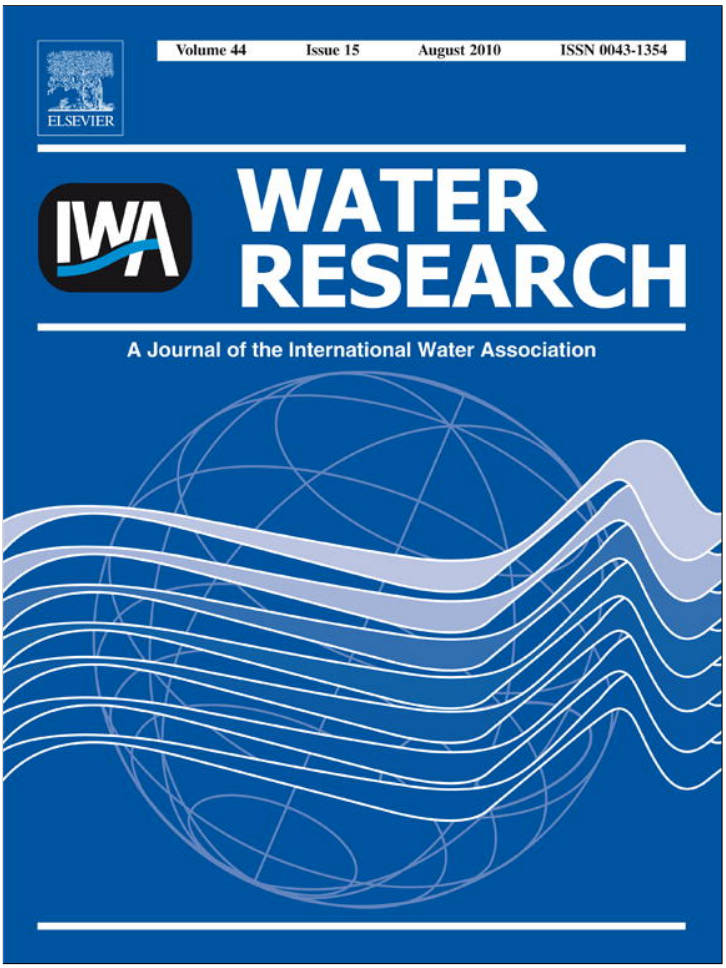

WATER RESEARCH 44 (2010) 4437–4450

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Toxicity removal efficiency of decentralised sequencing batch reactor and ultra-filtration membrane bioreactors

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ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form
31 May 2010
Accepted 4 June 2010
Available online 11 June 2010

Keywords:
AS-SBR
UF-MBR
Discharge quality
Ecotoxicity
Saltwater species

ABSTRACT

As a consequence of the Water Framework Directive and Marine Strategy Framework Directive, there is now more focus on discharges from wastewater treatment plants both to transitional and marine-coastal waters. The constraint to encourage sustainable water policy to prevent water deterioration and reduce or stop discharges has entailed new requirements for existing wastewater treatment plants in the form of advanced wastewater treatment technologies as further suggested by the Integrated Pollution and Prevention Control Bureau. A whole toolbox of physico-chemical and ecotoxicological parameters to investigate commercial and mixed domestic and industrial discharges was considered to check the efficiency of an Activated-Sludge Sequencing Batch Reactor (AS-SBR) and two Ultra-Filtration Membrane Biological Reactors (UF-MBRs) on a small scale decentralised basis. All discharges were conveyed into Venice lagoon (Italy), one of the widest impacted Mediterranean transitional environment. The UF-MBRs were able to provide good quality effluents potentially suitable for non-potable reuse, as well as reducing specific inorganic micro-pollutants concentration (*e.g.* metals). Conversely, the AS-SBR showed unpredictable and discontinuous removal abilities.

1. Introduction

The importance has recently been evidenced of producing higher quality treated wastewater within the perspective of zero emissions (OSPAR, 2007) along with the precautionary principle (Harremoës, 2000), not only to protect the receiving water body, but also to further support water recycling and reuse, covering end-of-pipe technologies for treatment of pollution immediately after it has been generated. In the European Union, the Water Framework Directive (2000/60/EC) (WFD, 2000) and the more recent Marine Strategy Framework Directive (2008/56/EC) (MSFD, 2008) suggested the adoption of a sustainable water policy to prevent water deterioration and reduce or stop discharges, emissions and losses of hazardous substances. Treated discharges from WasteWater Treatment Plants (WWTPs) must comply with Environmental Quality Standards defined under the WFD, entailing new requirements for existing WWTPs in the form of advanced wastewater treatment technologies. Various aspects must therefore be checked before selecting the optimal advanced treatment technology at a specific WWTP, including not only technical and economic values, but also environmental targets (*i.e.* physical, chemical and ecotoxicological goals) to be met that may play a leading role in the selection process (Høibye et al., 2008).

Sustainable development is at the forefront of today's policy agendas for technology developers who are involved in wastewater treatment. As indicated by the Integrated Pollution and Prevention Control directive (IPPC, 2008), recent Best Available Techniques (BAT) in wastewater management are oriented to water recycling as well as nutrients (N and P) and

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doi:10.1016/j.watres.2010.06.006

organics removal and, potentially, toxicity lowering. Moreover, it is increasingly expected the reduction/removal of growing concern inorganic (*e.g.* metals) and organic (*e.g.* pharmaceuticals) micro-pollutants (Verliefde et al., 2007; Abegglen et al., 2009; Santos et al., 2010). The general trend is to make river and sea outflows redundant with the reduction of requirements for large pipes, mostly by supporting on-site treatments and decentralisation procedures (Maurer et al., 2006; Weber et al., 2007). In particular, decentralised on-site wastewater treatment plants are spreading not only in rural and suburban communities, where sewerage systems are not available, but also in industrial, commercial and residential areas where water consumption rates necessitate considering alternative wastewater treatment scenarios to improve economic, social and environmental aspects related to water conservation and reclamation (Bakir, 2001; Ho and Anda, 2004).

In Italy, the best example of decentralisation is in the city of Venice. The city has no sewage system due to its geographical situation and historical characteristics, so untreated wastewater has been discharged directly into the surrounding Lagoon. In 1990, policy-makers and local authorities decided to urgently improve water quality and prevent pollution phenomena by requiring on-site WWTPs installation principally for arts and craft businesses, hospitals, tourist-related structures and restaurants. As a consequence of this 4493 WWTPs now exist, mainly septic tanks (80%), even if Activated-Sludge Sequencing Batch Reactor (AS-SBR) (≈1%) and Ultra-Filtration Membrane Biological Reactor (UF-MBR) (≈1%) facilities are increasing (MAV, 2007).

The integrated assessment of wastewater ecotoxicological effects has been recognised to be of major importance besides the physico-chemical characterisation, thus ecotoxicity testing is referred to as a useful way to identify potential environmental impacts to the receiving water environment (Mendonça et al., 2008). Current legislation including the WFD (2000), MSFD (2008), IPPC (2008) and Registration, Evaluation, Authorisation and restriction of CHemicals (REACH, 2006) as well as the Whole Effluent Toxicity (WET) approach (USEPA, 2004) and the Whole Effluent Assessment (WEA) (OSPAR, 2007) indicates that ecotoxicity testing is an integral part of the toolbox to investigate discharges in order to define a realistic assessment and management strategy.

The aim of this research was to check the efficiency of two advanced small scale decentralised wastewater treatment technologies, AS-SBR (Celis et al., 2008; Ben et al., 2009) and UF-MBR (Nosenzo et al., 2005; Radjenović et al., 2009), to increase the physico-chemical and ecotoxicological quality of effluents to be discharged into Venice lagoon, that is one of the widest Mediterranean transitional environment, boosting at the same time the general level of sustainability within the perspective of treated wastewater reclamation and reuse. Both commercial and mixed domestic and industrial (*i.e.* contaminated by metal and metallic micro-pollutants) wastewater samples were taken into consideration. Saltwater testing species were selected within the most widespread organisms already used in scientific literature for wastewater monitoring as well as required by national and international legislations. Bioluminescent bacteria (*Vibrio fischeri*) (Gutiérrez et al., 2002; ISO, 2007; Ricco et al., 2004) and two bivalve molluscs (*Crassostrea gigas* and *Mytilus galloprovincialis*), in order to allow the comparison of their relative sensitivities, were considered for this purpose (USEPA, 1995; RIKZ, 1999; SEPA, 2003; ASTM, 2004; OSPAR, 2007). Finally, traditional physico-chemical parameters were compared to toxicity data elaborated on the basis of the Libralato et al. (in press) scoring system and wastewater toxicity index to provide a whole integrated assessment of samples.

2. Materials and methods

2.1. Wastewater treatment plants

This research focused on three on-site decentralised WWTPs (AS-SBR, UF-MBR1 and UF-MBR2) located in Venice (Italy) historical centre, with the Venice lagoon as target receiving water body. The AS-SBR was installed in 1998, whereas the UF-MBR1 and UF-MBR2, in 2004 and 2005, respectively. Specifically, AS-SBR and UF-MBR1 were placed next to San Marco's square in the core of Venice, whereas UF-MBR2 was sited in Murano island that is a worldwide recognised district for its artistic glass production. AS-SBR and UF-MBR1 treated commercial wastewater characterised by sudden variations in influent load, while UF-MBR2 mixed domestic and metal-rich wastewater. The main specifications of the considered WWTPs are provided in Table 1. In addition, it must be said that all WWTPs are periodically required to manage and dispose excess sludge.

The AS-SBR operates on the basis of five sequential steps including feeding, mixing, aerobic reaction, settling and drawing (Metcalf and Eddy Inc., 2003), before the final discharge of treated wastewater as reported in Fig. 1. The UF-MBR still works on the principle of the activated-sludge process, but the secondary clarifier is replaced by a UF-membrane filtration system consisting of PolyVinyliDene Fluoride (PVDF) tubular membranes with a 0.12 μm particle cut off (A19, PCI, UK). The UF-MBR1 as shown in Fig. 2 has two interconnected aeration basins (named A and B) working simultaneously with two independent UF units, named A and B, respectively. The retentate is recirculated in the oxidation basin while the permeate is accumulated in the effluent tank, before the final discharge. The UF-MBR2 carries out the treatment process including screening and grinding, denitrification and aerobic oxidation prior to UF on PVDF membranes as reported in Fig. 3. The retentate is recirculated both in the denitrification and oxidation basins, while the permeate is sent to an activated carbon column to further improve effluent quality with special regard to colour and residual trace metals content, before the final discharge. The industrial component of the mixed wastewater was mainly composed of glass factory effluents that were rich in trace metal and metallic species. Before entering the equalisation basin, industrial wastewater was generally physico-chemically pre-treated as a first step of a larger multi-purpose plant.

2.2. Sample collection and handling

Wastewater samples were collected manually according to USEPA (2004) general guidelines. Influent was sampled in

Table 1 – Wastewater treatment plants main characteristics.

	Specification	Units	Values		
			AS-SBR	UF-MBR1	UF-MBR2
General	Daily flow rate	$m^3\,day^{-1}$	120	150	80–120
	Mixed liquor dissolved oxygen	$mg\,l^{-1}$	1.9–3.2	2.4–3.1	2.5–5.2
	MLSS	$g\,l^{-1}$	6–8	8–10	9–23
	MLVSS	$g\,l^{-1}$	4–6	6–8	7–13
	Sludge retention time	Day	30–40	50–75	150–300
	Sludge production	kg MLSS (kg CODi)$^{-1}$	0.4–0.6	0.09–0.12	0.06–0.09
	Operating temperature	°C	15–25	18–30	16–35
	Remote control		Yes	Yes	Yes
Denitrification	Basin area	m^2	–	–	18
	Minimum volume	m^3	–	–	–
	Maximum volume	m^3	–	–	90[a]
	Working volume	m^3	–	–	90
	Hydraulic retention time	h	–	–	18–27
Reaction/aeration	Basin area	m^2	32 + 32	38 + 38	73
	Minimum total volume	m^3	80	91[b]	87
	Maximum total volume	m^3	92	106[b]	145
	Working volume	m^3	84	100[b]	109
	Hydraulic retention time	h	18	16	22–33

i = Influent.
a Always operating at the maximum volume.
b Total volume.

WWTP feed tanks, whereas effluent was sampled after the final treatment and downstream of all entering wastewater before the final discharge. In the case of UF-MBR2, wastewater samples were also collected immediately after UF-membrane filtration.

Every sample was the result of 3 grab samples collected over a period of time not exceeding 6 h and homogenised to obtain a composite sample in order to reduce the variability of wastewater according to a time composite sampling procedure. Non-reactive pre-cleaned polyethylenterephtalate containers were completely filled (1 l), leaving no air-space between the content and the lid. Wastewater samples were cooled to 4 °C ± 1 °C for transport from the sampling site to the laboratory to minimise physico-chemical and biological changes. In the laboratory, discrete samples were mixed to produce composite samples. Wastewater aliquots for physical and chemical analyses were not processed further and stored at 4 °C ± 1 °C, providing their full characterisation 24–36 h after collecting. Conversely, ecotoxicological evaluations were carried out on salinity adjusted samples (OSPAR, 2007) by means of hypersaline brine addition, in order to simulate the potential adverse effects on the receiving saltwater environment (USEPA, 1995; Libralato et al., 2009).

Samples were named by a combination of the WWTP collection site identification letter (X = AS-SBR, Y = UF-MBR1 or Z = UF-MBR2), the treatment stage (i = influent, e = effluent only for X, p = permeate for both Y and Z and ac = activated carbon only for Z) and an integer number indicating the sequence in specimen collection. AS-SBR and UF-MBR1 were monitored for 8 weeks (from April to May) consecutively, whereas UF-MBR for 21 weeks (from January to August). Both monitoring periods provided one integrated sample per week considering influent, permeate and effluent after activated carbon filtration on a case-by-case basis.

2.3. *Chemical analyses*

The pH was measured via pHmeter HI 9025 Microcomputer (HANNA Instrument, Beverly, MA, USA), the salinity was checked with a refractometer (Atago, Japan) and the Dissolved Oxygen (DO) by a WTW multi-parametric device (Nova Analytics, Weilheim, Germany).

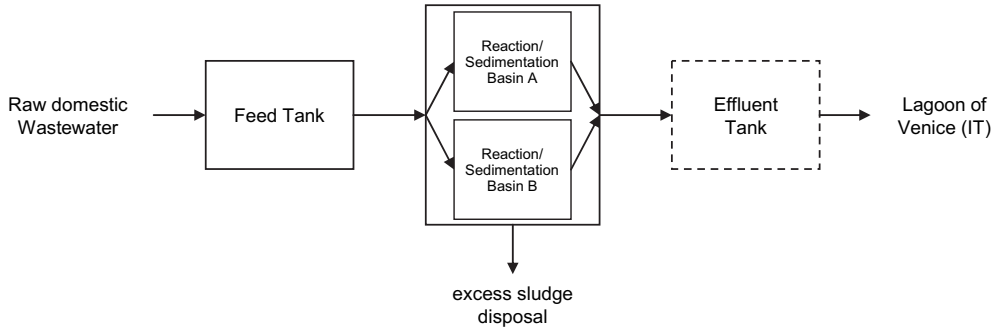


Fig. 1 – Flow chart of AS-SBR plant.

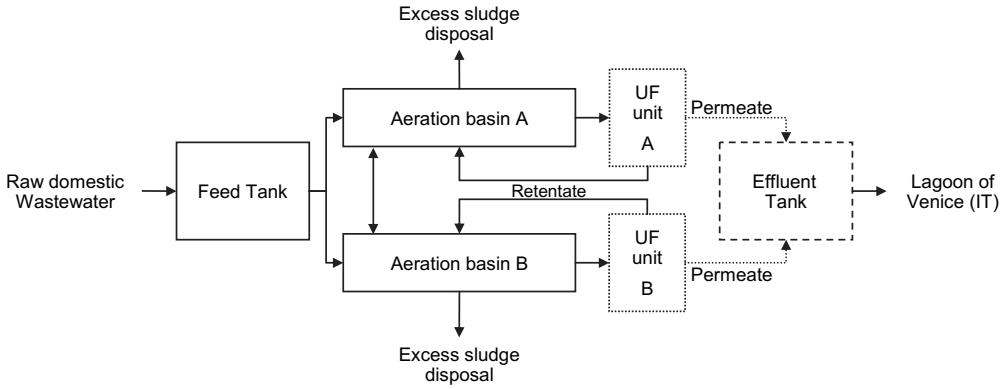


Fig. 2 – Flow chart of UF-MBR1 plant.

The determination of ionic species, chloride (Cl^-), nitrite ($N-NO_2^-$), nitrate ($N-NO_3^-$), ammonia ($N-NH_4^+$), phosphate ($P-PO_4^{3-}$) and sulphate ($S-SO_4^{2-}$), was performed using an Ion Chromatograph (IC) system (column Metrohm Metrosep A Supp 5150 × 4 mm, Metrohm 761 Compact IC, Switzerland) according to APHA (1998) methods. Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), total phosphorus (P_{TOT}), Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS) and raw wastewater Suspended Solids (SS) were analysed according to APHA (1998) methods.

The determination of metal and metallic elements such as aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), total chromium (Cr_{tot}), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), molybdenum (Sb), selenium (Se), vanadium (V) and zinc (Zn) was carried out according to USEPA (1992) and APHA (1998) methods using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Spectroflame Compact E, Spectro Analytical Instruments, Kleve, Germany). An ICP-OES multi-element standard solution (Merck 10580) was used for calibration and Quality Assurance/Quality Control (QA/QC) procedures. Only UF-MBR2 samples were checked for metal and metallic elements due to the origin of the treated wastewater.

2.4. *Toxicity tests*

Microtox® tests were performed using Gram-negative marine bioluminescent bacteria *V. fischeri*. The Azur Environmental (1998) 100% protocol was followed using Microtox® Model 500 Test System. This protocol allowed measurement of light outputs at a wavelength of 490 nm with readings after 5-, 15 and 30-min time exposure at 15 °C ± 1 °C to samples serial dilutions. Specifically, in this study only the 5-min data were taken into account to consider the effects derived from the maximum contact time. The light loss as a consequence of bacteria exposure to the toxic samples was the endpoint. Three replicates were performed for every sample dilution, including the control (dilution water) and reference toxicant. Light emission was recorded and the output data analysed using MicrotoxOmni® software Version 1.18 (Azur Environmental, 1998).

The bioassays with *C. gigas* and *M. galloprovincialis*, based on embryo-larval development abnormalities, were performed according to the methods proposed by ASTM (2004) modified to use gametes pools (Volpi Ghirardini et al., 2005; Libralato et al., in press). Conditioned adult oysters were purchased ready to use from the Guernsey Sea Farm Ltd

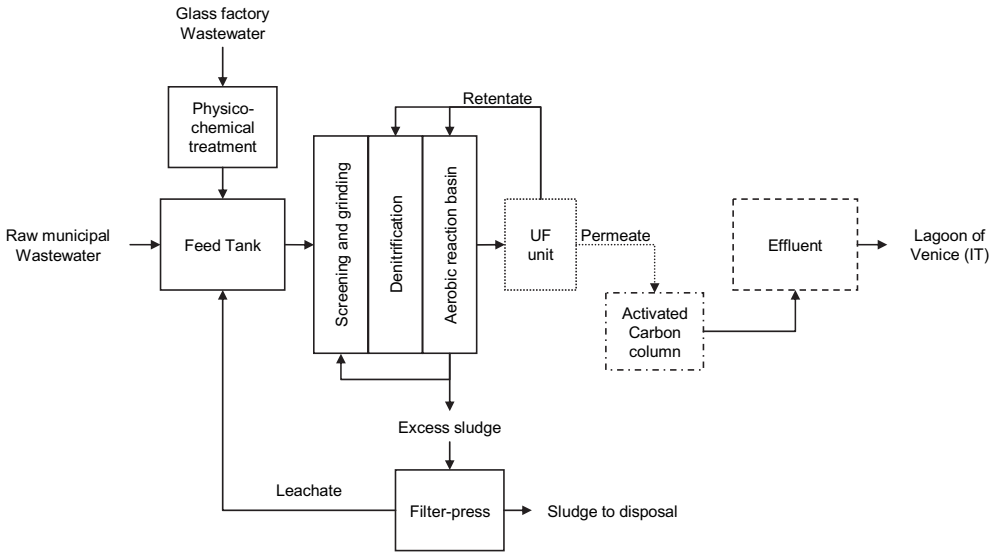


Fig. 3 – Flow chart of UF-MBR2 plant.

Table 2 – Species-specific Toxicity Scores (TS) organised in five classes for *C. gigas* and *M. galloprovincialis* embryotoxicity tests and *V. fischeri* 5-min luminescence inhibition test modified from Libralato et al. (in press).

Toxicity scores	Test organisms			TS Toxicity classes
	V. fischeri 5-min (A)	*C. gigas* (B)	*M. galloprovincialis* (C)	
	S ≥ TL	S > TL	S > TL	Absent (0)
	50 < S ≤ TL or TU50 < 1.22	50 < S ≤ TL or TU50 < 2.13	50 < S ≤ TL or TU50 < 2.48	Low (1)
	1.22 ≤ TU50 < 2.09	2.13 ≤ TU50 < 32.57	2.48 ≤ TU50 < 18.08	Medium (2)
	2.09 ≤ TU50 < 15.87	32.57 ≤ TU50 < 105.63	18.08 ≤ TU50 < 41.76	High (3)
	TU50 ≥ 15.87	TU50 ≥ 105.63	TU50 ≥ 41.76	Very high (4)

hatchery (Guernsey, UK), while wild mussels were collected during the breeding season (October–April) from the Adriatic sea. Sterile polystyrene micro-plates with lids (Iwaki Brand, Asahi Techno Glass Corporation, Tokyo, Japan) with 24 wells (3 ml) were used as test chambers. Dilution water was artificial seawater reconstituted according to ASTM (2004) at a salinity of 34. Toxicity tests were conducted in triplicate using at least five geometrically-scaled dilutions including the control (dilution water) and reference toxicant as reported in Libralato et al. (in press).

2.5. Data analysis and statistics

Microtox® EC50 values were obtained by linear regression between sample concentration and the fraction of light loss to light remaining (I) in a logarithmic scale where the EC50 corresponds to the sample concentration matching $I = 1$ with 95% confidence limits.

Bivalves toxicity data were expressed as EC50 values based on the Percentages of Effect (*i.e.* percentage of not normally developed larvae) (PE). EC50 values with 95% confidence limits

Table 3 – AS-SBR (X) physico-chemical results.

Parameters	Units	Sample	AS-SBR (X)								DR 24/08/1995	DM 12/06/2003
			1	2	3	4	5	6	7	8		
pH		i	7.77	7.81	7.91	7.92	7.42	8.06	7.84	7.40		6.0–9.5
		e	7.89	7.92	7.22	7.61	7.60	7.55	7.61	7.45		
SS	$mg\,l^{-1}$	i	176	184	148	216	252	112	125	115	–50%[a]	10
		e	6	272	104	44	68	68	66	100		
COD	$mg\,l^{-1}$	i	368	287	357	437	502	225	390	352	–75%[a]	100
		e	11	338	287	209	209	266	256	42		
Cl^-	$mg\,l^{-1}$	i	40	88	33	38	27	169	37	31		250
		e	55	70	91	71	76	69	63	93		
TKN	$mg\,l^{-1}$	i	37	31	37	35	33	37	25	37		15
		e	3	11	23	27	27	24	22	4		
$N–NH_4^+$	$mg\,l^{-1}$	i	20	14	13	23	24	22	22	20		2
		e	1	10	10	12	19	20	18	4		
$N–NO_2^-$	$mg\,l^{-1}$	i	0.12	<0.01	<0.01	<0.01	0.06	<0.01	0.10	0.77		–
		e	0.33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
$N–NO_3^-$	$mg\,l^{-1}$	i	0.20	0.01	0.15	<0.01	1.07	1.73	<0.01	<0.01		–
		e	13.90	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	15.08		
$P–PO_4^{3-}$	$mg\,l^{-1}$	i	1.75	2.33	1.56	0.70	2.33	9.88	1.05	1.23		–
		e	1.77	1.52	2.80	1.34	2.26	2.29	2.37	1.86		
P_{TOT}	$mg\,l^{-1}$	i	6	3	4	6	5	6	4	4		10
		e	2	6	6	3	3	6	5	4		
$S–SO_4^{2-}$	$mg\,l^{-1}$	i	8	9	12	6	9	3	9	9		500
		e	10	8	4	5	4	4	4	11		

i = Influent, e = effluent.
DR 24th August 1995 is about discharge limits from urban individual WWTPs in Venice lagoon.
DM 12th June 2003 is about treated wastewater reuse limits for non-potable purposes.
a Required decrease in the parameter concentration at the discharge compared to raw wastewater.

Table 4 – UF-MBR1 (Y) physico-chemical results.

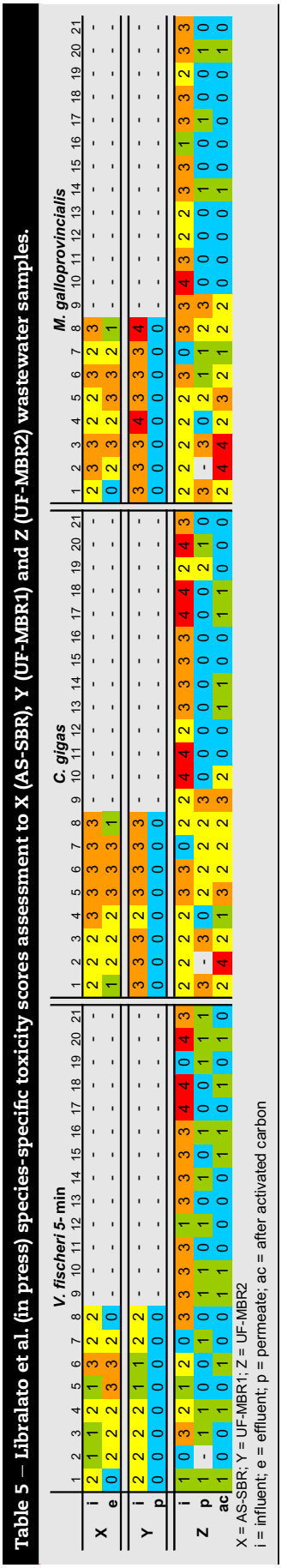
Parameters	Units	Sample	UF-MBR1 (Y)								DR 24/08/1995	DM 12/06/2003
			1	2	3	4	5	6	7	8		
pH		i	7.75	7.83	8.00	7.98	8.12	7.95	7.89	8.32		6.0–9.5
		p	7.89	7.79	7.92	7.85	7.86	7.79	7.83	7.77		
SS	$mg\,l^{-1}$	i	392	224	668	376	480	416	560	304	–50%[a]	10
		p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
COD	$mg\,l^{-1}$	i	769	500	903	797	1170	1530	1344	500	–75%[a]	100
		p	4	7	11	9	11	6	6	7		
Cl^-	$mg\,l^{-1}$	i	54	31	85	55	50	62	61	56		250
		p	67	56	234	44	44	60	61	56		
TKN	$mg\,l^{-1}$	i	87	33	71	64	47	26	36	30		15
		p	2	3	4	4	5	2	5	3		
$N–NH_4^+$	$mg\,l^{-1}$	i	17	33	14	37	20	19	22	24		2
		p	1.1	2.2	1.3	0.7	1.6	1.0	2.0	2.3		
$N–NO_2^-$	$mg\,l^{-1}$	i	0.09	0.37	0.13	<0.01	<0.01	<0.01	<0.01	0.42		–
		p	<0.01	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01		
$N–NO_3^-$	$mg\,l^{-1}$	i	0.73	0.02	0.19	<0.01	0.04	0.03	<0.01	0.06		–
		p	15.61	12.89	2.70	3.32	1.86	0.36	0.15	0.99		
$P–PO_4^{3-}$	$mg\,l^{-1}$	i	3.87	3.86	4.22	35.24	4.97	13.44	7.17	2.38		–
		p	3.29	4.29	3.05	4.76	4.76	3.46	3.53	3.65		
P_{TOT}	$mg\,l^{-1}$	i	9	7	12	44	37	8	8	8		10
		p	4	5	4	5	4	5	4	4		
$S–SO_4^{2-}$	$mg\,l^{-1}$	i	7	10	10	9	9	14	9	10		500
		p	11	10	16	11	11	12	12	10		

i = Influent, p = permeate.
DR 24th August 1995 is about discharge limits from urban individual WWTPs in Venice lagoon.
DM 12th June 2003 is about treated wastewater reuse limits for non-potable purposes.
a Required decrease in the parameter concentration at the discharge compared to raw wastewater.

were calculated by Trimmed Spearman–Karber statistical method (ASTM, 2004). Toxic Unit at 50% of the population exhibiting a response (TU50) was determined as 100/EC50 to provide values directly correlated to the toxicity magnitude. The Abbott's formula (ASTM, 2004) was considered to correct the responses for each treatment due to the effects in control tests. Moreover, in order to test the null hypothesis that the different treatments had no effect on larval development, the percentages of normal larvae at each concentration were compared to the controls using a one-way ANOVA after conducting Cochran's test for homogeneity of variance. If the data failed this test, an arcsin $P_{½}$ transformation was applied to the data to achieve homoschedasticity.

Toxicity data were elaborated according to Libralato et al. (in press) scoring system based on species-specific Toxicity Scores (TSs) and a final Wastewater Toxicity Index (WTI). The TSs have been defined in relation to (1) a separate-variance *t* test to verify if there is a significant difference ($p < 0.05$) in the mean organism response between the sample and the negative laboratory control and (2) the 90th-percentile of the Minimum Significant Difference (MSD) distribution according to Phillips et al. (2001). *V. fischeri*, *C. gigas* and *M. galloprovincialis* TSs have been displayed in Table 2. The WTI presents a five-class structure, each toxicity class is identified by a colour, a range of scores (0–4z, where z = number of TS available) and a synthetic judgement: absent (blue, 0), low (green, 1 – z), medium (yellow, z + 1 – 2z), high (orange, 2z + 1 – 3z) and very high (red, 3z + 1 – 4z), suggesting, in addition, the timing of the actions to be undertaken to improve the quality of the monitored discharge (from no action to urgency). The WTI is calculated as the sum of single species-specific TS values as follows: WTI = A + B + C + c, where A = 0–4, scoring from *V. fischeri* 5-min toxicity score, B = 0–4, scoring from *C. gigas* toxicity score, C = 0–4, scoring from *M. galloprovincialis* toxicity score, and c is an adjustment coefficient (if A ≠ 0 hence c = 0, whilst if A = 0 and B = {2,3,4} and C = {2,3,4} hence c(B,C) = 2; if only B or C is available, c[(B) or (C)] = 1). The application of WTI was performed considering both all toxicity data (*V. fischeri*, *C. gigas* and *M. galloprovincialis*) and just *V. fischeri* coupled one time with *C. gigas* and one time with *M. galloprovincialis* to observe how the presence of one or more than one sub-chronic endpoint as an index component would influence the final output.

The relationships between variables and the variation present in the dataset matrix were accounted via biplotting both the ordination component scores and the variable loading coefficients through Principal Component Analysis (PCA) based on the Pearson's correlation matrix, in order to identify the major discriminating variables associated with a given principal component. Normality of data and homogeneity of variance were previously checked. XLSTAT software, version 2008.4.01, a data analysis and statistical



application available for Microsoft Excel®, was used for data elaboration.

3. Results and discussion

3.1. AS-SBR vs UF-MBR1

AS-SBR and UF-MBR1 physico-chemical results for raw wastewater and final discharge are provided in Tables 3 and 4, respectively. Moreover, ecotoxicological data are shown in Table 5, as species-specific toxicity score judgements and as WTI in Table 6A–C. Both series of raw commercial wastewater samples presented similar physico-chemical characteristics, except for COD and SS that presented higher values in UF-MBR1. Nevertheless, the efficiency of these two treatment facilities could be suitably compared anyway.

Indeed, the COD and SS were much better removed by UF-MBR1 compared to AS-SBR. UF-MBR1 consistently provided high efficiency levels throughout the monitoring period, reducing COD by 99% and SS by 99.9%, whereas AS-SBR lowered both of them by less than 50% on average, as for ammonia and TKN. Phosphate and total phosphorus discharge concentrations were also improved better by UF-MBR1 rather than AS-SBR.

The assessment of toxicity data from Tables 5 and 6A–C revealed that AS-SBR and UF-MBR1 presented similar raw wastewater ecotoxicological characteristics, although the latter was slightly more toxic. From Table 5, the raw wastewater toxicity was identified in the range 2–3 and 3–4 for *C. gigas* and *M. galloprovincialis*, respectively, whereas in the range 1–3 for *V. fischeri*. Nevertheless, it was highlighted that UF-MBR1 effluent samples presented no toxicity according to each and every one testing species during all the monitoring period. On the contrary, the AS-SBR discharged effluents presenting toxicity in the range 0–3 (from no toxic to highly toxic). Sometimes, it has been evidenced that the discharged effluent presented the same or higher levels of toxicity than the corresponding untreated wastewater. The integration of species-specific toxicity judgements resulting in WTI, as shown in Table 6A–C, provided the final synthetic values stating the presence or absence of toxicity and its relative estimated magnitude. According to Table 6A summarising the integration of all toxicity data, the AS-SBR was shown to be less efficient than UF-MBR1 in toxicity reduction throughout all the monitoring period, with substantial unpredictable removal rates and some residual toxicity at the discharge (*i.e.* equal or higher that the influent). Conversely, UF-MBR1 removed toxicity in a continuous and efficient way, supporting the possibility for treated wastewater reclamation and reuse. The comparison of integrated toxicity data from Table 6B and C, where only one sub-chronic endpoint was considered at a time, confirmed the judgements expressed from Table 6A. The correlation analysis between the toxicity results from Table 6A and B indicated that there was no significant difference ($p < 0.01$) between *C. gigas* and *M. galloprovincialis* sensitivities towards the tested commercial wastewater (X(i,e) 1–8 and Y(i,p)1–8).

Moreover, the AS-SBR and UF-MBR1 performances were also compared to regulatory limits about effluent discharge

Table 6A – WTI application to X (AS-SBR), Y (UF-MBR1) and Z (UF-MBR2) samples considering all toxicity data (*V. fischeri*, *C. gigas* and *M. galloprovincialis*).

		WTI *V. fischeri* 5-min + *C. gigas* + *M. galloprovincialis* (z = 3)																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
X	i	6	6	6	7	6	9	7	8	-	-	-	-	-	-	-	-	-	-	-	-	-
	e	1	6	7	6	9	9	7	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	i	8	8	8	8	7	7	8	9	-	-	-	-	-	-	-	-	-	-	-	-	-
	p	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	i	5	6	7	6	6	8	0	8	8	11	10	5	8	9	9	7	11	11	6	11	9
	p	7	-	7	1	6	3	4	6	7	1	0	1	0	2	0	1	1	0	3	3	1
	ac	5	10	9	4	8	5	3	6	6	3	0	0	1	2	1	1	1	2	0	2	0

X = AS-SBR; Y = UF-MBR1; Z = UF-MBR2
i = influent; e = effluent; p = permeate; ac = after activated carbon
z = number of toxicity scores
# = adjusted with c = 2

from urban individual WWTPs in Venice lagoon (DR, 1995) and treated wastewater reuse for non-potable purposes (DM, 2003). Both limits have been displayed in the last two columns of Tables 3 and 4. The only two parameters taken into consideration by DR (1995) are SS and COD that are required to be removed from raw wastewater not less than 50% and 75%, respectively. The AS-SBR for both SS and COD did not always guarantee the above-mentioned removal rates, whereas the UF-MBR1 provided an outstanding performance.

Considering the DM (2003) about treated wastewater reuse for non-potable purposes, it could be observed that the relative regulatory limits for SS, COD, TKN and N–NH_4^+ were not always respected during the AS-SBR monitoring period. On the contrary, the UF-MBR1 complied with the regulatory limits for effluent reuse, except for samples Yp2,7,8 for N–NH_4^+ that presented a slight greater value than the respective threshold. In order to prevent future similar events, it was suggested the implementation of an activated carbon column (Long et al., 2008).

A biplot summarising the PCA results on chemical data for AS-SBR and UF-MBR1 wastewater samples weighted on WTI values from Table 6A is shown in Fig. 4. The first two principal components accounted for 48.60% and 18.10% of the variation, respectively. Thus 66.69% of the variation can be depicted by a two-axis ordination diagram. The biplot regarding components loadings suggested that the first component (F1) scores are influenced by the values of SS, COD, P_{TOT}, P–PO_4^{3-}, TKN and pH with positive loadings on the first axis. The second component (F2) was mainly influenced by N–NH_4^+ and P–PO_4^{3-} concentrations.

The ordination plot of component scores present in the F1–F2 biplot, as shown in Fig. 4, clustered wastewater samples in two main groups: all permeates (Yp1–8), Xe1–2 and Xe8 at the bottom left, AS-SBR effluents (Xe3–7) at the top left. Raw wastewater samples are scattered mostly on the right side of the plot, probably due to the high variability of their intrinsic characteristics. In accordance with WTI, the bottom left group consisted of good quality discharges from UF-MBR1, except for

Table 6B – WTI application to X (AS-SBR), Y (UF-MBR1) and Z (UF-MBR2) samples considering only *V. fischeri* and *C. gigas* toxicity data.

		WTI *V. fischeri* 5-min + *C. gigas* (z = 2)																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
X	i	4	3	3	5	4	6	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	e	1	4	4	4	6	6	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	i	5	5	5	4	4	4	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	p	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	i	3	3	5	4	4	5	0	5	5	7	7	3	6	6	6	6	8	8	3	8	6
	p	4	-	4	1	3	2	3	3	4	1	0	1	0	1	0	1	0	0	3	2	1
	ac	3	5	3	2	4	3	2	3	4	3	0	0	1	1	1	1	1	2	0	1	0

X = AS-SBR; Y = UF-MBR1; Z = UF-MBR2
i = influent; e = effluent; p = permeate; ac = after activated carbon
z = number of toxicity scores
# = adjusted with c = 1

Table 6C – WTI application to X (AS-SBR), Y (UF-MBR1) and Z (UF-MBR2) samples considering only *V. fischeri* and *M. galloprovincialis* toxicity data.

		WTI																				
		V. fischeri 5-min + *M. galloprovincialis* (z = 2)																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
X	i	4	4	4	4	3	6	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	e	0	4	5	4	6	6	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	i	5	5	5	6	4	4	5	6	-	-	-	-	-	-	-	-	-	-	-	-	-
	p	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	i	3	3	5	4	3	5	0	6	6	7	6	3	5	6	6	4	7	7	3	7	6
	p	4	-	4	1	3	1	2	3	4	1	0	1	0	2	0	1	1	0	1	2	1
	ac	3	5	5	3	4	3	1	3	3	1	0	0	0	1	1	1	0	1	0	2	0

X = AS-SBR; Y = UF-MBR1; Z = UF-MBR2
i = influent; e = effluent; p = permeate; ac = after activated carbon
z = number of toxicity scores
# = adjusted with c = 1

Xe2, whereas the top left one had discharges with residual toxicity even after treatment from AS-SBR.

3.1.1. *UF-MBR2*

UF-MBR2 physico-chemical data for mixed domestic and industrial influent, permeate and final discharge are summarised in Table 7. UF-MBR2 removed most of the COD (95%) during the biological treatment process, and to a lesser extent by activated carbon filtering, throughout all the monitoring period (1–21). The SS were also always completely removed (99.9%) from the final discharge (<0.01 mg l^{-1}), thanks to UF-membrane physical barrier.

Regarding N-based compounds, the ionised ammonia, that was reduced on average by 42%, evidenced the existence of two distinct treatment periods efficiency identified by two groups of samples: Z(p,ac)1–9 and Z(p,ac)10–21. This was probably due to the multi-purpose plant start-up period that occurred exactly in the 1–9 sampling. During the second period (Z(p,ac)10–21), $N–NH_4^+$ concentration was reduced more efficiently (79%) than in the first one. The TKN concentration indicated that organic nitrogen was more than halved (63% average removal), even though the efficiency was lower (49%) during the first period (Z(p,ac)1–9) than in the second (73%).

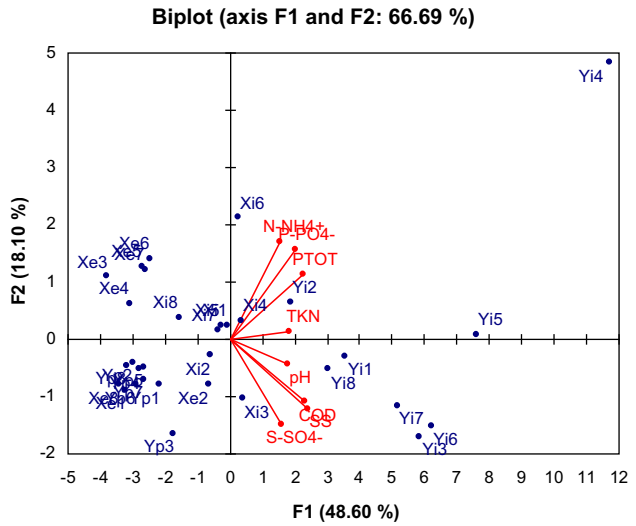


Fig. 4 – Principal component analysis biplot of chemical data with loadings and scores in the coordinates of the first two principal components (F1 and F2) weighed on WTI values according to Libralato et al. (in press) for X (AS-SBR) and Y (UF-MBR1) commercial wastewater considering influent (i), effluent (e, only for X) and permeate (p, only for Y) samples.

The concentration of metal and metallic contaminants presented the same trend as ammonia especially for Al, Ba, Cd, Mn, Ni and Zn, whereas the concentrations of As, Cr, Sb, Se and V were frequently under the relative detection limit values. The values of Co and V are not reported because their values were always below the relative detection limits, <7 μg l^{-1} and <2 μg l^{-1}, respectively. In particular, Cd and Ni concentrations were higher in the final effluent after activated carbon filtering than in the raw wastewater in the Zac1–9 period because, as discovered subsequently, the activated carbon filter was saturated and required backwashing. Indeed, Cd and Ni residual concentration in the following period, Zac10–21, after activated carbon cleaning, was significantly improved, as also occurred for Al, Ba, Fe, Mn and Zn.

The toxicity data elaborated according to Libralato et al. (in press) for mixed domestic and industrial wastewater samples were shown in Table 5 and Table 6A–C, as species-specific toxicity score results and WTI values, respectively. As for the chemical data, all three toxicity scores and WTI identified the existence of two distinct efficiency periods, summarising all chemical instances at one time. In particular, it was shown that after the starting up period (Z1–9), the UF-MBR2 provided very good quality effluents considering both permeate after UF filtration and permeate after activated carbon filtration, although the general toxicity level of untreated wastewater increased till the maximum upper value. On the basis of Table 5, untreated wastewater ranged between scores 0 and 4 for all toxicity bioassays considered. Scores for the starting up period (Z1–9) for both permeate and permeate samples after activated carbon filtration were particularly high, showing that sometimes the effluent toxicity was greater than the relative untreated wastewater specimen, thus correlating with chemical data presented above.

Table 7 – UF-MBR2 (Z) physico-chemical results.

Parameters	Units	Sample	UF-MBR2 (Z)																				DM 30/07/1999	DM 12/06/2003
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
pH		i	6.45	8.02	7.89	8.40	8.37	7.63	7.88	8.31	8.70	8.45	8.46	8.30	8.47	8.77	8.37	8.59	8.41	8.30	7.86	7.42	7.89	6.0–9.0	6.0–9.5
		p	7.57	–	7.81	7.10	6.94	6.28	6.71	7.70	6.06	7.99	7.73	7.45	7.89	6.86	7.62	8.02	7.70	7.45	8.24	8.24	8.00		
		ac	7.10	7.94	7.88	6.17	7.83	7.02	7.18	6.41	6.65	7.10	7.63	7.20	7.68	7.63	7.12	7.48	7.63	6.59	7.41	7.50	8.10		
SS	$mg\,l^{-1}$	i	–	–	–	9800	516	204	76	238	196	238	242	324	104	212	1076	94	208	234	732	202	324	35	10
		p	<0.01	–	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
		ac	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
COD	$mg\,l^{-1}$	i	216	214	146	1092	370	283	277	640	336	628	906	563	472	571	1024	512	577	532	943	519	525	120	100
		p	24	–	25	33	44	46	31	47	46	65	76	39	55	41	29	40	36	36	10	32	20		
		ac	11	9	19	15	21	30	13	15	16	25	45	28	32	27	17	27	26	30	11	24	10		
Cl^-	$mg\,l^{-1}$	i	34	3320	430	437	1499	2845	2222	269	417	85	86	<0.01	<0.01	222	619	114	88	91	493	73	110	300	250
		p	56	–	2103	878	1668	928	2232	1268	<0.01	998	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
		ac	85	2171	2032	<0.01	1648	1015	2144	1299	<0.01	1010	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
TKN	$mg\,l^{-1}$	i	35	13	20	149	54	23	16	64	51	69	82	30	53	83	226	118	71	73	2	63	68	10	15
		p	18	–	18	2	32	14	12	27	27	7	7	6	<0.01	5	1	2	2	7	5	5	5		
		ac	13	16	10	7	30	13	10	25	14	5	1	2	<0.01	5	1	3	5	2	5	2	4		
$N–NH_4^+$	$mg\,l^{-1}$	i	26	8	7	31	30	4	3	47	39	53	<0.01	28	49	65	91	72	53	60	1	61	62	2	2
		p	10.8	–	9.1	1.0	29.0	12.9	10.1	22.1	22.7	5.2	<0.01	2.2	<0.01	2.5	2.1	1.5	1.7	2.8	0.9	5.2	1.2		
		ac	11.5	14.8	9.2	0.8	28.8	11.2	11.0	23.5	11.5	1.8	<0.01	1.6	<0.01	2.1	1.4	2.6	1.9	2.4	3.0	1.9	2.2		
$N–NO_2^-$	$mg\,l^{-1}$	i	<0.01	<0.01	0.93	<0.01	<0.01	<0.01	<0.01	<0.01	0.34	0.46	0.30	0.70	<0.01	<0.01	<0.01	<0.01	0.47	0.40	<0.01	<0.01	<0.01	0.3	–
		p	<0.01	–	<0.01	<0.01	<0.01	1.85	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	<0.01	<0.01	<0.01	<0.01	0.00	0.10	<0.01	<0.01	<0.01		
		ac	<0.01	<0.01	<0.01	<0.01	<0.01	1.92	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.09	<0.01	<0.01	<0.01		
$N–NO_3^-$	$mg\,l^{-1}$	i	0.66	4.02	16.14	<0.01	0.15	12.15	<0.01	<0.01	0.15	<0.01	<0.01	0.33	<0.01	<0.01	<0.01	<0.01	<0.01	0.31	<0.01	<0.01	0.312	–	–
		p	13.01	–	10.60	30.10	0.06	4.51	8.05	1.38	2.88	5.19	<0.01	2.86	2.55	1.88	2.47	<0.01	3.27	0.13	3.45	<0.01	<0.01		
		ac	20.60	12.73	11.20	30.35	1.78	2.81	7.55	1.87	4.38	6.37	<0.01	3.52	1.41	1.72	1.95	<0.01	4.90	0.14	<0.01	<0.01	<0.01		
$P–PO_4^{3-}$	$mg\,l^{-1}$	i	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	3.67	2.54	4.05	3.50	<0.01	3.09	4.72	1.60	4.72	3.68	3.33	<0.01	1.83	3.39	0.5	–
		p	<0.01	–	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.77	<0.01	0.042	0.02	<0.01	0.051	<0.01	0.17	<0.01		
		ac	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.23	0.09	0.02	<0.01	0.06	<0.01	<0.01		
P_{TOT}	$mg\,l^{-1}$	i	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	5.64	3.20	4.06	4.00	<0.01	3.56	5.26	5.71	4.80	3.99	4.00	11.40	4.61	4.72	1	10
		p	<0.01	–	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.17	0.07	0.15	0.52	0.36	0.28	0.44	0.45	0.43		
		ac	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.12	0.88	0.45	0.72	0.64	0.28	1.40	4.04	1.43		
$S–SO_4^{2-}$	$mg\,l^{-1}$	i	1	87	22	21	16	26	38	15	12	12	10	21	22	11	7	11	12	14	6	6	21	500	500
		p	175	–	53	39	34	26	34	28	23	27	<0.01	22	20	18	19	17	22	21	27	27	33		
		ac	<0.01	54	48	41	34	26	35	29	25	27	<0.01	22	21	18	19	17	22	21	29	29	33		
Al	$\mu g\,l^{-1}$	i	–	151	247	6674	81	164	632	852	1037	1129	1507	430	67	1153	202	1054	1451	894	304	1047	1171	–	1000
		p	–	–	10296	31	207	5	47	3	754	5	5	3	9	2	8	10	15	12	3	17	31		
		ac	–	12276	10813	406	6941	117	45	2185	1440	315	54	65	47	62	17	23	38	36	12	48	46		
As	$\mu g\,l^{-1}$	i	–	5	6	10	5	5	6	<1	5	5	<1	8	8	7	16	5	5	5	9	10	11	1	20
		p	–	–	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1		
		ac	–	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1		
Ba	$\mu g\,l^{-1}$	i	–	91	161	1032	80	111	307	127	113	75	105	122	71	83	49	116	104	790	183	62	219	–	10,000
		p	–	–	162	31	57	42	75	44	32	40	41	52	35	210	74	68	60	51	59	46	55		
		ac	–	126	109	28	50	30	506	51	193	29	42	46	40	49	92	66	63	43	62	48	53		

Cd	$\mu g l^{-1}$	i	–	<1	<1	4	<1	<1	1	1	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	1	1	5
		p	–	–	11	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1		
		ac	–	13	12	<1	4	<1	2	4	4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1		
Cr_{tot}	$\mu g l^{-1}$	i	–	3	5	50	1	2	5	25	4	4	10	4	1	4	6	6	4	4	5	3	9	1	100
		p	–	–	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	<1	<1	<1	<1		
		ac	–	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1		
Cu	$\mu g l^{-1}$	i	–	22	8	67	7	14	30	50	39	43	35	88	14	41	18	39	32	42	20	37	87	50	1000
		p	–	–	8	<2	8	<2	<2	9	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2		
		ac	–	<2	<2	<2	<2	<2	<2	<2	9	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2		
Fe	$\mu g l^{-1}$	i	–	200	1352	28835	700	1990	7963	3620	1206	293	385	1096	381	523	450	275	1152	320	6978	800	7311	500	2000
		p	–	–	96	27	33	13	13	19	14	28	40	16	16	4	18	13	16	11	10	21	16		
		ac	–	41	39	22	21	12	2	8	124	20	25	10	6	6	8	6	6	3	9	5	9		
Mn	$\mu g l^{-1}$	i	–	70	27	113	84	115	166	50	87	20	22	94	66	26	407	22	24	22	79	18	24	500	200
		p	–	–	2039	127	<2	37	694	157	117	62	4	<2	3	<2	16	14	<2	32	<2	34	29		
		ac	–	2397	1896	157	385	51	1422	309	1162	72	15	11	4	3	80	47	23	6	44	28	27		
Ni	$\mu g l^{-1}$	i	–	18	7	21	17	19	17	6	15	4	3	12	8	4	9	4	4	4	19	4	7	100	200
		p	–	–	117	13	19	14	29	19	61	24	31	31	11	34	25	28	18	16	28	12	17		
		ac	–	162	150	17	77	15	30	110	184	29	35	34	18	15	20	22	21	17	17	16	19		
Sb	$\mu g l^{-1}$	i	–	<5	20	<5	<5	<5	<5	20	20	20	20	24	20	20	21	20	20	21	20	<5	<5	–	–
		p	–	–	<5	7	<5	<5	<5	<5	<5	<5	<5	11	12	<5	<5	15	<5	<5	<5	<5	13		
		ac	–	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	11		
Se	$\mu g l^{-1}$	i	–	<5	41	<5	<5	<5	<5	10	41	10	10	12	10	10	10	10	10	10	10	<5	<5	10	10
		p	–	–	<5	<5	<5	<5	<5	<5	<5	<5	11	10	12	<5	<5	11	13	18	14	15	17		
		ac	–	<5	<5	<5	<5	<5	8	<5	9	<5	14	8	15	14	7	7	13	15	16	19	18		
Zn	$\mu g l^{-1}$	i	237	134	81	1157	46	36	263	105	207	104	191	95	46	104	45	108	122	121	95	75	236	250	500
		p	–	–	7260	80	146	22	175	20	695	22	14	21	9	63	17	12	14	4	9	3	8		
		ac	–	10978	9141	386	1779	98	565	1620	1643	193	72	62	40	41	56	42	33	7	21	15	17		

i = Influent, p = permeate, ac = after activated carbon.
DM 30th July 1999 is about discharge limits in Venice lagoon.
DM 12th June 2003 is about treated wastewater reuse limits for non-potable purposes.

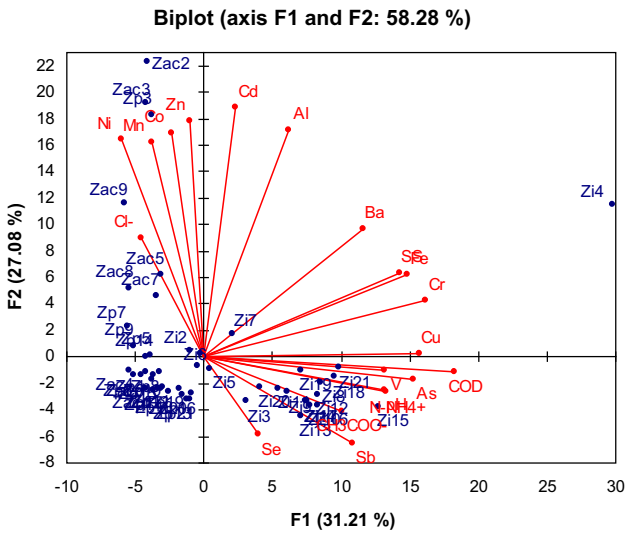


Fig. 5 – Principal component analysis biplot of chemical data with loadings and scores in the coordinates of the first two principal components (F1 and F2) weighed on WTI values according to Libralato et al. (in press) for Z (UF-MBR2) mixed metal and industries wastewater considering influent (i), permeate (p) and permeate after activated carbon filtration (ac) samples.

The integration of all species-specific toxicity judgements resulting in WTI, as displayed in Table 6A, provided interesting results. Indeed, it can be observed that there is a manifest difference in the efficiency of UF-MBR2 toxicity removal, allowing its exact distinction in the two previously mentioned performance periods. In particular, the first period was characterised by medium and highly toxic discharges, whereas the second one presented most of the time effluents with no or low toxicity levels. Thus, the UF-MBR2 after the starting up period significantly improved the final quality of the discharge.

Considering the WTI defined on the basis of two toxicity endpoints (*i.e. V. fischeri* and *C. gigas*; *V. fischeri* and *M. galloprovincialis*) as displayed in Table 6B and C, it could be observed that there is a general similarity between their sensitivities: the correlation analysis between the toxicity results elaborated on the two WTIs indicated that there was no significant difference ($p < 0.01$) between *C. gigas* and *M. galloprovincialis* sensitivities (91% of correlation) towards the tested mixed domestic and industrial wastewater samples (Z(i,p,ac)1–21).

Moreover, the UF-MBR2 performances were also compared to the very strict regulatory limits about effluent discharge from mixed domestic and industrial WWTPs in Venice lagoon (DM, 1999) and treated wastewater reuse for non-potable purposes (DM, 2003). Due to the above-mentioned problems occurred during the WWTP start-up, it was decided to comment results just from Zac10–21 samples. About DM (1999), it could be observed that all parameters complied with the relative regulatory limits except for N–NH_4^+ for Zac16,18,19,21, for P_{TOT} for Zac13,19,20 and for Se for Zac11,13,14,17–21, even though in a not meaningful way as stated by the absence/low toxicity of effluents in the second monitoring period (10–21). The same problems were found about the compliance with DM (2003) for treated wastewater reuse, where the same limits of DM (1999) are applied for both for N–NH_4^+ and Se. In conclusion, it could be said that UF-MBR2 effluents are not immediately suitable for discharge and/or reuse, but some actions should be taken to obtain the full regulatory compliance. Indeed, one of the main reasons of excess Se concentration in the final effluent might be associated to the fact that saturated activated carbon were not substituted, but only backwashed, as well as to the presence of potential great fluctuations in raw wastewater loads treated by the WWTP. The complete substitution of activated carbon filter will allow to improve Se removal as well as the slight excess of N–NH_4^+ concentrations compared to the relative regulatory limits (Jegadeesan et al., 2003; Long et al., 2008). Previous unpublished studies evidenced that regulatory requirements could fully complied via substituting the activated carbon column with a reverse osmosis treatment stage.

A biplot summarising PCA results concerning chemical data for UF-MBR2 wastewater samples weighted on WTI values from Table 6A is shown in Fig. 5. The first two principal components accounted for 31.21% and 27.08% of the variation, respectively. Therefore 58.28% of the variation can be depicted by a two-axis ordination diagram. The biplot regarding components loadings suggested that the F1 scores are influenced by high values of COD, total concentration of Cr, Cu, As, Fe, SS, N–NH_4^+, Ba and Sb in ascending order, which are clustered together and have positive loadings on the first axis. In addition, the loading of Cd, Zn, Al, Co, Ni, Mn on the F2 suggested that the second component scores could reflect the concentrations of these compounds in the samples.

Looking at the ordination plot of component scores in the F1–F2 biplot, it was found that wastewater samples could be clustered in three main groups: at the bottom left the good quality permeates (Zp10–21) and effluents after activated carbon filtering from the second monitoring period (Zac10–21), at the bottom right the raw wastewater specimen (Zi1–21), while at the top left the contaminated permeates and activated carbon filtered permeates from the first monitoring period (Zp1–9 and Zac1–9). This last group showed to be mainly influenced by the presence of some heavy metals (Ni, Mn and Zn) and chlorine.

4. Conclusions

This research focused on how to provide useful information to support innovation in the field of wastewater treatment to comply with most recent legislative trend, which makes the assessment of advanced wastewater treatment technologies a key issue for water sustainability and its potential for reuse. It has been evidenced that:

- ecotoxicological tools may be successfully used to discriminate between wastewater treatment technologies efficiency;
- the combined use of physico-chemical analyses and ecotoxicological issues might support potential effluent reclamation and reuse with the final aim of approaching the zero emissions discharge;
- it is worth to consider tools and approaches providing strategic integrated results on whole wastewater samples

and to select them on the basis of specific relative targets such as those related to receiving water body characteristics (*e.g.* transitional and marine-coastal waters);
- bacteria and bivalves toxicity outputs elaborated in the form of species-specific scoring and wastewater toxicity index offered immediate interesting tips for discriminating between the efficiency of AS-SBR and UF-MBR wastewater treatment technologies;
- UF-MBR technologies applied both to commercial and mixed domestic and metal industries wastewaters have shown to be able to provide superior quality effluents, as confirmed by physico-chemical analyses, even if some of the very strict regulatory limits were sporadically slightly exceeded (*i.e.* substantially unworthy under the ecotoxicological viewpoint);
- conversely, the AS-SBR facility did not attain the same level of efficiency of UF-MBR, displaying unpredictable and discontinuous performance in the final effluent quality.

Acknowledgements

This study was partly supported by a grant from Ingegnerie e Tecnologie Ecologiche srl (In.T.Ec. srl). The authors thank Andrea Scandella for chemical analyses.

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