Pharmaceutical active compounds fate and removal in Milan Nosedo WWTP: results of a 4 years research at full and pilot scale

F. Malpei[1], H. Bouju[2], G. Buttiglieri[3], S.Castiglioni[4], S. Colia[5], R. Mazzini[6], E. Zuccato[4]
[1]DIIAR Politecnico di Milano, Piazza L. da Vinci 32, Milano, 20133 Italy. francesca.malpei@polimi.it
[2] Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Grundenstrasse 40, Muttenz, 4132 Switzerland. helene.bouju@fhw.ch
[3]ICRA (Catalan Institute for Water Research). Scientific and Technological Park of the University of Girona. H2O Building. c/ Emili Grahit 101. Girona. E17003. Spain. gbuttiglieri@icra.cat.
[4]Istituto di Ricerche Farmacologiche Mario Negri, Via Giuseppe La Masa 19, 20156 Milano. zuccato@marionegri.it; sara.castiglioni@marionegri.it
[5]Metropolitana Milanese SpA –Servizio Idrico Integrato della città di Milano,Via del Vecchio Politecnico, 8 Milano, 20121 Italy. s.colia@metropolitanamilanese.it
[6]Milano Depur SpA, Via Lampedusa 13/D, Milano, 20141 Italy. mazzini@sibaspa.it

Abstract
The paper summarizes the results of researches realized at full and pilot scale by Istituto Mario Negri (IT) and Politecnico di Milano on the presence, fate and removal of pharmaceutical active compounds (PhACs) in the sewage of Milan Nosedo WWTP. Removal rates in Nosedo WWTP were higher than those measured in other treatment plants in Italy but many PhACs were still present in treated water, at concentration ranges spanning from few to several hundred ng·L^{-1}. Four sampling campaign were realized on the full plant conventional activated sludge plant (CAS) and a pilot membrane bioreactor (MBR), to compare the removal efficiency and sludge adsorption capacity and to study the effect of sludge retention time (SRT) on removal rates in MBR. Higher removal rates were observed for some PhACs in MBR, but not for the recalcitrant ones. Moreover, the operation of the MBR at four SRTs in the range 15-50 days did not demonstrate an effect of this parameter on PhACs removal. Adsorption on sludge of selected PhACs was evidenced.

Keywords: adsorption, Diclofenac, MBR, Ofloxacin, SRT

INTRODUCTION

Pharmaceutical substances removal from wastewater is an important issue since their discharge into the environment might damage ecosystems and impact human water supply quality. Nevertheless, knowledge about their fate in wastewater treatment plant is still scarce and pharmaceuticals discharge has not been regulated in Europe yet. Therefore monitoring of wastewater contamination is of particular interest for a reliable assessment of environmental risks, particularly in more sensitive situations such as very densely populated wastewater basins discharging into small receiving water bodies, such as the Lambro-Seveso-Olona rivers or agricultural wastewater reuse.
Since 2005 and for 4 years long, Istituto Mario Negri and DIIAR Politecnico di Milano have realized several separate or joint studies and researches regarding the presence and fate of pharmaceutical active compounds of human origin (PhACs) in Milan Nosedo influent and WWTP. The results obtained represents a relevant and consistent reference in the Italian panorama in terms of presence and concentration range of PhACs in municipal wastewater, variability along the yearly period, removal efficiencies and their variability along the yearly period and comparison of fate and removal of the full scale plants and a pilot scale MBR operating in parallel. In this paper the main results obtained along the whole research period are summarized and discussed.

MILAN NOSEDO WWTP

Milan Water and Wastewater Services are covering a 120 km^2 area and are managed by Metropolitana Milanese since 2003. Collection and treatment of municipal wastewater is organized in 3 areas, connected to 3 recent WWTPs: Milan S.Rocco (western area, in operation since

Dec.2004, Avg.DWF (dry weather flow) = 4.3 m^3s^{-1}); Milan Nosedo (central-eastern area, in operation since Oct.2004), Milan Est (eastern area, in operation since Apr.2005, Avg.DWF = 1.1 m^3s^{-1}). The public sewer system covers 1,460 km and the treatment capacity is of 2,550,000 PE.

The Nosedo WWTP is operated by *MilanoDepur SpA* in concession and receives approximately 50% of the overall Milan wastewater flow. Figure 1 shows the aerial view of the plant, equipped with 30 mm (coarse) and 3 mm (fine) grids, an activated sludge conventional secondary treatment (CAS) (4 modules and 32 lines in parallel, overall volume 358,100 m^3 including sedimentation tanks) plus sand filtration (20 cells with a total filtering surface of 2,442 m^2) and disinfection by peracetic acid.

In Table 1 the average yearly data in the period 2007-2010 are reported in terms of treated flow and influent/effluent concentration of some parameters. The effluent fully respects the agricultural reuse standards, according to Italian DPR 185/2003. Since 2005, more then $70 \cdot 10^6$ m^3year^{-1} (approx 50% of the effluent flow) are reused on a 37 km^2 agricultural district where corn, grass, grain and rice are cultivated, thus resulting in one the most relevant agricultural reuse reality in Europe.

Figure 1. Aerial view of Milan Nosedo WWTP

Table 1. Nosedo WWTP: average yearly treated flow, influent and effluent COD, Total N, TSS and *E. coli* in 2007-2010

	Average daily Inflow	**COD**		**TSS**		**Total N**		**E. Coli**
		IN	OUT	IN	OUT	IN	OUT	OUT
	$m^3 \cdot d^{-1}$	$g \cdot m^{-3}$	$g \cdot m^{-3}$	$g \cdot m^{-3}$	$g \cdot m^{-3}$	$g \cdot m^{-3}$	$g \cdot m^{-3}$	..
2007	377,759	281	<15	178	<5	27	6	<10
2008	372,521	270	<15	185	<5	28	7	<10
2009	407,407	291	<15	267	<5	27	6	<10
2010	431,754	282	<15	204	<5	27	6	<10

PhACs IN MILAN NOSEDO WWTP

A listing of "priority pharmaceuticals" for human use in Italy resulted in the selection of 26 pharmaceuticals, belonging to 11 therapeutic classes, the metabolites clofibric acid and demethyl diazepam and the natural estrogens 17β-estradiol and Estrone (Table 2). The pharmaceutical compounds (PhACs) prioritized were subsequently analysed in urban wastewater samples according to Castiglioni et al. (2005). Briefly, the pharmaceuticals were measured by reversed-phase liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), after combined extraction by two SPE columns, an Oasis MCX (60 mg, Waters Corp., Milford, MA) at pH 2.0 and a Lichrolut EN

(200 mg, Merck, Darmstadt, Germany) at pH 7.0. A Luna C8 column 50 mm × 2 mm i.d., 3 μm particle size (Phenomenex, Torrance, CA, USA) was used; the mass spectrometer was an Applied Biosystem-SCIEX API 3000 triple quadrupole ($Q_1q_2Q_3$) equipped with a turbo ion spray source (Applied Biosystems–Sciex, Thornhill, Ontario, Canada). Analysis was done in the multiple reaction monitoring mode, in positive and negative ionisation mode. Three deuterated internal standards were used: salbutamol-D_3, for quantification of the pharmaceuticals analyzed in the positive ion mode, and ibuprofen-D_3 and 17β-estradiol-D_2 for compounds analyzed in the negative ion mode. Recoveries of the pharmaceuticals generally exceeded 70%, variability of the method was below 8%, limits of quantification (LOQ) were lower than 1 ngL^{-1}, with few exceptions (amoxycillin 8.7 ng/L, 17β-estradiol 1.7 ngL^{-1}, and 17α-ethinylestradiol 4.8 ngL^{-1}). When analysis of the particulate was required, the filters containing the particulate obtained by filtration of the aqueous samples were dried at room temperature and extracted three times with 20 mL methanol under sonication. The supernatant was dried under an air stream, redissolved in 100 μL 0.01% acetic acid in MilliQ water and analyzed as described for the aqueous samples.

Table 2. List of "priority pharmaceuticals" (including the metabolites clofibric acid and demethyl diazepam and the natural estrogens 17β-estradiol and estrone).

Therapeutic class	Pharmaceutical	Sign	Therapeutic class	Pharmaceutical	Sign
Human antibiotics	Clarithromycin	CLA	Cardiovascular	Atenolol,	ATE
	Erythromycin	ERT		Enalapril	ENA
	Dehydro-Erythromycin	DHE	Urologic	*Sildenafil*	
	Lincomycin	LIN	CNS drugs	Carbamazepine	CBZ
	Ciprofloxacin	CPP		Diazepam	DZP
	Ofloxacin	OFL	Diuretics	Furosemide	FUR
	Vancomycin	VAN		*Hydrochlorothiazide*	
	Sulphamethoxazole,		Gastrointestinal	*Omeprazole*	
	Spiramycin, Amoxicillin			Ranitidine	RAN
Veterinary antibiotics	*Oleandomycin, Tylosin*		Lipid regulator	Bezafibrate,	BZF
	Tilmicosin,			Atorvastatin	ATV
	Oxytetracycline			Gemfibrozil	GEM
Anticancer	Cyclophosphamide	COF	Opiates	*Morphine,Codeine*	
	Methotrexate,Tamoxifen				
Broncho-dilators	Salbutamol	SAL	Natural estrogens	*17 β-Estradiol*	
				Estrone	EST
Anti-inflammatory	Ibuprofen	IBU	Estrogens	*17 α-Ethinylestradiol*	
	Ketoprofen	KET			
	Naproxen	NAP	Metabolites	*Clofibric Acid,*	
	Diclofenac	DF		*Demethyl diazepam*	

The analytical method was used to estimate removal rates of PhACs in the Nosedo treatment plant (Castiglioni et al., 2005; Zuccato et al., 2009) and to compare removal rates in some treatment plants in Italy (Castiglioni et al., 2006).

The total removal rates of the PhACs investigated in Nosedo were between 70 and 80% and were higher than those measured in other treatment plants in Italy, where removal rates were generally lower than 40%. Nevertheless, in Nosedo pharmaceuticals were still present in substantial amounts in treated water and sulphamethoxazole, ofloxacin, clarithromycin, vancomycin (antibiotics), atenolol (cardiovascular drug), furosemide, hydrochlorothiazide (diuretics), diclofenac (anti inflammatory) ranitidine (gastrointestinal drug), carbamazepine (anti-epileptic drug) and bezafibrate (lipid regulator) were the most abundant residual drugs.

RESEARCH ON PHAC$_S$ REMOVAL IN A PILOT MBR PILOT PLANT AND IN MILAN NOSEDO WWTP

From 2007 to 2010 a 280 L anoxic/aerobic pilot scale MBR pilot was operated in parallel to Milan Nosedo WWTP to evaluate the influence of process configuration and operational parameters on PhACs removal efficiencies as well as biodegradation/adsorption of these substances.
In fact, some studies have presented MBR as a process that leads to higher pharmaceuticals removal rates in comparison to conventional processes (Kreuzinger, *et al.*, 2004, Lesjean, *et al.*, 2005, Radjenovic, *et al.*, 2009). However, those compared CAS operated at standard SRT while MBR were operated at much higher SRT, or with synthetic feed.
In the following the main results obtained are summarized in terms of:

- comparison of full scale CAS and MBR removal efficiencies, when operating at similar SRT;
- comparison of MBR removal efficiency at variable SRT;
- comparison of adsorption isotherms of CAS and MBR sludge on levofloxacin.

A more ample description and discussion of the research results were published in Bouju et al. (2008, 2009, 2011) and Buttiglieri et al.(2010), including the evaluation of inhibitory effects of selected PhACs on CAS and MBR.

Materials and methods
The MBR influent was derived after the mechanical pre-treatments of the full plant and, to limit clogging of the membrane, pre-filtered with a 1 mm mesh filter bag which was manually cleaned regularly around two or three times per week. Its temperature was 7.7÷26.5°C and pH 7.5±0.3; average influent concentrations (for both MBR and WWTP) are represented in Table 3.

Table 3. Average MBR pilot plant and full scale WWTP influent concentration.

	COD		**NH_4^+**		**NO_2^-**		**NO_3^-**	
	mg_{COD} L^{-1}		mg_{NH4-N} L^{-1}		mg_{NO2-N} L^{-1}		mg_{NO3-N} L^{-1}	
	average	St.dev.	average	St.dev.	average	St.dev.	average	St.dev.
WWTP	252	90	16.8	5.4	0.35	0.31	0.92	1.71
MBR	264	120	16.6	5.2	0.32	0.21	0.70	1.00

The pilot (90 L anoxic tank, 190 L aerobic tank) contained a submerged hollow fibres micro-filtration membranes (Zenon-GE InfraWater Zee-Weed 10, surface area 0.93 m^2, nominal pore size of 0.2 µm). The permeate flow was around 0.3÷0.5 m^3 d^{-1} (13÷22 LMH). Scheme is presented elsewhere (Buttiglieri et al., 2005). The aerobic on anoxic TSS concentration ratio was kept constant and similar to that of the full scale plant (3.1 $gTSS_{AER}$ $gTSS_{DEN}^{-1}$ vs. 3.3 of Nosedo). This resulted in an average TSS concentration in the anoxic tank of 2.5 gTSS L^{-1} and of 4 gTSS L^{-1} in the aerobic tank. The MBR pilot plant was operated at different sludge ages between 15 and 82 days along the whole experimental period, working at lower, similar and higher SRT than the full scale WWTP. SRT in MBR was controlled by daily discharge of the corresponding amount (equal to $1/SRT_i$) of the volume of anoxic and aerobic tanks and adjusting the food to microorganism ratio (F/M) in order to maintain the MLSS concentration constant (Figure 2).
Sampling campaigns have been performed in springs 2007 (SRT_{MBR} = 35 d), 2008 (SRT_{MBR} = 15 d) and 2009 (SRT_{MBR} = 25 d) to limit differences due to external factors, such as human consumption and temperature. The last campaign (SRT_{MBR} = 50 d) was done in December 2010. All were started after at a period of at least $3xSRT_i$ days of stationary SRT operating conditions.
Sampling points were placed at the feeding points (MBR and CAS), the MBR permeate, the effluent of the biological treatment (CAS) and the final effluent (WWTP). Each campaign comprised 3 time-proportional 24 hours composite samples Immediately after collection, each sample was filtered on a 0.45µm fibreglass filter and frozen at -20°C until analyse. Analyses were

performed by means of HPLC/MS/MS by the Mario Negri Institute (Milan, Italy) as previously described (Table 3).

Adsorption isotherms of chemical grade Levofloxacin and Diclofenac (Sigma Aldrich) were determined on MBR and CAS sludges by a jar-test equipment filled with diluted sludge at 0.25 $gTSSL^{-1}$ at 0, 1, 5, 10 and 50 μgL^{-1} initial concentration. After 2 hours mixing (180 rpm) and centrifugation (2.400 g, 10 min) the supernatant was filtered on a 0.45 µm fibreglass filter and frozen at -20°C until analyses. Any biodegradation activity was previously inhibited by adding … HCl. The sludge specific surface of each sludge was previously determined according to Laurent et al. (2008). The values obtained as average of 10 duplicates were 172.2 +/- 11.5 $m^2\ g^{-1}{}_{TSS}$ for the MBR and 146.2 +/- 11.5 $m^2\ g^{-1}{}_{TSS}$ for the CAS sludge

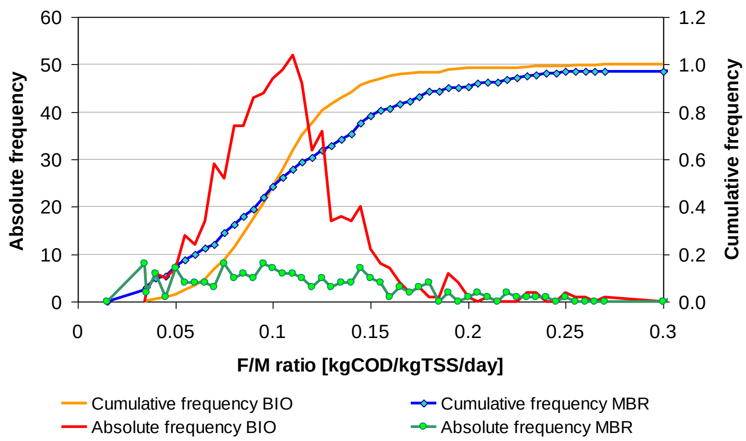

Figure 2. Absolute and cumulate frequencies of F/M values in MBR and full scale conventional activated sludge (BIO) (Buttiglieri et al., 2010)

Comparison of full scale CAS and MBR operating at similar SRT

The comparison was possible for the sampling campaign performed in 2009, when the full scale biological process was operated at 24 days and the MBR was operated at 25 days.

In Figure 3 the average removal rate measured on the CAS and the pilot MBR are reported. As the analyses were always done on the soluble fraction of the sample, the removal values do not take into account the possible contribution of PhACc adsorbed onto TSS in the influent/ effluent.

Removal rates of most compounds are similar for CAS and MBR considering the intrinsic accuracy of the analyses. Very recalcitrant compounds such as carbamazepine (CBZ) or Diazepam (DPZ) are not removed by CAS, nor by MBR. Considering the compounds present in concentration higher then 50 ngL^{-1}, three compounds (diclofenac, durosemide and ofloxacin/levofloxacin) are removed more in MBR then in CAS. This was also observed for lyncomicin and ranitidine (influent average concentration of 14 and 34 ngL^{-1} respectively) and for salbutamol (Cin < 10 ng L^{-1}).

Figure 4 shows the average value of the effluent concentration measured during the 4 sampling campaign (n = 12), for CAS and MBR, grouped by concentration ranges. Assuming as significant the difference between CAS and MBR effluent concentrations if the previous value are +/- 30% of the second, it can be concluded that estrone, benzafibrate, eritromycin, ofloxacin, ciproflaxacin, claritromicyn, atenolol, diclofenac and deydroeritromicyn are present at significantly lower concentration in MBR permeate, while atovarstatin, ibuprofen and ketoprofene are present in significantly lower concentration in CAS soluble effluent.

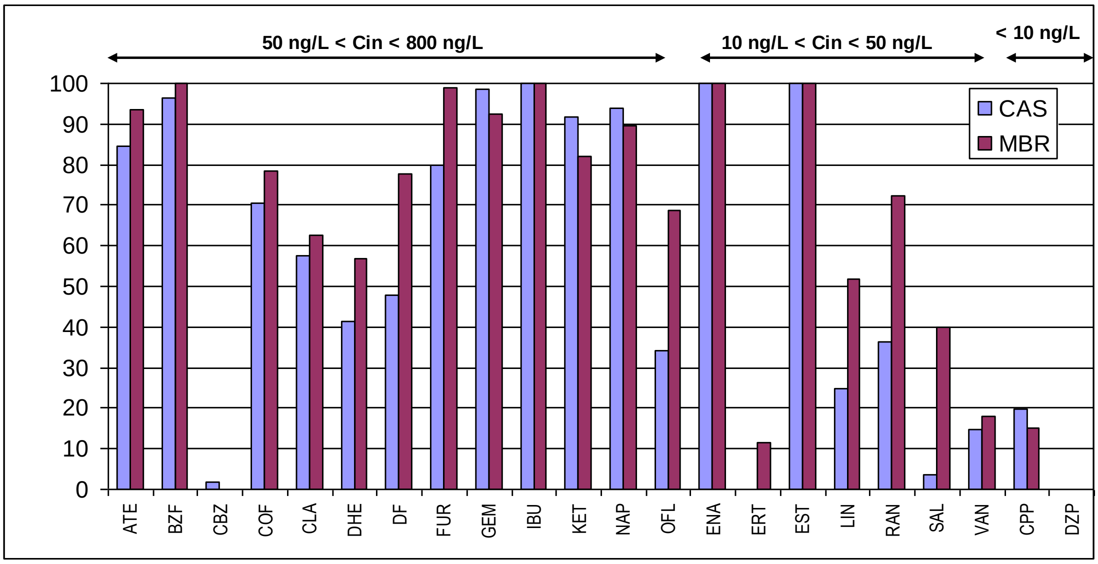

Figure 3. Average removal (%) measured on CAS and MBR operating at SRT (24-25 d)

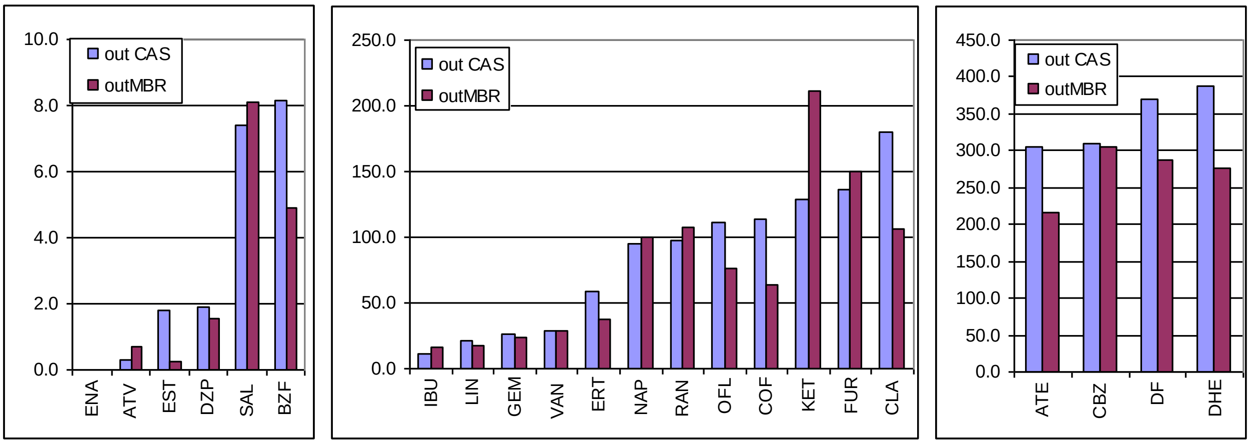

Figure 4. Average effluent concentration of PhACs (ngL^{-1}) from Nosedo CAS and MBR

Comparison of MBR removal at different SRT

The scope of the investigation was to study the dependence between of the removal efficiency in the MBR pilot plant and the SRT. Several studies point out the importance of operating at high SRT (Clara et al. 2005, Kreuzinger et al. 2004, Weiss & Reemtsma 2008, Kimura et al. 2007) to promote and enhance PhACs biodegradation. A positive correlation between PhACs removal and presence of AOB (ammonia oxidising bacteria) has been postulated by Tran et al. (2009), by cometabolism promoted by the ammonium monooxygenase enzyme.

In Figure 5 the average removal efficiency measured at 15, 25, 35 and 50 days SRT are reported.

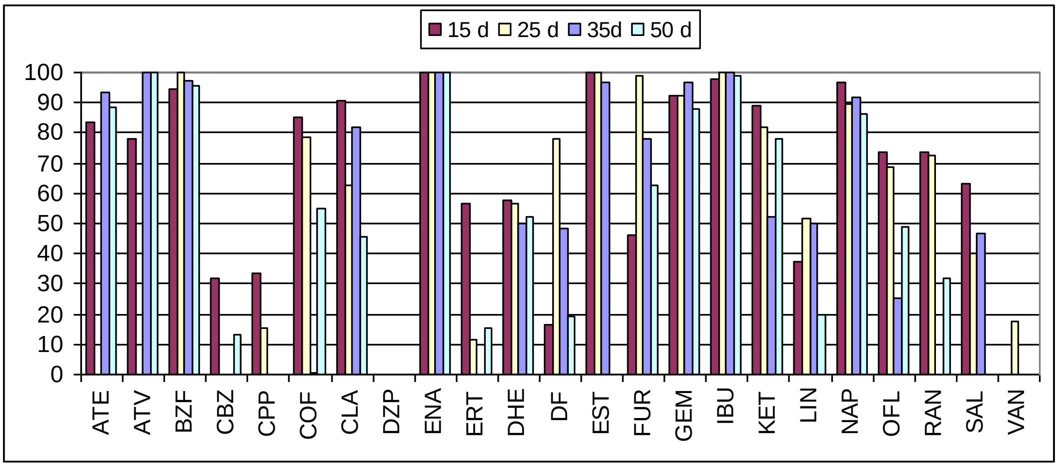

Figure 5 Average removal efficiencies (%) measured on MBR at different SRTs.

Figure 5 clearly shows the absence of a trend applicable to all pharmaceutical substances. diclofenac, furosemide and, to a lesser extent, lincomycin show a higher removal at 25 days rather then 15 days, and decreasing efficiency at 35 and 50 days. This behaviour could be possibly explained by the combined action of biodegradation and adsorption as removal mechanisms. ciproflaxcin, claritromycin, naproxen, ofloxacin and salbutamol tend to be less removed at increasing SRT.

Adsorption isotherm on CAS and MBR sludge

Adsorption isotherm of ofloxacin and diclofenac are shown in Figure 6. The data for ofloxacin fit very well a linear $X = \rho C_{eq}$ equation, that results from the Langmuir isotherm at infinite dilution of the solute. Both sludges have very similar values of the equilibrium constant k (1.32 – 1.40 $L \cdot TSS^{-1}$), in spite of the different specific surface. diclofenac isotherm (data not shown) on MBR was $X = 1.54C_{eq}$ ($r^2 = 0.90$). No adsorption of diclofenac was evidenced on CAS sludge.

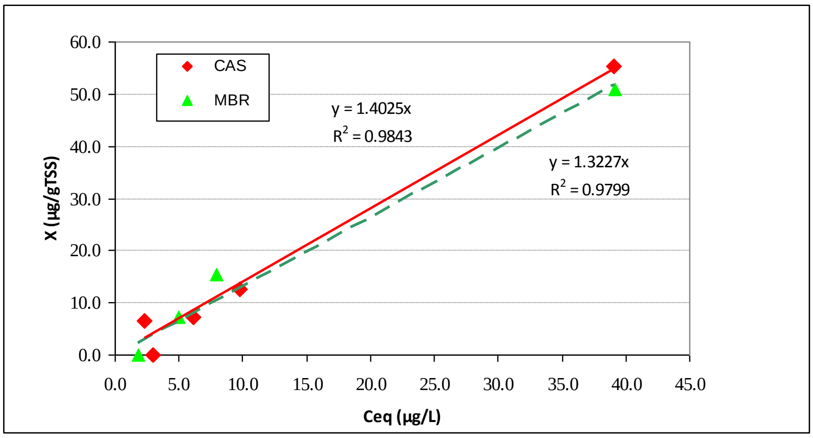

Figure 6 – Adsorption isotherm of Ofloxacin on CAS and MBR sludges

CONCLUSION

Removal rates in Nosedo WWTP were higher than those measured in other treatment plants in Italy but many PhACs were still present in treated water, at concentration ranges spanning from few to several hundreds ngL^{-1}. In the long term comparison of CAS and MBR performances it is possible to evidence relevant differences only for few compounds. No significant differences are measured for very recalcitrant (i.e. CBZ, DZP) or, on the other hand, readily removed compounds (i.e. Ibuprofen, Naproxen, Gemfibrozil, etc). Moreover, this study could also show that other operating parameters such as influent concentration and temperature do also affect the removal efficiency of partially removed compounds (Bouju, 2009), influencing the comparison between CAS and MBR. The operation of MBR at SRT in the range 15-50 d does not show a clear increasing or decreasing removal trend for a majority of the targeted compounds and, for the others, both increasing and decreasing trends are observed at increasing SRTs. This points out the fact that for some compounds adsorption on sludge, although limited in quantity, probably plays a role in the overall removal balance. Hence, the improvement of PhACs removal in MBR is rather limited and did not appear to be an essential alternative. Nevertheless, since MBR quality is of a far better quality than CAS, the use of MBR coupled to a tertiary treatment (i.e. NF, ozonation or GAC) might result in better removal of a wide range of PhACs and micropollutants.

ACKNOWLEDGEMENT
The authors are grateful to the staff of Vettabbia Scarl, company (Operation and Maintenance contract from Milanodepur spa) for the availability and the technical support to the research. This work was partly supported by a Marie Curie Early Stage Research Fellowship within the MBR-TRAIN project (contract number MEST-CT-2005-021050) and by the Italian MIUR – PRIN project 2007PM3TJM_002.

REFERENCES

Bouju H., Buttiglieri G. and Malpei F. (2008). The use of microcalorimetry to compare the biological activity of a CAS and a MBR sludge – Application to Pharmaceutical Active Compounds. *Water Science and Technology,* **58**(3) 529-535

Bouju H., Buttiglieri G. and Malpei F. (2009). Are MBRs really more efficient in removing pharmaceuticals? Comparison of a full scale conventional activated sludge process and a MBR pilot plant. 2nd International Conference on Occurrence, Fate, Effects, and Analysis of Emerging Contaminants in the Environment, 4-7 August 2009, Fort Collins, US

Bouju H., Buttiglieri G. and Malpei F. (2011). Influence of process type and operational parameters on pharmaceutical substances removal - comparison MBR and CAS. Micropol and Ecohazard 2011, 11-13 July 2011, Sydney, Australia

Buttiglieri G., Azzellino A., Bouju H. and Malpei F. (2010). Case study: Long term comparison between a full scale WWTP and a MBR pilot plant. IWA regional conference and exhibition on membrane technology and water reuse, 18-22 October 2010, Istanbul, Turkey

Buttiglieri G., Malpei F., Daverio E., Melchiori M., Nieman H., Lightart J. (2005). Denitrification of drinking water sources by advanced biological treatment using a membrane bioreactor. *Desalination,* **178** (1-3), 211-218.

Castiglioni S, Bagnati R , Fanelli R, Pomati F, Calamari D, Zuccato E. Removal of pharmaceuticals in sewage treatment plants in Italy. Environ. Sci. Technol. 2006, 40 : 357-363.

Castiglioni S, Bagnati R, Fanelli R, Calamari D, Zuccato E. (2005) A multiresidue analytical method using solid-phase extraction and HPLC-MS-MS to measure pharmaceuticals of different therapeutic classes in urban waste waters. J. Chromatography, 1092: 206-215.

Clara M., Kreuzinger N., Strenn B., Gans O. and Kroiss H. (2005) The solids retention time--a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, **39**, 97-106

Kimura K., Hara H. and Watanabe Y. (2007) Elimination of Selected Acidic Pharmaceuticals from Municipal Wastewater by an Activated Sludge System and Membrane Bioreactors. *Environmental Science & Technology*, **41**, 3708-3714

Kreuzinger N., Clara M., Strenn B. and Kroiss H. (2004) Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. *Water Science and Technology*, **50**, 149-156

Tran N.H, Urase T. and Kusakabe O. (2009). The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. *Journal of Hazardous Materials*, **171** (1–3), 1051–1057.

Weiss S. and Reemtsma T. (2008) Membrane bioreactors for municipal wastewater treatment - A viable option to reduce the amount of polar pollutants discharged into surface waters? *Water Research*, **42**, 3837-3847Tran N.H., Urase T., Kusakabe O. (2009) The characteristic of enriched nitrifier culture in the degradation of selected pharmaceutically actoive compounds. J. of hazardous Materials, 171, 1-3, 1051-1057..

Zuccato E, Castiglioni S, Mazzini R (2009). L'inquinamento ambientale da farmaci e il depuratore di Nosedo. Ingegneria Ambientale; 38 : 353-357.