

Biological treatability of raw and ozonated synthetic penicillin formulation effluent

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Abstract Chemical pre-treatment of synthetic Procaine Penicillin G (PPG) effluent with ozone (applied dose = 1440 mg/h; treatment duration = 60 min) at pH = 7 was investigated. Successive biological treatability studies were performed with raw, ozonated penicillin formulation effluent and synthetic readily biodegradable substrate as simulated domestic wastewater. The PPG effluent additions were adjusted to constitute approximately 30% of the total COD in the reactor. Ozonation of PPG effluent resulted in practically complete removal of the parent pollutant accompanied by 40% COD abatement. Speaking for the raw PPG effluent, prolonged acclimation periods were necessary to obtain significant COD removal efficiencies. Batch activated sludge treatment experiments and respirometric studies have demonstrated that the selection of true retention time is extremely crucial for having high amount of slowly hydrolysable substrate or complex wastewater, like pharmaceutical effluent. The effect of ozonation time on biological treatability performance of PPG has been evaluated in the study. Pre-ozonation of PPG effluent did not improve its ultimate biodegradability.

Keywords Activated sludge treatment; chemical oxidation; ozonation; penicillin formulation effluent; Procaine Penicillin G

Nomenclature

F/M ratio	Food to microorganisms ratio
OUR	Oxygen uptake rate [$\text{ML}^{-1}\text{T}^{-1}$]
$S_{AA\text{initial}}$	Initial acetic acid COD concentration [MCODL^{-1}]
$S_{AA\text{utilized}}$	Utilized acetic acid COD concentration [MCODL^{-1}]
$S_{T\text{initial}}$	Initial total COD concentration [MCODL^{-1}]
$S_{T\text{utilized}}$	Utilized total COD concentration [MCODL^{-1}]
$S_{WW\text{initial}}$	Initial wastewater COD concentration [MCODL^{-1}]
$S_{WW\text{utilized}}$	Utilized wastewater COD concentration [MCODL^{-1}]
Y_{HWW}	Growth yield on wastewater [MCODM COD^{-1}]
Y_{STO}	Storage yield on acetic acid [MCODM COD^{-1}]

Introduction

Recently, the presence of pharmaceuticals in sewage effluent – unmodified or in the form of metabolites – has been reported and documented as an emerging risk to the biotic environment (Anon (Editorial), 2000; Ternes *et al.*, 2003). Some available data on antibiotics indicate their capability to exert toxic effects to living organisms even in the ng/L – $\mu\text{g/L}$ concentration range (Halling-Sorensen, 2000). Among all other pharmaceutical drugs and substances, the literature on antibiotics is much more developed due to its serious irreversible effects on the aquatic and terrestrial environment (Kümmerer *et al.*, 2000; Reinthaler *et al.*, 2003).

The toxic effects of antibiotics on several organisms, e.g. bacteria, algae, water flea (*Daphnia magna*), have been found not only in high concentrations, but also in low

concentrations and in chronic tests. For instance, Richardson and Brown (1985) examined a number of antibiotics such as ampicillin, erythromycin, sulphamethoxazole, tetracycline and penicilloyl groups for their biodegradability during sewage treatment. Their study indicated that these antibiotics were practically non-biodegradable and had the potential to survive sewage treatment. This property poses a persistent of these compounds in the environment and potential for bio-accumulation (Wollenberger *et al.*, 2000). Studies concerning antibiotics in the aquatic environment have also clearly shown that elimination in municipal sewage treatment plant is often incomplete.

Chemical oxidation, especially ozonation, has already been demonstrated to be an effective means of removing refractory and/or toxic chemicals from water and wastewater (Masten and Davies, 1993; Scott and Ollis, 1995; Alvares *et al.*, 2001). Dozens of papers have postulated that ozonation can at least partially remove chemical oxygen demand (COD) and dissolved organic carbon (DOC) content of industrial pollutants if optimised properly with respect to pH, feeding rate and applied dose (Prado and Esplugas, 1998; Ghaly *et al.*, 2001; Balcioglu and Arslan, 2001; Chu and Wong, 2003; Pena *et al.*, 2003). For the installation of full-scale ozonation facilities, process kinetics have to be well known and optimised, otherwise ozonation would be exceedingly costly and time consuming to achieve a substantial degree of degradation. Ozonation is expected to be an effective tool for the partial oxidation of potentially refractory and/or toxic pharmaceuticals, for instance antibiotics. Until now, only few studies dealing with the ozonation of antibiotics in water and wastewater have been published (Zwiener and Frimmel, 2000; Balcioglu and Otker, 2002; Ternes *et al.*, 2003). None of these studies have investigated ozonation characteristics, i.e. process optimisation and kinetics, in detail.

Aerobic treatment systems have been employed for the treatment of pharmaceutical wastewater including activated sludge systems (Anderson, 1980; Bernard and Gray, 2000; Gohary *et al.*, 1995). Gohary *et al.* (1995) performed the biological treatment of wastewater from pharmaceutical and chemical company. The wastewater was very acidic and contained high concentrations of organic compounds and total solids. The results obtained indicated that biological treatment using extended aeration activated sludge or biological filters followed by activated sludge process significantly removed the organic pollutants in wastewater approximately 95 per cent of COD and BOD reduction. Rosen *et al.* (1998) also found that the biological treatment of chemical synthesis based pharmaceutical wastewater provided high removal of COD and toxicity.

In the present experimental work the biological treatability of raw and pre-ozonated Procaine Penicillin G (PPG) effluent has been investigated. PPG has been selected as the refractory model pollutants due to its low price and relatively high consumption rate. The treatment performance of ozonation has been evaluated by COD, active ingredient and characteristic UV absorbance measurements. The ultimate biodegradability of the compounds has been studied by performing biological treatability and respirometric tests that have been conducted using a mixed culture acclimated to PPG.

Materials and methods

Synthetic penicillin formulation effluent

The antibiotic formulation Procaine Penicillin G (PPG) injectable suspension (solubility in water: ≥ 100 g/L; activity units 300,000/mL; molecular weight of Penicillin G potassium salt = 372.5 g/mol; molecular structure = $C_{16}H_{17}KN_2O_4S$; molecular weight of Procaine = 236.3 g/mol; molecular structure = $C_{13}H_{20}N_2O_2$) was purchased from a pharmaceutical company located in Istanbul, Turkey (Figure 1).

Experimental analyses

All analyses on collective environmental parameters was performed as defined in *Standard Methods* (1998) except the COD measurements. COD measurements were performed as described in the method proposed by ISO 6060 (1986). For COD determination, samples were filtered through 0.45 μm membranes. The analytical survey also used Whatman GF/C glass-fibre filters for suspended solids (SS), and Volatile suspended solids (VSS) measurements. OUR measurements were conducted with a Man-term RA-1000 continuous respirometer with PC connection. Raw and ozonated PPG was traced using an iodometric back titration procedure as described in USP 26 – 425, (1998). The UV absorbance of raw and ozonated penicillin formulation effluent at the characteristic wavelength of PPG ($\lambda = 291 \text{ nm}$) was measured on a Shimadzu model double beam spectrophotometer in 1 cm quartz cuvettes.

The ozone reactor

The ozonation system of the present study comprised an 800 mL capacity borosilicate glass bubble column used as the reactor unit. A coarse sintered gas dispersion disc delivered the $\text{O}_3 + \text{air}$ mixture from the reactor bottom at a flow rate of 1.3 mL/min. Ozone was generated from air using a PL1 GI model pilot scale ozone generator with a maximum production capacity of 20 g/h O_3 . The applied ozone dose ranged between 600–2600 mg/min. For a typical run, the reactor was initially charged with PPG solution and thereafter continuously ozonated in semi-batch mode with respect to ozone introduction. The initial COD (COD_0) was adjusted to 600 mg/L if not otherwise indicated, however, for certain experiments, the PPG formulation effluent was prepared at different initial COD values ($= 200, 300, 400$ and 500 mg/L) in distilled, de-ionized water. The reaction pH adjustment was made with appropriate phosphate buffers to keep the reaction pH constant at the desired value. Teflon tubing was used for all connections from the ozone generator to the reaction vessel. All excess (unreacted) gaseous ozone leaving from the reactor top was collected in two gas washing bottles connected in series and filled with 10% KI solution in order to determine the amount of off-gas ozone production. Two other gas washing bottles filled with only 2% KI solution were placed in another ozonation line in order to determine the exact ozone input rate prior to adjustment of the appropriate ozone dose. Ozone was fed to one of the lines (input line or ozonation line) by means of a three-way valve. The ozone dose was set as 1440 mg/(L \times h) for all ozonation experiments. The input and off-gas ozone concentrations were determined iodometrically (IOA, 1987).

Ozonation experiments were principally carried out for 1 h, corresponding to ozone feeding rates varying between 720–2880 mg/h and to ozone doses in the range of 900–3600 mg/L (for $\text{COD}_0 = 600 \text{ mg/L}$). To maintain constant pH values throughout the ozonation experiments, synthetic PPG formulation effluent was prepared in pH = 7 (0.1 M KH_2PO_4 , 0.1 M NaOH) buffered, de-ionized water. Preliminary ozonation tests have confirmed that the pH did not change (decrease) more than +0.2 pH units throughout the experiments.

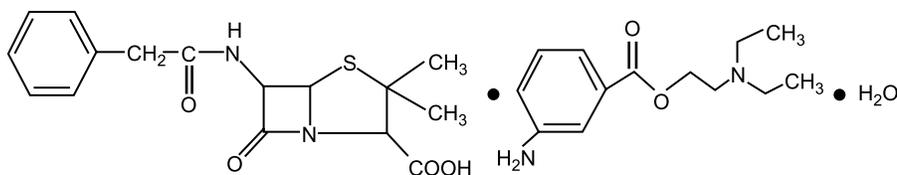


Figure 1 Molecular structure of Procaine Penicillin G

Biological reactors

Biological treatability studies were conducted in three laboratory-scale fill-and-draw reactors. The reactors were operated at a sludge age of 10 days and a hydraulic retention time (HRT) of one day for a period of 8 months. A synthetic wastewater consisting of an appropriate mixture of acetic acid, propionic acid, ethanol, glutamic acid and glucose has been prepared in accordance with Henze (1992) to simulate the readily biodegradable COD fraction in domestic sewage. This synthetic wastewater served as the main substrate in all biodegradation experiments. One of the reactors was fed with the synthetic wastewater alone, which was supplemented in the second reactor with PPG effluent, in the third reactor with the ozonated PPG effluent. The PPG effluent additions were adjusted to constitute approximately 30% of the total COD in the reactor.

The respirometric tests were started with the biomass seeding alone to obtain the initial endogenous oxygen uptake rate (OUR) level. At the desired F/M ratios, samples were added on the biomass in the reactor, and the OUR data was monitored.

Experimental results

Pretreatment with ozone

PPG formulation wastewater was first subjected to ozonation at $\text{pH} = 7$ and an ozone dose of 30 mM (applied feeding rate = 1440 mg/h). Ozonation was performed at $\text{pH} = 7$ as for the fact that penicillin formulation effluent pH is typically in the range of 6–8. In order to keep the reaction pH constant throughout the reaction, the aqueous PPG solutions were first buffered at $\text{pH} = 7$ using phosphate salt solutions. Figure 2 displays the changes in PPG (a), COD (a) content and PPG active ingredient as a function of ozonation time.

As was expected, the removal rate of PPG active ingredient and its functional groupings was appreciably faster than the COD abatement rate. The COD content of the wastewater decreased from 600 mg/L to 400 mg/L, whereas PPG removal was practically complete after applying an ozone dose of 1800 mg/(L h) to the wastewater.

Biological treatability studies

Three laboratory-scale fill-and-draw reactors with a retention time of 24 hours for a period of 8 months were operated for biological treatability experiments. Food and micro-

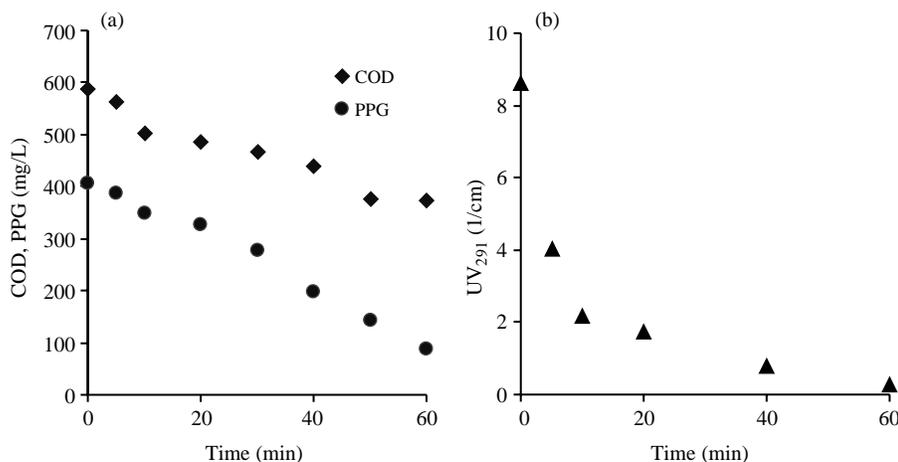


Figure 2 COD, PPG (a) and UV₂₉₁ (b) abatement during ozonation of PPG formulation effluent

organism ratios (F/M) were between 0.29–0.35 mg COD/mg VSS.day for three reactors acclimated to three different effluents. Control reactor was fed only synthetic wastewater with overall COD removal of 91% and a microbial product of 9%. For PPG and ozonated PPG reactors, 30% of PPG or ozonated PPG and 70% synthetic wastewater added in the reactors for initial COD. The performance data obtained through the study for PPG and ozonated PPG was shown that COD removal was over 80% for both. Figure 3 shows the changing of COD concentration versus time in a cycle when 600 and 1200 mg COD/l PPG effluent was fed into system. COD removal efficiencies for 8 hours were 34% and 43% respectively for PPG effluent of 600 and 1200 mg/l. However more than 80% of COD removal efficiency for 24 hours evaluation was obtained for both experiments. This shows that selection of true retention time is extremely crucial for having high slowly hydrolysable substrate or complex wastewater.

Additionally, respirometric tests were performed with relevant acclimated biomass (to PPG, ozonated PPG effluents), seeding alone to obtain the level of initial endogenous oxygen uptake rate (OUR). Several respirometric batch tests were conducted at desired F/M ratios, with only synthetic wastewater and PPG, ozonated (5, 20, 40, 60 minutes) PPG effluents added to the same amount of synthetic wastewater. In all respirometric tests, synthetic wastewater was arranged such as acetic acid is a sole carbon source (Table 1). For PPG experiment, the system was fed with only 1200 mg/l COD at the beginning. Hydrolysis of penicillin formulation wastewater occurred in the system in four steps (Figure 4). It is very obvious that the retention time is extremely important for this type wastewater. Removal efficiency is 53% for approximately 15 hours whereas in the acclimation reactor having 24 hrs retention time, the overall efficiency was 80% after very long acclimation period as more than 8 months.

Six sets of respirometric measurements were conducted using ozonated reactor sludge. These experiments included only synthetic wastewater, 5, 20, 40, 60 minutes ozonated penicillin formulation wastewater with synthetic wastewaters and finally just 20 minutes ozonated penicillin formulation wastewater (Table 1; Figure 5). It was observed that 5 and 20 min. ozonated wastewater with synthetic addition on the biological reactor gave an inhibition effect on maximum specific growth rate because of a decrease on the first plateau. But after a while the system recovered with synthetic wastewater removal efficiency of 100% and ozonated penicillin wastewater removal efficiency of more than 55% for both. There is no inhibition effect on maximum growth rate for 40 minutes ozonation. Approximately the same efficiency was obtained with the 5 and 20 minutes ozonation. There is an inhibition effect for 60 minutes ozonation. The removal efficiency was decreased approximately 10%. 100% synthetic removal occurred

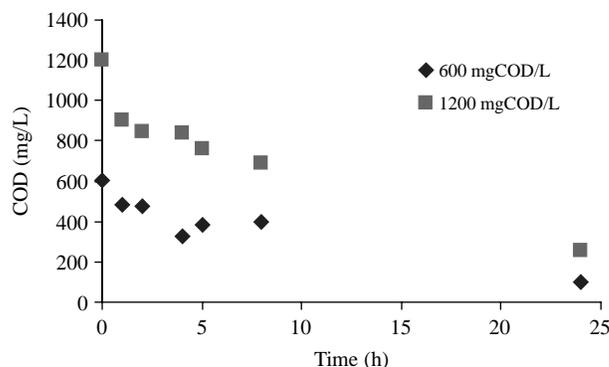


Figure 3 COD abatement rates in the biological reactor

Table 1 Evaluation of biological treatability performance of raw, ozonated PPG effluent compared with that of synthetic domestic wastewater control

Model Coefficient	Synthetic	PPG	Ozonated with t20	Synthetic with t5	Synthetic with t20	Synthetic with t40	Synthetic with t60
F/M (g COD/g VSS)	0.21	0.98	0.08	0.23	0.42	0.12	0.17
Y _{STO} (g COD/ g COD)	0.72	-	-	0.72	0.72	0.72	0.72
Y _{HWW} (g COD/ g COD)	-	0.65	0.65	0.65	0.65	0.65	0.65
S _{Tinitial} (mg COD/L)	435	1200	87	631	481	251	285
S _{Tutilized} (mg COD/L)	435	640	52	545	456	218	218
S _{AAinitial} (mg COD/L)	435	-	-	435	435	200	200
S _{AAutilized} (mg COD/L)	435	-	-	435	435	200	200
S _{WWinitial} (mg COD/L)	-	1200	87	196	46	51	85
S _{WWutilized} (mg COD/L)	-	640	52	110	28	18	18
Removal Efficiency for S _T (%)	100	53	60	86	87	87	77
Removal Efficiency for S _{AA} (%)	100	-	-	100	100	100	100
Removal Efficiency for S _{WW} (%)	-	53	60	56	60	35	21

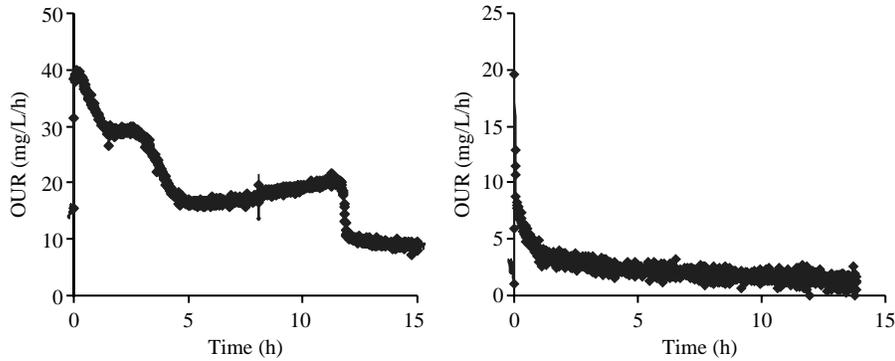


Figure 4 Respirometric data for raw and 20 min-ozonated PPG effluents

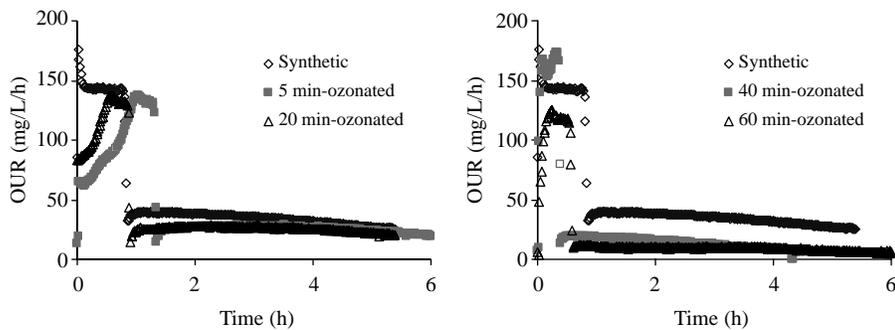


Figure 5 Respirometric data for 5, 20, 40, 60 minutes ozonated PPG and synthetic domestic effluent

and 21% ozonated wastewater removal has obtained. In conclusion, long terms of ozonation could cause inhibition, but after a while the system recovers itself. The same efficiencies (60%) were obtained for only 20 minutes ozonated and 20 minutes ozonated with synthetic wastewater.

Conclusion

In the present experimental work the biological treatability of raw and pre-ozonated Procaine Penicillin G (PPG) effluent has been investigated. Prolonged acclimation periods were necessary to obtain significant COD removal efficiencies for the raw PPG effluent. Biological treatability studies have demonstrated that the selection of true retention time is extremely crucial for complex wastewater bearing high amounts of slowly hydrolysable substrate, such as pharmaceutical effluent. COD removal efficiency is 34% for 8 hours and 53% for 15 hours activated sludge treatment, whereas in the acclimation reactor having 24 hrs retention time, the overall treatment efficiency was 80% after a long acclimation period.

Biological treatment of 5 and 20 min ozonated PPG effluent mixed with synthetic domestic wastewater caused inhibition of the maximum specific growth rate, but after a while the system recovered resulting in synthetic domestic wastewater removal efficiency of 100% and more than 55% COD removal efficiency for 5–20 min ozonated PPG effluents. No inhibition effect on maximum specific growth rate was observed for 40 min ozonated PPG wastewater, whereas further ozonation for up to 60 min caused decrease in treatment efficiency, emphasizing the need for ozone dose (ozonation time) optimization. In conclusion, in contrast to previous studies, ozonation did not improve the ultimate biodegradability of the investigated pharmaceutical effluent.

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