0.58$, $p = 0.00$), fecal enterococci and somatic bacteriophages ($r = 0.64$, $p = 0.00$), fecal enterococci and sulfite-reducing clostridia ($r = 0.55$, $p = 0.00$) and between somatic bacteriophages and sulfite-reducing clostridia ($r = 0.45$, $p = 0.01$).

Table 3 | Spearman's correlation results on the comparison between the microorganisms indicators evaluated in the secondary and reclaimed effluents

	Spearman's correlations (*p* bilateral ≤0.05)			
	E. coli	**Fecal enterococci**	**Somatic bacteriophages**	**Spores of sulfite-reducing clostridia**
Secondary effluent				
E. coli	1	$r = 0.81$ $p = 0.00$	$r = -0.53$ $p = 0.74$	$r = 0.36$ $p = 0.02$
Fecal enterococci	$r = 0.81$ $p = 0.00$	1	$r = -0.02$ $p = 0.90$	$r = 0.48$ $p = 0.00$
Somatic bacteriophages	$r = -0.53$ $p = 0.74$	$r = -0.02$ $p = 0.90$	1	$r = 0.30$ $p = 0.06$
Spores of sulfite-reducing clostridia	$r = 0.36$ $p = 0.02$	$r = 0.48$ $p = 0.00$	$r = 0.30$ $p = 0.06$	1
Reclaimed effluent				
E. coli	1	$r = 0.90$ $p = 0.00$	$r = 0.65$ $p = 0.00$	$r = 0.58$ $p = 0.00$
Fecal enterococci	$r = 0.90$ $p = 0.00$	1	$r = 0.64$ $p = 0.00$	$r = 0.55$ $p = 0.00$
Somatic bacteriophages	$r = 0.8$ $p = 0.00$	$r = 0.64$ $p = 0.00$	1	$r = 0.45$ $p = 0.01$
Spores of sulfite-reducing clostridia	$r = 0.8$ $p = 0.00$	$r = 0.55$ $p = 0.00$	$r = 0.45$ $p = 0.01$	1

r: correlation coefficient; p: significance level.

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The results described are in agreement with the results obtained in other studies performed in secondary and reclaimed effluents (Mandilara *et al.* 2006; Costán-Longares *et al.* 2008) and indicate that among the indicators evaluated there is only a high (strong) and also significant correlation between *E. coli* and fecal enterococi.

Therefore, the bacterial indicators do not adequately reflect the removal of either pathogenic viruses or protozoa achieved in disinfection procedures used to obtain the reclaimed effluent and, in order to evaluate the microbiological risk of the use of the reclaimed effluent, it is necessary to analyze the three types of indicator microorganisms (bacteria, virus and protozoa).

CONCLUSIONS

The results obtained show that the reclamation treatments evaluated present different efficiencies in indicator microorganism removal depending on the type of microorganism, while the WWTP treatments present a similar removal for all the indicators evaluated.

The average removal of *E. coli* and the fecal enterococci was higher than that of somatic bacteriophages and sulfite-reducing clostridia in the reclamation treatments evaluated. This confirms the great resistance of virus and spores to chlorination at the dose evaluated in this study.

The Spearman's correlations indicate that there are no significant correlations between bacterial indicators and somatic bacteriophages, and between bacterial indicators and spores of sulfite-reducing clostridia. Therefore, it is not suitable to use any of the bacterial indicators evaluated to predict the content of virus or spores of sulfite-reducing clostridia and it is recommended to include the monitoring of the three types of indicator microorganisms (bacteria, virus and protozoa) in order to evaluate the microbiological quality of the reclaimed effluent.

It has to be taken into account that the operational regime of the WRP can affect the microbiological quality of the reclaimed effluent.

In respect to the 'legal' content of the reclaimed effluent, it should be noted that this resource can be reused for several purposes.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the CENIT project SOSTAQUA and the University and Research Grants and Fellowships Agency (AGAUR) of the Government of Catalonia.

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First received 22 November 2011; accepted in revised form 14 August 2012. Available online 9 October 2012

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