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# Biological treatability of raw and ozonated penicillin formulation effluent

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**Abstract**

In the present study, oxidative pre-treatment of pharmaceutical wastewater originating from the formulation of the penicillin *Sultamycillin Tosylate Diydrate* via ozonation at varying pH and ozone feed rates was investigated. Biological treatability studies were performed with a synthetic wastewater alone and supplemented with raw and ozonated penicillin formulation effluents. The highest COD (34%) and TOC (24%) removal efficiencies were obtained at pH 11.0, whereas the $BOD_5$ value increased from 16 mg $l^{-1}$ to 128 mg $l^{-1}$ after 40 min of ozonation, corresponding to an applied ozone dose of 1670 mg $l^{-1}$ and 33% relative ozone absorption. The studies showed that no degradation of raw penicillin fraction (30% of total COD) occurred, and degradation of the synthetic wastewater being completely treatable without penicillin addition, was inhibited by 7%. Upon 40 min ozonation, the synthetic wastewater could be completely oxidized and at the same time 35% of ozonated penicillin wastewater removal was obtained. Respirometric studies were conducted in parallel and produced results indicating a 22% decrease in the total oxygen consumption rate established for raw penicillin formulation effluent compared to the results obtained from the aerobic batch reactor. No inhibition of the synthetic fraction was observed for the 40 min-ozonated penicillin formulation effluent, biodegradability of the 60 min-ozonated penicillin effluent decreased possibly due to recalcitrant oxidation product accumulation. The modeling study provided experimental support and information on inhibition kinetics in activated sludge model no. 3 (ASM3) by means of respirometric tests for the first time.


*Keywords:* ASM3; Biological treatability oriented modeling; Ozonation; Penicillin formulation effluent; Substrate storage

## 1. Introduction

Bulk pharmaceutical substances consist of structurally complex organic chemical compounds that are produced within several steps under a variety of precise conditions [1]. Most of the substances involved in the chemical synthesis are listed as priority pollutants [2,3]. These organic and inorganic compounds that are considered as the principal environmental concern of this sector are generated during the synthesis and formulation steps of production. Hence special attention has recently been devoted to the effective treatment of pharmaceutical formulation effluent [4,5]. The wide application of antibiotics in human and veterinary medicine has led to large-scale dissemination of refractory and even toxic pollutants in the environment. In many countries, a multitude of extremely resistant antibiotics have been found in treated sewage, industrial effluent, the aquatic environment and even in drinking water [6]. These products are discharged into receiving water bodies due to incomplete removal in industrial and municipal treatment plants [7].

Most known practices and technologies have appeared to be inappropriate for pharmaceutical effluent treatment and hence there is a need for more advanced and effective oxidative treatment processes such as the Fenton's reagent ($Fe^{2+}/H_2O_2$/acidic pH), ozonation at elevated pH, or $H_2O_2$-assisted ozonation [8,9] all being categorized and described as advanced oxidation processes (AOPs). A huge body of scientific literature has already been devoted to integrated chemical and biochemical treatment of pollutants originating from different industrial sectors to remove recalcitrance

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**Nomenclature**

| | |
|---|---|
| $b_H$ | endogenous respiration rate of heterotrophs ($T^{-1}$) |
| $b_{STO}$ | endogenous respiration rate of storage products ($T^{-1}$) |
| $BOD_5$ | biochemical oxygen demand ($ML^{-1}$) |
| COD | chemical oxygen demand ($ML^{-1}$) |
| $f_{SI}$ | fraction of the soluble metabolic products |
| $f_I$ | fraction of the particulate metabolic products |
| F/M ratio | food to micro-organisms ratio |
| $k_{STO}$ | maximum rate of storage ($T^{-1}$) |
| $K_O$ | half saturation constant of oxygen ($ML^{-1}$) |
| $K_S$ | half saturation constant of substrate ($ML^{-1}$) |
| $K_{STO}$ | half saturation constant of storage ($MCODMCOD^{-1}$) |
| OUR | oxygen uptake rate ($ML^{-1}\,T^{-1}$) |
| $O_{3A}$ | ozone absorption rate ($ML^{-1}$) or efficiency (%) |
| $O_{3,in}$ | input ozone concentration ($ML^{-1}$) |
| $O_{3,out}$ | off-gas ozone concentration ($ML^{-1}$) |
| $S_O$ | oxygen concentration ($ML^{-1}$) |
| $S_S$ | readily biodegradable substrate concentration ($MCODL^{-1}$) |
| $S_{T_{initial}}$ | initial soluble COD concentration ($MCODL^{-1}$) |
| $S_{T_{utilized}}$ | utilized soluble COD concentration ($MCODL^{-1}$) |
| $X_H$ | concentration of heterotrophic biomass ($ML^{-1}$) |
| $X_{STO}$ | concentration of storage products ($ML^{-1}$) |
| $Y_H$ | growth yield ($MCODMCOD^{-1}$) |
| $Y_{STO}$ | storage yield ($MCODMCOD^{-1}$) |

*Greek letter*

| | |
|---|---|
| $\mu_H$ | maximum growth rate ($T^{-1}$) |

and/or toxicity from wastewater [10–12]. Among the studied AOPs, the applied pre-oxidation steps did not always lead to a significant biodegradability enhancement [13,14]. Some industrial pollutants required extreme doses of oxidant and/or extended reaction periods [15].

Recently, respirometry has been introduced as a useful tool for the identification of organic fractions with different biodegradation characteristics in wastewater [16,17] for the assessment of the appropriate values of coefficients defining process kinetics and stoichiometry [18,19] and for model calibration of experimental data defining the response of activated sludge in wastewater treatment [20]. Toxicity can have inhibitory effects on biodegradation, which can be measured via respiration. However, respirometric applications have so far been quite limited in testing the toxicity effects of different pollutants on activated sludge processes. Consequently, no practical application of a toxicity control strategy for activated sludge systems is presently available the literature. The degree of toxicity depends on the diversity of life forms in the process, which depends on several factors including inhibitory substance species and concentration, pH, influent type and strength, amount of biomass present in the system, and the extent of microbial acclimatization. Recent studies have indicated that the acclimatization is the major phenomenon by which micro-organism mitigates the toxic effects of inhibitors [21–25].

Major progress has been achieved in the modelling of activated sludge with the introduction of different substrate and biomass fractions. Activated sludge model no. 1, (ASM1), represents the pioneering effort of such *mechanistic models*, also introducing respirometry as an essential modelling complement [26]. Recently, the concept of substrate storage was introduced into activated sludge modeling through activated sludge model no. 3, (ASM3), which triggered substantial research showing that storage mechanism plays an important role under dynamic substrate conditions experienced in treatment plants [27].

The purpose of the present work was to demonstrate the efficiency of ozonation at three different pH's on effluent from the penicillin formulation stage of a pharmaceutical industry. According to our knowledge, this is the first time that ASM3 is used to evaluate the effect of chemical oxidative pre-treatment on biodegradability of industrial effluent. The changes in biotreatability of penicillin formulation effluent subjected to ozonation at two different doses were studied in more detail. To meet the need for a means of assessing inhibition of ready biodegradability, a combination of $BOD_5$ measurements and respirometric experiments with an acclimated microbial consortium was investigated. $BOD_5$ measurements rather served to screen the optimum pre-treatment period for subsequent biodegradation and respirometric inhibition experiments.

## 2. Materials and methods

### 2.1. Analyses

All analyses of conventional environmental parameters were performed as defined in standard methods [28] except COD, colour and TOC analyses. For COD, colour and TOC determination, samples were first filtered through 0.45 μm Millipore membranes. COD was measured as described in the method proposed by ISO 6060 [29], whereas colour (i.e. optical density) of raw and treated samples were monitored using a Pharmacia LKB Novaspec II model colorimeter at λ = 436 nm wavelength in 1 cm glass cuvettes. TOC was measured on a 1505 Type Ionics Model Total Carbon Analyzer employing standard potassium hydrogen phytalate calibration for an expected TOC range of 100–500 mg $l^{-1}$.

The analytical survey also used Whatman GF/C glass-fibre filters for suspended solids (SS), and volatile suspended solids (VSS) determination. Oxygen uptake rate (OUR)

measurements were conducted by means of a Manoterm RA-1000 continuous respirometer with a PC connection.

### 2.2. Ozonation experiments

All ozonation experiments have been conducted in duplicate and average experimental results have been presented. One liter-penicillin wastewater samples were ozonated for 5, 10, 20, 40 and 60 min in a borosilicate glass bubble column at semi-batch mode wherein the ozone + oxygen gas mixture was continuously sparged at a rate of 2 l $min^{-1}$ through a fritted dispersion disc with a diameter of 5 cm. Ozone was produced by a corona discharge PCI GL-1 model pilot scale ozone generator with a maximum capacity of 20 g $h^{-1}$ ozone. 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 exact $O_3$ input rates. The applied ozone dose per time and volume of wastewater was set as 2760 mg $l^{-1}$ $h^{-1}$ for all experiments. The ozone transfer efficiency (i.e. the absorbed ozone $O_{3A}$, %) was determined by measuring the gas phase inlet ($O_{3,in}$) and outlet ($O_{3,out}$) ozone per volume of wastewater (units in mg $l^{-1}$) for each pre-ozonation experiment iodometrically [30];

$$O_{3A}(\text{mg l}^{-1}) = O_{3,in}(\text{mg l}^{-1}) - O_{3,out}(\text{mg l}^{-1}) \quad (1)$$

$$O_{3A}(\%) = \frac{O_{3A}(\text{mg l}^{-1})}{O_{3,in}(\text{mg l}^{-1})} \times 100 \quad (2)$$

The ozone mass transfer coefficient for the semi-batch ozone reactor was determined as 0.22 $min^{-1}$ in acidic pure water by employing the indigo colorimetric method [28]. The same method was also used for the determination of residual, liquid phase ozone in the reaction solution.

### 2.3. Biodegradation experiments

The biodegradation experiments were conducted in three laboratory-scale fill-and-draw reactors, which were 3 l in volume. The reactors were operated at a sludge age of 10 days and a hydraulic retention time (HRT) of 2 days for a period of 4 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 [31] 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 raw penicillin formulation effluent and in the third reactor with the ozonated effluent. The penicillin effluent additions were adjusted to constitute a 30% of the total COD in the reactor to represent the conditions of the pharmaceutical factory.

The response of the biomass acclimated to the mixture of synthetic wastewater, raw and ozonated (40 and 60 min ozonated at pH 11) penicillin formulation wastewaters, was also evaluated in terms of respirometric measurements. Tests were started with biomass seeding alone to obtain first the OUR, attributed to the level of initial endogenous respiration of the biomass. Then substrate samples were added on the biomass in the reactor for the monitoring of induced OUR profile until the initial endogenous respiration level was achieved to obtain the total biodegradable substrate. Model simulations were performed using ASM3 model [27] with AQUASIM program developed by Reichert [32].

Biological treatability studies were conducted with five sets of respirometric batch experiments with appropriate F/M ratios ranging between 0.16 and 0.23 g COD g $VSS^{-1}$. Two of these sets were run with synthetic wastewaters, which were used as control tests to determine the behaviour of the biomass under non-inhibitory conditions. The third batch experiment was performed with raw penicillin formulation and synthetic wastewater mixture at a ratio of 3:7 on COD basis. The fourth and the fifth sets were conducted with a mixture of synthetic wastewater and penicillin formulation effluent ozonated for $t$ = 40 min ($t_{40}$) and $t$ = 60 min ($t_{60}$) at $pH_o$ = 11, respectively. The same COD ratio was applied for the last two sets. All of the biodegradation experiments conducted with penicillin formulation wastewaters were designed to have the same amount of synthetic wastewater as the control sets.

Table 1
Matrix representation of activated sludge model no. 3 (ASM3)

| | Component (process) | | | | | | Rate |
|---|---|---|---|---|---|---|---|
| | $S_O$ ($O_2$) | $S_S$ (COD) | $S_I$ (COD) | $X_I$ (COD) | $X_H$(COD) | $X_{STO}$ (COD) | |
| Storage of $S_S$ | $-(1-Y_{STO})$ | $-1$ | | | | $Y_{STO}$ | $k_{STO}\frac{S_O}{K_O+S_O}\frac{S_S}{K_S+S_S}X_H$ |
| Growth on $X_{STO}$ | $-\frac{(1-Y_H)}{Y_H}$ | | | | 1 | $-1/Y_H$ | $\mu_H\frac{S_O}{K_O+S_O}\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}X_H$ |
| Endogenous respiration | $-(1-f_I-f_{SI})$ | | $f_{SI}$ | $f_I$ | $-1$ | | $b_H\frac{S_O}{K_O+S_O}X_H$ |
| Respiration of $X_{STO}$ | $-1$ | | | | | $-1$ | $b_{STO}\frac{S_O}{K_O+S_O}X_{STO}$ |

Table 2
Ingredients of the penicillin formulation effluent[a]

| Formulation content | Function |
|---|---|
| Sultamycillin tosylate dihydrate | Active agent |
| Explotap, dried | Additive |
| Methocel E-15 | Additive |
| Colloidal silicone diokside | Ainding agent |
| Magnesium stearate | Filling agent |
| Microcrystalline cellulose, dried | Binding agent |

[a] Exact amounts were strictly confidential.

The OUR results obtained from respirometric batch experiments were simulated with ASM3 [27]. The model involves four main processes for carbon removal in aerobic systems as; storage of biodegradable substrate, growth on stored products and the endogenous respiration of biomass and storage polymers. The stoichiometric relations and the kinetics describing the model are given in Table 1. Hydrolysis process has been omitted in the model since the penicillin formulation wastewaters do not contain particulate COD, which is described as the slowly biodegradable fraction in ASM3.

### 2.4. Characteristics of the penicillin formulation effluent

The effluent used in this study was penicillin wastewater from the ALFASID® formulation process (active ingredient: Sultamycillin tosylate dihydrate; molecular weight: 802.85 g mol$^{-1}$; chemical formula: $C_{25}H_{30}N_4O_9S_2{\cdot}2H_2O$). The formulation effluent was supplied by a pharmaceutical company located in Istanbul, Turkey. The selected formulation rinse water corresponded to approximately 30% of the total daily effluent (150 m$^3$ d$^{-1}$), of which 48% was process water. The collected wastewater was stored in plastic carboys at 2–4 °C before use for upto 4 weeks. Prior to each experiment, the effluent suspensions were filtered through a glass fiber 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 wash waters) are summarized in Tables 2 and 3, respectively.

Table 3
Environmental characterization of the penicillin formulation effluent

| Parameter | Value |
|---|---|
| Total COD (mg l$^{-1}$) | 710 |
| Soluble COD (mg l$^{-1}$) | 690 |
| TOC (mg l$^{-1}$) | 200 |
| $BOD_5$ (mg l$^{-1}$) | 15 |
| TKN (mg l$^{-1}$) | 85 |
| TP (mg l$^{-1}$) | 11 |
| Detergents (mg l$^{-1}$) | 25 |
| $Cl^-$ (mg l$^{-1}$) | 95 |
| Alkalinity (mg $CaCO_3$ l$^{-1}$) | 55 |
| Colour (cm$^{-1}$)[a] | 0.01 |
| pH | 6.85 |

[a] Measured at wavelength λ = 436 nm in 1 cm glass cuvettes.

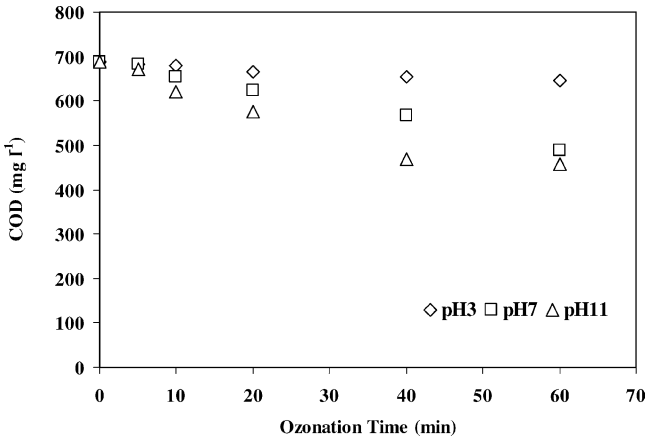


Fig. 1. COD abatement during ozonation of penicillin formulation effluent at pH = 3, 7 and 11 and for 1 h (ozone feed rate = 2750 mg l$^{-1}$).

## 3. Experimental results and discussion

### 3.1. Pretreatment with ozone

During all ozonation experiments, no attempts were made to control the pH of the formulation effluent. Ozonation pH was adjusted to the desired value and decreased throughout the reaction period as a consequence of acidic product formation. Consequently, during ozonation experiments that were run at elevated pH ($pH_o$ = 11.0), no second pH adjustment was required prior to biodegradation tests due to the fact that the pH decreased to the required, neutral values.

Fig. 1 displays COD abatement rates at three different initial pH values of 3.0, 7.0 and 11.0 for an ozonation period of 60 min. At the end of the ozonation period, the overall COD removal efficiency increased from only 6% at pH = 3 to 23% and 34% for ozonation at pH = 7 and pH = 11, respectively. As was theoretically expected, the reaction pH had a distinct effect upon COD abatement kinetics, which increased with increasing pH values as a consequence of accelerated ozone decomposition. This observation indicated that the indirect, free radical type reaction mechanism was responsible for effective oxidation [33]. It is believed that at elevated pH the major oxidizing agent for the degradation of formulation effluent is the hydroxyl radical ($^{\bullet}OH$), since no residual, liquid phase ozone was detectable at pH > 7.0 in the reaction medium of real and synthetic industrial wastewater [34,35] suggesting complete ozone decomposition.

Evaluation of Fig. 2 presenting changes in absolute ozone absorption rates ($O_{3A}$, mg l$^{-1}$) and percent ozone absorption efficiencies ($O_{3A}$, %) as a function of ozonation time or applied ozone dose ($O_{3,in}$, mg l$^{-1}$), obtained for an ozonation experiment run at $pH_o$ = 11, gives the indication of a linear relationship between ozone input and ozone absorption rates (shown as mg l$^{-1}$ and percent $O_{3A}$ values in Fig. 2). However, only 30–35% of the total introduced ozone was absorbed (consumed) at the end of the reaction. The ozone absorption efficiency $O_{3A}$ was high at the beginning of the reaction (i.e.

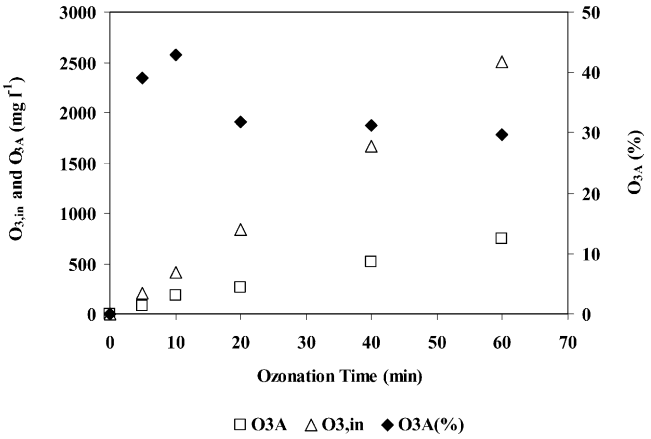


Fig. 2. Changes in relative (in %) and absolute (in mg $l^{-1}$) ozone absorption rates ($O_{3A}$) during ozonation of penicillin formulation effluent as a function of applied ozone dose at pH = 11 and for 1 h (=2750 mg $l^{-1}$ $O_3$).

the first 10 min of ozonation), because the pH was high and hence ozone decomposition was very fast during this initial reaction period. Upon extended ozonation, the pH decreased to neutral values (pH = 6.8–7.2) and ozone decomposition leveled off speculatively due to acid accumulation (Fig. 3). Without pH control, these results suggest that ozone decomposition is mainly governed by the reaction pH and not by its transfer efficiency. Therefore, no direct correlation between oxidation efficiency, ozone absorption and ozone decomposition seemed plausible.

At the end of the ozonation experiment run at $pH_o$ = 11, COD was reduced by 34% or 233 mg $l^{-1}$ (Fig. 1) and 742 mg $l^{-1}$ of the applied $O_3$ was absorbed in the reaction solution. As is evident in Fig. 3, TOC abatement remained relatively low (24% overall reduction) throughout the ozonation period, and a parallel decrease in pH was observed. However, decrease in TOC values was actually not the purpose of the present work. Partial oxidation and pre-treatment of the

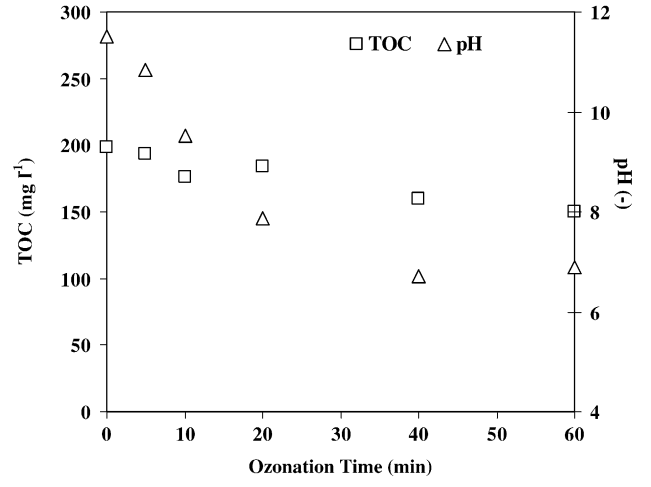


Fig. 3. Changes in TOC and pH values during ozonation of penicillin formulation effluent as a function of ozonation time at pH = 11 and for 1 h (=2760 mg $l^{-1}$ $O_3$).

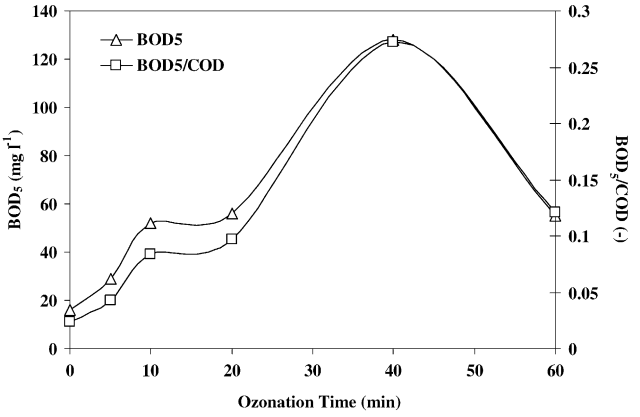


Fig. 4. Changes in $BOD_5$ and $BOD_5$/COD values during ozonation of penicillin formulation effluent as a function of ozonation time at pH = 11 and for 1 h (=2760 mg $l^{-1}$ $O_3$).

penicillin formulation ingredients to more "biocompatible" compounds instead of ultimate mineralization down to oxidation end products such as carbonate, inorganic salts and $H_2O$, was aimed in our study. In fact, the partial oxidation rates were still appreciably higher than total oxidation rates, as is obvious from the TOC abatement rates displayed in the same figure.

Fig. 4 presents changes in the $BOD_5$ content and the $BOD_5$/COD ratio of penicillin formulation effluent as a function of ozonation time. These parameters may also be evaluated as a crude index of biodegradability. From the figure it can be seen that the $BOD_5$/COD ratio, was improved from only 0.02 of the original, untreated wastewater (original $BOD_5$ = 17 ± 2 mg $l^{-1}$) to 0.12 at the end of the 60 min-ozonation period reaching a maximum value of 0.27 ($BOD_5$ = 128 mg $l^{-1}$) after 40 min ozonation that corresponded to an ozone input rate (ozone dose) of 1680 mg $l^{-1}$. Obtained results revealed that a relative increase in biochemical oxygen demand values was apparent for the pre-ozonated wastewater, indicating that ozonation at a specific, "optimum" dose may have positive effects on its biological treatability. The appearance of a distinct, maximum biodegradability ratio and a maximum $BOD_5$ has already been evidenced in previous experimental studies that reported a critical ozone dose in the range of 1–3 mg $O_3$ per mg initial COD to achieve best results in terms of biodegradability improvement [8,33]. In the present study, the optimum ozone dose that was required to achieve the highest measured $BOD_5$ value was 1.9 mg $O_3$ mg $COD_o^{-1}$ and hence lies in this pre-established, recommended range for pre-treatment purposes.

### 3.2. Biological treatability studies

Results of model simulations obtained for five sets of biodegradation experiments are depicted in Fig. 5. The model can be fitted to produce reliable predictions of the respirometric behaviour of the effluent samples during biodegradation. Table 4 elucidates the total amount of soluble COD ($S_{T_{initial}}$)

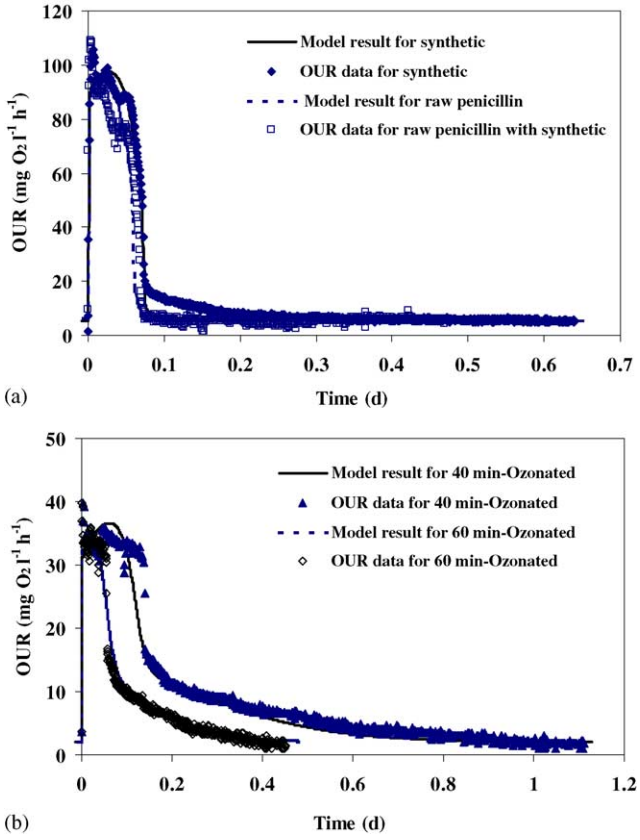


Fig. 5. Experimentally observed and modeled OUR profiles obtained for: (a) synthetic wastewater, raw penicillin formulation effluent and synthetic wastewater mixture, (b) 40- and 60 min-ozonated penicillin formulation effluent and synthetic wastewater mixtures.

fed to the reactors during batch experiments, the amount of utilized COD ($S_{T_{utilized}}$) and the values of kinetic and stoichiometric parameters obtained for each wastewater mixture.

Process stoichiometry of the applied model has been defined in accordance with ASM3 and the previously published literature [36,37]. A storage yield, $Y_{STO}$, of 0.78 g COD (g COD)$^{-1}$ and a heterotrophic growth yield, $Y_H$, of 0.67 g cell COD (g COD)$^{-1}$ have been estimated, resulting in a net biomass yield of 0.61 g cell COD (g COD)$^{-1}$. The stoichiometric coefficients, $f_{SI}$, fraction of the soluble metabolic products, and $f_I$, fraction of the particulate metabolic products, have been chosen as 0.10 g COD (g COD)$^{-1}$.

The values obtained for substrate storage rate, $k_{STO}$, and for the maximum heterotrophic growth rate, $\mu_H$, of synthetic wastewater bearing five different readily biodegradable COD components, were in accordance with those values reported for acetic acid [38]. This result is obviously a direct consequence of the high acetic acid content of the synthetic wastewater. The substrate storage affinity constant, $K_s$, 40 mg l$^{-1}$, appeared to be appreciably higher than those given for acetic acid as 3 mg l$^{-1}$in the scientific literature [36]. The $K_s$ value of the present work is more comparable to typical $K_s$ values of domestic wastewater, implying that some biodegradable COD components being present in the effluent mixture were utilized at a lower rate than the volatile fatty acid and alcohol fractions [39].

According to the OUR profiles presented in Fig. 5a,b the addition of raw penicillin formulation wastewater to the synthetic wastewater at a volumetric ratio of 30% resulted in a 22% reduction in the total oxygen consumption. Results of the model simulation done for this batch test have indicated that the kinetic coefficients established for substrate storage and growth processes were identical to those obtained for synthetic wastewater, except that the amount of utilized substrate was decreased at a rate of 10%. Conclusively, some of the organic matter present in raw penicillin formulation wastewater was not biodegradable and also its addition hindered the degradation of the synthetic wastewater in the mixture. The readily biodegradable COD fraction of the synthetic wastewater component could only be utilized upto 90%, due to the formation of non-biodegradable, complex compounds. This trend was also observed in the acclimation reactor that was fed with a raw penicillin formulation and synthetic wastewater mixture; where no degradation of the penicillin formulation wastewater occurred and the degradation of the synthetic wastewater was inhibited by 7% in terms of COD removal. However, in the absence of the penicillin formulation wastewater, the synthetic wastewater was completely biodegradable.

Upon close inspection of the oxygen utilization rates established for the synthetic wastewater that was mixed with 40 min-ozonated penicillin formulation effluent, it is evident

Table 4
ASM3 model coefficients obtained for synthetic wastewater, raw and ozonated penicillin formulation wastewaters

| Model coefficient | Synthetic | Synthetic with raw | Synthetic with $t_{40}$ | Synthetic with $t_{60}$ |
|---|---|---|---|---|
| F/M (g COD (g VSS)$^{-1}$) | 0.16 | 0.23 | 0.21 | 0.21 |
| $S_{T_{initial}}$ (mg COD l$^{-1}$) | 300 | 420 (300 + 120) | 418 (300 + 118) | 380 (300 + 80) |
| $S_{T_{utilized}}$ (mg COD l$^{-1}$) | 300 | 270 | 341 | 161 |
| $b_H$ (d$^{-1}$) | 0.20 | 0.20 | 0.10 | 0.10 |
| $b_{STO}$ (d$^{-1}$) | 0.20 | 0.20 | 0.10 | 0.10 |
| $Y_{STO}$ (g COD (g COD)$^{-1}$) | 0.78 | 0.78 | 0.78 | 0.78 |
| $Y_H$ (g cell COD (g COD)$^{-1}$) | 0.67 | 0.67 | 0.67 | 0.67 |
| $k_{STO}$ (d$^{-1}$) | 9.2 | 9.2 | 9.2 | 9.2 |
| $K_S$ (mg COD l$^{-1}$) | 40 | 40 | 40 | 40 |
| $\mu_H$ (d$^{-1}$) | 3.2 | 3.2 | 3.2 | 3.2 |
| $K_{STO}$ (g COD (g cell COD)$^{-1}$) | 0.002 | 0.002 | 0.7 | 0.4 |

that the synthetic domestic wastewater content could be completely oxidized and at the same time 35% removal of the 40 min-ozonated penicillin effluent fraction was achieved. These results were in complete agreement with those obtained in the long-term operated acclimation reactor. The modelling studies showed that the biomass acclimated to ozonated effluent would go through the endogenous respiration processes at slower rates with endogenous respiration rate of heterotrophs, $b_H$ and that of storage products, $b_{STO}$ values of 0.1 $d^{-1}$ than biomass fed with raw penicillin formulation wastewater. Reliable simulation results were obtained with the same kinetic parameters applied for synthetic wastewater but the rate of the growth process was reduced by the high affinity constant, $K_{STO}$, of 0.7 g COD (g cell COD)$^{-1}$. This observation is most likely due to the different storage compounds generated from the degraded portion of ozonated penicillin formulation wastewater, which are harder to utilize for heterotrophic growth.

The 60 min-ozonated penicillin formulation effluent exerted an inhibitory effect upon synthetic wastewater substrate degradation. Only 161 mg $l^{-1}$ COD of the synthetic wastewater substrate having an initial COD of 300 mg $l^{-1}$ could be utilized upon the addition of 60 min-ozonated penicillin formulation effluent, corresponding to a 46% decrease in substrate utilization. This effect is in complete accordance with the information reported in literature for the inhibitory effect of prolonged ozonation periods on successive biological treatment [40]. Although the kinetic parameters of substrate storage obtained for 60 min ozonated wastewater were the same as that of synthetic wastewater, the inhibiting behaviour could be observed on heterotrophic growth with a high $K_{STO}$ value of 0.4 g COD (g cell COD)$^{-1}$.

Noteworthy is in particular the observation that the $BOD_5$ values of the samples ozonated for 40 min were significantly higher than those of raw and 60 min-ozonated penicillin formulation effluent being in complete agreement with the respirometric findings (Fig. 4).

## 4. Summary and conclusions

This study provides an evaluation of the biological treatability of raw and ozonated penicillin formulation effluent bearing *Sultamycillin Tosylate Diydrate* as the penicillin active ingredient. Ozonation appeared to be an efficient pretreatment process with 34% COD and 24% TOC removal at elevated ($pH_o$ = 11.0) and a parallel increase in the $BOD_5$ content from 16 mg $l^{-1}$ to 128 mg $l^{-1}$ being observed after 40 min ozonation corresponding to an ozone dose of 1670 mg $l^{-1}$.

Biological treatability studies have shown that in addition to the non-biodegradable character of raw penicillin formulation wastewater, synthetic wastewater degradation was inhibited by the addition of penicillin formulation wastewater, most probably as a consequence of non-biodegradable complex formation.

A 40 min-ozonation had a positive effect the biodegradation characteristics of the initially marginally biodegradable and inhibitory penicillin formulation effluent. While the synthetic wastewater could be completely oxidized, the COD content of 40 min-ozonated formulation effluent could be removed by 35%.

Prolonged ozonation of the penicillin formulation effluent did not provide satisfactory results in terms of biocompatibility. A 60 min-ozonation of the penicillin formulation effluent appeared to reduce the COD removal rate of the synthetic effluent fraction by 46% during successive biodegradation. The heterotrophic growth was also inhibited by the oxidation products formed after ozone “overdosing”.

Ozonation has apparently found its place in integrated biotic–abiotic treatment. However, the results of the present study have indicated that although ozonation can be a preferable process for partial oxidation of organic matter present in pharmaceutical effluent, the application of ozonation for pretreatment purposes needs careful consideration. The applied ozone dose had to be optimized to achieve the desired ultimate removal rates without inhibiting a succeeding biological treatment stage.

The study presented herein also provided experimental support and information regarding activated sludge modelling using respirometric methods and inhibition kinetics. Further research should be conducted on the assessment of specific inhibition kinetics for pharmaceutical industry wastewaters originating from antibiotic formulation being most well known for their inhibitory nature. The description type and mechanism of inhibition still needs additional experimental studies including specific data on storage products.

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