



Toxicity-directed approach of polyester manufacturing industry wastewater provides useful information for conducting treatability studies

Roberto A. Caffaro-Filho^{a,*}, Dione M. Morita^b, Roger Wagner^c, Lucia R. Durrant^a

^a Microbial Systematics and Physiology Laboratory, Food Sciences Department, Faculty of Food Engineering, State University of Campinas, Brazil

^b Hydraulic and Sanitary Engineering Department, Politechnic School, University of São Paulo, Brazil

^c Food Analysis Laboratory, Food Sciences Department, Faculty of Food Engineering, State University of Campinas, Brazil

ARTICLE INFO

Article history:

Received 9 April 2007

Received in revised form 11 June 2008

Accepted 17 June 2008

Available online 26 June 2008

Keywords:

Toxicity

Polyester manufacturing wastewater

Biological treatment

Respirometry

Organic chemical industry

ABSTRACT

A broader characterization of industrial wastewaters, especially in respect to hazardous compounds and their potential toxicity, is often necessary in order to determine the best practical treatment (or pre-treatment) technology available to reduce the discharge of harmful pollutants to the environment or publicly owned treatment works. Using a toxicity-directed approach, this paper sets the base for a rational treatability study of polyester resin manufacturing. Relevant physical and chemical characteristics were determined. Respirometry was used for toxicity reduction evaluation after physical and chemical effluent fractionation. Of all the procedures investigated, only air stripping was significantly effective in reducing wastewater toxicity. Air stripping in pH 7 reduced toxicity in 18.2%, while in pH 11 a toxicity reduction of 62.5% was observed. Results indicated that toxicants responsible for the most significant fraction of the effluent's instantaneous toxic effect to unadapted activated sludge were organic compounds poorly or not volatilized in acid conditions. These results led to useful directions for conducting treatability studies which will be grounded on actual effluent properties rather than empirical or based on the rare specific data on this kind of industrial wastewater.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

For many decades, industrial effluents were often discharged to publicly owned treatment works (POTWs) to be fully treated, along with sewage. However, toxicants found in industrial wastewaters may inhibit the biological activity of the treatment plant biomass, causing process upsets and making their way into the waters [1]. There is also the possibility of volatilization of the toxicants in sewers, bar racks, sedimentation tanks and aeration basins before biodegradation. Toxicants can also accumulate in the produced sludge, causing potential hazard risks for plant operators and public health, as well as sludge disposal problems [2]. In developed countries, more than 30 years ago, these practical and environmental concerns led to regulations that demanded pretreatment at the source of industrial wastewaters before discharge in POTWs [3]. Although this is a worldwide tendency nowadays, Brazilian POTWs still struggle with toxic industrial wastes and there's no consensus about a broad pretreatment program concerning hazardous wastes.

In order to determine the best practicable pretreatment technology available to eliminate or reduce the discharge of hazardous

pollutants to POTWs, the concern of industries to better know and understand the nature of their effluents has grown. This led to the necessity of a broader characterization of industrial wastewaters, especially in respect to hazardous compounds and their potential toxicity. The early approach for wastewater analysis and monitoring for toxicity was on a chemical-specific basis. The chemical specific approach alone has many shortcomings, including the inability to identify synergistic effects or the bioavailability of the toxin(s). The more contemporary whole-effluent approach to toxicity involves also the use of toxicity tests to measure the toxicity of wastewaters [4].

Industrial effluents are often contaminated by a multitude of chemicals. Since often no a priori knowledge of relevant toxicants exists, chemical analysis alone is not an appropriate tool for treatability assessment. Instead, a linkage of effect data and hazardous compounds is required. For that purpose, the concept of toxicity-directed wastewater approach was developed, which is based on a combination of fractionation procedures, bioassays and chemical analytical methods [5]. Identification of chemical classes associated with the measured biological endpoint is frequently achievable [6]. Thus, if the goal is to obtain information for effective effluent toxicity reduction with a given treatment or pretreatment, toxicity-directed wastewater approach can be the best available option.

A protocol for toxicity identification evaluation (TIE), which consists of a series of fractionation procedures followed by a bioassay

* Corresponding author at: R. Monteiro Lobato 80, Cep 13083-862, Campus Zeferino Vaz, Campinas, SP, Brazil. Tel.: +55 11 3521 2173; fax: +55 11 3521 2173.

E-mail address: caffaro.filho@gmail.com (R.A. Caffaro-Filho).

that are carried out systematically to determine the sources of effluent toxicity and the potential causative toxicant(s), was proposed by the United States Environmental Protection Agency (USEPA) [7] for complex aquatic mixtures such as industrial effluents. Wastewater fractionation is also the first step of a protocol for toxicity reduction evaluation (TRE) of effluents to POTW [8]. Since TRE tests provide information about the physical and chemical properties of the most significant toxicants in a given wastewater, this knowledge aid the evaluation of control techniques both for its treatment and pretreatment [9].

Fractionation procedures are simple techniques, requiring relatively basic reagents and equipment. If applying fractionation with the aim of selecting treatment strategies, one must keep in mind what will be feasible and cost-effective in large scale. Hazardous organic and inorganic pollutants can be removed from effluents via the following mechanisms: biodegradation, sorption, volatilization, chemical oxidation and chemical flocculation [4]. Nonpolar organic pollutants tend to adsorb to wastewater biomass. Potentially toxic metals can be removed by chelating agents, such as ethylenediaminetetraacetic acid (EDTA). Air stripping can remove volatile organic compounds (VOC) and ammonia. Chemical coagulation, flocculation and sedimentation can remove heavy metals and trace organics via adsorption. There are simple fractionation procedures that can achieve results similar to these mentioned pretreatment processes [10].

There are several possible bioassays that can be used in toxicity-directed analysis and the right choice is critical for assuring representativeness of results. The most widely used and studied biotests for wastewater toxicity evaluation for aerobic treatment are bioluminescence and respirometric methods, mainly because they are practical and have short-term duration. The bioluminescence method most thoroughly used is the Microtox[®] assay (Azur Environmental, Carlsbad, CA). The Microtox[®] assay is based on the naturally occurring luminescent marine bacterium *Vibrio fischeri*. The luminescence of *V. fischeri* is repressed in the presence of toxicants, and thus it responds to toxicants in a "lights-off" fashion [1]. The basis for respirometric tests is that the respiration rate of activated sludge or sludge organisms can be reduced in the presence of toxicants. The most common measure of the bacterial respiration rate is the oxygen uptake rate. Many respirometric methods are well-established and several standardized tests have existed for a long time. One example is the Organization for Economic Co-operation and Development (OECD) no. 209 inhibition test [11].

Using a toxicity-directed approach, this work sets the base for a rational treatability study of polyester resin manufacturing wastewater. Specific literature on this kind of effluent is rare and mainly based on chemical-specific analysis [12]. Relevant physical and chemical characteristics were determined. Respirometry was used for toxicity reduction evaluation after physical and chemical effluent fractionation. Information about the physical and chemical properties of the most relevant toxicant(s) to biological treatment was retrieved. These results led to valuable directions for conducting treatability studies which will be grounded on actual effluent properties rather than empirical or based on the rare specific data on this kind of industrial wastewater.

2. Materials and methods

2.1. Effluent sampling

The effluent consists of water produced by esterification reactions which differ in raw materials. This water, since formed in contact with reactants and products, is heavily contaminated and is inherent in producing the product [13]. Each polyester resin

reaction is made according to commercial demand and generates respective process water. The characteristics of process waters from different resins produced can vary depending on reaction conditions and raw materials. Different reactions for same polyester resin production generate basically the same process water. In order to assure sample representativeness, a survey of year-round polyester resin production by type was made, since production is directly correlated with effluent generation. Eight different process water lines were chosen for effluent sample composition. Six of them were responsible for approximately 70% of overall production, in slightly different proportions. Two other process water lines were selected for effluent sample composition because they were suspected to be more toxic than the average because of higher organic content (as measured by COD) and preliminary treatability results. These two water lines together were responsible for approximately 13% of overall year production. Hence, the eight process water lines chosen were responsible for more than 80% of total year production. None of the process waters that were left out of sample composition were responsible for more than 3.5% of total year production. For effluent sample composition, the process waters lines were mixed in proportion to their respective year production contribution. The two process water lines supposed to be more toxic were mixed in proportion to their maximum month production observed (12 and 10%). The process water lines were kept separate under refrigeration. Effluent sample composition was always made immediately before any analysis or fractionation procedure.

2.2. Physical and chemical analysis

Unless otherwise stated, all physical and chemical analyses were made accordingly to Standard Methods [14]. Chemical oxygen demand (COD) analyses were made by the open reflux method. Since effluent contains toxic pollutants and its production occurs at high temperature (200 °C), it was assumed that seeding would be necessary for effluent biochemical oxygen demand (BOD) analysis. Seeding was made through inoculation of BOD dilution water with 0.2 mL L⁻¹ of activated sludge adapted for a month to increasing concentrations of effluent up to 20% (v/v).

The following analytical methods were used:

- Phenols: chloroform extraction method and the 4-aminoantipyrine colorimetric method;
- Nitrogen (ammonia): distillation, titrimetric method;
- Nitrite: colorimetric method;
- Nitrogen (organic): Macro-Kjeldahl method;
- Total phosphorus: sulfuric acid/nitric acid digestion + ascorbic acid method;
- Total reactive phosphorus: ascorbic acid colorimetric method;
- Oil and grease: Soxhlet extraction method;
- Solids: gravimetric method.

Conductivity was measured by an Orion conductivity meter, model 150.

Gas chromatography/mass spectrometry (GC-MS) was used for analysis of volatile organic compounds (VOCs). Two aliquots of wastewater were separated. One of them had its pH adjusted to 3 and the other one to 11. 10 mL of sample were transferred to a 24 mL extraction vial (Chromatography Research Supplies) sealed with a silicon/PTFE septum. VOCs were extracted by solid phase micro-extraction (SPME) in vial headspace, using a 10 mm long Carboxen-PDMS coated fiber with 75 μm film thickness (Supelco, Bellefonte, PA, USA). The SPME fiber was pre-conditioned at 280 °C for 60 min in the GC injector. After immersion of the vial with sample in a bath at 30 °C for 15 min, the needle of the SPME system was introduced in the vial through a septum and exposed to the

headspace for 45 min at 30 °C, with magnetic stirring. The fiber was removed and inserted in the GC injector. VOCs were separated by gas chromatography (SHIMADZU, GC-17A) and detected by electron ionization (+70 eV) mass spectrometry (SHIMADZU, GC-MS QP-5000). Thermal desorption of VOCs from SPME fiber was made at 290 °C, in splitless mode for 2 min and after that period, the valve maintained a division of 1:20. The fiber was maintained in the injector for 10 min to eliminate memory effect. A DB-1 (30 m × 0.25 mm × 0.25 μm) nonpolar silica capillary column was used for compounds separation (J&W Scientific, Folsom, California, USA). Carrier gas was helium at 1.0 mL min⁻¹ constant flow rate. Column temperature was held at 30 °C for 5 min, then followed a temperature program from 30 to 80 °C at 1.5 °C min⁻¹ and from 80 to 200 °C at 6 °C min⁻¹, being held at 200 °C for 5 min. The GC-MS interface temperature was 240 °C. The mass spectrometer scan mode operation was 35–350 amu. Detector voltage was 1.2 kV.

2.3. Fractionation procedures

All the procedures conducted reflect feasible physical and chemical pretreatment options. The procedures were made immediately before biotesting or at the day before, according to required reaction time. All of them were performed at room temperature (≈25 °C):

- Neutralization: neutralization of approximately 200 mL of effluent was done through addition of 0.1 mol L⁻¹ NaOH solution, until the pH was 7.0 ± 1.0.
- Air stripping: air stripping was made in pH 3, 7 and 11 [7]. Approximately 200 mL of effluent were adjusted to the desired pH and left for approximately 15 h in 500 mL Erlenmeyers with magnetic stirring. Then, air was pumped through a porous rock for 45 min (≈3 L min⁻¹ rate). Before biotesting, the pH was adjusted to 7.0 ± 1.0.
- Activated carbon adsorption and filtration: a 200 mL aliquot of effluent was left for approximately 15 h in a 500 mL Erlenmeyer with magnetic stirring after addition of 2 g L⁻¹ of powdered activated carbon (PAC). After that, the Erlenmeyer content was filtered through a GF/C glass fiber filter with 1.2 μm particle retention size (Schleicher & Schuell).
- EDTA chelation: a 200 mL aliquot of neutralized effluent was magnetically stirred for 10 min in a 500 mL Erlenmeyer after addition of 2.0 g L⁻¹ of EDTA sodium salt. The sample was not filtered before biotesting.

2.4. Respirometric toxicity tests

The biologic material used was the mixed liquor of a laboratory sequencing batch reactor with 3 L of working volume. This reactor was filled with mixed liquor from an activated sludge pilot plant treating domestic sewage and maintained for 4 days, the time necessary for performing all the tests. At the end of each day, mixing and aeration were stopped. After decanting, half of reactor volume was discarded and the same volume of fresh media was added. Media consisted of yeast extract and peptone in concentrations of 500 and 150 mg L⁻¹, respectively. The toxicity test procedure is an adaption of the OECD no. 209 method [11]. Assays were carried out as batch tests in 500 mL glass bottles at room temperature. The pH was 7.0 ± 1.0. The measurement of dissolved oxygen for oxygen uptake rate (OUR) calculation was made by the membrane electrode method with a YSI Model 55 Handheld Dissolved Oxygen System (Yellow Springs, Ohio), following the manufacturer instructions for calibration. Each assay had a working volume of 400 mL, being 200 mL of reactor mixed liquor and 200 mL of control or test solution. Air was pumped through a porous rock (≈3 L min⁻¹ rate) and mixing was achieved by magnetic stirring. After addition of

mixed liquor and control or test solution, the vessel was aerated until dissolved oxygen was close to saturation. Then, aeration was stopped and values of dissolved oxygen were registered at 1 min intervals, during the following 10 min. Control solution consisted of yeast extract and peptone in the same concentration of reactor feeding. Test solutions consisted of: baseline effluent; effluent after neutralization; air stripped effluent in pH 3, 7 and 11; effluent after activated carbon adsorption and filtration; and effluent after chelating agent addition. Given the OUR as the slope of dissolved oxygen concentration by time, it is possible to establish, for all the tested solutions, instantaneous inhibition effect (*I*) of the respiration rate occurring in the control [15]:

$$I(\%) = \left(\frac{1 - \text{OUR}_t}{\text{OUR}_c} \right) \times 100 \quad (1)$$

where OUR_t is the tested solution OUR (whether baseline or fractionated effluent) and OUR_c is the control OUR. The comparison of the baseline effluent (*I_b*) and fractionated (*I_f*) solutions inhibition effects obtained with Eq. (1) gives the inhibition effect reduction (IR) due to physical and chemical treatment:

$$\text{IR}(\%) = \left(\frac{1 - I_f}{I_b} \right) \times 100 \quad (2)$$

In order to assess the magnitude of effluent inhibition, different concentrations of the baseline effluent were tested on the first experiment day. A concentration that had an inhibition effect close to 50% was adopted for the next two test days, for baseline effluent testing and also for fractionated solutions. Tap water was used for dilution. The volatile suspended solids (VSS) of each testing was not measured. Precautions were taken so that the amount of biomass was always the same when all comparisons were made. This was achieved through repeating control and baseline effluent testing immediately before each battery of fractionated solutions tests at any given day of experiment.

3. Results and discussion

3.1. Physical and chemical effluent characterization

The composite effluent was clear, lightly yellow, with no visible suspended solids or floating material. Table 1 shows the results of the effluent physical and chemical characterization analyses. Nutrients (N and P) were analyzed to assess if they would have to be added when conducting a treatability study.

Table 1
Physical and chemical effluent characterization

Parameter	Unit	Result
pH	–	3.0 to 3.1
Total solids (TS)	mg L ⁻¹	386 ± 15
Volatile solids (VS)	mg L ⁻¹	276 ± 23
Fixed solids (FS)	mg L ⁻¹	60 ± 10
Total suspended solids (TSS)	mg L ⁻¹	4 ± 1
Volatile suspended solids (VSS)	mg L ⁻¹	3 ± 1
Fixed suspended solids (FSS)	mg L ⁻¹	1 ± 0.5
Conductivity	μS cm ⁻¹	94 ± 1
Chemical oxygen demand (COD)	mg O ₂ L ⁻¹	83.933 ± 2.050
Biochemical oxygen demand (BOD ₅)	mg O ₂ L ⁻¹	13.508 ± 793
Oil and grease	mg L ⁻¹	16 ± 2
Phosphorous	mg L ⁻¹	1 ± 0.2
Reactive phosphorous	mg L ⁻¹	<0.2
Nitrogen (ammonia)	mg NH ₃ -NL ⁻¹	4.7 ± 0.6
Nitrogen (nitrite)	μg NO ₂ ⁻ -NL ⁻¹	11 ± 1
Total Kjeldahl nitrogen	mg NL ⁻¹	8 ± 1
Phenols	μg phenol L ⁻¹	122 ± 4

Three samples analyzed for each parameter.

Important information is given by the results from physical and chemical characterization. The BOD5/COD ratio is 0.16, indicating that the readily assimilable organic matter is only a small fraction of the total organic content of the effluent. The results also show a C:N:P ratio (C as BOD5) of 100:0.06:0.007. Nutrient addition will be needed for effluent biological treatment.

However, bottom line conclusions about the sources effluent toxicity are not possible to be drawn from this physical and chemical characterization.

Phenols and their derivatives are well known organic toxicants in organic chemical industry effluents [13,16], but they were not significantly detected by the method used. If detected, a more elaborate phenol analysis (by chromatography) would have been done.

As expected, condensation process waters had almost no salt content (as deduced by the extremely low conductivity) and also low solids content. The high organic content is typical of organic chemical industry effluents, as seen by the high COD and BOD.

3.2. Respirometric tests

In Table 2 the results of the respirometric toxicity tests of each effluent fraction are shown.

The baseline effluent concentration used in toxicity tests should be close to the IC50 (concentration causing 50% respiration inhibition in relation to the control OUR) [9]. The IC50 in the first experiment day was between 25 and 50% of effluent (v/v), since these tested concentrations inhibited the OUR in 31.1 and 67.5%, respectively. The effluent concentration chosen for testing on subsequent days was 40%, which inhibited the OUR in 63.2% on the second day of experiment and in 66.4% on the third day. On the fourth and last day of experiment, the baseline effluent concentration was decreased to 30% (v/v) and it inhibited the OUR in 63.7%. These data suggest an increase in toxic effects caused by the same concentration of effluent as the days went by.

The increase in toxic effects could be explained either by a decrease or a weakening of the biomass in the laboratory reactor, after several aliquots taken for the tests. There was probably no time for all species reposition by growth in less than 24 h between each battery of tests. However, this fact did not affect the outcome of the tests, since at the beginning of each battery of tests in each day, control and baseline effluent were tested again, and subsequent tested fractioned solutions were compared with that results, obtained at that same day. This guaranteed that the biomass present in compared tests had the same concentration and characteristics.

This approach was, in our opinion, better than using specific oxygen uptake rate (SOUR) for comparisons. SOUR is not grounded on the nature of the microbial community, but only in indirect biomass quantification (VSS), and it is well known that bioreactors are very dynamic systems [17]. Not using SOUR and VSS analyses possibly avoided deviations caused by indirect biomass quantification.

The effluent, being condensate water resultant of polymerization reactions, is probably easily buffered by the mixed liquor

contents. This can be the reason why neutralization of baseline effluent had almost no effect for toxicity reduction.

EDTA chelation fractionation test, which can indicate the presence of toxic metals [10], had practically no toxicity reduction effect. It appears that potentially toxic metals which are present in reaction catalysts are not present in effluent in an acute toxic concentration or were already complexed. Besides the EDTA sodium salt added (2.0 g L^{-1}), the toxic metals could also adsorb on mixed liquor biomass. However, heavy metals, even in very low wastewater concentrations, can accumulate on biomass and eventually get to a toxic concentration, causing plant upset. But this phenomenon can only be assessed in long term pilot plant studies.

Adsorption in PAC followed by filtration had little toxicity reduction. The filtration test gives an indication of whether the causative toxicant(s) is associated with solids. Thus, it appears that toxicants were not present significantly in the suspended solids. Some high log Kow organic toxicants such as aromatics may be significantly involved in wastewater toxicity of industries of the same Standard Industrial Classification System (SIC) and subcategorization of polyester manufacturing industries [16]. This do not appear to be the case in the effluent tested, given the low toxicity reduction observed with PAC treatment, since aromatics are known to be removed by adsorption in activated carbon [18]. However, if these organic toxicants are present even in acute subtoxic levels, they can also accumulate in sludge, causing the same problems as heavy metal accumulation. Again, long term studies should be performed to address this kind of potential hazard.

Air stripping tests provide information about whether the effluent toxicity is caused by volatile or oxidizable compounds. Since changing of pH can change chemical characteristics of compounds, this test was carried out at ambient, acid and basic conditions [7]. The most relevant toxicity reduction was observed with this type of fractionation technique. Air stripping at pH 3 (close to the effluent's original pH) reduced toxicity in 13.3%. There was a positive correlation between increasing values of pH and toxicity reduction. However, this correlation was not linear. From pH 3 to 7 there was a toxicity reduction surplus of roughly 5% and with another four pH units increase, toxicity reduction increased more than 44%. The result observed with this last procedure was remarkable, since this fraction is related to a decrease of approximately 60% in effluent toxicity. Thus, the toxicant(s) eliminated with air stripping in pH 11 responded for a very significant fraction of effluent instantaneous toxic effect to unadapted activated sludge.

3.3. Suppositions about identity of toxicant(s)

Suppositions were made about the identity of the effluent toxicant(s) eliminated with basic pH air stripping. Oxidizable compound hypothesis was initially discarded because the same amount of oxygen was pumped in the three air stripping tests. If oxidizable compounds were responsible for the observed toxic effects, there would probably be a more similar toxicity reduction among the air stripping tests.

Ammonia is a toxic pollutant that exhibits the physical and chemical properties that would lead to its exclusion by air stripping in basic conditions. It is commonly found and frequently removed by stripping in refinery wastewaters. While acidification fixes it in form of ammonium ion, in more basic pH it is volatilized as the gas ammonia [19]. However, the concentration of nitrogen (ammonia) found in the effluent ($4.7 \pm 0.6 \text{ mg NH}_3\text{-NL}^{-1}$) was very low and thus insufficient to cause the observed toxic effect [20]. The ammonia hypothesis was then discarded.

Another possibility was investigated, whether the toxicant(s) was volatilized or it was only hydrolyzed in alkaline conditions and

Table 2
Effluent fractions respirometric test results

Fraction	OUR _t	r ²	OUR _c	I _f (%)	I _b (%)	IR (%)
Neutralization	0.423	0.9991	1.114	62.0	63.7	2.7
Air stripping/pH 3.0	0.143	0.9995	0.337	57.6	66.4	13.3
Air stripping/pH 7.0	0.154	0.9979	0.337	54.3	66.4	18.2
Air stripping/pH 11.0	0.253	0.9969	0.337	24.9	66.4	62.5
PAC adsorption and filtration	0.129	0.9998	0.337	61.9	66.4	6.8
EDTA chelation	0.399	0.9995	1.114	64.2	63.7	-0.8

OUR_t: tested solution oxygen uptake rate; OUR_c: control oxygen uptake rate; I_f: inhibition of control respiration by fraction; I_b: inhibition of control respiration by baseline effluent; IR: toxicity reduction by fractionation procedure.

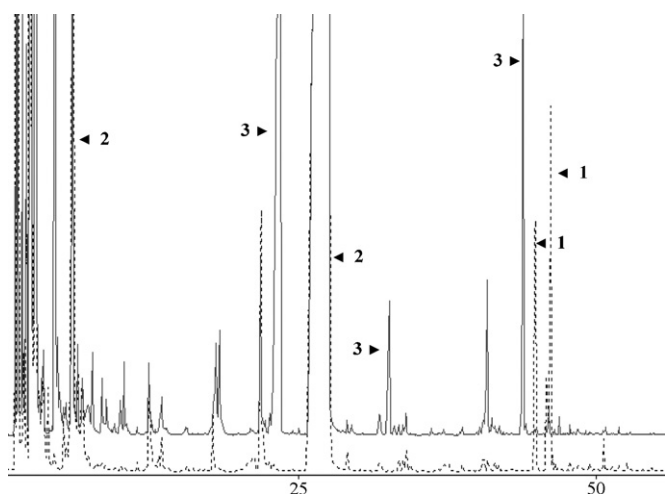


Fig. 1. Superposition of effluent headspace chromatograms in pH 3 (traced line) and in pH 11 (continued line). [1] VOCs stripped in pH 3 but not significantly stripped in pH 11; [2] VOCs stripped in both pH 3 and 11; [3] VOCs stripped only in pH 11.

lost its toxicity. One more respirometric assay, in exactly the same conditions previously described, was made with an effluent sample that had its pH elevated to 11 and after mixing was neutralized. The result was a toxicity reduction of less than 3% and this hypothesis was also discarded.

The remaining and most consistent hypothesis for toxicant(s) identity was that it was a volatile organic compound which is poorly stripped in acid to ambient pH and volatile at basic pH. It is more likely that a group of VOCs were responsible for the toxic effect observed. In fact, GC–MS analysis showed different VOCs in pH 11 which were not detected in pH 3 (Fig. 1). These are potential responsible for toxicity in the studied wastewater.

3.4. Confirmation of toxic effects to acclimated biomass

Questions rose if there would be any change in the observed results if the activated sludge biomass was adapted first to the effluent, and whether the results were reproducible in different conditions. Another sample collection and effluent composition was made in the same way as already described, 5 months after the first results were obtained. Biomass from the same pilot plant activated sludge was again brought to laboratory and incubated in an orbital shaker for 4 weeks, since laboratory reactor conditions previously described favored toxics volatilization. The culture was fed only with OECD synthetic sewage [11] in the first week. Besides synthetic sewage, the culture was fed with increasing concentrations of composite effluent in subsequent weeks, up to 20% (v/v) at the last week, when COD removal achieved more than 90% in 6 days. This adapted biomass was then used in respirometric assays both with the recently collected effluent (new sample) and the one used in the previous tests (former sample). The conditions were similar as already described except that control test was fed with OECD concentrated synthetic sewage in the same F/M ratio (as COD) of the baseline effluent test, something that was not assured in the first experiments. OECD synthetic sewage was also present in test solution bottles, in concentration (as COD) similar to sewage, to meet adequate nutrient requirements of biomass which could not be met by effluent alone. Finally, only air stripping fractionation procedures in pH 3 and 11 were tested. Baseline effluent and fractionated solutions were tested in a concentration of 40% (v/v), which was very close to IC50 for the former sample, but for the new sample, it inhibited in 66% the observed control OUR. The results are shown in Table 3.

Table 3
Effluent fractions respirometric test results of second experiment

Fraction	OUR _t	r ²	OUR _c	I _f (%)	I _b (%)	IR (%)
Air stripping/pH 3 (former sample)	0.125	0.9983	0.237	47.3	54.3	12.9
Air stripping/pH 11 (former sample)	0.164	0.9991	0.237	30.8	54.3	43.3
Air stripping/pH 3 (new sample)	0.129	0.9989	0.237	45.6	66.0	30.9
Air stripping/pH 11 (new sample)	0.155	0.9994	0.237	34.6	66.0	47.6

OUR_t: tested solution oxygen uptake rate; OUR_c: control oxygen uptake rate; I_f: inhibition of control respiration by fraction; I_b: inhibition of control respiration by baseline effluent; IR: toxicity reduction by fractionation procedure.

Baseline effluent toxicity was still observed with acclimated biomass. Air stripping in basic pH again showed to reduce about half of the baseline effluent toxicity for both samples. Thus, toxicity reduction with this fractionation procedure could be reproduced and the VOCs stripped in basic conditions were still significantly toxic even to adapted activated sludge.

There was an increase in toxicity reduction with air stripping in pH 3 in the new sample compared to the former sample. That could be explained by the maintenance of the former sample in laboratory for several months. Since the original pH of the process waters is acid, toxic compounds which are volatilized in acid pH may have already been gone from the former sample, even in tight capped containers. However, even in the new sample, stripping in basic conditions was still more effective in toxicity reduction.

3.5. Treatability study directions

Wastewater treatment of organic chemicals, plastics and synthetic fibers industries often requires combination of several stages of conventional as well as advanced methods of treatment, being air stripping one of the recommended technologies [16]. Pretreatment is especially important in treating organic chemical plant effluents.

Although final identification of compounds causing toxicity in the wastewater is of great interest, it is not essential for achievement of the initial objective proposed, which was to obtain information for effective effluent toxicity reduction with a given fractionation procedure. For that purpose, toxicity-directed wastewater approach was a very effective tool.

The information retrieved strongly suggests that aerobic biological treatment of polyester manufacturing wastewater would be very difficult without any pretreatment. Results obtained suggest that air stripping after pH adjustment to basic conditions can be effective for toxicity reduction to aerobic biomass. The design of treatability studies on this kind of wastewater should take into account this information.

In treatability studies of effluents specific to esterification processes in synthetic resins industry, chemical oxidation techniques were suggested to provide partial oxidation and in this way, to enhance wastewater biological treatability [21]. Wet air oxidation was tested as pretreatment of a variety of wastewaters, including polymer manufacturing [22].

Advanced methods of oxidation combined with biological treatment were adopted in a treatability study of a polyester manufacturing effluent very similar to the one described in this work [12]. It was concluded that it was technically possible but inevitably costly, due to expensive chemicals requirements for chemical oxidation.

None of the above mentioned treatability studies assessed the potential toxic effects of effluent fractions to biomass. By using this toxicity-directed approach, one can avoid empiricism and the difficulty of finding specific literature on the effluent being studied. Treatability studies design can be based on relevant data retrieved

from the particular wastewater that is being studied. This can be very valuable in organic chemical industry wastewater treatment, which should be addressed in a case-by-case manner [13]. Besides, the data that led to valuable pretreatment directions for the effluent studied was obtained by simple, inexpensive and rapid procedures that could be used by almost any laboratory around the world since sophisticated equipment is not required.

The volatile compounds that enter wastewater treatment plants are a potential health threat to plant operators [9]. In this particular case, they showed to be toxic even to the potential plant biomass. Many other toxicological studies should be conducted to accurately assess the risks, but the removal of toxic volatile compounds from this wastewater is recommended before entrance in any kind of aerated system to prevent serious health consequences that may occur due to their volatilization.

4. Conclusions

- The toxicity-directed approach was a very effective tool for reaching the initial objectives of the study, which was to gain knowledge of feasible and efficient means for reducing effluent toxicity to aerobic biomass.
- Air stripping in pH 11 reduced effluent toxicity in 62.5%. The toxicant(s) removed by this fractionation procedure were responsible for more than half of the observed inhibition to unadapted activated sludge. Since ammonia and alkaline hydrolysis were discarded, the toxicants were supposed to be volatile organic compounds poorly or not stripped in acid conditions.
- The toxicants stripped in pH 11 were significantly toxic even to adapted activated sludge.
- By using a toxicity-directed approach, both empiricism and the difficulty of finding specific literature on the particular kind of wastewater studied could be avoided for designing treatability studies.
- It was possible to retrieve relevant information on effluent properties by the use of procedures, tests and analyses that are simple, inexpensive and not time consuming.

Acknowledgements

The present work was supported by the Coordination of Superior Level Staff Perfectioning (CAPES) of the Education Ministry of Brazil. We thank Prof. Rosana Filomena Vazoller (Biomedical Sciences Institute), from University of São Paulo, who greatly contributed to this work with her teaching, direction and insights. Thanks also to Prof. Ranulfo Monte Alegre (Food Engineering Department, FEA, State University of Campinas) for kindly lending the dissolved oxygen probe.

References

- [1] S. Ren, Assessing wastewater toxicity to activated sludge: recent research and developments, *Environ. Int.* 30 (2004) 1151–1164.
- [2] United States Environmental Protection Agency, Fate of priority pollutants in publicly owned treatment works, Final report, v. 1–2, USEPA, Washington, DC, 1982.
- [3] United States Environmental Protection Agency, EPA'S National Pretreatment Program, 1973–2003: Thirty Years of Protecting the Environment, Office of Water, USEPA, Washington, DC, 2003.
- [4] G. Tchobanoglous, F.L. Burton, H.D. Stensel, *Wastewater Engineering, Treatment and Reuse*, Fourth ed., Metcalf and Eddy, Inc. McGraw Hill, Boston, 2003.
- [5] W. Brack, Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? *Anal. Bioanal. Chem.* 377 (2003) 397–407.
- [6] L.M. Hewitt, C.H. Marvin, Analytical methods in environmental effects directed investigations of effluents, *Mut. Res.* 589 (2005) 208–232.
- [7] United States Environmental Protection Agency, Methods for Aquatic Toxicity Identification Evaluation—Phase I, Toxicity Characterization Procedures, USEPA, Duluth, MN, 1988.
- [8] United States Environmental Protection Agency, Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants, Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, Ohio, 1989.
- [9] G. Bitton, *Wastewater Microbiology*, Wiley-Liss, Inc., New York, NY, 1994.
- [10] W.S. Hall, Introduction to toxicity identification and toxicity reduction evaluations, in: D.L. Ford (Ed.), *Toxicity Reduction: Evaluation and Control*, Technomic Publishing Company Inc., Lancaster, PA, 1992, pp. 17–30.
- [11] Organization for Economic Co-operation and Development, Activated sludge, respiration inhibition test, Guidelines for Testing of Chemicals, OECD Method 209, Organization for Economic Co-operation and Development, Paris, France, 1984.
- [12] S. Meriç, I. Kabdasli, O. Tünay, D. Orhon, Treatability of strong wastewaters from polyester manufacturing industry, *Water Sci. Technol.* 39 (1999) 1–7.
- [13] P.S. Minor, Organic chemical industry's waste waters, *Environ. Sci. Technol.* 8 (1974) 620–625.
- [14] L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), *Standard Methods for the Examination of Water and Wastewater*, 20th ed., American Public Health Association/American Water Works Association/Water Environment Federation, United Book Press, Inc., Baltimore, Maryland, 1998.
- [15] L. Mrafkova, D. Goi, V. Gallo, I. Colussi, Preliminary evaluation of inhibitory effects of some substances on aerobic and anaerobic treatment plant biomasses, *Chem. Biochem. Eng. Q.* 17 (2003) 243–247.
- [16] United States Environmental Protection Agency, Development Document for Effluent Limitations Guidelines and Standards for the Organic Chemicals and Plastics and Synthetic Fibers, Point Source Category, vol. I, USEPA, Washington, DC, 1987.
- [17] A. Fernández, S. Huang, S. Seston, J. Xing, R. Hickey, C. Criddle, J. Tiedje, How stable is stable? Function versus community composition, *Appl. Environ. Microbiol.* 65 (1999) 3697–3704.
- [18] G.J. O'Brien, Estimation of the removal of organic priority pollutants by the powdered activated carbon treatment process, *Water Environ. Res.* 64 (1992) 877–883.
- [19] W.W. Eckenfelder Jr., *Industrial Water Pollution Control*, Third ed., McGraw Hill, Boston, 2000.
- [20] P.B. Dorn, Case histories—the petroleum refining industry, in: D.L. Ford (Ed.), *Toxicity Reduction: Evaluation and Control*, Technomic Publishing Company Inc., Lancaster, PA, 1992, pp. 183–224.
- [21] O. Tünay, S. Erden, D. Orhon, I. Kabdasli, Treatability of 2,4-D production wastewater, *Water Sci. Technol.* 30 (1994) 73–78.
- [22] D. Mantzavinos, R. Hellebrand, A.G. Livingston, I.S. Metcalfe, Reaction mechanisms and kinetics of chemical pretreatment of bioresistant organic molecules by wet air oxidation, *Water Sci. Technol.* 35 (1997) 119–127.