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RESEARCH ARTICLE

The relevance of physicochemical and biological parameters for setting emission limit values for plants treating complex industrial wastewaters

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Abstract The influents of plants treating complex industrial wastewaters from third parties may contain a large variety of often unknown or unidentified potentially harmful substances. The conventional approach of assessing and regulating the effluents of these plants is to set emission limit values for a limited set of physicochemical parameters, such as heavy metals, biological oxygen demand, chemical oxygen demand and adsorbable organic halogen compounds. The objective of this study was to evaluate the relevance of physicochemical parameters for setting emission limit values for such plants based on a comparison of effluent analyses by physicochemical and biological assessment tools. The results show that physicochemical parameters alone are not sufficient to evaluate the effectiveness of the water treatment plants for removing hazardous compounds and to protect the environment. The introduction of toxicity limits and limits for the total bioaccumulation potential should be considered to supplement generic parameters such as chemical oxygen demand and adsorbable organic halogens. A recommendation is made to include toxicity screening as a technique to consider in the determination of best available techniques (BAT) during the upcoming revision of the BAT reference document for the waste treatment industries to provide a more rational basis in decisions on additional treatment steps.

Keywords Toxicity screening · Potentially bioaccumulating substances (PBS) · Effluent regulation · Waste treatment industry · Best available techniques (BAT)

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Introduction

Industrial wastewaters require specific treatment systems that enable the removal and/or detoxification of the hazardous constituents that are dissolved or suspended in the wastewater. The wastewater treatment (WWT) is often done on site in a dedicated plant designed to treat a specific type wastewater. In some cases, complex industrial wastewaters are transported to a specialized waste (water) treatment plant (WTP) designed to treat a variety of complex wastewaters from different sources. These specialized WTPs accept a wide and varying range of wastewaters and sludges from third parties (e.g. textile industry, chemical industry, surface treatment of metals, paint production, cleaning activities in different industrial sectors, etc.). The incoming streams can be contaminated with a high variety of potentially hazardous chemicals, e.g. acids/alkalis, metals, salts, organic compounds, etc. Wastewater treatment in these specialized plants typically involves a sequence of technical unit processes that should remove various types of contaminants from a variety of input streams, e.g. cyanide destruction, chromium reduction, two stage metal precipitation, pH adjustment, solid filtration, biological degradation, carbon adsorption, sludge dewatering, coagulation/flocculation and others (Joint Research Centre 2005). The selection and sequence of unit processes is determined by the characteristics of the accepted input streams and the required effluent quality. Treatment should guarantee a final effluent that is not hazardous to the aquatic ecosystem, and this is evaluated by the assessment of the effluent quality. This is commonly done by defining emission limit values (ELVs) for individual polluting substances or for generic indicators of pollution. In this study, the effluent quality was also evaluated by toxicity screening and measurement of the bioaccumulation potential, and the relevance of the different parameters for the assessment of the WWT effectiveness with respect to removal of hazardous compounds is discussed.

The conventional approach of using physicochemical measurements to asses and control emissions of harmful substances is found in the Industrial Emissions Directive (IE Directive, Directive 2010/75/EU) and previously in the Integrated Pollution Prevention and Control Directive (IPPC Directive, Directive 2008/1/EC). Installations for the treatment of complex, hazardous industrial wastewaters from third parties fall within the scope of these directives if their capacity exceeds 10 tonnes per day. According to the directives, the environmental permit conditions of such installations have to be based on the best available techniques (BAT), as defined in Article 3 of the IE Directive and as determined in the European BAT reference documents (BREFs). The permit conditions should include ELVs for the polluting substances that are listed in Annex II of the IE directive (see Table 1) and also for other polluting substances, which are likely to be emitted from the installation concerned in significant quantities, having regard to their nature and their potential to transfer pollution from one medium to another. The ELVs have to be based on the BAT and may be supplemented or replaced by equivalent parameters or technical measures ensuring at least an equivalent level of environmental protection. In this study, additionally, toxicity screening and bioaccumulation potential (potentially bioaccumulating substances, PBS) were used to evaluate the possible environmental impact of the effluents.

The BAT for waste treatment activities are determined in the European BREF for the waste treatment industries (Joint Research Centre 2005). BREFs are the result of an exchange of information within a dedicated technical working group (TWG) (Schoenberger 2009). They offer guidance to those who have to make specific decisions on BAT in implementing the IPPC and IE Directive, i.e. industrial operators, policy makers, permit writers and society at large. The BREF for the waste treatment industries provides 'emission values associated with the use of BAT' for a number of parameters, including values for emissions to water (see Table 2). These generic values are a reference point for setting ELVs for all

Table 1 Polluting substances for water listed in Annex II of the Industrial Emissions Directive

1	Organohalogen compounds and substances which may form such compounds in the aquatic environment
2	Organophosphorus compounds
3	Organotin compounds
4	Substances and mixtures which have been proved to possess carcinogenic or mutagenic properties or properties which may affect reproduction in or via the aquatic environment
5	Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances
6	Cyanides
7	Metals and their compounds
8	Arsenic and its compounds
9	Biocides and plant protection products
10	Materials in suspension
11	Substances which contribute to eutrophication (in particular nitrates and phosphates)
12	Substances which have an unfavourable influence on the oxygen balance (and can be measured using parameters such as BOD, COD, etc.)
13	Substances listed in Annex X to Directive 2000/60/EC

waste treatment activities covered by the BREF, including WTPs treating complex industrial wastewaters from third parties. In the concluding remarks, the BREF states that the generic wastewater parameters (Table 2) are insufficient for the evaluation of the effluent quality of plants for physicochemical treatment of wastewaters. As the range of accepted wastewater streams in these plants is very wide, the input to the WWT can contain almost any hazardous compound, and the list of potentially relevant contaminants that should be controlled is almost infinite. This is especially the case for the organic compounds. The number of inorganic hazardous compounds is more limited (heavy metals, cyanides, chlorides, fluorine, etc.), and they are more easily measured by chemical analytical methods. Due to a lack of data in the information exchange process within the TWG of the BREF, it was not possible to identify emission values associated with the use of BAT for all potentially relevant parameters (Joint Research Centre 2005). In this study, toxicity screening and PBS were used as complementary instruments for an overall effluent quality control as a supplement to the generic parameters identified in the BREF.

Direct toxicity assessment (DTA) procedures for whole effluent assessment (whole effluent toxicity, WET) were introduced in the 1970s and a nice review on the subject can be found in the OSPAR document (Ospar Commission 2000). The aim is to use risk assessment tools in the effluent quality assessment and to predict adverse effects on the aquatic ecosystem. In short, when using WET assessment, relevant aquatic organisms are directly exposed to the wastewater, and the

Table 2 Emission values associated with the use of BAT for the waste treatment industries (wastewater parameters only) (ppm) (Joint Research Centre 2005)

Water parameter	Emission values associated with the use of BAT (ppm)
COD	20–120
BOD	2–20
Heavy metals (Cr, Cu, Ni, Pb and Zn)	0.1–1
Highly toxic heavy metals:	
As	<0.1
Hg	0.01–0.05
Cd	<0.1–0.2
Cr(VI)	<0.1–0.4

acute toxic effects of the effluent are quantified and used for quality assessment. Over the years, the number of protocols and the experience in performing tests on effluents has grown rapidly. Experience in effluent testing soon indicated that even discharges that had passed chemical quality criteria imposed by competent authorities were acutely toxic to aquatic life (Heber et al. 1996). Studies on effluents from different industrial sectors, including, e.g. metal processing, pulp and paper, textile and chemical industries, illustrate the usefulness of complementary biological assessment because physicochemical analyses alone often do not provide sufficient information on the potential harmful effects of effluents to the aquatic environment (e.g. Teodorović et al. 2008; Picado et al. 2008; Sponza 2003). In other cases, e.g. in a study on landfill leachates (Pablos et al. 2011) and on cork-boiling wastewater (Mendonça et al. 2007), good correlations were found between physicochemical measurements and toxicity tests. In these cases, the toxicity is related to the specific measured parameters. In general, however, the identity and/or toxicity of numerous wastewater contaminants is unknown, and their toxicological impact cannot be predicted. By assessing WET through DTA, the additive, synergistic or antagonistic effects are measured without chemical characterization (Ospar Commission 2000; Williams et al. 1993; Tonkes et al. 1999). WET/DTA is now used in many countries as an additional condition for environmental permits (Belgium, Holland, Germany, UK and USA), limiting the maximum tolerable toxic load of the effluents. In many countries, the use of acute and chronic toxicity tests, as well as tests for mutagenicity, biodegradation and bioaccumulation are now used for a more sound environmental risk assessment of complex effluents (Power and Boumphrey 2004; Chapman 2000; Gartiser et al. 2010a; Gartiser et al. 2010b; Mendonça et al. 2009). In general, the term DTA is used when relevant biological tests are directly performed on (fractions of) the wastewater, and the toxic effects are used for quality assessment.

Next to their use within risk assessment, test batteries and/or single biotests can be used as warning/detection systems. In the present study, a toxicity assessment using a very sensitive biotest was used as a screening instrument to alert to the presence of acute toxic compounds in the organic fraction of the effluent after the WWT. While the presence of inorganic hazardous compounds is easily measured by classic chemical analytical methods and emission values associated with the use of BAT are provided by the BREF, it is not possible to identify and quantify the almost infinite list of organic compounds in effluents of specialized WWT treating complex wastewaters from third parties. Therefore, in this study, overall toxicity screening was applied to the organic fraction of the effluent as an alert for the presence of acute toxic substances. Based on previous experimental results on organic extracts of 33 complex waste samples, it was concluded that microtox is a suitable tool for direct toxicity assessment of the organic fraction. The test results were predictive for both acute and chronic biological responses (Deprez et al. 2012; Weltens and Maes 2010; Weltens et al. 2012). Although the bacterial assay is a measure for bacterial toxicity, it is therefore also indicative for toxicity to a broader panel of biological targets. Extended literature data show the sensitivity of the microtox assay to a broad panel of chemicals (Nacci et al. 1986) and its higher sensitivity for organic compounds (Niemrycz et al. 2007; Doherty 2001). Moreover, the microtox test is fast, cheap and easy to perform, as described in literature (Parvez et al. 2006).

The objective of this pilot study was to evaluate the relevance of physicochemical parameters for setting emission limit values for plants treating complex industrial wastewaters from external parties and their validity to evaluate the effectiveness of these plants with respect to removal of hazardous compounds. This evaluation is based on effluent analyses of different plants using physicochemical assessment to evaluate the presence of inorganic compounds and using both physicochemical and biological assessment tools to evaluate the presence of organic (hazardous) compounds in the effluent. Effluent samples of 10 Flemish WTPs, each accepting industrial wastewaters from different and varying third parties, were collected and evaluated according to the BREF parameters (heavy metals for the inorganic compounds, chemical oxygen demand (COD), biological oxygen demand (BOD) and adsorbable organic halogens (AOX) for the organic compounds). Heavy metals are persistent, and toxic pollutants that need to be controlled in all cases as they are the most problematic inorganic hazardous compounds present in wastewaters. Where relevant, other physicochemical parameters (cyanides, fluorides, chlorides, etc.) could easily be measured and used to efficiently control the removal of inorganic compounds from the wastewater.

For assessing the organic content of the final effluent, COD and BOD are commonly used to measure the remaining total and biodegradable fraction of the organic compounds, respectively, in the final effluent. These group parameters both include toxic and non-toxic compounds. In Flanders, also AOX is a commonly measured parameter in effluent quality assessment, as an indication for halogenated organic compounds that adsorb to active carbon. Organohalogens are persistent organic compounds, often with chronic effects on ecological and human targets. In this study, the effluent quality with respect to the organic compounds was complementary assessed by toxicity screening in the acute microtox of the organic C18 extracts from the effluents. This provides an indication for the effective removal of organic acutely hazardous compounds from the wastewater. Next to toxicity, also the amount of PBS in the wastewater was determined as these compounds are a potential risk for the environment even if they are not or only slightly toxic. The relevance of limiting the parameters PBS, toxicity, BOD, COD and AOX to protect the aquatic environment from organic pollution is evaluated.

Materials and methods

Effluent sampling

Effluents samples were collected at 10 specialized WTPs (A–J), each treating a complex variety of industrial wastewaters from third parties (various industrial sectors). All plants were located in the Flemish region of Belgium and used a wastewater treatment system involving a sequence of unit processes, including usually a primary physicochemical treatment, followed by a biological treatment and finally a carbon adsorption step. For each plant, two to five samples were taken over a period of 6 months (see Table 3). Each sample consisted of several subsamples, a 1-l sample for the determination of PBS, a 1-l sample for microtox analysis and additional samples for the physiochemical analyses. The samples were collected in glass recipients that were completely filled up and firmly closed. The recipients in which the samples for determination of PBS were collected contained 1 ml $AgNO_3$ (1 g/l solution). Samples were cooled during transport and stored in a cooled space until they were analyzed. Determination of PBS was done within a period of 5–7 days after sampling.

Table 3 Overview of the samples taken per plant

	Plant									
Month	A	B	C	D	E	F	G	H	I	J
April 2011	x		x		x		x	x	x	
May 2011	x		x	x	x	x	x	x	x	
July 2011	x	x	x	x	x	x			x	
September 2011	x	x	x	x	x		x	x	x	x
October 2011	x	x	x	x	x	x	x	x	x	x
Total sampling moments	5	3	5	4	5	3	4	4	5	2

C18 extraction

The adsorbable apolar fraction was extracted from the 1 l effluent samples using C18 columns (Isolute, 10 g sorbent, 70 ml). The eluate from this C18 column was again collected and is further called the C18-eluate.

The compounds that adsorbed to the C18 column were desorbed using methanol as a solvent. Methanol was then substituted by 1 ml dimethyl sulphoxide (DMSO) which then contained the equivalence of 1 l sample/ml (1,000 mleq/ml). This extract is further called the C18-extract.

Determination of PBS

The method is described by Leslie (2006) and Leslie and Leonards (2005). In principle, the method involves a solid phase microextraction (SPME) of the effluent using biomimetic fibres with 100 μm polydimethylsiloxane coating. These fibres extract from the surrounding effluent the organic compounds that can enter the biological cells due to their lipid solubility. The fibres are cleaned at 250 °C prior to use.

Three replicate samples of the wastewater of 250 ml each are prepared. The 250-ml recipients are filled to the brim (no headspace) and a fibre is added. They are stirred continuously by electromagnetic stirring for 24 h. After the contact period, the fibre is inserted into the gas chromatograph (specs), and organics are released by thermic desorption in splitless mode. Analysis of the organics is performed by FID detection.

When organics are released from the fibre, this shows like a bulk peak on the chromatogram. The surface area is correlated to a standard curve using 2,3-dimethylnaphtalene as a reference. Results are expressed in equivalent molar concentration of this reference substance as potentially bioaccumulating substances (PBS; millimoles per litre).

Based on an interlaboratory study on the use of SPME as a screening method for PBS, the following classification of effluent samples was proposed (Leslie, 2006): <5 mmol/l (very low level of PBS), 5–20 mmol/l (low level of PBS), >20 mmol/l (high level of PBS) and >40 mmol/l (narcotic toxicity expected).

Toxicity testing

The acute toxicity for the Microtox bacteria was measured in both the C18 extracts (samples of all sampling periods) and the C18 eluates (samples from one sampling period only).

In these tests, the bioluminescent bacterium *Vibrio fischeri* is used as a test organism and exposed to the samples. The test protocol is based upon the protocol described in ISO 11348-3 with some adaptations. The toxicity is measured as a decrease in the bioluminescent signal in comparison to the control values after 0, 5, 15 and 30 min of exposure. The effect is calculated as percent inhibition of luminescence at these exposure times when compared to control. For the final interpretation, the results at 30 min are used. Phenol is used as a reference substance to verify the test quality. Only results from valid tests were used.

The tests were carried out using a Microtox M500 analyzer (SDI Europe, UK) according to the Microtox Manual (1992) standard procedure for the phenol controls and the 90 % or standard procedure for the leachable fractions. The procedure was adapted for DMSO samples: *V. fischeri* were exposed to 1 % of DMSO extracts in Microtox Diluent® (5 μl sample in 500 μl diluent; 1 % DMSO was used as a negative control). DMSO had no adverse effect on light emission at this concentration.

When a concentration dependent inhibition was seen, the EC_{50} value was derived.

Limit screening test The microorganisms are exposed to 90 % of the C18-eluates or to 1 % of the DMSO extracts. The results are expressed as percent inhibition of the bioluminescence when compared to control (% INH).

Final test Toxic samples (i.e. those showing >50 % inhibition in the screening test) were further investigated. As none of the analyzed C18-eluates showed toxicity in the screening test, final test was only performed on theC18-extracts. Eleven of the extracts showed a toxicity higher than 50 %. A one half dilution series of these extracts in DMSO was prepared and used for testing (at 1 %) and the concentration–effect relationship could be defined. From this, the EC_{50} values are derived, i.e. the concentration that causes 50 % inhibition of the bioluminescence. Results are expressed in percent of the extract needed for 50 % inhibition.

The limit value for microtox toxicity for this purpose is not defined yet and should be further discussed and validated. Assuming that general effects are seen in a concentration range between $EC_{50}/3$ to $EC_{50}*3$, it is proposed to classify samples with an EC_{50} below 0.3 % extract (<3 leq/l) in the microtox as toxic loaded samples because the acute toxicity would also be seen in the original wastewater.

Physicochemical parameters

The physicochemical analysis involved a determination of COD, BOD, AOX and metals (Cr, Cu, Ni, Pb, Zn, As, Hg, Cd and Cr^{VI}). The analyses were done using the Flemish WAC methods (VITO 2012), which are based on the respective ISO standards (ISO 6060: 1989 and ISO 15705:2002 for COD, ISO 5815: 2003 for BOD, ISO 17294-1 and ISO 17294-2 for metals and ISO 9562: 2004 for AOX).

Statistical analysis

In order to determine the correlations between PBS, toxicity and the physicochemical parameters, Pearson correlation coefficients were calculated. Correlations are considered to be significant at a significance level of 0.05. The effects of the physicochemical parameters (BOD, COD and AOX) on PBS and toxicity were analyzed using linear regression analysis. The significance level of the regression analysis (p) and the R^2 indicate to what extent the variance of PBS and toxicity is explained by the physicochemical parameters.

Results

PBS, toxicity and physicochemical parameters

The measured values for PBS (millimoles per litre), toxicity (INH, %) and the physicochemical parameters (BOD, COD and AOX, milligrams per litre) for each WTP are represented in Fig. 1. The measured metal concentrations are summarized in Table 4.

Physicochemical parameters

Concentrations of metals (Cr, Cu, Ni, Pb, Zn, As, Hg, Cd and Cr^{VI}) were low in all effluents (Table 4). The measured values are all well below the maximum value of the emission values associated with the use of BAT as determined in the BREF (Table 2) and often below the reporting limit of the analytical method. It can therefore be concluded that the removal of these metals from the wastewaters is effective.

BOD is an indicator for organic compounds that are readily biodegradable. These compounds can be toxic or not but—unless they are very highly acute toxic—they are of relatively low risk to the environment because of their short half-life. In effluents of WTP, the BOD values are expected to be low as the biological treatment is designed to remove biodegradable compounds. An elevated BOD is associated with an impairment of the biological treatment step (possibly due to toxicity of the influent for the active sludge). BOD emission values associated with the use of BAT range between 2 and 20 ppm (Table 2). The measured BOD values in this study were indeed in the BAT range for most samples (Fig. 1). Only in plant F the mean value exceeds the 20-ppm level. In plants C, H and I, the BOD values occasionally exceed the 20-ppm level. Overall, it can be concluded that the biodegradable fraction is removed efficiently from the influent. In cases where BOD exceeds the BAT value, this is an indication that the WTP is not designed, operated or maintained according to

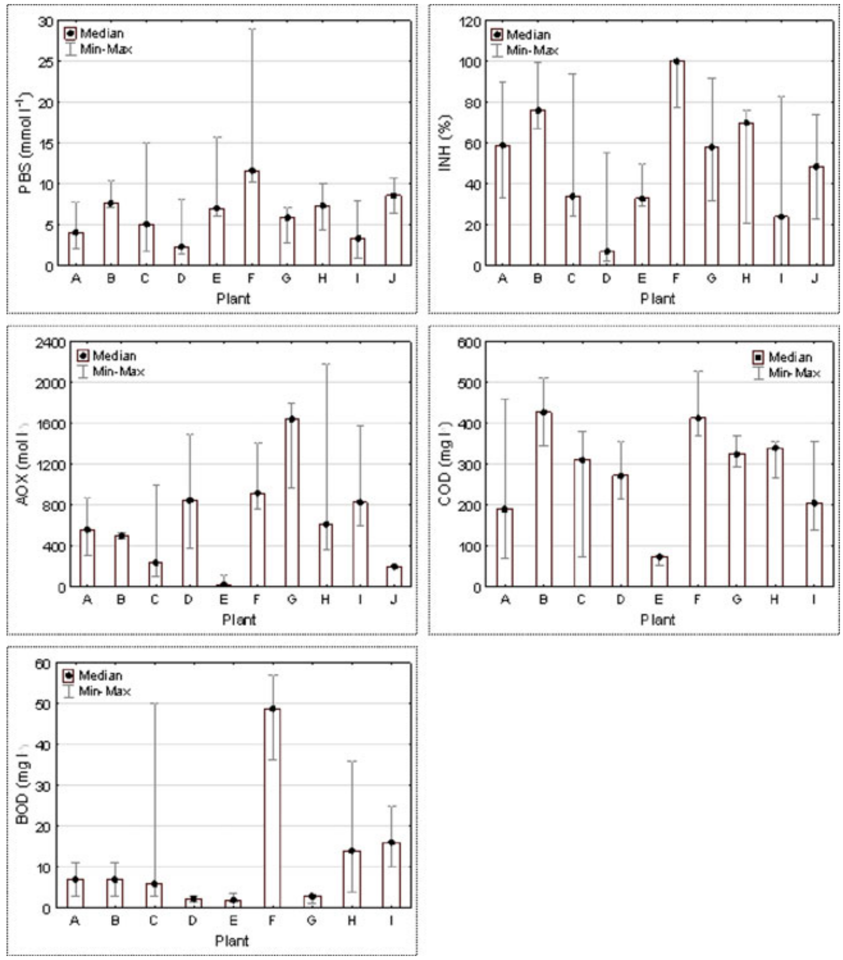

Fig. 1 Mean of measured values (n=2–5) for PBS, toxicity (INH), BOD, COD and AOX at each WTP. *Error bars* represent min and max values

the BAT principles, and measures should be taken to improve the biological removal of BOD in the WTP. BOD is considered as a meaningful parameter for the evaluation of the WWT—biological unit efficiency.

COD is a measure for the total amount of organics. These compounds can either be toxic or not. COD emission values associated with the use of BAT range between 20 and 120 ppm (Table 2). The measured COD values in this study were in most cases above the upper BAT value of 120 ppm, except for plant E (Fig. 1). The results between different sampling periods are variable, reflecting a different effectiveness in removing organic chemicals from the wastewaters and/or variations in the composition of the accepted (incoming) wastewater streams. In order to reach the BAT values determined in the BREF, additional measures are necessary. These can include technical measures to improve the removal of organic compounds from the wastewater or the implementation of acceptance procedures to limit the input of organic compounds via the incoming wastewater streams. As not all COD is hazardous to the environment, however, the environmental benefit of such extra measures is questionable.

AOX covers a large group of halogenated organic substances that can be adsorbed from water onto activated carbon. AOX are a subclass of organics that are included in COD. AOX can consist of simple or complex organic molecules as dioxins/furans with a large variety of toxic properties often chronic toxic effects (reproduction impairment and endocrine disruption). Most AOX are chlorine-containing molecules, but bromo- and iodo-AOXs also occur. Many AOX compounds are toxic, persistent and have a tendency to bioaccumulate (Swedish Pollutions Release and Transfer Register 2009). AOX emission values associated with the use of BAT are not specified in the BREF for the waste treatment industries (Table 2). In Flanders, this parameter is used as an extra screening indicator for release of potentially dangerous substances. A value of 400 mg l^{-1} is often used as a limit value. The present results show that AOX values in the effluents of WTP are highly variable, ranging from 20 to 2,180 mg l^{-1}. The relevance of AOX as an additional generic parameter can be questioned because AOX is already included in COD and the hazardous potential of a mixture "AOX" cannot be predicted.

Table 4 Number of measurements and measured concentrations of nine metals; only concentrations above the reporting limit are presented

Parameter	As	Cd	Cr	Cr^{6+}	Cu	Hg	Ni	Pb	Zn
Total number of measurements	41	41	40	15	41	41	41	41	41
Number of measurements below reporting limit	37	39	27	15	36	34	10	38	21
Concentrations (mg l^{-1})	0.0057	0.0049	0.0158		0.145	0.00003	0.469	0.0081	0.415
	0.0016	0.0043	0.0142		0.0895	0.00004	0.247	0.17	0.832
	0.0104		0.0179		0.0722	0.00007	0.169	0.2	0.85
	0.0103		0.0178		0.12	0.00005	0.152		0.0352
			0.0234		0.14	0.00005	0.457		0.0249
			0.0311			0.000112	0.302		0.0426
			0.0175			0.00002	0.317		0.466
			0.0108				0.0662		0.549
			0.02				0.0778		0.289
			0.02				0.0615		0.195
			0.22				0.0801		0.0281
			0.0124				0.187		0.0205
			0.01				0.0505		0.0338
							0.16		0.159
							0.194		0.28
							0.0409		0.45
							0.0761		0.52
							0.0335		0.0419
							0.0745		0.0665
							0.0237		0.111
							0.0208		
							0.0455		
							0.0311		
							0.0483		
							0.073		
							0.049		
							0.065		
							0.0355		
							0.029		
							0.0238		
							0.0547		

Toxicity screening

In the C18 extracts, significant acute toxicity to bacteria was often measured (Fig. 1), indicating that removal of organic hazardous compounds is not always effective. First toxicity was measured in limit tests on the 1 % DMSO extracts. These extracts contain per millilitre the organic load that has been extracted from 1 l effluent samples (1 leq/ml). The extracts are tested at 1 %, and the highest test concentration in the microtox is therefore ten times more concentrated than the original sample (10 mleq/ml). In almost 50 % of the cases (18 out of 40), a toxic signal was measured at this concentration (i.e. >50 % inhibition). Extracts that have less than 50 % effect at this high test concentration are considered as not containing a relevant amount of toxic substances.

Like the organic parameters (COD and AOX), the toxicity (% INH) varied considerably within and between the various WTPs, reflecting the variability in influent quality and/or the variability in the effectiveness of the WTP in removing toxic compounds from the incoming wastewater.

To quantify the toxicity, the acute toxicity was further measured on dilution series for 11 available DMSO extracts that showed a toxic signal >50 % in the limit test. This allowed to derive EC_{50} values for these samples (Table 5). There is not yet an established limit value for this purpose. In the present study, $EC_{50} \geq 0.3$ % is proposed as a limit for acceptable toxic

Table 5 Toxicity values [percent inhibition in microtox by 1 % extract (limit test)] and EC50 values for microtox (i.e. percent extract that inhibits the bioluminescence by 50 %) of effluent samples with >50 % inhibition in the limit test (selection of samples by availability)

Plant	Percent INH for 1 % extract	EC50 (% extract)
A	67	0.45
A	90	0.26
B	76	0.44
B	100	0.23
C	94	0.12
D	55	0.97
F	100	0.017
F	77	0.32
F	100	0.005
G	92	0.165
I	83	0.25

load. At this concentration, acute effects for microtox are expected in the original sample. Using this EC_{50} value as a classifier (≥0.3 % extract), seven out of 11 samples are considered as acute toxic samples. An elevated level of toxicity clearly demonstrates that the removal of hazardous substances by the WWT is not sufficient to guarantee that the final effluent is not hazardous to the receiving environment.

Also, the acute toxicity of the C18-eluate to microtox was measured in one series of samples. No acute toxicity for microtox was measured in these eluate fractions of the effluents. This was also seen in previous studies where microtox often was the least sensitive biotest for leachates (Deprez et al. 2012).

PBS

PBS is a measure for bioaccumulating compounds. These are organic compounds that are able to enter the biomembrane and have the potential to bioaccumulate. Substances that can bioaccumulate (and show up as PBS) are in any case harmful for the environment, regardless of their intrinsic toxicity. PBS values ranged from 1.0 to 29.0 mmol l^{-1} (Fig. 1). Based on the classification as proposed by Leslie (2006), 16 measurements were <5 mmol/l (very low level of PBS), 24 measurements were between 5 and 20 mmol/l (low level of PBS), one measurement was >20 mmol/l (high level op PBS) and no measurements were >40 mmol/l (narcotic toxicity expected). Overall, it can be concluded that the values for PBS were at the low or very low level.

Like the other parameters also, the PBS values varied in and between the various WTPs, reflecting the variability in the influent quality and/or the variability in the efficiency of the WTP in removing bioaccumulating compounds from the incoming wastewater.

Relation between physicochemical parameters, PBS and toxicity

Pearson correlation coefficients (r) are given in Table 6. No significant correlation could be seen between AOX and PBS or toxicity (INH). Significant but very weak correlations were seen: BOD is significantly correlated with PBS (r=0.43) and toxicity (r=0.42), and COD is correlated with toxicity (r=0.43). In order to further explore the relationships between the physicochemical parameters and PBS and toxicity, linear regression analyses were performed on paired data (Fig. 2). In this figure, only the regression equations significant at the 0.05 level are plotted. Although again the three significant regression equations show up, only a small part of the variance in toxicity or PBS (ca. 18 %) is predicted by the independent variables COD or BOD. It is concluded that elevated levels of AOX, BOD and COD in effluent are therefore not necessarily an indication for a high level of environmental impact, and low levels of AOX, BOD and COD do not guarantee a low level of environmental impact.

Discussion

The BAT for waste treatment, as described in the BREF for the waste treatment industries (Joint Research Centre 2005), include several techniques that should limit the discharge of toxic and/or bioaccumulating substances through the effluent of waste treatment plants. These include general management techniques, implementation of (pre)acceptance and sampling procedures and technical measures related to the treatment processes. The BREF provides emission values associated with the use of BAT for only a limited number of water parameters (COD, BOD and heavy metals). These values are a reference point for setting ELVs. The BREF concludes, however, that these parameters are not sufficient for the evaluation of the effluent quality of plants for treatment of complex wastewaters. Since the range of accepted wastewaters in these plants is very wide, the influent can contain almost any

Table 6 Pearson correlation coefficients between PBS (millimoles per litre), toxicity (inhibition, INH %) and physicochemical parameters (AOX, COD and BOD, milligram per litre)

	PBS	INH	AOX	COD	BOD
PBS	–	*0.44*	−0.06	0.18	*0.43*
INH		–	−0.02	*0.43*	*0.42*
AOX			–	*0.41*	0.29
COD				–	*0.46*
BOD					–

Italicized coefficients are significant at 0.05

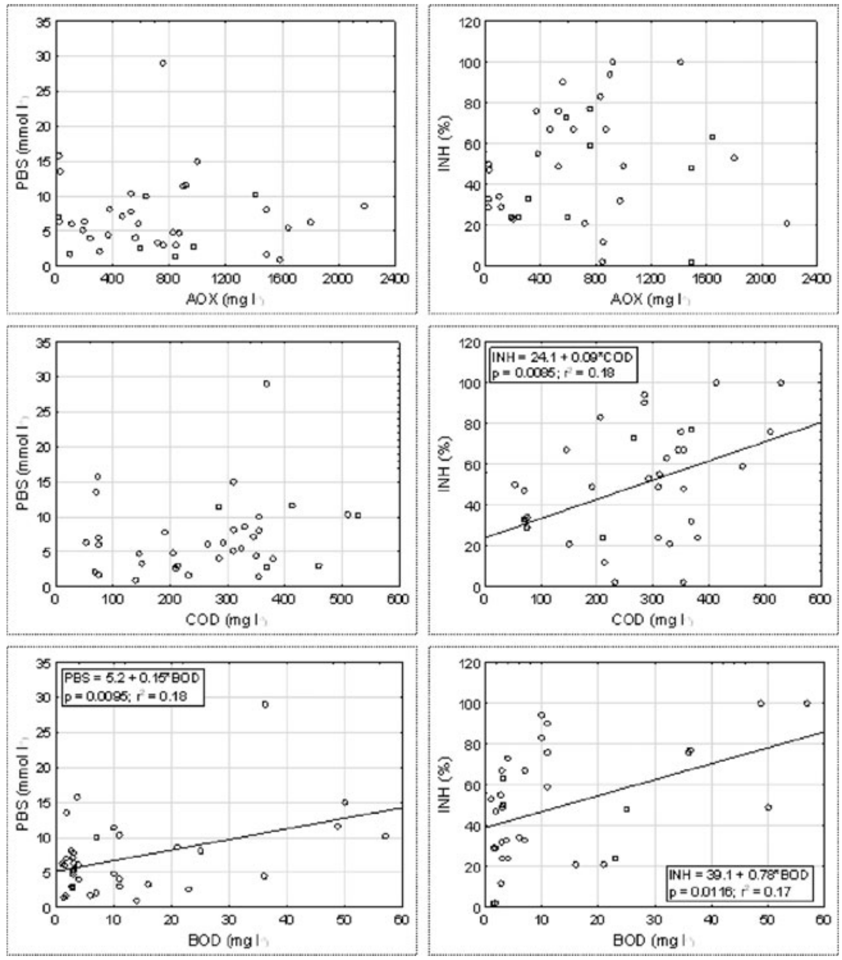

Fig. 2 PBS and toxicity (INH) as a function of physicochemical parameters (AOX, COD and BOD); significant regression equations ($p < 0.05$) are plotted

hazardous component, and it is practically impossible to identify all relevant contaminants and set emission limit values for them. It is however clear that additional parameters are needed. In Flanders, also the generic parameter AOX is commonly used for evaluation of the efficiency of the WTP.

In this paper, the effluent quality of WTPs treating complex industrial wastewaters from third parties was assessed using both biological and physicochemical assessments. For all parameters that were measured (toxicity, PBS, COD, BOD, AOX and heavy metals), the data show a high variability within and between various WTPs. This variability is not only explained by differences in the applied treatment processes but also by differences and variations in the composition of the accepted wastewater streams. WTPs do not produce a constant effluent quality, but the effluent quality is dependent upon the influent quality.

Levels of heavy metals and BOD were in most cases below the emission levels associated with the use of BAT. This implies that, as far as these parameters are concerned, the installations are designed, operated and maintained according to the BAT principles. From the results, it can be concluded that both BOD and heavy metal measurements in the influent give relevant information on the performance of the WWT with respect to effective removal of these specific compounds. Emission limit values for these parameters are therefore considered useful for environmental permits and quality control.

Levels of COD were often higher than the BAT levels determined in the BREF. Also, AOX levels often exceeded the reference value that is used in Flanders. In order to reach the BAT levels or the reference values for these parameters, additional measures are necessary. These can include technical measures related to the treatment process (e.g. additional adsorption units) or the implementation of more stringent acceptance procedures. Looking at the paired data (Fig. 2), COD was elevated in 28 cases, indicating that the removal of organic compounds was not always effective. The remaining organic fraction was however not always acute toxic, and the toxicity limit for bacteria of $EC_{50} \geq 0.3$ % extract was exceeded in only seven cases. The PBS limit (>20 mM) was exceeded in only one case. Based on reduction with respect to acute toxicity of the organic fraction of the wastewater for bacteria and bioaccumulation potential of the organics, there is a need for further remediation of the effluents organic content in eight cases. Unlike WET, the microtox toxicity screening is not meant to provide a full risk assessment for the aquatic ecosystem, but it is used as a fast and robust warning system to alert to the release of acutely hazardous compounds after WWT. The chronic toxicity of the organic fraction was however not measured; it would be relevant to include also biological screening tests to control and limit the amount of endocrine disrupting or genotoxic substances present in the final effluent.

The acute toxicity of the inorganic fraction was not measured in this study, but from the consistently low concentrations of heavy metals in the final effluents, it seems that these hazardous compounds are effectively removed during WWT. Inorganic parameters can be measured more easily and faster by chemical analytical methods than by the use of bioassays. Microtox is often the least sensitive test method for aquatic matrices, and all other bioassays need exposure times of at least 48 h.

The paired data sets that were produced in this study clearly show that neither COD nor AOX are on their own a straightforward measure for acute toxicity to bacteria and PBS. Wastewaters not only with high COD and low toxicity (see Fig. 2) were seen but also wastewaters with low COD (e.g. Fig.1: plant D) and high acute toxicity in the Microtox assay. This confirms the conclusions in earlier studies (Ospar Commission 2000) that there is no strict relationship between group parameters and ecotoxic effects, as was also observed in other studies for other types of industrial effluents (Teodorović et al. 2008; Picado et al. 2008; Sponza 2003). The generic physicochemical parameters can be used to evaluate the effectiveness in removal of the total organic content, but they are not sufficient to evaluate the effectiveness in removal of the acute *hazardous* compounds. In theory, this could be solved by using emission limit values for individual hazardous compounds in addition to emission limit values for the group parameters. In practice, however, it is impossible to identify all potentially relevant compounds in the wastewaters, especially in the case of the type of wastewater treatment plants considered in this study, because they treat a variety of wastewaters from external parties containing a very large range of (often unidentified and even unknown) contaminants. Therefore, the conventional approach of controlling emissions by using a limited set of physicochemical and generic parameters is for these types of plants not sufficient to protect organisms in receiving waters. This study showed that not all compounds included in the COD or AOX group parameters represent acute toxicity to bacteria or bioaccumulation potential, and thus, the environmental benefit of extra measures needs further investigation considering chronic toxic effects and PBS properties for all levels in the aquatic food chain.

High toxicity indicates that toxic compounds are still present in the effluent after the WWT. In order to enable specific remediation measures, the causing agent(s) need to be identified. Toxicity can be backtracked using toxicity identification and evaluation: f.i. the toxic extract can be fractionated based on different HPLC retention times, and the toxicity of the different fractions can then be measured again using the microtox test. This points out which fraction(s) contain the toxic compounds. The isolated fraction can then be further characterized using relevant chemical analytical techniques like gas chromatography–mass spectrometry. By testing the toxicity of the identified compounds, it is confirmed whether they are the actual causing agent(s) for effluent toxicity.

Based on this rationale, we strongly recommend to consider toxicity screening for the complex organic fraction of these wastewaters and PBS measurement in the determination BAT during the upcoming revision of the BREF for the waste treatment industries. Toxicity screening and PBS measurements have the advantage that no individual identification of the organic compounds in the mixture is needed. The overall acute toxicity and bioaccumulation potential are alerts for the presence of hazardous organic substances in the final effluent. The methods are fast, cheap and practical and improve the quality assessment with relevant extra information. Generic parameters like COD and AOX are still needed to evaluate the effectivity in removal of the total organic (halogen) content from the wastewater. The quality of the organic fraction of the effluent is more efficiently controlled by introducing limit values for its total acute toxic and PBS loads in addition to limit values for COD and AOX. Threshold values for toxic load and PBS, which supplement the ELVs for generic physicochemical parameters, would highly improve the assessment of the environmental protection efficiency of the WWT. Also, the implementation of additional biotests to screen for chronic toxicity is strongly advised.

Conclusions

WTPs accept a wide variety of industrial wastewaters from third parties and are using complex wastewater treatment systems that are designed to remove in principle any contaminant by using a sequence of unit processes, including a primary physicochemical treatment, followed by a biological treatment and finally a carbon adsorption step.

The BREF for the waste treatment industries determines emission values associated with the use of BAT for heavy metals, BOD and COD. These are a reference point in setting emission limit values, but the BREF clearly states that this set of parameters is not sufficient to control the presence of all potentially harmful compounds in the effluent. Additional instruments are needed to evaluate the performance of the WWT.

Emission limit values for heavy metals and BOD are relevant because these parameters allow to assess the performance of the WTP with respect to removal of the highly toxic metals and the performance of the biological treatment step. COD and AOX provide information on the total organic content of the final effluent, but given the results of this study, the COD and AOX are on their own, not a sufficient measure to assess the potential environmental impact of the effluent. The introduction of toxicity limits and PBS limits should be considered to supplement these generic parameters.

In the present study, only the acute toxicity of the C18 extracts was screened with microtox, but screening tests for chronic effects would provide useful information to predict environmental adverse effects. Screening for hazardous properties is particularly relevant for the organic fraction of the effluent as this complex mixture cannot be characterized by other methods. Inorganic fraction of effluents can easily be characterized by chemical analytical tools, as the number of compounds is limited, and their ecotoxic properties are well known.

Toxic responses were seen in the C18 extracts, indicating that the removal of (acute) hazardous organic substances in the WTP is not sufficient in all cases. The suggested limit value for toxic load needs further elaboration and validation and also the use of screening tests for chronic toxicity should be investigated, but the added value of the introduction of an ELV for toxic load is clearly demonstrated in this study.

The BREF for the waste treatment industries was adopted in 2005 under the IPPC Directive, and a review of the BREF under the IE Directive is scheduled to start in 2013. The introduction of direct toxicity assessment as a technique to consider in the determination of BAT is highly recommended for a safer effluent and a more rational basis in decisions on additional treatment steps.

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