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Short Communication

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SUMMARY

The wide application range of antibiotics in human and veterinary medicine has led to large-scale dissemination of refractory and even toxic pollutants in the environment. These organic and inorganic compounds, considered to be the principal environmental concern in this sector, are generated during the synthesis and formulation steps of production. In the present study, the oxidative pre-treatment of penicillin formulation effluent containing Sultamicillin Tosylate Diydrate (molecular weight: 802.85 g/mol; initial COD = 685 mg/L; initial TOC = 199 mg/L) via ozonation at varying pH (3 - 11.5) and ozone feed rates (127 - 2750 mg/h) was investigated. The biodegradability of raw and pre-ozonated penicillin formulation effluent was also questioned in separate biotreatment experiments.

KEYWORDS: Penicillin formulation effluent, chemical pre-treatment, ozonation, activated sludge inhibition, biodegradability.

INTRODUCTION

Most known treatment practices and technologies have appeared to be inappropriate for wastewaters containing refractory and/or toxic chemicals such as pharmaceutical residues. Hence more advanced treatment processes, such as Fenton's oxidation, ozonation, H_2O_2 - assisted ozonation or photochemical degradation, have to be applied for the "destructive" pre-treatment of such wastewaters. A huge body of scientific literature has already been devoted to the integrated chemical and biochemical treatment of pollutants originating from different industrial sectors to remove recalcitrance and/or toxicity from wastewater [1-3]. However, some industrial pollutants require extreme doses of oxidants and/or extended reaction periods to achieve an acceptable degree of ultimate oxidation.

Toxicity control based on respirometry is an underdeveloped area in the field of activated sludge treatment systems. Respirometric techniques have been widely used to test the toxic effects of different pollutants on activated sludge processes. Until now, no practical application of a respirometry–based toxicity control of activated sludge treatment is currently being reported in the scientific literature [4-8].

The main purpose of this work was to demonstrate the applicability of ozonation as a pre-treatment process to improve the ultimate biodegradability of wastewater originating from the penicillin formulation process of a pharmaceutical industry. The biodegradability improvement of penicillin formulation effluent subjected to ozonation was evaluated using conventional and respirometric methods.

MATERIALS AND METHODS

All analyses of conventional parameters were performed as defined in the Standard Methods [9], except the COD measurements. They have been done as described in ISO 6060 [10]. For soluble COD determination, the samples were filtered through 0.45 μm membrane filters. The analytical survey also used Whatman GF/C glass-fibre filters for suspended solids (SS) and volatile suspended solids (VSS) measurements.

One L aliquots of penicillin wastewater were ozonated up to 60 min in a borosilicate glass bubble column at semi-batch mode, wherein the ozone/oxygen gas mixture was continuously sparged through a fritted dispersion disc with a diameter of 5 cm at a rate of 1.3 $L.min^{-1}$. Ozone was produced by a corona discharge PCI GL-1 model pilot scale ozone generator with a maximum capacity of 20 $g.h^{-1}$. Teflon tubing was used for all connections from the ozone generator to the reaction vessel. All excess (unreacted) gaseous ozone leaving the column was collected in two gas washing bottles connected in series and filled with 10% KI solution, whereas two other gas washing bottles with 2% KI solution were directly placed after the gas introduction line to determine and calibrate the O_3 input rates exactly. Beforehand, the exact ozone input of 10%

corresponding to an ozonation rate of 2760 $mg.L^{-1}h^{-1}$ was selected for the experiments. The ozone transfer efficiency (i.e. absorbed ozone O_{3A}, %) was determined by measuring the input and off-gas concentrations of each pre-ozonation experiment iodometrically [11]. The mass transfer coefficient of ozone in the semi-batch reactor was determined to be 1.0 min^{-1} in acidic pure water using the indigo spectrophotometric method. This method was also used for the determination of the residual, liquid-phase ozone in the reaction solution.

Biological treatability studies were performed with synthetic wastewater [12] as well as raw and ozonated penicillin formulation effluents. Three fill-and-draw reactors were simultaneously fed with synthetic wastewater as well as raw and ozonated samples. The sludge age and hydraulic retention time were set as 10 and 2 days, respectively, for a period of three months. Oxygen Uptake Rate (OUR) measurements were conducted with a Manoterm RA-1000 continuous-mode respirometer and a PC connection. The tests were started with biomass seeding alone to obtain the initial endogenous OUR level. The samples were added to the OUR reactor at the desired food-to-microorganism (F/M) ratio, and, thereafter, the OUR data were continuously monitored. A nitrification inhibitor (Formula 2533™, Hach Company) was added to the reactors working at neutral pH and ambient temperature of 16°C.

RESULTS AND DISCUSSION

Penicillin Formulation Effluent

The penicillin formulation effluent used in this study was wastewater from the ALFASID® formulation process (active ingredient: sultamicillin tosylate dihydrate; molecular weight: 802.85 $g.mol^{-1}$; chemical formula: $C_{25}H_{30}N_4O_9S_2.2H_2O$), which was supplied by a pharmaceutical company located in Istanbul, Turkey. The selected effluent corresponded to approximately 30 % of the total daily effluent (150 $m^3.d^{-1}$), of which 52 % was wastewater of domestic nature. The collected effluent was stored at 2-4 °C before use for up to four weeks. Prior to each experiment, the effluent suspensions were filtered through a glass fibre filter with a pore size of 1.2 µm to obtain a clear reaction solution. The penicillin formulation composition and corresponding effluent characterization (i.e. the mixture of all obtained formulation effluents) are summarized in Tables 1 and 2, respectively.

TABLE 1
Ingredients of the penicillin formulation ALFASID®.

Formulation content	Function
Sultamicillin tosylate dihydrate	active agent
Explotab dried	additive
Methocel E15	additive
Colloidal silicone dioxide	binding agent
Magnesium stearate	filling agent
Microcrystalline cellulose dried	binding agent

TABLE 2
Conventional characterization of the penicillin formulation effluent.

Parameter	Unit	Value
Total COD	$(mg.L^{-1})$	685
Soluble COD	$(mg.L^{-1})$	643
TOC	$(mg.L^{-1})$	199
BOD_5	$(mg.L^{-1})$	16
TKN	$(mg.L^{-1})$	85
TP	$(mg.L^{-1})$	11
pH	-	6.85
Alkalinity	mg $CaCO_3.L^{-1}$	55
Detergents	$(mg.L^{-1})$	25
Cl^-	$(mg.L^{-1})$	95
Colour*	cm^{-1}	0.010

* Measured as absorbance at wavelength λ = 436 nm.

Ozonation Experiments

Preliminary ozonation experiments have shown that significant COD and TOC removals were only obtained at elevated pH values (at pH 11.5) [13]as a consequence of enhanced ozone decomposition into free radicals (data not shown). It is believed that at elevated pHs the major oxidizing agent for the degradation of formulation effluent was •OH [14], since no residual ozone was detectable at pH>7.5 in the reaction medium. No attempts were made to control the pH of the formulation effluent during ozonation, which decreased to pH 7.3-7.5 at the end of the reaction period. Thus, no further pH adjustment was required prior to biodegradation tests.

At the end of the ozonation period 233 mg L^{-1} (34%) COD was removed and 742 mg L^{-1} of the applied O_3 was absorbed in the reaction solution (Figure 1). TOC abatement remained rather low (24% overall reduction) throughout the ozonation period; however, its decrease was actually not the purpose of this work.

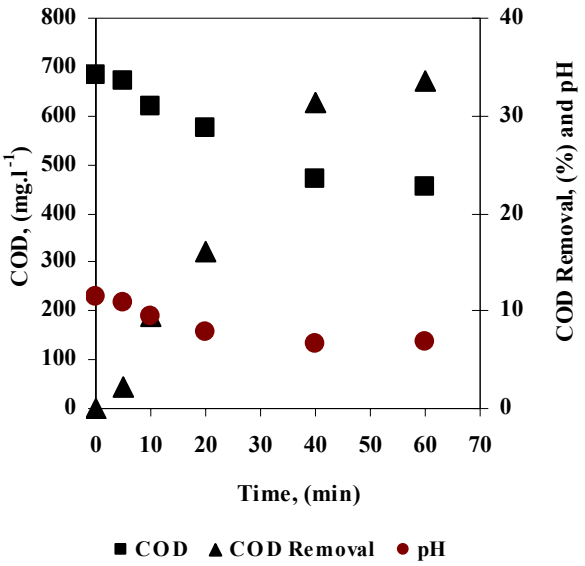

FIGURE 1 - COD and TOC removal during ozonation of penicillin formulation effluent at pH=11.5.

Biological Treatability Experiments

The biological treatability studies performed with biomass that was acclimated to wastewater containing raw penicillin formulation effluent showed that no degradation of the raw penicillin effluent fraction occurred. On the other hand, degradation of the synthetic wastewater fraction of effluent mixture containing the pre-ozonated penicillin effluent sample was inhibited by 25 %. Upon 40 min ozonation, the synthetic wastewater fraction in the mixed effluent could be completely oxidized and at the same time 35 % of the pre-ozonated penicillin formulation effluent was obtained (Table 3). In order to observe a shock-loading effect, a separate test was performed with biomass being acclimated to synthetic wastewater only. The results have indicated that the raw penicillin effluent inhibited the COD removal efficiency of the synthetic wastewater fraction by 16%. Moreover, no inhibitory effect was observed when the biomass being acclimated to synthetic domestic wastewater was pre-ozonated at pHs 7 and 11.

TABLE 3
COD removal efficiency obtained in the acclimated reactors.

Reactor No	Wastewater Type	Influent COD ($mg.L^{-1}$)	Removal Efficiency (%)	
			Synthetic	Penicillin
R1	Synthetic	600	97	-
R2	Synthetic + raw penicillin	1010 (870, 140)	73	0
R3	Synthetic + 40 min ozonated penicillin	840 (745, 95)	97	35

( ) Synthetic and penicillin wastewaters concentrations in the reactor, respectively

For respirometric evaluation, another test was performed with the acclimated biomass seeding alone to obtain the level of initial endogenous OUR. At the desired F/M ratios, three respirometric studies were carried out with synthetic domestic wastewater and the raw + 40 min pre-ozonated penicillin formulation effluent mixtures. The analysis of the OUR profiles gave parallel and reliable results. The inhibition of total oxygen consumption was found to be 22% for the wastewater mixture containing raw effluent, whereas no inhibition occurred for the wastewater with 40 min pre-ozonated effluent (Table 4 and Figure 2).

TABLE 4
Respirometric evaluation results.

Wastewater Type	COD	Total Consumed O_2	Inhibition	Removal of Penicillin Fraction
	($mg.L^{-1}$)	($mg.L^{-1}$)	(%)	(%)
Synthetic	300	150	0	-
Synthetic + Raw penicillin	420	117	22	0
Synthetic + 40 min ozonated penicillin	410	167	0	36

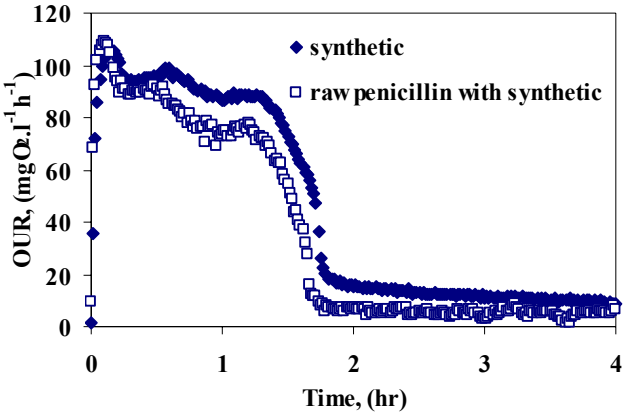

FIGURE 2 - Respirometric data obtained for batch experiments with synthetic wastewater and raw penicillin formulation effluent.

CONCLUSIONS

The study provides information about the biological treatability of raw and pre-ozonated sultamicillin tosylate diydrate-bearing penicillin formulation effluents. Ozonation was found to be an efficient pre-treatment process with 34% COD and 24% TOC removal at pH 11.5, with an increase in the BOD_5 value from 16 $mg.L^{-1}$ to 128 $mg.L^{-1}$ after 40 min of ozonation.

Biological treatability studies have shown that in addition to the non-biodegradable character of raw penicillin formulation effluent, synthetic wastewater degradation was also inhibited with the addition of penicillin effluent, possibly due to the generation of non-biodegradable chemical complexes.

Ozonation up to 40 min enhanced the biodegradation characteristics of penicillin formulation effluent. While the synthetic wastewater could be completely oxidized, pre-ozonated penicillin wastewater was found to have a COD removal rate of 35%.

Further research should be conducted on the assessment of specific inhibition kinetics for pharmaceutical industry wastewaters. The type and mechanism of microbial inhibition needs to be further studied in detail. The model calibrations should be done in order to determine inhibition kinetics for each industrial wastewater investigated.

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