Evaluation of acute ecotoxicity removal from industrial wastewater using a battery of rapid bioassays

Jan Dries, Dominique Daens, Luc Geuens and Ronny Blust

ABSTRACT

The present study compares conventional wastewater treatment technologies (coagulation–flocculation and activated sludge) and powdered activated carbon (PAC) treatment for the removal of acute ecotoxicity from wastewater generated by tank truck cleaning (TTC) processes. Ecotoxicity was assessed with a battery of four commercially available rapid biological toxicity testing systems, verified by the US Environmental Protection Agency. Chemical coagulation–flocculation of raw TTC wastewater had no impact on the inhibition of the bioluminescence by Vibrio fischeri (BioTox assay). Subsequent biological treatment with activated sludge without PAC resulted in BioTox inhibition-free effluent (<10% inhibition). In contrast, activated sludge treatment without PAC produced an effluent that significantly inhibited (>50%) (i) the bioluminescence by Photobacterium leiognathi (ToxScreen3 test kit), (ii) the photosynthesis by the green algae Chlorella vulgaris (LuminoTox SAPS test kit), and (iii) the particle ingestion by the crustacean Thamnocephalus platyurus (Rapidtoxkit test kit). The lowest inhibition was measured after activated sludge treatment with the highest PAC dose (400 mg/L), demonstrating the effectiveness of PAC treatment for ecotoxicity removal from TTC wastewater. In conclusion, the combination of bioassays applied in the present study represents a promising test battery for rapid ecotoxicity assessment in wastewater treatment.

Key words | activated sludge, coagulation–flocculation, powdered activated carbon, tank truck cleaning, whole effluent toxicity

INTRODUCTION

The tank truck cleaning (TTC) process involves cleaning of tank truck interiors. The wide range of transported cargo, ranging from food products to hazardous chemicals, results in a complex wastewater with variable composition and characteristics. The discharge limits for treated TTC effluents are currently mostly based on chemical parameters, such as chemical oxygen demand (COD). Several studies, however, indicate that results from both routine and advanced chemical analyses are unable to predict the potential ecological impact of complex effluents, emphasizing the need for whole effluent toxicity testing (WET) (Hernando et al. 2005; Rosa et al. 2010; Huybrechts et al. 2014).

In previous studies with a particular TTC wastewater, we found that conventional treatment technologies such as chemical coagulation and activated sludge treatment did not suffice for complete detoxification, resulting in a significant residual ecotoxicity in the final effluent (De Schepper et al. 2010; Dries et al. 2013). An advanced treatment step, such as powdered activated carbon treatment (PACT), is probably needed for complete removal of the toxicity in these cases (Eckenfelder et al. 2009). In the PACT process, powdered-activated carbon (PAC) is directly added to the effluent doses of 20–500 mg/L (Çeçen & Aktas 2012).

WET is commonly performed with green algae, daphnids, and fish as representatives of different trophic levels in the aquatic food chain (Escher & Leusch 2012). Major drawbacks of these tests, however, are the high cost, the need for trained personnel for the cultivation of the organisms and the large exposure times. To date, the bacterial bioluminescence inhibition test using Vibrio fischeri is the only standardized cost-effective and rapid toxicity assay that is widely applied for screening purposes in aquatic toxicity analysis (Parvez et al. 2006; Ma et al. 2014). The relative sensitivity of the assay using Vibrio fischeri in comparison with algae and daphnids varies according to the compound...
classes present in effluent (Girotti et al. 2008). These observations highlight the need to expand the array of rapid assays that can be combined for toxicity applications in wastewater treatment.

The objectives of the present study were (i) to compare conventional wastewater treatment technologies with the PACT process for the removal of acute ecotoxicity from real TTC wastewater and (ii) to evaluate the response of a battery of four commercially available rapid bioassays, based on distinct inhibition principles.

METHODS

Sampling

The full-scale TTC wastewater treatment plant is located in the harbor region of Antwerp, and consists of the following steps: oil separation, primary coagulation–flocculation followed by dissolved air flotation, activated sludge treatment including sedimentation, and finally tertiary sand filtration (for the removal of suspended solids). At regular time intervals, we collected raw wastewater samples before coagulation–flocculation, and effluent samples after biological treatment. The grab samples were kept cool (at 4 °C) and in the dark before use.

Laboratory-scale treatment

The raw wastewater samples were treated by coagulation–flocculation in two phases. In a first phase, the coagulation was performed in 500 mL glass beakers using a range of coagulant doses. The coagulant was PAX-XL19, a water solution of polyaluminum chloride provided by the TTC company. Increasing concentrations of PAX from 0 to 100 mg/L were added while the samples were intensively mixed (200 rpm, using a Lovibond jar-tester). Subsequently, the mixing rate was lowered (to 40 rpm), the pH was adjusted to approximately 8.0–8.2, and a flocculant was added. The flocculant was anionic Optifloc 330 (Kemira Chemicals) provided by the TTC company, and the dose applied was about 0.8 mg/L. In the final step, the flocculated samples were allowed to settle for 30 min. In the second phase, we repeated the coagulation–flocculation for each wastewater sample on 20 L batches using only one PAX dose, based on the results from the first phase. The samples that were chemically treated in this second phase, were subsequently fed to three parallel sequencing batch reactors (SBR 1, 2, and 3) inoculated with activated sludge originating from the TTC company. The applied SBR cycle simulated the operation of the full-scale plant. Each 6 h cycle consisted of nine steps: initialization (1 min), two aerobic feed phases (28 min each) followed by two aerobic reaction phases (1 h 29 min each), post-anoxic mixing (30 min), refresh (5 min aeration), settling (45 min), and discharge (45 min). The starting mixed liquor volume in the SBRs was 10 L, and 620 mL of wastewater was added every cycle; 250 mL of the mixed liquor was wasted daily to obtain a sludge retention time of approximately 40 days. In aerated steps, the aeration was continuous, and dissolved oxygen was above 2 mg/L at all times.

Pulsorb FG5 PAC (Chemviron Carbon) was added daily to SBR 2 (influent PAC dose of 80 mg/L) and SBR 3 (400 mg/L). No PAC was added to SBR 1.

Chemical analyses

Turbidity was measured using a portable turbidimeter (Hach model 2100P), and expressed as nephelometric turbidity units (NTU). COD and soluble COD (sCOD, i.e. COD analysis after filtration over a 0.45 μm glass fiber filter) were determined using micro-COD tubes (Hannah Instruments, Temse, Belgium). Mixed liquor suspended solids were measured gravimetrically after three consecutive centrifugation/washing cycles to remove the dissolved salts, and drying overnight at 105 °C.

Rapid ecotoxicity tests

A battery of four different commercially available acute toxicity tests was applied in the current study. Each assay was performed according to the instructions supplied by the test manufacturer, as described briefly below.

The BioTox kit (Abotox Oy, Finland) uses freeze-dried naturally luminescent Vibrio fischeri. The test protocol involved combining 500 μL of test samples (with adjusted salinity of 2% sodium chloride) with 500 μL of reconstituted bacteria. After a contact time of 50 min at 15 °C, the decrease of light intensity was measured with a portable tube luminometer (Berthold Technologies Junior LB 9509). The inhibitory effect is compared to a negative control (2% sodium chloride) to give the percentage inhibition.

The ToxScreen3 test kit (CheckLight Ltd, Israel) uses freeze-dried luminescent Photobacterium leiognathi and two proprietary assay buffers: one for detecting heavy metals (Pro-Metal Buffer) and the other for organic pollutants (pro-organic buffer). The test procedure involved combining 800 μL of test samples with 200 μL of assay
buffer and 10 μL of hydrated bacteria. The decrease of light intensity was measured after a contact time of 15 min at 30 °C. The relative inhibitory effect for each sample is calculated by comparing the extent of luminescence change to a negative control (clean water with assay buffer).

The LuminoTox SAPS test kit (Lab-Bell Inc., Canada) uses whole algae (Chlorella vulgaris) hereafter referred to as SAPS. The test is performed in the dark by exposing 100 μL of SAPS solution to 2 mL of test sample for 10 min (at ambient temperature). SAPS are then activated at a wavelength of 470 nm in a dedicated fluorometer. Some of the absorbed energy is emitted as fluorescence at wavelengths longer than 700 nm, which is the signal measured both in background water and effluent samples. Decreases in fluorescence as a result of toxic contamination are expressed as percent inhibition. Prior to analysis, SAPS were activated in room light for 90 min at ambient temperature.

The Rapidtoxkit (MicroBioTests, Inc., Belgium) uses larvae of the anostracan crustacean Thamnocephalus platyurus. The bioassay was performed in test tubes using T. platyurus hatched from cysts (hatching was initiated 30–45 h prior to performing the test). The T. platyurus were exposed to samples for 1 h at 25 °C, after which a suspension of red microspheres was added. The organisms ingest the microspheres, resulting in a deep red color in their digestive tracts. Stressed organisms either fail to take up particles or ingest at a much lower rate. The presence or the absence of colored microspheres in the digestive tract of the larval crustaceans was observed under a stereomicroscope. The total number of T. platyurus in the control (standard freshwater) wells and the number of T. platyurus that have taken up the red particles were counted. The fraction of larval crustaceans affected by the sample is defined as the percent inhibition.

**Statistical analyses**

Analysis of variance (ANOVA) and Tukey’s multiple comparisons of means were performed using the R software environment for statistical computing and graphics (http://www.r-project.org). A paired t-test for comparison of means was performed using the Data Analysis tool in Microsoft Excel 2010.

**RESULTS AND DISCUSSION**

**Selection of the test battery**

From 1995 to 2013, the US EPA Environmental Technology Verification Program verified the performance of nearly 500 innovative environmental technologies (http://www.epa.gov/etv/vt-ams.html). Table 1 gives an overview of the 12 commercially available rapid biological toxicity testing systems that were included in the verification program. Exposure times between the test organisms and the test samples in these systems varies from 5 min to 2 h, which is significantly less than the conventional acute ecotoxicity assays using daphnids (48 h) or green algae (72 h).

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Organism</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>AbraTox</td>
<td>Vibrio fischeri</td>
<td>Bioluminescence</td>
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<td></td>
<td>BioTox</td>
<td>Vibrio fischeri</td>
<td>Bioluminescence</td>
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<td>Deltatox</td>
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<td>Bioluminescence</td>
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<td></td>
<td>Microtox</td>
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<td>Bioluminescence</td>
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<tr>
<td></td>
<td>ToxScreen³</td>
<td>Photobacterium leiognathi</td>
<td>Bioluminescence</td>
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<td></td>
<td>Toxi-Chromotest</td>
<td>Escherichia coli</td>
<td>β-Galactosidase activity</td>
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<td>ToxTrak</td>
<td>Different species</td>
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<td>Mixed culture</td>
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<td>LuminoTox PECs</td>
<td>Chloroplast membranes</td>
<td>Photosynthesis</td>
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<td>Photosynthesis</td>
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<tr>
<td></td>
<td>Rapidtoxkit</td>
<td>Thamnocephalus platyurus</td>
<td>Particle ingestion</td>
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</table>

The four toxicity tests in bold were applied in the current study.
Most listed bioassays are based on a bacterial biosensor system such as the bioluminescent *Vibrio fischeri*. In contrast, only one test system, with two variants, is based on green plants (LuminoTox). In order to cover a wide range of ecological effects, we selected a test battery (highlighted in bold in Table 1) consisting of two distinct bacterial sensors, one algal assay and one crustacean-based system. With respect to the ToxScreen³ test, only results obtained with the pro-organic buffer are reported. The assay using the pro-metal buffer yielded no significant inhibition in any of the samples, suggesting that metal toxicity was not an issue for this particular effluent (results not shown).

**Removal of Vibrio fischeri** bioluminescence inhibition by conventional treatment technologies

Over a period of about 4 months, we collected seven batches of raw TTC wastewater after oil separation in the full-scale treatment plant. The average total COD, sCOD, and turbidity of these raw wastewater samples were 1268 ± 328 mg/L, 1023 ± 334 mg/L and 163 ± 70 NTU, respectively.

Treatment of raw wastewater by coagulation–floculation in the laboratory removed 8–25% of the total COD (Figure 1; \( p < 0.001 \)), yielding clear water with an average turbidity of 5 ± 1 NTU. In contrast, the average sCOD removal was not significant at about 5% (Figure 2; \( p > 0.05 \)). The inhibition of the *Vibrio fischeri* bioluminescence by the raw wastewater, measured using the BioTox assay, varied strongly from 39 to 84% for the different samples, with an average of 58%. Coagulation–floculation had no impact on the bioluminescence inhibition (Figure 2). In contrast, several authors report a significant reduction of bioluminescence inhibition from diverse industrial effluents, including TTC wastewater, after treatment with either ferric chloride or aluminum salts (e.g. Gotvajn et al. 2009; Dries et al. 2013). Our results can be explained by the fact that the measured bioluminescence inhibition was entirely associated with the filtered fraction of the raw wastewater (results not shown), which was not removed by the coagulation–floculation step (Figure 2).

Subsequent activated sludge treatment, without PAC addition, removed 93% of the remaining sCOD (Figure 2). Final sCOD concentrations were comparable to the values obtained after activated sludge treatment in the full-scale installation, and are well below the Flemish discharge limits for TTC effluents in surface water (i.e. 10-day average of 300 mg/L). Activated sludge treatment also removed 86% of the BioTox bioluminescence inhibition (Figure 2). In agreement with our results, a number of researchers report a significant decrease in bioluminescence inhibition after activated sludge treatment of both domestic and industrial wastewaters (Araújo et al. 2005; Katsoyiannis & Samara 2007; Rosa et al. 2010; Ma et al. 2011; Dries et al. 2013; Zhao et al. 2014).

**Response of the battery of rapid toxicity tests and evaluation of the PACT process**

Table 2 shows the effect of PAC treatment on the response of the battery of rapid bioassays for the three laboratory-scale SBR reactors and the full-scale plant. The response of the BioTox assay was well below 10% inhibition in all effluent samples, corresponding to an absence of toxicity. These results indicate that activated sludge treatment, without PAC addition, was sufficient for the removal of the *Vibrio fischeri* bioluminescence inhibition from this...
particular TTC wastewater (see also Figure 2). Similarly, Huybrechts et al. (2014) only found a significant bioluminescence inhibition when TTC effluent samples were 10 times concentrated. In contrast, the response of the three other bioassays was significantly different from the non-toxic negative controls (Table 2). These results indicate (i) that ecotoxic effects were still present in the treated effluent and (ii) that the assays applied were more sensitive to TTC effluent toxicity than the Vibrio fischeri inhibition test. In agreement with our observations, Ulitzur et al. (2002) reported the higher sensitivity of the ToxScreen test for a series of organic toxicants, in comparison with the Microtox test. Like the Vibrio fischeri inhibition test, the LuminoTox algal photosynthesis inhibition test aims at identifying general ecotoxic effects (Bellemare et al. 2006). Comparative responses of these two assays toward (industrial) effluents have not been reported before. The Rapidtoxkit, finally, uses the same test organism (Thamnocephalus platyurus) as the 24 h mortality Thamnotoxkit test which has been reported to have comparable sensitivity for wastewater as the Microtox test (Nalecz-Jawecki & Persoone 2006; Mendonça et al. 2009). Our results confirm that comparative sensitivities of different test organisms for environmental samples cannot be generalized, but should be investigated for each individual case (Ma et al. 2014). To the best of our knowledge, this is the first report on the application of a battery of EPA-verified rapid biological toxicity testing systems in wastewater treatment.

ANOVA results (Table 2) indicate that PAC treatment had significant impact on the effluent COD (p < 0.001), on the response of the Lumintox SAPS test (p < 0.001) and on the response of the ToxScreen³ test (p < 0.05). The Rapidtoxkit yielded inhibition values above 50% in all effluent samples, but the impact of PAC treatment was not statistically significant (p = 0.25). The results of the pairwise comparison of means according to Tukey’s procedure, for the different parameters and reactors are summarized in Table 2. The highest COD and inhibition values were found in the laboratory-scale reactor without PAC, and in the effluent of the full-scale plant where PAC was not applied. Conversely, the lowest effluent COD and ecotoxicity were recorded in the laboratory-scale SBR treated with the highest PAC dose (400 mg/L). These results clearly demonstrate the effectiveness of PAC dosing on ecotoxicity removal from TTC wastewater, as observed in earlier studies for other types of wastewaters (Bundschuh et al. 2011; Margot et al. 2013).

### CONCLUSIONS

In the present study, we found that primary coagulation–flocculation and activated sludge treatment did not suffice for the removal of ecotoxic effects from TTC wastewater. The PACT process on the other hand resulted in lower effluent COD values and significantly decreased the inhibition response of a battery of rapid ecotoxicity assays. The biological toxicity testing systems ToxScreen³, LuminoTox SAPS, and Rapidtoxkit were more sensitive to TTC effluent toxicity than the Vibrio fischeri-based BioTox inhibition test. The combination of rapid assay systems based on distinct inhibition principles and using a variety of biosensors holds great promise for time-efficient monitoring of wastewater treatment processes. In order to validate the test battery applied in the present study, future research should investigate the comparative sensitivity with the common WET battery using green algae, daphnids, and fish.

### ACKNOWLEDGEMENT

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