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Photocatalytic inhibitory effect of immobilized TiO_2 semiconductor on the growth of *Escherichia coli* studied by acoustic wave impedance analysis

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Abstract

The effect of immobilized TiO_2 photocatalyst on the growth of *Escherichia coli* was investigated with acoustic wave impedance (AWI) analysis technique for the first time. The motional resistance variation (ΔR_1) curves under different growth conditions were obtained and compared. By fitting ΔR_1 versus time curves toward the impedance response model, three growth kinetic parameters (A, μ_m and λ) for different growth situations were gained. The results showed the lag time (λ) was prolonged, and the maximum specific growth rate (μ_m) and the asymptote (A) were decreased in the presence of UV light and TiO_2 photocatalyst. In addition, the quantificational relationships between the growth parameters and TiO_2 dosage were also investigated, which reflect the inhibitory effect of TiO_2 under a certain light intensity on the growth of *E. coli*. The three kinetic growth parameters (A, μ_m and λ) obtained from the impedance response model are close to those obtained from the Gompertz popular growth model. These results showed that the AWI analysis technique adapts to study the effect of TiO_2 photocatalyst on the growth of bacteria on the qualitative and quantificational level.

Keywords: Photocatalytic inhibition; TiO_2 photocatalyst; Growth process; Acoustic wave impedance analysis; Impedance response model

1. Introduction

In 1985, Matsunaga et al. reported for the first time the antibacterial effect of TiO_2 photocatalytic reactions [1]. Since then, the biological effect of TiO_2 photocatalyst has attracted increasing attention worldwide. So far, research work on the photobiological action of TiO_2 has been intensively conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae and cancer cells [2–9]. However, all these reports have focused their interests only upon the study of antibacterial results. As to the photocatalytic inhibitory effect of TiO_2 on the growth of bacteria, i.e. effect on the growth kinetic parameters, we are not aware of any published studies. But to study the response of bacteria to TiO_2 photocatalytic reactions in more detail and understand the biological effect of TiO_2 photocatalyst on bacteria further, investigating the effect of TiO_2 photocatalytic reactions on the growth kinetic parameters of bacteria and describing the effect quantitatively is significant. This paper is aimed at performing this aspect of work.

Acoustic wave impedance analysis (AWI) is another kind of piezoelectric sensing technique. It can provide multidimensional and real-time information to reflect some physical and chemical properties of the investigated system. It can be analyzed according to the Butterworth–Van Dyke (BVD) equivalent electrical circuit model (Fig. 1a), composed of a motional arm and a static arm in parallel. The motional arm contains three equivalent circuit elements in series, i.e. motional resistance (R_1), motional inductance (L_1) and motional capacitance (C_1), while the static arm only contains the static capacitance (C_0). All of these parameters have distinct physical meanings [10–14]. AWI analysis technique has been successfully applied to many fields, such as the study of DNA–platinum-based drug interaction mechanism [15], the determination of α-amylase [16] and rheumatoid factor [17], and monitoring of mutagenic process [18].

In this paper, we took *Escherichia coli* as model bacteria and the effect of immobilized TiO_2 photocatalyst on the growth of *E. coli* was studied by AWI analysis technique for the first time. The study was based on the following fact: the physical and chemical properties of culture system would change due to the growth of *E. coli* in culture medium, this causes the variations of equivalent circuit parameters of piezoelectric quartz crystal (PQC) accordingly.

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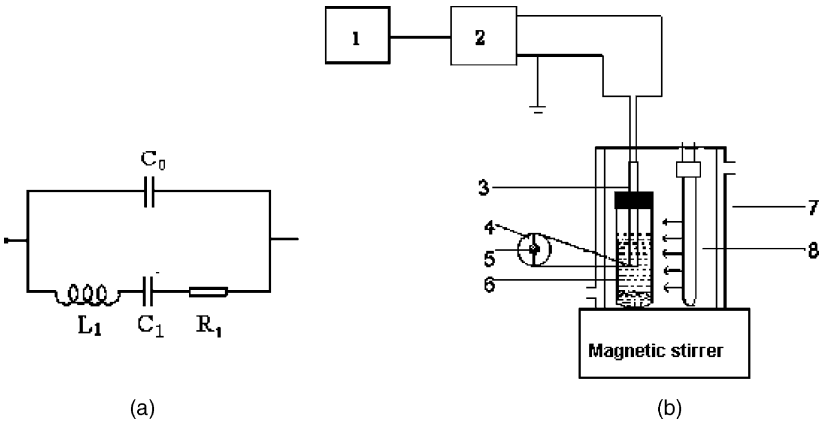

Fig. 1. (a) Electrical equivalent circuit for the PQC sensor: C_0 is the static capacitance, and L_1, C_1 and R_1 are the motional inductance, motional capacitance and motional resistance, respectively. (b) Schematic diagram of the experimental setup: (1) personal computer; (2) HP4192A LF impedance analyzer; (3) PQC sensor; (4) quartz crystal; (5) gold electrode; (6) the detection cell with TiO_2 photocatalyst immobilized on the inside surface; (7) thermostatic bath; (8) UV light.

The changing process of equivalent circuit parameters could reflect the growth process of *E. coli*. Thus, by monitoring the different changing process of impedance parameters in the absence/presence of UV-activated TiO_2, we could investigate the inhibitory effect of TiO_2 photocatalyst on the growth of bacteria.

2. Materials and methods

2.1. Reagents

Nanosized TiO_2 power (primary anatase, 30 nm mean particle size, 40 m^2/g surface area) was purchased from Central South University (Changsha, China). All chemicals used were analytical reagent grade. Double-distilled and sterilized water was used throughout. The composition of the culture medium for *E. coli* was as follows: beef extract, 3 g; peptone, 10 g; sodium chloride, 5 g; distilled water, 1000 ml. The pH value of medium was adjust to 7.2 by 0.1 mol/l NaOH. The medium was sterilized by autoclaving at 121 °C for 15 min.

2.2. Bacterial culture

E. coli (DH 5α) was obtained from the College of Life Science of Hunan University (Changsha, China). Four loops of *E. coli* on slant agar were inoculated into a 50 ml sterilized conical glass flasks containing 25 ml of medium and were cultured aerobically at 37 °C for about 18 h. The culture gave an approximate concentration of 3.8×10^8 cells/ml determined by the PPC method.

2.3. Materials and instrumentation

The AT-cut 9 MHz piezoelectric quartz crystals (12.5 mm in diameter) with a gold electrode (6 mm in diameter) on each side were purchased from National 707 Factory (Beijing, China). The gold-coated quartz crystal was first washed with acetone and then sterilized by autoclaving at 121 °C for 15 min before use.

The experimental setup employed in this study is shown in Fig. 1b. It consists of an AWI analysis system, a 6 W ultraviolet lamp and a thermostatic jacket. The AWI analysis system comprises a HP4192A LF impedance analyzer, which one side connected to the terminal contacting liquid of PQC, and the other side connected with a personal computer in which a user program was written in Visual Basic 6.0 to control the analyzer and to acquire admittance data. The equivalent circuit parameters were obtained at a time interval of ca. 2 min and displayed in the Visual Basic form during experiments. The peak wavelength of the ultraviolet lamp is 356 nm and the light intensity was measured by a UV power meter (Photoelectric Instrument Company of Beijing Normal University). The incident UV light intensity was approximately 40 μW/cm^2.

2.4. Immobilization of TiO_2 photocatalyst

The immobilization of TiO_2 was similar to the procedures described by Huassain et al. [19] with modifications. Requisite amount of nanosized TiO_2 was weighed and dispersed in 5 ml of double-distilled water. The suspension was subsequently homogenized by sonication for 15 min, and then 0.5 ml of the solution was introduced into the detection cell to cover uniformly the internal wall of the detection cell. Finally, the suspension was evaporated to dryness under vacuum with warm air blow around the external surface of the detection cell. The detection cell was sterilized in an oven at 450 °C for 2 h before use.

2.5. Procedures

Fifteen microliters of stock *E. coli* cells suspension and 15 ml sterilized medium were added to detection cell and

mixed thoroughly. Then the PQC sensor was immersed. The detection cell was stuffed with a rubber plug and placed in a thermostatic jacket at $37 \pm 0.1\,^{\circ}C$. Meanwhile, the ultraviolet lamp was turned on and the variation of the impedance parameters was monitored by AWI analysis system. The culture system was stirred slightly at a time interval of ca. 15 min.

3. Results and discussion

3.1. Response theory of the AWI analysis

When the PQC sensor was used in gas, Sauerbrey equation [20] displays a linear relationship between the mass change (Δm) of the PQC and the frequency shift (Δf_m):

$$\Delta f_m = -\left(\frac{2{f_0}^2}{(\rho_Q \mu_Q)^{1/2}}\right)\left(\frac{\Delta m}{A}\right) \quad (1)$$

where f_0 is the fundamental resonant frequency of PQC, ρ_Q and μ_Q are the density and the shear modulus of quartz crystal, respectively, and A is the area of the quartz plate.

In the liquid, the physical and chemical properties of the solution can also cause the frequency to change [13]. It can be expressed as follows:

$$\Delta f_L = -\frac{f_0^{3/2}(\rho_L \eta_L)^{1/2}}{(\pi \rho_Q \mu_Q)^{1/2}} \quad (2)$$

where ρ_L and η_L are the viscosity and density of the liquid, respectively.

Therefore, the total frequency shift (Δf) is the sum of Δf_m and Δf_L:

$$\Delta f = \Delta f_m + \Delta f_L \quad (3)$$

In the AWI analysis, the motional resistance (R_1) is related linearly to the $(\rho_L \eta_L)^{1/2}$ of the liquid [10]:

$$R_1 = \frac{(2\pi f_0 \rho_L \eta_L)^{1/2} A}{\kappa^2} \quad (4)$$

where κ represents the electromechanical coupling factor.

If Δf is dominated by the net changes in the viscosity and density of liquid, as for a presently used 9 MHz piezoelectric quartz crystal, the slope of Δf versus ΔR_1 is calculated to be 9.45 Hz/Ω according to Calvo et al. [21]. As for a practical system, the ratio of Δf versus ΔR_1 is often greater than 9.45 Hz/Ω, because measured value of Δf may contain the contributions from other factors, such as mass absorption.

3.2. Typical response curves during the normal growth process

The typical response curves of impedance parameters (Δf, ΔR_1, ΔC_0) during the normal growth of *E. coli* without TiO_2 and UV light are shown in Fig. 2. It can be seen that ΔR_1 almost keeps a constant value during the initial 5 h to form the first plateau, then increases continuously for about 3 h and finally reaches a stable level to form the second plateau. The total signal size of ΔR_1 is about 30 Ω. The result reflects the variation of the viscosity–density of culture system during the growth of *E. coli*. In addition, it can be noted that the shape of ΔR_1 response curve is similar to the theoretical growth curve of bacteria. The first plateau phase corresponds to the lag phase of the growth of bacteria, the quickly increasing phase corresponds to the exponential growth phase and the second plateau phase corresponds to bacterial growth saturation phase.

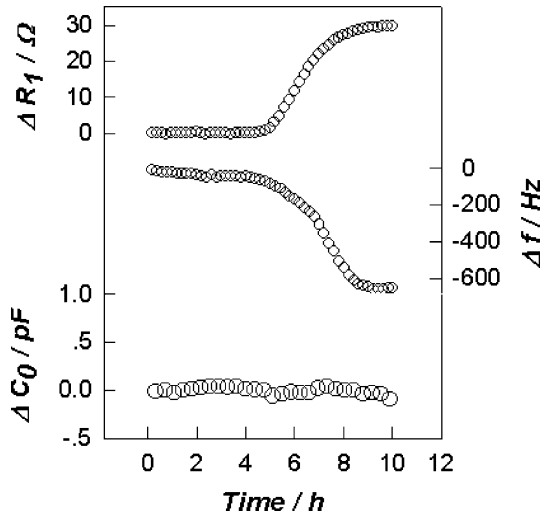

Fig. 2. Time courses of simultaneous response of ΔR_1, Δf and ΔC_0 during the normal growth of *E. coil* without TiO_2 and UV light. The initial cell concentration of the detection system is 3.8×10^5 cells/ml and the volume of the medium is 15 ml.

It also can be seen from Fig. 2 that Δf decreased for about 664 Hz and ΔR_1 increased for about 30 Ω. The ratio of Δf and ΔR_1 is 22.1 Hz/Ω. The value is larger than 9.45 Hz/Ω and indicates that the decrease of f originates not only from the increase in viscosity–density of the culture system, but also from the adsorption of the culture components onto the gold electrode of the PQC sensor. However, ΔR_1 still reflects well the variation in viscosity–density of culture system according to Eq. (4). So the change of ΔR_1 was used to analyze the effect of TiO_2 photocatalyst on the growth of bacteria.

It can also be found that ΔC_0 keeps a constant value basically during the normal growth process. This result showed that the dielectric constant of the quartz almost has no change.

3.3. ΔR_1 response curves during the growth of E. coli in the presence of TiO_2 photocatalyst

The effect of TiO_2 photocatalyst under UV light on the growth of *E. coli* was investigated and the response curve of ΔR_1 is shown in Fig. 3c. In the presence of illuminated TiO_2, ΔR_1 curve displays similar changing trend. The first plateau

lasts ca. 8 h, and then after a rapid increase in ΔR_1 for about 9 h, the second plateau appears. The total signal size of ΔR_1 is about 14 Ω. But by comparison with curve (a) obtained from the normal growth condition, we could find the difference between these two curves from following aspects. First, the lasting time of the first plateau is different, the continuance of this phase in curve (c) is much longer than that in curve (a). It is because that the viable cell concentration is less than that in the normal growth case due to the photocatalytic reactions of TiO_2, and the surviving bacteria need a period time to regain the growth capability. So the time of *E. coli* getting into the exponential phase is prolonged and then the lasting time of the first plateau is longer. Secondly, the signal size of ΔR_1 is different, smaller ΔR_{max} value is obtained in curve (c). It may be that the amount of *E. coli* getting into the exponential phase is smaller than that in normal growth condition, thus the variation of viscosity–density is less than that of normal growth; hence the ΔR_{max} value is smaller. Thirdly, the slope of ΔR_1 curve in the great increasing phase is different. It can be seen from Fig. 3 that ΔR_1 increases slower in curve (c). There are two possible reasons, the first reason is that photocatalytic action of TiO_2 influences a specific biochemical step that is vital for bacterial propagation, and thus cellular metabolism and growth mechanism is influenced. So the growth rate is slower than the normal growth rate. The second reason is that the nutriment in the medium is decomposed by TiO_2 photocatalytic reactions and lead to the decrease of the amount of available nutriment during the growth of the bacteria. It also can slow down the growth rate of bacteria. Due to these two reasons, the variation rate of the viscosity–density is decreased and caused the smaller slope of ΔR_1 curves.

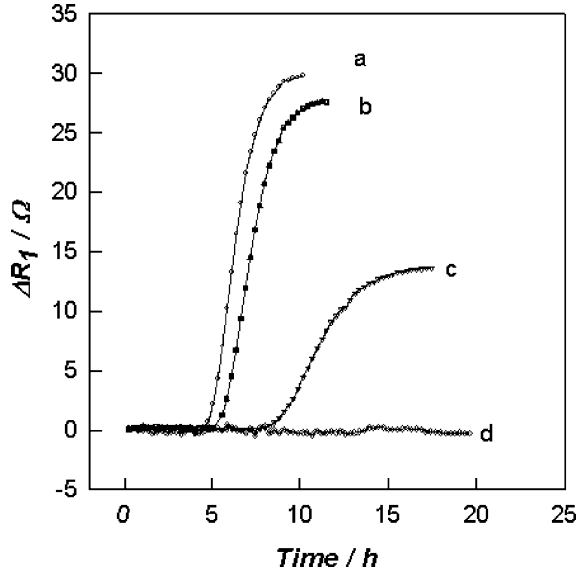

Fig. 3. ΔR_1 response curves during the growth of *E. coli* under different experimental conditions. The initial cell concentration of the detection system is 3.8×10^5 cells/ml and the volume of the medium is 15 ml. The incident UV light intensity was approximately 40 μW/cm^2. (a) Cell + medium without TiO_2 and UV light; (b) cell + medium with UV light; (c) cell + medium with TiO_2 (1.0 mg) and UV light; (d) only medium with TiO_2 (1.0 mg) and UV light.

Since ΔR_1 response curve is similar to the theoretical growth curve of bacteria, the above results showed that the lag time is prolonged, and the maximum number of bacteria and the growth rate are reduced due to TiO_2 photocatalytic reactions. In short, the growth of *E. coli* was inhibited in the presence of TiO_2 photocatalyst.

To investigate the effect of immobilized TiO_2 on the medium, we also carried out the experiment only with the fresh medium without bacteria under the same experimental conditions and the result is shown in Fig. 3d. It can be seen that the ΔR_1 response curve keeps a beeline basically. That is to say, the viscosity–density of the solution dose not change obviously within 20 h in the presence of UV-activated TiO_2. Therefore, curve (c) dose not include the effect of TiO_2 on the medium.

Control experiments were run with UV light only. The ΔR_1 response curve is Fig. 3b. It suggests that the growth of *E. coli* was still inhibited under only light irradiation condition. But the inhibitory effect of UV light was much less than that of TiO_2 photocatalyst under UV light with the same light intensity.

3.4. Effect of TiO_2 dosage on the photocatalytic inhibitory activity

Under the same experimental conditions, the influence of TiO_2 dose on the ΔR_1 response curves was examined. Fig. 4 contains the relevant data. It can be noticed from this figure that the slope of ΔR_1 curve in the great increasing phase and the single size of ΔR_1 are decreased, but the lasting time of the first plateau is increased with an increase in TiO_2

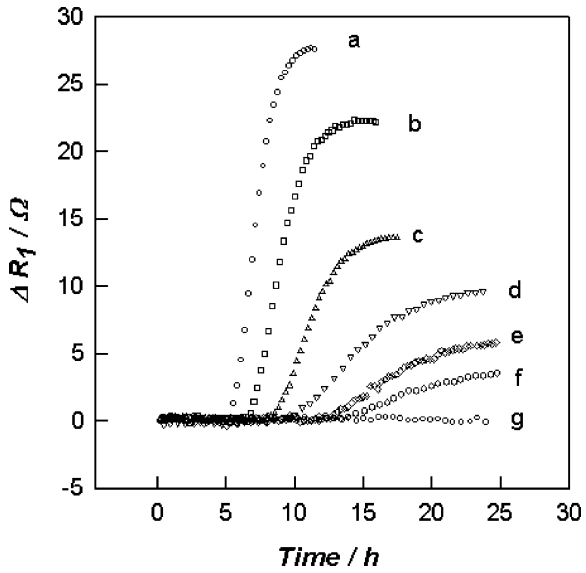

Fig. 4. Influence of TiO_2 dose on the ΔR_1 response curves: (a) 0.0 mg; (b) 0.5 mg; (c) 1.0 mg; (d) 1.5 mg; (e) 2.0 mg; (f) 2.5 mg; (g) 5.0 mg. The other experimental conditions are same as in Fig. 3.

dose. The result showed that the growth of *E. coli* was inhibited due to the effect of TiO_2 photocatalytic reactions, and the photocatalytic inhibitory effect was stronger when larger dose of TiO_2 was used. It could be noticed that ΔR_1 response nearly did not change within 24 h when high dosage of TiO_2 was used, which indicates *E. coli* was inactivated totally and did not grow again when a high dosage of TiO_2 was used.

3.5. AWI response model and the kinetic parameters of bacterial growth estimation

In general, a typical bacterial growth curve can be described by Gompertz model [22]:

$$\ln\left(\frac{N}{N_0}\right) = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (5)$$

where t is the culture time. A with no dimension, μ_m in h^{-1} and λ in h are the asymptote, maximum specific growth rate and lag time, respectively; each of them has its specific biological meanings. Each changes with the change of the growth situation of bacteria.

To examine the relationship between ΔR_1 response and $\ln(N/N_0)$, the cell concentration was determined by PPC method during the AWI analysis. The results showed that there is a linear relationship between ΔR_1 and $\ln(N/N_0)$:

$$\Delta R_1 = k\ln\left(\frac{N}{N_0}\right) \quad (6)$$

where k is a coefficient in Ω.

From Eqs. (5) and (6), a new impedance response model which reflecting the relationship between ΔR_1 and the bacterial growth kinetic parameters can be obtained as follows:

$$\Delta R_1 = A'\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (7)$$

By taking A', A, μ_m and λ as estimation parameters, the non-linear fitting program embedded in Sigmaplot Scientific Graphing Software version 2.0. is used to obtained the fitted values and the quality of the fitting can be evaluated by the relative sum of the residual square (q_r) defined as:

$$q_r = \frac{\sum_1^n(\Delta R_{fit} - \Delta R_{exp})^2}{\sum_1^n(\Delta R_{exp})^2} \quad (8)$$

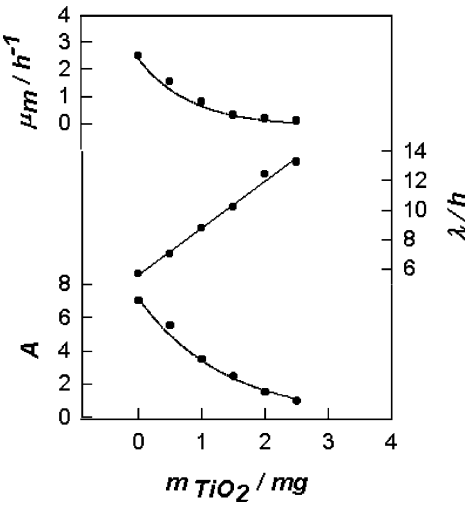

Fig. 5. Relationship between λ, μ_m, A and TiO_2 dosage, respectively. The samples are the experimental data. The line is the fitting data.

where ΔR_{fit} represents the fitted value, ΔR_{exp} is the experimental response value and n is the number of the response signal points.

By fitting the experimentally obtained values of ΔR_1 in Figs. 3 and 4 to the derived model, the fitted kinetic parameters and q_r were obtained and shown in Table 1. Compared with the kinetic parameters obtained from the normal growth case, the lag time (λ) is prolonged, and the maximum specific growth rate (μ_m) and the asymptote (A) are decreased in the presence of UV light and TiO_2 photocatalyst. Since these three parameters reflect the growth situation of bacteria. The change in the three parameters shows that the growth of *E. coli* is inhibited by the photocatalytic activity of TiO_2 and the inhibitory effect is more serious with an increase in TiO_2 dose. The satisfactory value of q_r in all cases shows that the ΔR_1 versus time curves can be used to reflect the growth situations of *E. coli* in the presence/absence of UV light and TiO_2 and this impedance response model can reasonably describe the kinetic characteristic in the growth process.

To investigate the effect of TiO_2 dosage on the photocatalytic inhibitory activity in more detail, the curves of λ, μ_m and A versus TiO_2 dose were plotted, respectively. The results are shown in Fig. 5.

Table 1
Results obtained by fitting the ΔR_1 response values in Figs. 3 and 4 to Eq. (7)

Experiment	A'	μ_m	λ	A	k	q_r
Without TiO_2 and UV light	30.1396	3.0469	5.0243	7.5888	3.9716	6.21×10^{-5}
With UV light only	27.9983	2.4945	5.7083	6.9905	4.0052	5.83×10^{-5}
With illuminated TiO_2 (0.5 mg)	22.3821	1.5315	7.0273	5.5360	4.0430	1.04×10^{-4}
With illuminated TiO_2 (1.0 mg)	13.8042	0.7797	8.7526	3.4793	3.9775	2.62×10^{-4}
With illuminated TiO_2 (1.5 mg)	9.8803	0.3041	10.2078	2.4716	3.9976	4.88×10^{-4}
With illuminated TiO_2 (2.0 mg)	6.0669	0.1816	12.4272	1.5249	3.9786	2.43×10^{-3}
With illuminated TiO_2 (2.5 mg)	3.8413	0.1038	13.2641	0.9660	3.9761	5.54×10^{-3}

Table 2
Bacterial kinetic growth parameters obtained from two models

Experiment	A		μ_m		λ	
	Proposed	Gompertz	Proposed	Gompertz	Proposed	Gompertz
Without TiO_2 and UV light	7.5888	7.6275	3.0469	3.1104	5.0243	5.0671
With UV light only	6.9905	7.0765	2.4945	2.5015	5.7083	5.5522
With illuminated TiO_2 (0.5 mg)	5.5360	5.6443	1.5315	1.6089	7.0273	7.1233
With illuminated TiO_2 (1.0 mg)	3.4793	3.3757	0.7797	0.7113	8.7526	8.8416
With illuminated TiO_2 (1.5 mg)	2.4716	2.5627	0.3041	0.3539	10.2078	10.3174
With illuminated TiO_2 (2.0 mg)	1.5249	1.2516	0.1816	0.1307	12.4272	12.8045
With illuminated TiO_2 (2.5 mg)	0.9660	1.1508	0.1038	0.0829	13.2641	13.5899

From Fig. 5, it can be seen that there is a linear relationship between λ and TiO_2 dosage, while an exponential relationship exists between μ_m, A and TiO_2 dosage, respectively. The regression equations were obtained as follows:

$$\lambda = 5.60 + 3.17m \qquad (r = 0.992) \tag{9}$$

$$\mu_m = 2.55e^{-1.20m} \qquad (q_r = 4.03 \times 10^{-3}) \tag{10}$$

$$A = 7.25e^{-0.73m} \qquad (q_r = 3.84 \times 10^{-3}) \tag{11}$$

where m is the dosage of TiO_2 in mg.

From Eqs. (7) and (9)–(11), a quantitative equation reflecting the relationship between ΔR_1 response and TiO_2 dosage was derived as follows:

$$\Delta R_1 = 7.25k \exp\{-0.73m - \exp[e^{1-0.47m}(1.96 + 11.1m - 0.35t) + 1]\} \tag{12}$$

We calculated the value of k in all cases and the results are shown in Table 1. One can see that the value of k was approximately 4, so we taken k as 4 Ω and then Eq. (12) can be expressed as:

$$\Delta R_1 = 29.00 \exp\{-0.73m - \exp[e^{1-0.47m}(1.96 + 11.1m - 0.35t) + 1]\} \tag{13}$$

This equation quantificationally reflects the photocatalytic inhibitory effect of TiO_2 on the growth of *E. coli*. In fact, we can obtain different quantitative relationships corresponding to different type of TiO_2 and different light intensity by AWI technique. And with these relationships, we can quantificationally know the impact of TiO_2 on the growth of bacteria and predict the growth trend under the given TiO_2 dosage or light intensity, which is useful for the optimal use of the TiO_2 photocatalyst and the rational design of TiO_2 photocatalytic reactors. So the AWI analysis technique can be used to investigate the inhibitory effect of TiO_2 under different conditions on the qualitative and quantificational level.

3.6. Comparison of AWI technique with the traditional PPC method

In this study, the growth situations of *E. coli* under different culture conditions were also examined by PPC method. By fitting the experimentally obtained values of cell concentration to the Gompertz model, the bacterial growth parameters were obtained and compared with those obtained from the impedance response model. Table 2 contains the relevant data. One can seen that the parameters of bacterial growth obtained from these two models are close, which indicates that AWI technique can be used to study the effect of TiO_2 on the growth of bacteria.

4. Conclusion

In this study, AWI analysis technique has been adopted successfully to study the effect of immobilized TiO_2 photocatalyst on the growth of *E. coli*. The present work extended previous work by others in two ways. First, the growth process is monitored in real time by AWI technique. So far, the antibacterial effect of TiO_2 has been investigated mainly by microbial methods (such as PPC method). These methods are not only cumbersome and time-consuming, but also cannot provide the process information in real time. Whereas, in our work, AWI analysis technique combined the microbial growth with the change of the physical–chemical properties in the culture system during the growth process and was used to monitor the growth process of bacteria (not only just adapt to *E. coli*) in the presence of UV-activated TiO_2 in real time. Secondly, the present work studied the inhibitory effect of TiO_2 on the growth of bacteria in more detail. Three growth kinetic parameters (A, λ and μ_m) under different growth conditions have been investigated by fitting the experimental data towards the impedance response model, The results showed the lag time (λ) is prolonged, and the maximum specific growth rate (μ_m) and the asymptote (A) are decreased in the presence of UV light and TiO_2 photocatalyst. Besides, we also discussed the inhibitory effect of TiO_2 under the experimental conditions on the quantificational level. Compared with the microbial methods, AWI analysis technique not only can provide real-time growth process information in the presence of UV-activated TiO_2, but also can study the inhibitory effect of TiO_2 on the bacterial growth on the qualitative and quantificational level. Therefore, this work not only examined the effect of TiO_2 on bacterial growth in more detail, but also provided a new

method (AWI analysis technique) for studying the biological effects of TiO_2 photocatalytic reactions.

Acknowledgements

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