Enhanced inactivation of *E. coli* bacteria using immobilized porous TiO$_2$ photoelectrocatalysis

Nir Baram$^a$, David Starosvetsky$^a$, Jeana Starosvetsky$^b$, Marina Epshtein$^b$, Robert Armon$^b$, Yair Ein-Eli$^{b,*}$

$^a$ Department of Materials Engineering, Technion-Israel Institute of Technology, 32000 Haifa, Israel
$^b$ Civil and Environmental Engineering, Technion-Israel Institute of Technology, 32000 Haifa, Israel

**Abstract**

Immobilized TiO$_2$ nanotube electrodes with high surface areas were grown via electrochemical anodization in aqueous solution containing fluoride ions for photocatalysis applications. The photoelectrochemical properties of the grown immobilized TiO$_2$ film were studied by potentiodynamic measurements (linear sweep voltammetry), in addition to the calculation of the photocurrent response. The nanotube electrode properties were compared to mesoporous TiO$_2$ electrodes grown by anodization in sulfuric acid at high potentials (above the microsparking potential) and to 1 g/l P-25 TiO$_2$ powder. Photocatalyst films were evaluated by high resolution SEM and XRD for surface and crystallographic characterization. Finally, photoelectrocatalytic application of TiO$_2$ was studied via inactivation of *E. coli*. The use of the high surface area TiO$_2$ nanotubes resulted in a high photocurrent and an extremely rapid *E. coli* inactivation rate of $\sim 10^6$ CFU/ml bacteria within 10 min. The immobilized nanotube system is proven to be the most potent electrode for water purification.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Over the last few decades, titanium dioxide has been the main material used for photocatalytic degradation of organic compounds for water purification. Various studies have shown that using TiO$_2$ in photocatalysis is an efficient process for the removal of a large variety of hazardous chemicals [1–4]. The mechanism of photocatalysis in the presence of TiO$_2$ is the enhanced formation of hydroxyl (OH$^\bullet$) radicals which are active in oxidation processes and have a significant effect on the chemical oxidation of organic compounds in the environment. Complete mineralization of many organic substances is possible in aqueous systems, when sufficient OH$^\bullet$ radical flux can be generated.

A wide variety of preparation methods of TiO$_2$ exist, and intensive efforts are being devoted to fabrication of high surface area TiO$_2$ photocatalysts. One of the most common methods to obtain a high surface area is by using powdery TiO$_2$. In this regard, it is generally well acknowledged that titania of Degussa P-25, produced by hydrolysis of TiCl$_4$, is considered to be a superior commercial photocatalyst [5]. Two limiting problems arise when TiO$_2$ powder is being used: environmental problems from insufficient filtration [6,7], and a lack of ability to reuse and regenerate the material. Those problems can be effectively solved using embedded catalysts fabricated via the implementation of a few methods, such as gelation [8,9], sputtering [10], and electrochemical oxidation [11,12]. Thus, the catalysts can be repeatedly used and easily removed [13,14]. Another major obstacle in the development of photocatalytic reactors is the relatively low efficiency of light utilization due to high recombination rates of electrons and holes [15]. This drawback can be overcome by applying an anodic potential to the high surface area photocatalyst anode and suppressing the recombination of electrons and holes [14,16,17]. Recently, we demonstrated in a short communication that anodically electrochemically grown titania from molten salts eliminated *E. coli* bacteria in a short fairly time [18].

Electrochemical polarization of titanium is an effective method for growing TiO$_2$ because of the good mechanical adhesion and the electrical conductivity of the titanium metal substrate. The parameters that most affect the TiO$_2$ characteristics are the electrolyte type, concentration, and pH value. Using different electrolytes and applied potentials can alter the microstructure, morphology, and thickness of the grown oxide layer [18–21]. Phosphoric and sulfuric acids are very common electrolytes in various degrees of dilution. The addition of fluoride ions to the electrolyte during anodization changes the mechanism of growth leading to the formation of long and smooth nanotubular morphology [22,23].
In this report, immobilized nanotubular TiO$_2$ with high surface area was grown by anodization of Ti in aqueous solution containing fluoride ions. The photocurrent response was measured and compared to mesoporous oxide layers that were grown by anodization of Ti in sulfuric acid solution. The efficiency and kinetics of the photoelectrocatalytic devices having immobilized TiO$_2$ as an anode were studied. The efficiency of *E. coli* inactivation was experimentally compared to Degussa P-25 powder TiO$_2$.

2. Experimental

2.1. Anodization of Ti

Titanium foils (99.2% purity, Alfa Aesar), 0.5 mm in thickness, were mechanically polished and then etched in a HF: HCl: HNO$_3$ solution, followed by rinsing with deionized water. Ti anodization was carried out at a constant potential of 20 V in a Hewlett Packard 6035A system power supply for 2 h at room temperature in 1 M Na$_2$SO$_4$ + 0.5 wt% NaF solution with a platinum counter electrode, as was previously described by Macak et al. [24]. Subsequent to the anodization process, the samples were thermally treated at 450 °C for 3 h in ambient air (unprotected atmosphere).

Immobilized titania was also prepared by anodizing in 0.5 M sulfuric acid at a constant current of 100 mA/cm$^2$ until final potentials of 110 and 150 V was reached. Such formation was conducted subsequent to the cleaning procedure described above.

2.2. Electrode characterization

Potentiodynamic evaluation of the Ti/TiO$_2$ electrodes (linear sweep voltammetry) in a 0.01% NaCl solution was performed in a scan rate of 5 mV s$^{-1}$, using a potentiostat/galvanostat model 263A (EG&G Princeton Applied Research). The potentials were measured relative to a standard calomel reference electrode.

The surface morphology of the formed TiO$_2$, pore size, and distribution were characterized using high resolution scanning electron microscopy (HRSEM) on a LEO 982 Gemini microscope equipped with a field emission gun (FEG–SEM). X-ray diffraction measurements (XRD) were acquired in order to determine the TiO$_2$ phase using a conventional X-ray powder diffractometer (Philips X’Pert Diffractometer, Eindhoven, The Netherlands) with a CuKa tube, operated at 40 mA and 40 kV.

2.3. Microbiological studies

Microbiological studies were carried out with *E. coli* bacteria (strain CN13) suspension prepared in 0.01% NaCl solution with initial concentration of $\sim$10$^6$ CFU/ml. Measurements were conducted in three cells: electrochemical cell and two control cells (the preparation procedure along with the experimental system was previously described [18]). The photocatalysis electrochemical cell included the Ti/TiO$_2$ electrode with a surface area of 25 cm$^2$ (working electrode) under UV light illumination, a platinum counter electrode and standard calomel electrode (SCE reference electrode). A UV lamp (30 W UV lamp, $\lambda = 360$ nm) with a light intensity of 3 mW/cm$^2$ was mounted over the cell. An anodic potential in the range of 0.2–5 V$_{SCE}$ (V vs. standard calomel electrode) were applied potentiostatically at the working Ti/TiO$_2$ electrode. Once an anodic current has been stabilized (about 100 s) UV illumination was turned on for 60 min. Similar studies, without polarization were conducted while using TiO$_2$ nanopowder (Degussa P-25) in concentration of 1 g/l solution. During UV illumination, tested bacterial suspensions in the cells was periodically probed. The residual *E. coli* concentration was enumerated by growth at 36 °C on plates with R$_2$A agar medium (total plate count method).

3. Results and discussion

3.1. Electrode characterization

The surface morphology and film thickness of the immobilized nanotubular TiO$_2$ produced in NaF containing solution can be seen in the top and cross-section micrographs in Fig. 1. The nanotubular TiO$_2$ is composed of long shape and narrow nanotubes with a mean pore size of 75 nm. From the cross-sectional micrograph obtained from the mechanically bent sample, it can be seen that the oxide layer has a thickness of $\sim$2 μm. XRD pattern (Fig. 2) shows that the formed oxide has the undesired amorphous microstructure due to the relatively low potential being used during the anodization (20 V). As known, amorphous structure provides recombination centers which reduce the charge carriers concentration and thus, photocatalytic effect is being drastically reduced [25]. Subsequent to a thermal treatment for 3 h at 450 °C, the microstructure has been transformed into the crystallographic anatase phase [26] which is the most reactive crystal structure of the TiO$_2$ [27].

In addition to the immobilized nanotubular TiO$_2$, a two immobilized mesoporous oxide layers were grown in 0.5 M H$_2$SO$_4$ solution at a constant current density of 100 mA/cm$^2$ until final potentials of 110 and 150 V were reached. Titania growth rate in this solution is shown in Fig. 3 for the specimen, being anodized up to 150 V. The samples having final potentials of 150 V (HS150V) and 110 V (HS110V) are marked in the graphs. High resolution SEM micrographs shown in Fig. 3, as well, indicate that the surface is covered with pores with a wide diameter distribution of 50–500 nm. The final potential, being reached during the anodization process, is above the microsparking potential threshold, causing the formation of "wormlike" porous structure [28], as opposed to the nanotubular structure formation being formed due to a chemical etching by the fluoride ions. Since the formation of HS150V sample is longer, having higher final potential (150 V), the oxide layer has higher density of pores than the HS110V specimen obtained at lower potential of 110 V. Nevertheless, the mesoporous oxide layers are assumed to have smaller surface area than the nanotubular TiO$_2$ due to difference in the structure and density of the pores. The XRD patterns shown in Fig. 3 reveal that the HS110V TiO$_2$ crystallographic phase is anatase, while increasing the anodization potential to 150 V led to the formation of a rutile phase in addition to the existing anatase phase.

3.2. Electrochemical characterization

The nanotubular TiO$_2$ photocatalyst was studied under linear sweep voltammetry conditions in the dark and under UV illumination (Fig. 4a). The current–voltage curve obtained from the TiO$_2$ electrode in the dark exhibits polarization current only above a potential of 2 V$_{SCE}$ with a final current density of 50 μA/cm$^2$ at 4 V$_{SCE}$. This typical electrochemical behavior is a result of electrolysis of water. Water electrolyzing decomposition occurs at a more positive potential than the theoretical one ($E^\circ = 1.229$ V), due to the high over-potential effect of the TiO$_2$ semiconductor. These results demonstrate a typical characteristic of n-type semiconductors [29].

Under UV illumination electrons and holes are generated. The electrons are transferred from the anode to the counter electrode via the external circuit; the holes are transferred to the surface of the TiO$_2$ and react with the solution [30] and therefore, the current density is linearly increased until a stabilization at a potential of 2 V$_{SCE}$. Further increase in the potential yields a behavior observed in the dark up to a potential of 4 V$_{SCE}$, attributed to the water electrolysis. This phenomenon is better seen by measuring the photocurrent.
3383

Fig. 1. Top and cross-sectional high resolution SEM micrographs of TiO₂ grown via anodization in 1 M Na₂SO₄ + 0.5 wt% NaF in a constant potential of 20 V for 2 h.

Photocurrent values were obtained by calculating the difference between the total current under UV illumination and the dark current (Eq. (1)).

\[ I_{\text{Ph}} = I_{\text{Total}} - I_{\text{Dark}} \]  

The photocurrent increases linearly, from OCP until saturation at a potential of about 2 V SCE, as shown in Fig. 4b and according to Gärtner model [31]. Similar behavior is also observed with the mesoporous TiO₂, HS110V and HS150V, shown in Fig. 5a; electrolysis of water above potential of 2 V SCE is detected. However, the current density under UV illumination is smaller than the measured current density of the nanotubular TiO₂ and these affect the measured photocurrent (Fig. 5b). Similar behavior is observed in all forms of TiO₂; a linear increase in the photocurrent until saturation. Of the three TiO₂ electrodes, the nanotubular TiO₂ possesses the highest saturation photocurrent. This behavior indicates that the charge transfer and conduction is more effective through the nanotubes than the mesoporous film, most probably due to the increased surface area of the nanotubular structure.

3.3. Disinfection studies

As known, the mechanism of photocatalysis in the presence of TiO₂ involves the enhanced formation of OH• radicals, which actively participate in the oxidation processes. Hydroxyl radicals (OH•) have a significant effect on the chemical oxidation of organic compounds in the aqueous media [1,32]. Complete mineralization of many organic substances is possible in aqueous systems when a sufficient flux of OH• radicals can be generated [2,33]. TiO₂ behaves as a classical semiconductor [34] and when it is illuminated by light with a wavelength of less than 400 nm it generates excess of electrons in the conduction band (\(e_{\text{cb}}\)) and positive holes in the valence band (\(h_{\text{vb}}\)). H₂O molecules or OH⁻ groups adsorbed on a TiO₂ particle surface react to form OH• radicals according to the following.
Photocatalysis inactivation of *E. coli* can be divided into three different regions that were previously discussed in the literature by Benabbou et al. [35]: (a) initial incubation period is called the “shoulder”, where the hydroxyl radicals begin to attack the bacteria; (b) the second period is the main part of the photocatalysis where fast inactivation of the bacteria is occurred; (c) “tail” region where the process is being decelerated. There are several possible explanations for this deceleration such as screening effect of the bacteria or a competition between the bacteria and the organic byproducts, released to the solution by the inactivated bacteria, on the hydroxyl radicals. This type of inactivation kinetic process divided into three steps was recently fitted by Marugán et al. [36] to the modified Hom model (Eq. (3)).

\[
\log \frac{C}{C_0} = -k_1[1 - \exp(-k_2t)]^{k_3}
\]

where \(C\) is the *E. coli* concentration at time \(t\), \(C_0\) is the initial concentration, \(k_1\), \(k_2\), and \(k_3\) are kinetic constants. This model can be solved using Microsoft Excel tool called GlnaFIT [36,37] which covers various kinetic models including this one. Using this tool, three parameters that represent the three steps can be obtained: \(S_1\) (shoulder length) is the incubation time of the first step, \(k_{max}\) is the slope of the second step and \(n_{res}\) is the starting point of the third deceleration step. Calculating these three parameters is very important for understanding the kinetic of inactivation *E. coli*. Achieving short incubation period and fast inactivation, without the deceleration period is essential for an efficient photocatalysis process.

The capabilities of the grown Ti/TiO\(_2\) substrates to inactivate *E. coli* was studied using 0.01% NaCl solution having a pH value of 5.2 and under an anodic potential of 1.5 V\(_{SCE}\). Chloride containing salt (NaCl) was used as an electrolyte due to its small photocatalytic inhibition effect, compared with other inorganic ions such as NO\(_3^−\) and SO\(_4^{2−}\), as was outlined in Rincon and Pulgarin work [38]. The effect of different anion species was also investigated in photoelectrocatalytic experiments. The results (to be published elsewhere), similarly to the results obtained from solutions having low Cl\(^−\) concentrations, indicate that the use of same and low concentration of NaNO\(_3\) did not affect the photocatalytic rate, as well. Thus, anions (such as chloride and nitrate) concentration is substantially low to have any impact on *E. coli* inactivation. In addition to the immobilized TiO\(_2\), this experiment, without the application of any potential, was also conducted using dispersed 1 g/l P-25 powder TiO\(_2\) which is being considered as the most common photocatalyst today. The dependence of *E. coli* concentration on time is presented in Fig. 6 along with the calculated kinetic parameters in Table 1. These results demonstrate the power of fast *E. coli* inactivation when using the nanotubular TiO\(_2\). Notably, the nanotubular TiO\(_2\) possesses the fastest inactivation rate (highest \(k_{max}\) in the second step) and complete inactivation of *E. coli* was achieved after 15 min only. It is important to note that the nanotubular TiO\(_2\) does not show any deceleration in the end of the process. On a contrary,
complete elimination was achieved after 60 min when HS150V and HS110V TiO2 were used and after 30 min when powder TiO2 was used. In addition, all of them demonstrate deceleration in the last step as opposed to the nanotubular TiO2. From these results, it is clear that the nanotubular TiO2 is the most efficient photocatalyst with the fastest elimination rate than any other immobilized photocatalysts, as was expected from the photocurrent measurements. Furthermore, the surface area of the nanotubular TiO2 is estimated to be as 30 times larger than the actual surface area, because of the tubular structure [39]. This leads to a 3 orders of magnitude difference in the surface area between the nanotubular and the powder TiO2 and still, the nanotubular TiO2 has faster inactivation rate than P-25 powder TiO2 due to the anodic potential. The main advantage of using powder TiO2 is the short incubation period in the first step. The powder is uniformly dispersed in the solution and therefore, the initial reaction between the E. coli and the TiO2 is faster leading to a shorter incubation time. The HS150V has faster inactivation rate than the HS110V TiO2 in the second step and $k_{\text{max}}$ is twice as large, but both of them are not as efficient as the nanotubular TiO2. All three immobilized photocatalysts have similar incubation time with main differences in the inactivation and deceleration kinetics. Although the photocurrents were measured in a solution without the presence of E. coli bacteria, there is a clear correlation between the photocurrents values and the inactivation rates. In conclusion, the combination of increased surface area and anodic potential leads to the fastest inactivation rate observed in the nanotubular TiO2 compared with mesoporous and powder TiO2.

The effect of the anodic potential applied on illuminated nanotubular TiO2 on bacterial deactivation was studied. Various anodic potentials in the range of 0.2–5 V were applied on the TiO2 electrode and the “residual E. coli concentration–time” dependence was detected (Fig. 7a). “Current–time” curves measured at applied potentials can be seen in Fig. 7b. These results clearly show an enhancement in the E. coli inactivation rate from 30 min under 0.2 V SCE anodic potential to 10 min under anodic potential of 3 V SCE anodic potential for complete inactivation. In addition, $k_{\text{max}}$ is increased as well, as can be seen in the calculated parameters in Table 2. Raising the anodic potential increases the depth of the space charge region and suppresses the electrons—holes recombination, and therefore, an enhanced photocatalysis rate is achieved. On a contrary, from the photocurrent results presented in Fig. 4b it was assumed that increasing the anodic potential above 2 V SCE will not result in an increased inactivation rate. In previous work it was demonstrated that bacteria in solution having pH values of 3–9 are negatively charged and therefore, can be attracted by the positive anodic potential [40,41]. As a result, the effect of enhanced photocatalysis is mainly observed in a decreased incubation period along

### Table 1

<table>
<thead>
<tr>
<th>Photocatalyst type</th>
<th>$S_i$ [min]</th>
<th>$k_{\text{max}}$</th>
<th>$\log(n_{\text{max}})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanotubular TiO2</td>
<td>4.99 ± 0.90</td>
<td>1.39 ± 0.12</td>
<td>0.42 ± 0.3</td>
</tr>
<tr>
<td>HS150V</td>
<td>4.78 ± 2.51</td>
<td>0.81 ± 0.20</td>
<td>0.12 ± 0.30</td>
</tr>
<tr>
<td>HS110V</td>
<td>4.94 ± 1.20</td>
<td>0.37 ± 0.02</td>
<td>0.12 ± 0.30</td>
</tr>
<tr>
<td>P-25 powder TiO2</td>
<td>2.75 ± 1.07</td>
<td>1.00 ± 0.22</td>
<td>0.1 ± 1.02</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Anodic bias [V]</th>
<th>$S_i$ [min]</th>
<th>$k_{\text{max}}$</th>
<th>$\log(n_{\text{max}})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.82</td>
<td>2.35</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.37</td>
<td>2.42</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.98</td>
<td>2.81</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.14 ± 2.79</td>
<td>1.42 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.49 ± 2.19</td>
<td>1.10 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>6.00 ± 2.59</td>
<td>0.62 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>
with increased currents when the anodic potential is increased up to 5 \textit{V}_{SCE}. Moreover, it can be seen that the measured currents under potential of 3 \textit{V}_{SCE} decay when the inactivation is finished to the same currents under potential of 2 \textit{V}_{SCE} as was expected from the currents measurements in Fig. 4a. Increasing the anodic potential up to 4 and 5 \textit{V}_{SCE} lead to higher current density in correlation with highly ordered nanotubular TiO\textsubscript{2} having a high surface area showed sodium sulfate solution resulted in a nanotubular structure. The porous titania structure, while the addition of fluoride ions to a rather, it causes water splitting.

It is important to note that in order to evaluate the effect of the applied potential, studies were conducted with an applied anodic potential up to 8 V on the Ti/TiO\textsubscript{2} electrode only, without UV illumination. Under these conditions, no significant changes in the bacteria concentration were observed. Thus, the anodic potential has no influence by itself on the inactivation of the bacteria, but rather, it causes water splitting.

4. Conclusions

Various methods of growing immobilized porous oxide layers were studied. Anodization of Ti in sulfuric acid formed a mesoporous titania structure, while the addition of fluoride ions to a sodium sulfate solution resulted in a nanotubular structure. The highly ordered nanotubular TiO\textsubscript{2} having a high surface area showed an increased photocurrent response, and therefore, an enhanced photodegradation rate of \textit{E. coli} bacteria. The inactivation rate was faster compared to the mesoporous TiO\textsubscript{2}, as was expected from the photocurrent measurements, without a deceleration period at the end of the process. The nanotubular photocatalyst also showed better inactivation rate than P-25 powder TiO\textsubscript{2} with applied anodic potential of 1.5 \textit{V}_{SCE}, in spite of, the difference of three orders of magnitude in the surface area. The only advantage of the powder over nanotubular titania was the shorter incubation time of the photocatalysis due to the dispersion of the powder in the solution.

Increasing the anodic potential up to 3 \textit{V}_{SCE} revealed an enhancement in the inactivation rate of \textit{E. coli} using the nanotubular TiO\textsubscript{2}. However, above this potential only the incubation time has decreased without any significant change in the inactivation kinetic, as was expected from the photocurrent measurements. The shorter incubation time was accompanied with an increased current density. Using high surface area immobilized nanotubular TiO\textsubscript{2} is a promising and powerful tool for efficient photocatalysis for water disinfection. This anodically formed immobilized TiO\textsubscript{2} is expected to be easily removed and thus, repeatedly used, as compared to powder photocatalysts and still achieve high inactivation efficiency with complete elimination of 10\textsuperscript{6} CFU/ml \textit{E. coli} after 10 min only under 3–5 \textit{V}_{SCE}.

References