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Examination of Photocatalyzed Chlorophenols for Sequential Photocatalytic-Biological Treatment Optimization

Liliana Bobirică, Constantin Bobirică and Cristina Orbeci *

Department of Analytical Chemistry and Environmental Engineering, University Politehnica of Bucharest, 1–7 Polizu, 011061 Bucharest, Romania; l_bobirica_ro@yahoo.com (L.B.); c_bobirica@yahoo.com (C.B.)
* Correspondence: cristina.orbeci@upb.ro; Tel.: +40-7212-598-75

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Abstract: Given the known adverse effect of chlorophenols for the aquatic environments which they can reach, the development of efficient methods both technically and economically to remove them has gained increasing attention over time. The combination of photocatalytic oxidation with biological treatment can lead to high removal efficiencies of chlorophenols, while reducing the costs associated with the need to treat large volumes of aqueous solutions. Therefore, the present paper had as its main objective the identification of the minimum photocatalytic oxidation period during which the aqueous solutions of 4-chlorophenol and 2,4-dichlorophenol can be considered as readily biodegradable. Thus, the results of photocatalytic oxidation and biodegradability tests showed that, regardless of the concentration of chlorophenol and its type, the working solutions become readily biodegradable after up to 120 min of irradiation in ultraviolet light. At this irradiation time, the maximum organic content of the aqueous solution is less than 40%, and the biochemical oxygen demand and chemical oxygen demand (BOD/COD) ratio is much higher than 0.4. The maximum specific heterotrophic growth rate of activated sludge has an average value of 4.221 d^{-1} for 4-chlorophenol, and 3.126 d^{-1} for 2,4-dichlorophenol. This irradiation period represents at most half of the total irradiation period necessary for the complete mineralization of the working solutions. The results obtained were correlated with the intermediates identified during the photocatalytic oxidation. It seems that, working solutions initially containing 4-chlorophenol can more easily form readily biodegradable intermediates.

Keywords: photocatalytic oxidation; chlorophenols; titanium dioxide membrane; biological treatment; biodegradability

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1. Introduction

Chlorophenols represent one of the largest groups of phenols used by industry [1], and therefore, they have the highest chance to reach natural water bodies through industrial contaminated streams [2]. Due to their specific characteristics, which place them in the category of toxic organic compounds, they have a negative effect on the aquatic ecosystem, and also on human health [3]. For this reason, a wide range of methods for removing them from aqueous solutions have been developed over time, the most known being adsorption on various adsorbents [4], and advanced oxidation processes [5,6] of which photocatalytic oxidation has received special attention lately [7–11]. Although many of these methods have been shown to be effective in removing chlorophenols from many aqueous systems, their large-scale application is often difficult both technically and economically.

Biological treatment methods are generally cheaper compared with physical or chemical treatment methods [12,13]. Large-scale activated sludge treatability studies have shown promising results in concomitant degradation of chlorophenols, co-substrate, and phosphorus [14]. However, the effectiveness of the biological treatment depends heavily on the type of chlorophenol, its initial concentration, co-substrates, electron acceptors, reactor type, and operating conditions [15]. It seems that the presence of chlorophenols affects the activated sludge involved in the degradation of organic material especially in terms of inhibition of microbial growth [16,17], which could greatly prolongs the necessary period for organic material mineralization [18]. In this context, combining an advanced oxidation treatment with a biological treatment appears to be a promising solution in terms of rate of removal of recalcitrant organic compounds, technical and economic feasibility, and environmental friendliness. In this respect, Goel et al. [19] used an integrated photocatalytic and biological treatment for 4-chlorophenol removal from wastewater with good results in terms of reducing the time required for its complete mineralization compared with the use of biological treatment alone. Suryaman and Hasegawa [20] used a photocatalytic-biological as well as a biological-photocatalytic sequential integrated treatment for removing a mixture of 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and pentachlorophenol from tap water with the best results for the second combination. Given the excellent results obtained with regard to the complete and rapid removal of phenol derivatives from their highly dilute solutions [21], the application of a sequential three-step treatment such as photocatalytic oxidation-biological treatment-photocatalytic oxidation could be opportune in the sense of developing some energy-saving technologies for the complete removal of recalcitrant organic compounds from aqueous systems. There have also been numerous attempts to couple the photocatalytic treatment simultaneously with the biological one, with superior results to the sequential coupling systems [22]. It should be mentioned that the biodegradability of the initial organic compound as well as of the intermediates formed during the degradation process plays a key role in achieving a maximum efficiency of the treatment system. Although intense efforts have been made to characterize various aqueous systems containing a wide range of recalcitrant organic compounds, including chlorophenols [23–25], in terms of their biodegradability, there are still ambiguities regarding its connection with the other characteristics of the treatment system (i.e., operating conditions of reactors such as pH, temperature, hydraulic retention time, microorganisms acclimatization, etc.).

Therefore, the variation in biodegradability of the organic content of an ultraviolet (UV) photocatalytic reactor equipped with a TiO_2/Ti photocatalytic membrane over the irradiation period of the initial aqueous solutions of 4-chlorophenol and 2,4-dichlorophenol was studied in this work. The work aims to identify the minimum necessary irradiation period so that the degradation intermediates derived from the photocatalytic oxidation pre-treatment step to be considered as readily biodegradable in the next step of biological treatment with activated sludge.

2. Results and Discussion

2.1. Photocatalytic Oxidation

The results obtained from the study on the photocatalytic degradation of 4-chlorophenol (Figure 1) and 2,4-dichlorophenol (Figure 2) show that the irradiation time until a complete degradation of chlorophenols is reached increases with their initial concentration. Thus, the irradiation time increases by about 100 min from an initial concentration of 4-chlorophenol of 50 mg O_2/L to an initial concentration of 300 mg O_2/L. For the same initial concentrations of 2,4-dichlorophenol, the increase in irradiation time is about 200 min. The explanation could be that at high concentrations of organic compound, its excess molecules adsorbed on the photocatalyst membrane can decrease the formation of hydroxyl radicals by competitively decreasing the number of hydroxyl ions adsorbed on it [25]. Moreover, during photocatalytic degradation the aryl-Cl bond cleavage takes place which leads to the release of chloride ions (Cl^-) in the aqueous solution [22]. The more chlorine atoms grafted onto the aromatic ring, the more chloride ions generated during photocatalytic degradation. It is known that chloride ions decrease photocatalytic activity because they can interact with holes ($h^+{}_{vb}$) and hydroxyl radicals

(HO•) forming their own radicals (Cl•, Cl_2^-•, and $HOCl^-$•) which are less reactive [26]. Therefore, these types of interaction (Equations (1)–(7)) lead to a decrease in the number of reactive species such as hydroxyl radicals (HO•) and superoxide radicals (O_2^-•) which are effectively involved in the degradation of the organic material [27,28]. This may be one of the reasons why the degradation of 2,4-dichlorophenol is slower than that of 4-chlorophenol.

$$\text{Photocatalyst} + h\nu \rightarrow e^-_{cb} + h^+_{vb}. \tag{1}$$

$$H_2O + h^+_{vb} \rightarrow HO\bullet + H^+ \tag{2}$$

$$O_2 + e^-_{cb} \rightarrow O_2^-\bullet \tag{3}$$

$$Cl^- + h^+_{vb} \rightarrow Cl\bullet \tag{4}$$

$$Cl^- + Cl\bullet \rightarrow Cl^-\bullet \tag{5}$$

$$HO\bullet + Cl^- \rightarrow HOCl^-\bullet \tag{6}$$

$$HOCl^-\bullet + H^+ \rightarrow Cl\bullet + H_2O \tag{7}$$

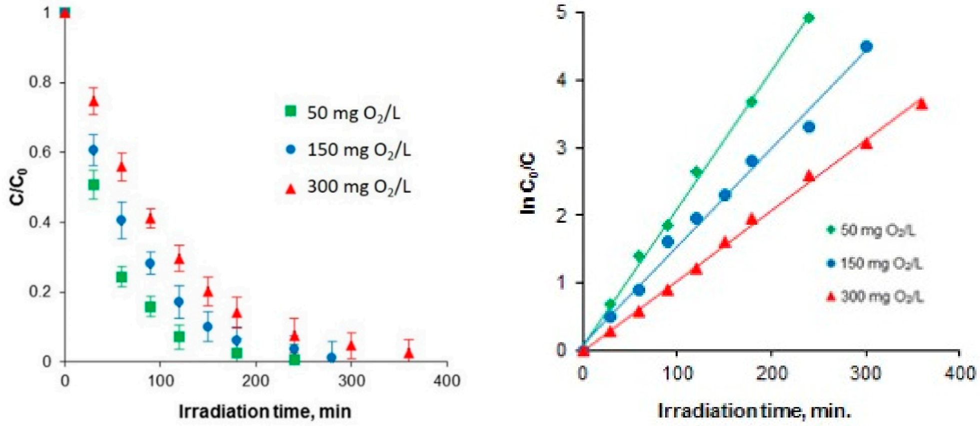

Figure 1. Kinetics of 4-chlorophenol photocatalytic degradation as a function of its initial concentration. Error bars represent the calculated standard deviation for the experimental data.

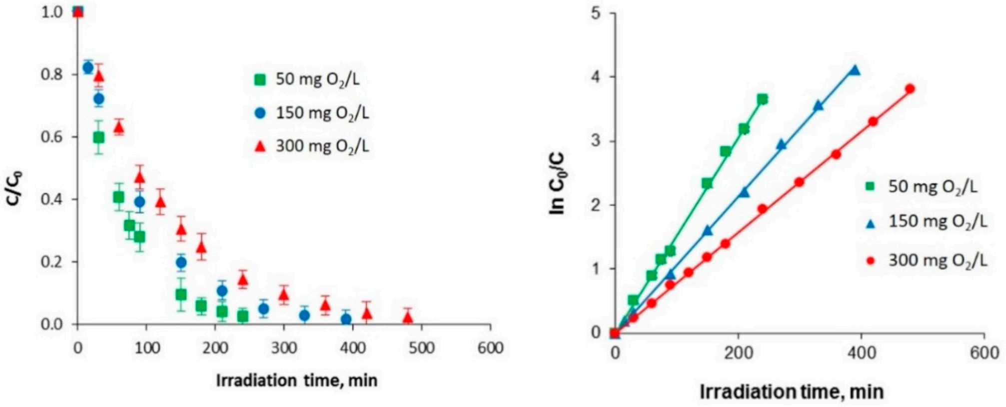

Figure 2. Kinetics of 2,4-dichlorophenol photocatalytic degradation as a function of its initial concentration. Error bars represent the calculated standard deviation for the experimental data.

The photocatalytic oxidation of 4-chlorophenol and 2,4-dichlorophenol follows a pseudo-first order kinetics, which is described by Equation (8) [29].

$$\ln\left(\frac{C_0}{C}\right) = k_{obs} \times t \tag{8}$$

where C_0 is the initial concentration of the organic material in the aqueous solution (mg/L), C is the residual concentration of the organic material after irradiation at time t (mg/L), k_{obs} is the observed pseudo-first order rate constant, which is related to the total reaction (min^{-1}), and t is the irradiation time (min). k_{obs} is determined from the slope of the straight lines of the $\ln(C_0/C)$ versus irradiation time plots (Figures 1 and 2). The kinetic parameters are shown in Table 1. The data obtained after fitting indicate that the rate of photocatalytic degradation of 4-chlorophenol is approximately 1.3 times higher than that of 2,4-dichlorophenol, regardless of their initial concentration. The difference between the rate constants for different initial chlorophenol concentrations can be explained based on the competitive system that characterizes the photocatalytic degradation reaction. Thus, the organic compound molecules compete with the peroxo groups for the surface of the photocatalyst [30]. As the concentration of organic compound increases, more and more molecules will be adsorbed on the photocatalyst surface, while fewer reactive species will be generated in the system, leading to a decrease in the reaction rate.

Table 1. Kinetic parameters of photocatalytic degradation.

Kinetic Parameter	**Chlorophenol Initial Concentration (mg O_2/L)**		
	50	150	300
k_{obs}, min^{-1}	*4-chlorophenol*		
	0.0203	0.0144	0.0103
	2,4-dichlorophenol		
	0.0153	0.0107	0.0079

2.2. *Biodegradability of Photocatalyzed Chlorophenols*

To establish the biodegradability, the ratio between the biochemical oxygen demand and chemical oxygen demand (BOD/COD) was determined for the initial chlorophenol solutions, respectively for the solutions pretreated by photocatalytic oxidation (Figure 3). As can be seen, the BOD/COD ratio increases as the irradiation time increases regardless of the type of chlorophenol and its initial concentration. The threshold value of the BOD/COD ratio of 0.4, from which the solution is considered to become readily biodegradable [31,32], is exceeded (or at least reached) after 10 min of irradiation in the case of 4-chlorophenol solutions with an initial concentration of 50 mg/L, and after 60 min of irradiation in the case of 4-chlorophenol solutions with an initial concentration of 300 mg/L. The same result was also obtained in the case of the solution of 2,4-dichlorophenol with an initial concentration of 50 mg/L, but for an initial concentration of 300 mg/L the BOD/COD ratio threshold value is exceeded after a longer irradiation time. It should be noted that the value of the BOD/COD ratio is higher in the case of the 4-chlorophenol solution compared to the 2,4-dichlorophenol solution, regardless of their initial concentration. Moreover, it seems to be a clear difference between the values of the BOD/COD ratio as a function of initial concentration of the chlorophenol solution. In this respect, the BOD/COD ratio increases with decreasing the initial chlorophenol concentration regardless of the irradiation time or the type of chlorophenol.

The quantification of the degree of inhibition of the activated sludge by chlorophenols and the intermediates formed in the photocatalytic oxidation step was carried out on the basis of respiration inhibition tests, and was determined according to Equation (9) [33].

$$\text{Inhibition} = \left(1 - \frac{SOUR_P}{SOUR_0}\right) \cdot 100 \tag{9}$$

where $SOUR_p$ represents the specific oxygen uptake rate of the activated sludge in the presence of the test substance, and $SOUR_o$ represents the specific oxygen uptake rate of the activated sludge in the presence of the control substance.

Based on the experimental results on the specific rate of oxygen consumption (SOUR), it was established that the degree of inhibition decreases with increasing photocatalytic oxidation time, both for 4-chlorophenol and 2,4-dichlorophenol (Figure 4). This result suggests that the intermediates resulting from photocatalytic oxidation have an increasingly reduced inhibitory effect as the irradiation time increases. It can be seen that there are considerable differences between the three working solutions with different concentrations of chlorophenols in terms of reducing the degree of inhibition with increasing irradiation time. This may be due to the toxicity of the intermediates formed in the first minutes of irradiation. Therefore, the higher the initial concentration of chlorophenols, the higher the concentration of intermediates formed, and thus a much more emphasized inhibitory effect.

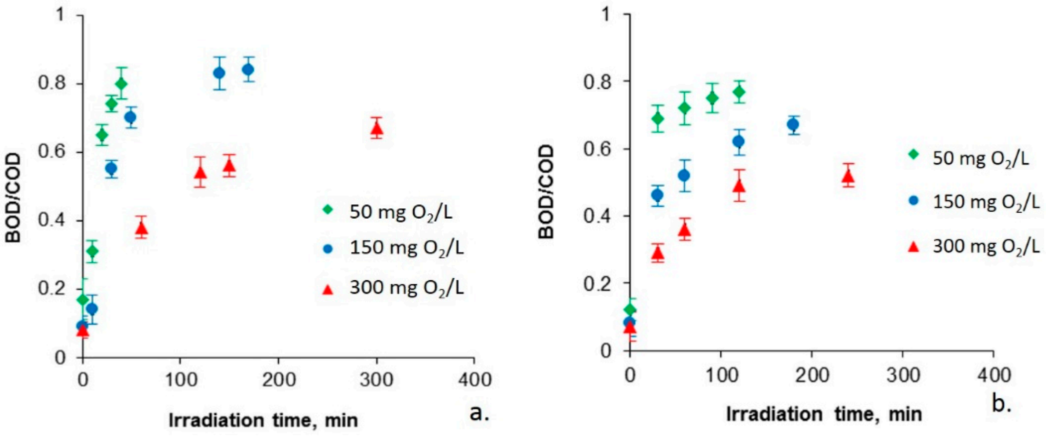

Figure 3. Biodegradability of chlorophenols: (**a**) 4-chlorophenol, (**b**) 2,4-dichlorophenol. Error bars represent the calculated standard deviation for the experimental data.

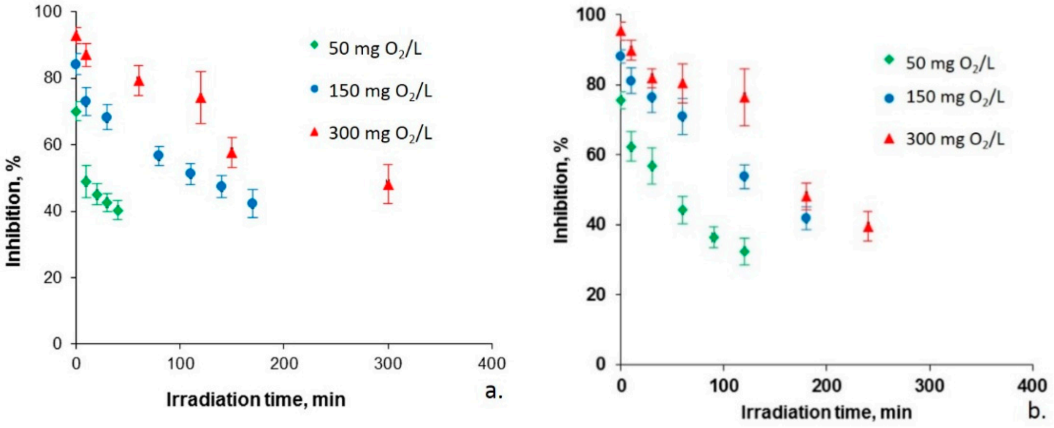

Figure 4. Evolution of the degree of inhibition of activated sludge by chlorophenols and degradation intermediates as a function of irradiation time: (**a**) 4-chlorophenol, (**b**) 2,4-dichlorophenol. Error bars represent the calculated standard deviation for the experimental data.

The results shown above could be explained based on the analysis of intermediates formed during the photocatalytic oxidation of the initial chlorophenols. As can be seen from Figure 5, the relative abundance of intermediates formed in the case of photocatalytic oxidation of 4-chlorophenol, indicates a massive formation of hydroquinone in the first 60 min of irradiation, simultaneously with the sharp decrease in 4-chlorophenol content. Also, the relative abundance of chlorocatechol formed in the first minutes of irradiation decreases after the first 60 min of irradiation to almost zero, with the simultaneous formation of catechol.

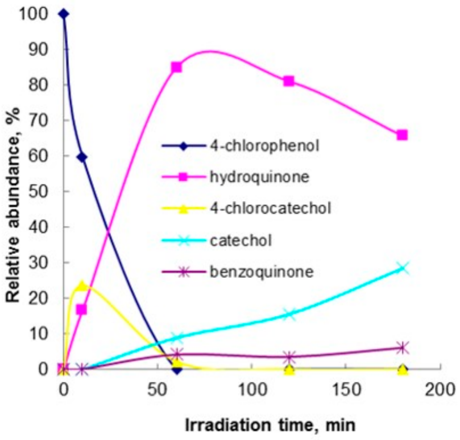

Figure 5. Intermediates identified during the photocatalytic oxidation of 4-chlorophenol working solution of 300 mg O_2/L.

In the case of photocatalytic oxidation of 2,4-dichlorophenol (Figure 6), the main intermediate formed after the first 60 min of irradiation is 2-chlorohydroquinone. At higher irradiation times, other intermediates such as 4-chlorophenol and 3,5-dichlorocatehol begin to appear in the photocatalyzed solution, along with 2-chlorohydroquinone. However, their relative abundance begins to decrease after 120 min of irradiation, simultaneously with the formation of hydroquinone.

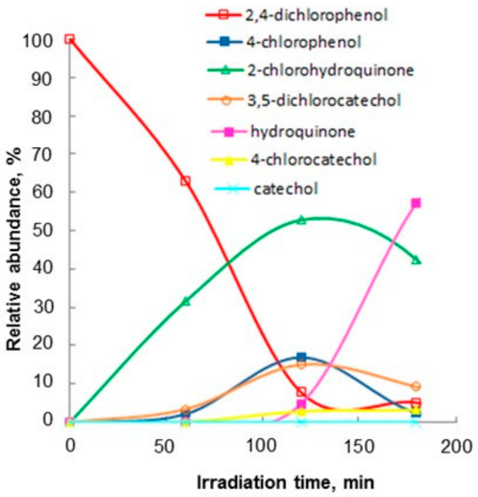

Figure 6. Intermediates identified during the photocatalytic oxidation of 2,4-dichlorophenol working solution of 300 mg O_2/L.

The formation of chlorinated intermediates in the first minutes of photocatalytic oxidation such as chlorocatechol in the case of photocatalytic oxidation of 4-chlorophenol, and 2-chlorohydroquinone in the case of photocatalytic oxidation of 2,4-dichlorophenol, could lead to the keeping of a high level of toxicity of the working solutions, which could explain the high degree of inhibition of microorganisms in the solutions resulting after the first 60 min of photocatalysis. The formation after the first 60 min of irradiation of 4-chlorophenol and 3,5-dichlorocatehol, in the case of photocatalytic oxidation of 2,4-dichlorophenol, leads to an increase in the toxicity of the working solutions and, therefore, to the keeping of a degree of inhibition of microorganisms over a longer period of time. This could explain the keeping of a higher degree of inhibition in the case of irradiated solutions for longer periods of time derived from 2,4-dichlorophenol, compared to solutions derived from 4-chlorophenol (Figure 4). The subsequent formation of hydroxylated intermediates, which can be more easily biodegraded, results in a decrease in the degree of inhibition of photocatalyzed solutions. However, the persistence of the hydroquinone-benzoquinone equilibrium at long irradiation times probably continues to confer some toxicity to the aqueous system [34], as evidenced by the fact that the BOD/COD ratio remains at approximately 0.8 even at long irradiation times regardless of the type of chlorophenol. Based on the results shown above regarding the intermediates formed during the photocatalytic oxidation of 4-chlorophenol and 2,4-dichlorophenol, speculative degradation mechanisms have been proposed for each chlorophenol (Figures 7 and 8).

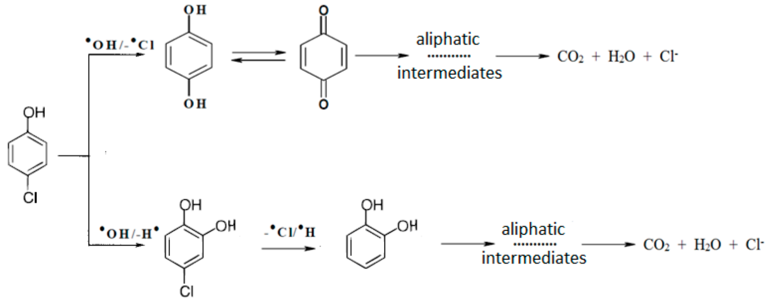

Figure 7. Speculative photocatalytic degradation pathways of 4-chlorophenol.

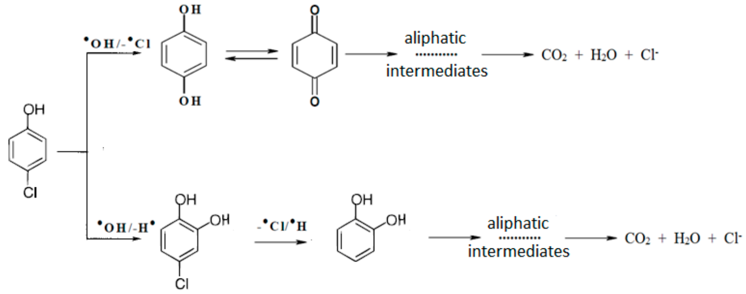

Figure 8. Speculative photocatalytic degradation pathways of 2,4-chlorophenol.

To further highlight the impact of the studied chlorophenols and the intermediates formed during photocatalytic degradation on the activated sludge used in this work, the determination of the maximum specific heterotrophic growth rate ($\hat{\mu}_m$) was carried out from oxygen uptake rate (OUR) measurements in batch experiments. Its value was calculated according to the model proposed by Ekama et al. [35].

$$\hat{\mu}_m = K_{ms} Y_H \tag{10}$$

where K_{ms} is the maximum readily biodegradable substrate uptake rate (mg COD/mg AVSS/d), and Y_H is the yield coefficient for heterotrophs (mg VSS/mg COD). AVSS (active volatile suspended solids), VSS (volatile suspended solids).

$$K_{ms} = \frac{1}{1 - f_{cv} Y_H} OUR_i 24 \left(\frac{V_{ww} + V_{mL}}{f_{av} X_V V_{mL}} \right) \tag{11}$$

where f_{cv} is the COD/VSS ratio of the sludge (mg COD/mg VSS), OUR_i is the initial oxygen uptake rate (mg/L/h), V_{mL} is the total volume of the mixture consisting of active sludge and working solution containing chlorophenols (L), V_{ww} is the volume of the working solution containing chlorophenols (L), X_V is the volatile suspended solids (VSS) concentration of the mixture added to the batch experiment (mg VSS/L), f_{av} is the active fraction of the VSS, and 24 is the number of hours per day. The active fraction of the VSS (f_{av}) was calculated by using the equation proposed by Ekama et al. [36].

$$f_{av} = \frac{OUR_i}{(f_{cv} + 4.57f_n)(1 - f_{EH})b_{HT}X_V} \quad (12)$$

where f_n is TKN/VSS (total Kjeldahl nitrogen/volatile suspended solids) ratio of the activated sludge (mg N/mg VSS), f_{EH} is the unbiodegradable fraction of heterotrophic microorganisms in endogenous respiration and death regeneration models, b_{HT} is the heterotrophic microorganisms endogenous respiration and death rates at temperature T (d^{-1}). The values for f_{cv}, f_n, and f_{EH} were set at 1.5 mg COD/mg VSS, 0.10 mg N/mg VSS, and 0.20 [36].

$$b_{HT} = 0.24(1.029)^{(T-20)} \quad (13)$$

where T is the working temperature (°C). The working temperature was 20 ± 2 °C and, therefore, the value of b_{HT} was set at 0.24 d^{-1} [36].

The yield coefficient for heterotrophs (Y_H) was calculated according to Equation (14) [37].

$$y_H = \frac{1 - \frac{\Delta O_2}{\Delta COD}}{f_{cv}} \quad (14)$$

where ΔO_2 is the mass of oxygen utilized (for substrate consumption) per liter of beach mixture (mg O_2/L), and ΔCOD is the COD consumed per liter of beach mixture (mg COD/L).

As can be seen from the results obtained (Figure 9), the maximum specific heterotrophic growth rate ($\hat{\mu}_m$) increases with increasing the irradiation time, for both chlorophenols studied, up to close to the specific value of the activated sludge maintained in optimal conditions from the nutritional point of view, namely 4.626 d^{-1} (average value). The obvious difference between the two types of chlorophenols supports the ones shown above in connection with their different toxicity as well as the intermediates formed during the photocatalytic degradation.

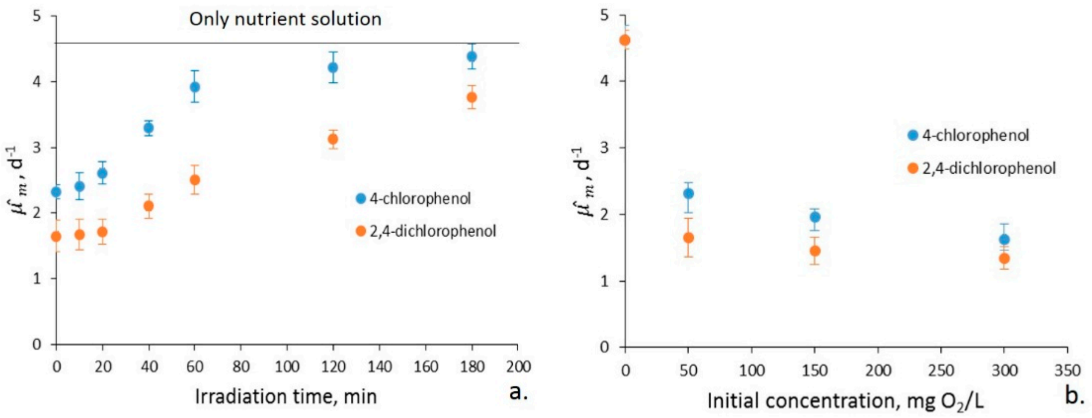

Figure 9. Evolution of the maximum specific heterotrophic growth rate of activated sludge depending on: (**a**) irradiation time (chlorophenol initial concentration of 300 mg O_2/L), (**b**) the initial substrate concentration (without irradiation). Error bars represent the calculated standard deviation for the experimental data.

3. Materials and Methods

3.1. Chemicals and Reagents

Both 4-chlorophenol (C_6H_5ClO) and 2,4-dichlorophenol ($C_6H_4Cl_2O$) used in the experiments were of analytical grade, and were purchased from Fluka Chemicals. Their main physical and chemical properties are presented in Table 2. Hydrogen peroxide (H_2O_2) solution of 30% purchased from Sigma-Aldrich was used in the photocatalytic oxidation experiments as hydroxyl radicals precursor. The pH of the aqueous solution was adjusted with sulfuric acid 1 N (H_2SO_4) purchased from Sigma-Aldrich. Potassium dichromate ($K_2Cr_2O_7$), silver sulfate (Ag_2SO_4), potassium hydrogen phthalate ($C_8H_5KO_4$) and sulfuric acid 95–97% of analytical grade (purchased from Sigma-Aldrich) were used in the chemical oxygen demand (COD) analysis. Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$), sodium dihydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), ammonium chloride (NH_4Cl), calcium chloride hexahydrate ($CaCl_2 \cdot 6H_2O$), glutamic acid ($C_5H_9NO_4$), urea (CH_4N_2O), and glucose ($C_6H_{12}O_6$) of analytical grade (purchased from Sigma-Aldrich) were used in the biochemical oxygen demand (BOD) analysis and to prepare the nutrient solution used to feed the activated sludge. The pH of the aqueous solution in the biodegradability experiments was adjusted with sodium hydroxide 1 M (NaOH) purchased from Sigma-Aldrich. Both the diethyl ether solution and anhydrous sodium sulfate of analytical grade (purchased from Sigma-Aldrich) were used in the extraction experiments for photocatalytic oxidation intermediates identification. Distilled water was used in all experiments.

Table 2. Physical and chemical properties of 4–cholorophenol and 2,4-dichlorophenol.

Property	4-Cholorophenol	2,4-Dichlorophenol
Formula	C_6H_4ClOH	$C_6H_4Cl_2O$
Density (25 °C)	1.306 g/cm^3	1.38 g/cm^3
Molecular weight	128.56 g/mol	163.00 g/mol
Solubility in water (20 °C)	27.1 g/L	4.5 g/L

3.2. Photocatalytic Oxidation Experiments

The experiments were carried out in a UV photocatalytic reactor equipped with an external centrifugal pump for continuous recirculation of the working solution during the irradiation period. The photocatalytic reactor was designed in such a way that the UV lamp, the photocatalytic membrane (cylindrical shape, 10 cm × 30 cm), and the water cooling jacket were coaxially positioned. The simplified scheme of the photocatalytic reactor is presented in Figure 10. The photocatalytic reactor volume was 1.5 L and it was operated at a recirculation flow rate of the working solution of 1 L/min. The power of the UV lamp was 120 W. The photocatalytic membrane (PHOTOPERM BIT/313) was made from titanium dioxide (anatase form) deposited on titanium, TiO_2 (30 ± 3%)/Ti, and was kindly supplied by Bellobono Innovative Technologies (Milan, Italy). The deposition of the TiO_2 on to the pretreated titanium support was performed by plasma-grafting technique. After preparing the working solution, its pH was adjusted to 3 (optimal value established from previous studies) [38]. A hydrogen peroxide/chlorophenol molar ratio of 1 was used.

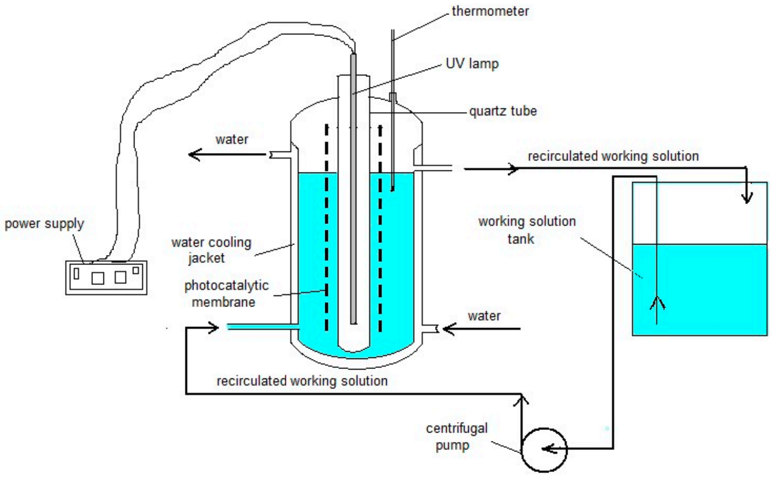

Figure 10. The simplified scheme of the photocatalytic reactor.

In order to monitor the evolution of the organic content of the working solution during the irradiation period, samples of 10 mL were taken from the reactor at predetermined irradiation times. At the end of each irradiation time, the samples were filtered and subjected to COD analysis according to the APHA 5220 D standard method (closed reflux, colorimetric method) [39]. The analyses were performed by using a Hach Lange LT 200 thermostat and Hach Lange DR 3800 spectrophotometer. It should be noted that after each experiment the photocatalytic membrane was regenerated by washing with distilled water (in which hydrogen peroxide of 30% was added) for at least 2 h, in the presence of UV radiation. Each experiment was carried out in triplicate.

3.3. Biodegradability Experiments

The biodegradability experiments were based on both OUR measurements in batch experiments and BOD analysis. The oxygen uptake rate measurements were performed in six Winkler bottles of 300 mL to which was attached the oxygen sensor in such a way that it is no longer possible for air to enter the bottle. So as not to disturb the biological process, the pH of the working solutions was adjusted to 7 with 1 M sodium hydroxide (NaOH) solution. The dissolved oxygen concentration was measured with a WTW Stirrox G oxygen sensor (WTW InoLab Multi 740). The pH in the system was measured with a WTW Sentix 82 pH electrode (WTW InoLab Multi 740). The tests were performed under continuous mixing following a modified OECD procedure [40]. In each bottle was introduced 20 mL of activated sludge with an average concentration of 3800 mg SS/L and 1900 mg VSS/L, 4 mL of working solution (containing as appropriate, chlorophenols, oxidation intermediates, or sodium acetate used as control), and this was diluted to 300 mL with a nutrient solution of which composition is described in the following. The Velp Scientifica FOC 225E thermostatic unit was used to maintain the working temperature at 20 ± 2 °C. The active sludge was kindly supplied by a local wastewater treatment plant and kept under stabile conditions by providing optimal amounts of oxygen and nutrient solution. The nutrient solution has the mass ratio between macronutrients of C:N:P = 100:5:1. It was prepared from 2.452 g of glucose, 3 g of sodium acetate, and 0.2 g of urea to which micronutrients were added such as 5 mL $MgSO_4 \cdot 7H_2O$ solution of 22.5 g/L, 20 mL $FeCl_3 \cdot 6H_2O$ solution of 0.25 g/L, 2 mL $CaCl_2 \cdot 6H_2O$ solution of 27.5 g/L, and 2 mL pH 7.2 buffer solution (formed from appropriate amounts of KH_2PO_4, $K_2HPO_4 \cdot 3H_2O$, $Na_2HPO_4 \cdot 12H_2O$, and NH_4Cl).

In the first Winkler bottle, endogenous respiration was determined, so that in this case the bottle contained only activated sludge and nutrient solution, without organic substrate. Exogenous respiration was determined in the next two Winkler bottles. To determine the exogenous respiration rate, oxygen consumption over time was measured by the activated sludge in contact with a readily biodegradable organic substrate (sodium acetate). In the last three Winkler bottles, exogenous respiration inhibition was determined. The rate of inhibition of exogenous respiration was the decrease in the rate of oxygen consumption by activated sludge in contact with a potentially inhibitory substance (chlorophenol or oxidation intermediates).

The determination of suspended solids (SS) was carried out by drying at 105 ± 2 °C a pre-filtered active sludge suspension sample through a glass-fiber filter until the constant mass was reached.

$$\mathrm{SS} = \frac{(\mathrm{m}_1 - \mathrm{m}_0)}{\mathrm{V}} \cdot 1000 \tag{15}$$

where m_1 represents the mass of the crucible with the active sludge sample (mg), m_0 represents the mass of the empty crucible (mg), and V represents the volume of the sample taken into measurements (mL). All determinations were performed in triplicate and presented as average values. Next, the samples were measured for volatile suspended solids (VSS) by calcination at 550 ± 2 °C for 30 min.

$$\mathrm{VSS} = \frac{(\mathrm{m}_2 - \mathrm{m}_0)}{\mathrm{V}} \cdot 1000 \tag{16}$$

where m_2 represents the mass of the crucible with the activated sludge sample from SS measurements (mg), m_0 represents the mass of the empty crucible (mg), and V represents the volume of the sample taken into measurements (mL). All determinations were performed in triplicate and presented as average values.

The biological oxygen demand (BOD) analysis were carried out in Winkler bottles in which the working solution, inoculum (activated sludge), and nutrient solution were introduced. The dissolved oxygen concentration was measured at the initial time and after five days of storage at a temperature of 20 ± 2 °C. The Velp Scientifica FOC 225E thermostatic unit was used to maintain the working temperature during the five days of storage. In parallel, control samples were run in the same conditions in which a solution of glucose-glutamic acid was used instead of working solution. The dissolved oxygen concentration was measured by using a WTW Stirrox G oxygen sensor (WTW InoLab Multi 740). Each experiment was carried out in triplicate.

3.4. Identification of Photocatalytic Oxidation Intermediates

The identification of photocatalytic oxidation intermediates was performed by gas chromatography coupled with mass spectroscopy (GC-MS) analysis using an Agilent 6890 gas chromatograph coupled with Agilent 5973 N mass spectrometer. It was used a HP−5MS GC column with 30 m × 0.32 mm, i.d., and thick-film substrate of 25 μm. The column temperature program was: 80 °C for 3 min, 370 °C for 7 min; heating rate of 15 °C/min. The solvent used for extraction was diethyl ether. The working solution/solvent extraction ration was 5/1. The extractions were performed in a single step using as a dehumidifier anhydrous sodium sulfate.

4. Conclusions

The biodegradability of intermediates formed during the process of photocatalytic oxidation of 4-chlorophenol and 2,4-dichlorophenol was studied in this work. The results obtained showed that the biodegradability of the intermediates formed depends on the type of chlorophenol subjected to photocatalytic oxidation, its initial concentration, and the irradiation time. It appears that 4-chlorophenol forms readily biodegradable products after a shorter irradiation time than 2,4-dichlorophenol. However, in both cases, the organic content of the aqueous solution subjected to the photocatalytic treatment becomes biodegradable at most half of the irradiation time necessary for a complete mineralization.

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