

## An investigation on TiO<sub>2</sub>-assisted photo-oxidation of thymine

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### Abstract

The photo-oxidation ( $\lambda_{\text{irr}} = 365 \text{ nm}$ ) of the biologically important compound thymine, catalysed by the semiconductor TiO<sub>2</sub>, was investigated in an aqueous suspension. The photocatalytic process was found to conform to a Langmuir adsorption isotherm. The influence of the initial thymine concentration, amount of TiO<sub>2</sub> and pH on the photocatalytic rate was studied. A mechanism involving the oxidation of thymine induced by  $\cdot\text{OH}$  and  $\text{H}^+$ , leading to the formation of thymine glycol, is proposed.

*Keywords:* TiO<sub>2</sub>; Photocatalysis; Thymine

### 1. Introduction

Semiconductor-assisted photocatalysis is an active area of research [1]. It has found application in solar energy conversion [2] and wastewater treatment processes [3,4]. The degradation of a number of organic pollutants, such as phenol [5], haloaromatics [6] and dyes [7], using semiconductor-mediated photoprocesses has been carried out. Recently, studies involving the modification of the semiconductor surface leading to changes in the photodegradation efficiency have been reported [8].

However, semiconductor-photocatalyst-induced reactions of biomolecules have received little attention. The investigation of biomolecules in this manner is important in understanding the photo-oxidation ability of these compounds in a heterogeneous environment (biological interfaces). A study of this nature may be extended to yield information on the role played by the electrostatic and electronic interactions with the metal. Furthermore, the site-specific modification of DNA via light-driven processes may be potentially useful in the development of novel methods for analysing DNA sequences [9]. Different photosensitizers, e.g. porphyrins, acridines and metal polypyridyl complexes, have been used to enhance the cleavage of DNA. One drawback in the use of such photosensitizers to initiate cleavage is that they remain present once their role has been carried out and may subsequently react with the DNA base or sugar radicals produced [10]. Hence, a semiconductor photocatalyst approach may

serve as a simpler and neater method of photogenerating potential oxidizing intermediates ( $\cdot\text{OH}$ ,  $h\nu_{\text{vb}}^+$ ,  $\text{O}_2^{\cdot-}$ ).

The photo-oxidation of pyrimidine bases is relevant to the direct effect of ionizing radiation on DNA, involving radical cation intermediates. In this work, the TiO<sub>2</sub>-assisted photo-oxidation of a pyrimidine base, thymine, is reported for the first time. This forms a part of our studies on the TiO<sub>2</sub> photo-oxidation of pyrimidine bases.

### 2. Experimental details

Thymine, NaOH, HClO<sub>4</sub> and all other chemicals used were of AnalaR grade. Water, distilled from a Kilburn still, was redistilled over alkaline permanganate in an all-glass vessel and used for solution preparation. The titanium dioxide (TiO<sub>2</sub>) photocatalyst was Degussa P-25. This material is mainly anatase, and has a BET surface area of 50 m<sup>2</sup> g<sup>-1</sup> and a mean particle size of 30 nm.

Irradiations were performed with a 125 W medium-pressure Hg lamp (Mysore Lamps, India). Aqueous suspensions of TiO<sub>2</sub> containing thymine in a polymerization tube, with an inlet for O<sub>2</sub> and with constant magnetic stirring, were irradiated for different intervals of time. All the experiments were carried out in the presence of O<sub>2</sub> as a scavenger for  $e_{\text{cb}}^-$ . The pH of the solutions was measured with a Toshniwal CL-46 pH meter. The progress of the reaction was followed by monitoring the disappearance of the pyrimidine base at 265 nm. The amount of thymine decomposed with respect to time was calculated. The rate constants  $k$  (s<sup>-1</sup>) were evaluated from plots of  $\log(\text{absorbance})_{265}$  vs. irradiation time, and the

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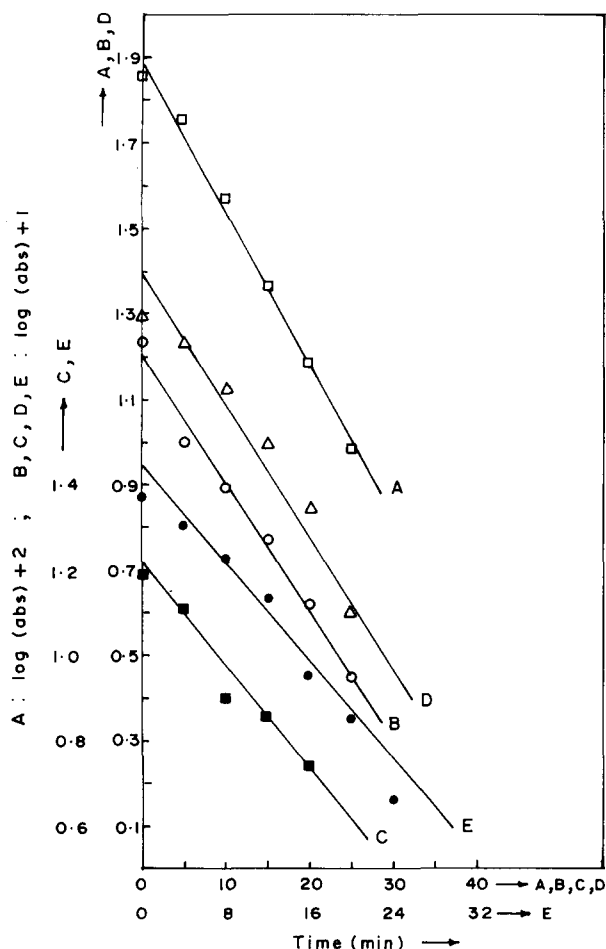


Fig. 1. Influence of  $[\text{thymine}]_0$  on the photocatalytic degradation rate (0.008 g  $\text{TiO}_2$  per 75 ml; pH 5.0): A,  $[\text{thymine}]_0 = 1.0 \times 10^{-4} \text{ mol dm}^{-3}$ ; B,  $[\text{thymine}]_0 = 1.5 \times 10^{-4} \text{ mol dm}^{-3}$ ; C,  $[\text{thymine}]_0 = 2.0 \times 10^{-4} \text{ mol dm}^{-3}$ ; D,  $[\text{thymine}]_0 = 2.5 \times 10^{-4} \text{ mol dm}^{-3}$ ; E,  $[\text{thymine}]_0 = 3.0 \times 10^{-4} \text{ mol dm}^{-3}$ .

Table 1  
Dependence of the photocatalytic degradation rate on  $[\text{thymine}]_0$  (pH 5.0; weight of  $\text{TiO}_2$ , 0.008 g per 75 ml)

$[\text{Thymine}]_0 \times 10^4$ ( $\text{mol dm}^{-3}$ )	Rate $\times 10^7$ ( $\text{mol dm}^{-3} \text{ s}^{-1}$ )
1	1.36
1.5	1.65
2.0	2.04
2.5	2.94
3.0	3.22

initial rates of decomposition of thymine were calculated by multiplying the rate constants with the appropriate initial thymine concentrations  $[\text{thymine}]_0$ .

The product thymine glycol was determined as follows. The irradiated solution was centrifuged and extracted with dichloromethane. It was then evaporated. The resulting solid was recrystallized from ethanol. The melting point and  $R_f$  value (paper chromatography) were determined; these were in close agreement with literature values [11], confirming the formation of thymine glycol.

### 3. Results and discussion

The effect of the initial concentration of thymine, amount of  $\text{TiO}_2$  and pH on the photocatalytic degradation (oxidation) rate was studied. From separate experiments, it was observed that the dark adsorption of thymine on  $\text{TiO}_2$  was negligible.

#### 3.1. Influence of $[\text{thymine}]_0$

At a fixed weight of  $\text{TiO}_2$  and pH, the photocatalytic experiments were carried out at different initial concentrations of thymine ( $[\text{thymine}]_0$ ). A plot of  $\log(\text{absorbance})_{265}$  vs. irradiation time from such experiments yields straight lines (Fig. 1); from the slopes, the rate constants  $k$  ( $\text{s}^{-1}$ ) can be evaluated. The  $[\text{thymine}]_0$  vs. rate data are given in Table 1. Experiments carried out with various initial concentrations of thymine ( $1 \times 10^{-4} \text{ mol dm}^{-3}$  to  $3 \times 10^{-4} \text{ mol dm}^{-3}$ ), at the natural pH of the solution, show that the rate of degradation of thymine ( $\text{rate} = k(\text{s}^{-1}) \times [\text{thymine}]_0$ ) initially increases with an increase in  $[\text{thymine}]_0$  and then remains almost constant beyond a certain level (Fig. 2), indicative of Langmuir kinetics. This is confirmed by the linear plot of  $1/\text{rate}$  vs.  $1/[\text{thymine}]_0$ , with an intercept on the ordinate (Fig. 3), in agreement with the equation

$$\text{rate} = \frac{k'K[\text{thymine}]_0}{1 + K[\text{thymine}]_0}$$

$$\frac{1}{\text{rate}} = \frac{1}{k'K[\text{thymine}]_0} + \frac{1}{k'}$$

where  $k'$  is a proportionality constant and  $K$  is the equilibrium constant or adsorption coefficient. The values of  $k'$  and  $K$  obtained from the intercept and slope of this plot are  $0.12 \text{ mol dm}^{-3} \text{ s}^{-1}$  and  $0.02445 \text{ dm}^3 \text{ mol}^{-1}$  respectively.

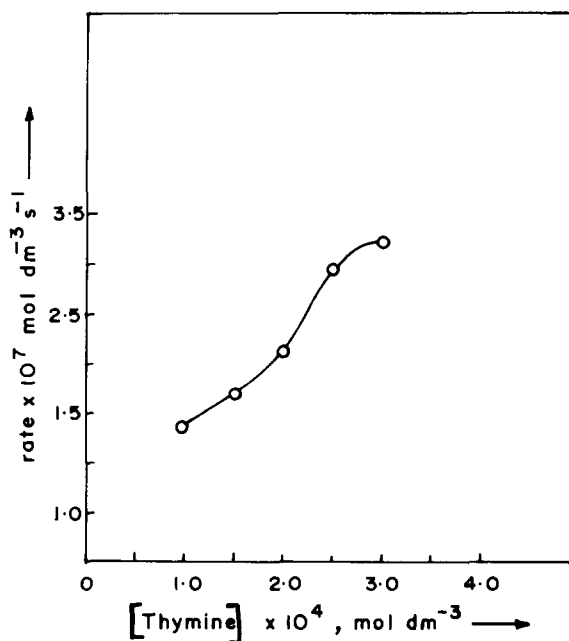


Fig. 2. Rate vs.  $[\text{thymine}]_0$  (0.008 g  $\text{TiO}_2$  per 75 ml; pH 5.0).

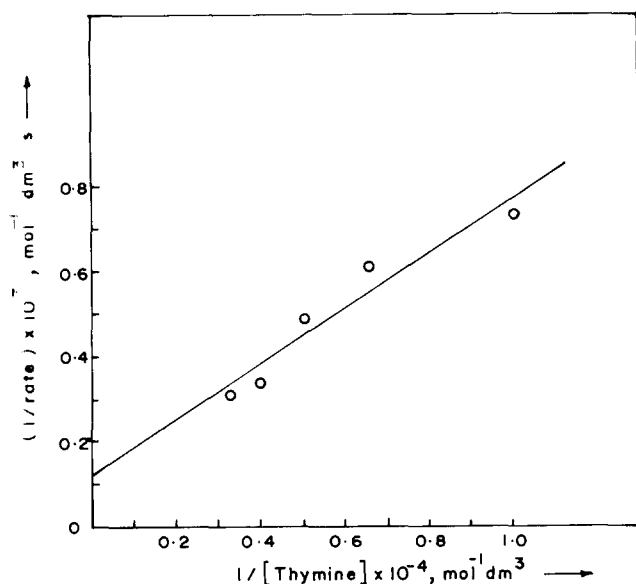
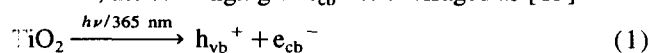


Fig. 3. Langmuir adsorption isotherm (0.008 g TiO<sub>2</sub> per 75 ml; pH 5.0).

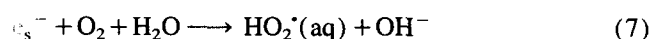
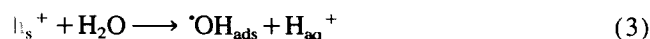
Band gap irradiation of the semiconductor TiO<sub>2</sub> leads to the generation of valence band holes and conduction band electrons [12]. Due to the presence of O<sub>2</sub> in the reaction solution, the scavenging of e<sub>cb</sub><sup>-</sup> is envisaged as [13]



Hence, it is clear that the recombination of h<sub>vb</sub><sup>+</sup> and e<sub>cb</sub><sup>-</sup> is prevented, enabling h<sub>vb</sub><sup>+</sup> to oxidize the pyrimidine base. Furthermore, the <sup>•</sup>OH and O<sub>2</sub><sup>•-</sup> produced (steps (3) and (6), see below) are powerful oxidants [14] and can also oxidatively degrade thymine.

In homogeneous solution, the <sup>•</sup>OH-induced oxidation of thymine by pulse radiolysis [15] has been reported. In the present work, thymine glycol was found to be the product formed. Thymine glycol was also identified as a product in the <sup>•</sup>OH-induced oxidation of thymine [15], indicating that a similar mechanism occurs in both cases.

The mechanistic scheme in the present work can be described as follows



Thymine glycol can originate from the attack of the C<sub>5</sub>–C<sub>6</sub> double bond by <sup>•</sup>OH (step (5)). This has been well established by electron spin resonance (ESR) studies [16]. Indeed, it has been reported that <sup>•</sup>OH is the main active species causing damage to the pyrimidine base [17]. Two isomeric radicals, namely 5-hydroxy-6-yl and 6-hydroxy-5-yl, of thy-

mine are the expected intermediates. These radicals differ in their redox properties [18]. In an earlier study using pulse radiolysis, it was established that the 5-yl radical is oxidizing and the 6-yl radical is reducing in nature [18]. Hence, in the present work, of the two primary redox equivalents generated, namely h<sub>vb</sub><sup>+</sup> and e<sub>cb</sub><sup>-</sup>, it is reasonable to foresee the oxidation of the 6-yl thymine radical by the powerful oxidant H<sup>+</sup> [19]. It is also possible that, based on the comparable oxidative ability of h<sup>+</sup> and SO<sub>4</sub><sup>•-</sup>, the oxidation of thymine by the loss of an electron from the C<sub>5</sub>–C<sub>6</sub> double bond to give the corresponding radical cation intermediates, leading finally to thymine glycol (by subsequent reaction of the radical cation with water), may occur [15]. Indeed, the involvement of such radical cations in the reaction of thymine with SO<sub>4</sub><sup>•-</sup> has been established [15].

### 3.2. Effect of amount of catalyst

The effect of the amount of catalyst TiO<sub>2</sub> on the photodegradation rate was investigated. Experiments were conducted with various amounts of catalyst powder (0.005–0.035 g per 75 ml) at constant [thymine]<sub>0</sub> (1 × 10<sup>-4</sup> mol dm<sup>-3</sup>). The rate increases with an increase in the amount of catalyst and remains almost constant above a certain level (Fig. 4, Table 2). This can be interpreted as follows. As the amount of catalyst is increased, the number of photons absorbed and the number of dye molecules adsorbed are increased owing to an increase in the number of catalyst particles. The density of particles in the area of illumination also increases and so the

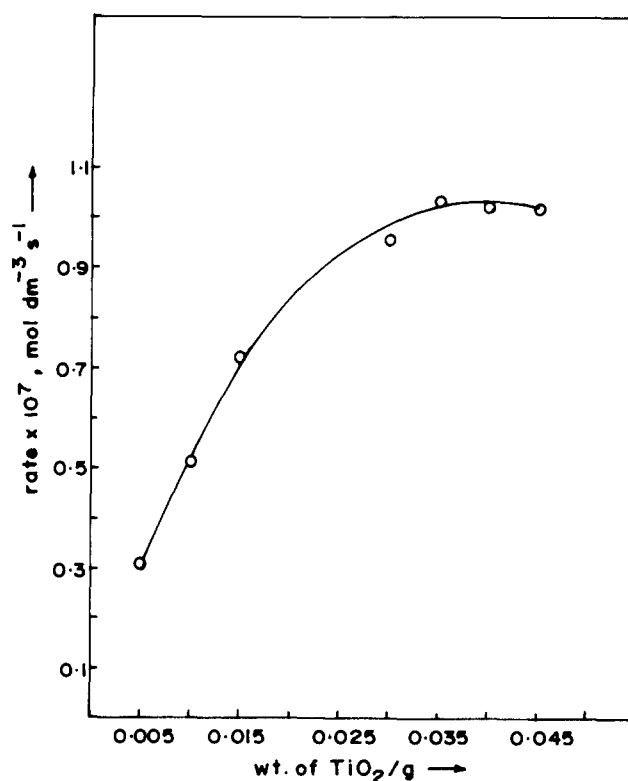


Fig. 4. Dependence of the photocatalytic degradation rate on the amount of TiO<sub>2</sub> ([thymine]<sub>0</sub> = 1 × 10<sup>-4</sup> mol dm<sup>-3</sup>; pH 5.0).

Table 2

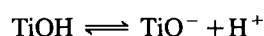
Dependence of the photocatalytic degradation rate on the amount of TiO<sub>2</sub> (pH 5.0; [thymine]<sub>0</sub> = 1 × 10<sup>-4</sup> mol dm<sup>-3</sup>)

Weight of TiO <sub>2</sub> (g) per 75 ml	Rate × 10 <sup>7</sup> (mol dm <sup>-3</sup> s <sup>-1</sup> )
0.005	0.307
0.010	0.511
0.015	0.720
0.030	0.950
0.035	1.030
0.040	1.010
0.045	1.020

rate is enhanced. At a certain level, the number of available substrate molecules is insufficient for adsorption by the increased number of TiO<sub>2</sub> particles, i.e. although more area is available, for constant [thymine]<sub>0</sub>, the number of substrate molecules present in the solution remains the same. Hence, above a certain level, additional catalyst powder is not involved in catalyst activity and the rate tends to level off.

### 3.3. Effect of pH

The role of pH on the photocatalytic degradation rate was studied at the pH values 3, 5, 6 and 8 at constant [thymine]<sub>0</sub> (1 × 10<sup>-4</sup> mol dm<sup>-3</sup>) and weight of TiO<sub>2</sub> (0.02 g per 75 ml) (Fig. 5, Table 3). The rate is at a maximum at pH 3 and then continuously decreases with increasing pH up to 8. This is indicative of the significant role of the surface properties of the photocatalyst TiO<sub>2</sub>, i.e. the acid–base properties of the metal oxide surface have a considerable influence on the photocatalytic activity with respect to pH. A similar influence of pH on the initial photocatalytic degradation rate was noted for the TiO<sub>2</sub>-catalysed photoreactions of trichloroacetate [20] and phenol [21]. In the case of trichloroacetate, the reaction was observed only below p*H*<sub>zpc</sub> of TiO<sub>2</sub> (3.5–6.7). In the case of phenol, a maximum rate of degradation was observed only at pH 3.3. The pH-dependent behaviour in the present study can be explained by the nernstian shift of the band edges to more negative values with increasing pH, leading to a decreasing oxidation potential of h<sub>ν</sub><sup>+</sup> at high pH [22]. Furthermore, the increase in photocatalytic effect in acidic solutions can be explained on the basis of the following dissociation mechanism of TiO<sub>2</sub>. The surface of a TiO<sub>2</sub> crystal is covered with TiOH in water



Since the dissociation does not proceed in acidic solutions [21], the photocatalytic effect is at a maximum at pH 3.

## 4. Conclusions

It has been shown that the TiO<sub>2</sub> photo-oxidation of thymine conforms to a Langmuir isotherm. The influence of the weight of TiO<sub>2</sub> on the photodegradation rate exhibits saturation

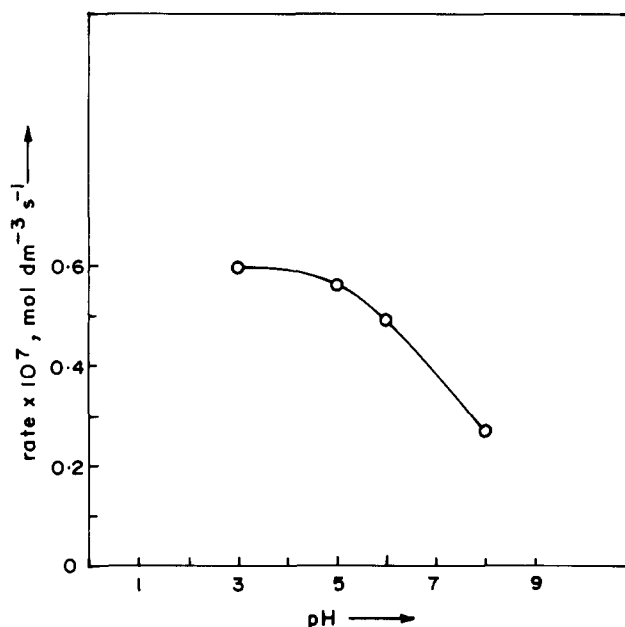


Fig. 5. Effect of pH on the photocatalytic degradation rate of thymine (0.02 g of TiO<sub>2</sub> per 75 ml; [thymine]<sub>0</sub> = 1 × 10<sup>-4</sup> mol dm<sup>-3</sup>).

Table 3

Effect of pH on the photocatalytic degradation rate (weight of TiO<sub>2</sub>, 0.02 g per 75 ml; [thymine]<sub>0</sub> = 1 × 10<sup>-4</sup> mol dm<sup>-3</sup>)

pH	Rate × 10 <sup>7</sup> (mol dm <sup>-3</sup> s <sup>-1</sup> )
3	0.59
5	0.56
6	0.49
8	0.27

behaviour. The marked role of pH can be explained in terms of the decreased oxidation potential of h<sub>ν</sub><sup>+</sup> at high pH and the dissociation of TiOH into TiO<sup>-</sup>. The rate is at a maximum at pH 3. A mechanism involving the oxidation of thymine by h<sub>ν</sub><sup>+</sup> and <sup>•</sup>OH is envisaged, via a radical cation intermediate of thymine, mimicking the direct effect of ionizing radiation on DNA.

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