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Supporting Information

Inactivation of *E. coli* using a Novel TiO₂ Nanotube Electrode

Submitted by

Amir Ahmadi, Tingting Wu*

Department of Civil and Environmental Engineering

The University of Alabama in Huntsville

Huntsville, AL, 35899, USA

* Corresponding author: Tel: +1-256-824-6423; email: Tingting.Wu@uah.edu

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Scavenger method for ROS detection:

Nitro blue tetrazolium salt (NBT) was used to detect the possible formation of superoxide anion $({}^{\circ}O_{2}{}^{\circ})$ and generation of singlet oxygen $({}^{1}O_{2})$ was monitored by the loss of Furfuryl Alcohol (FFA).¹ Sodium azide (NaN₃) was also added in selected runs as a ${}^{1}O_{2}$ scavenger.² Tert-butyl alcohol (TBA) and 4-chlorobenzoic acid (*p*CBA) were used to detect the generation of hydroxyl radicals (${}^{\circ}OH$)^{3,4}. Moreover, it was reported that alcohols with an α hydrogen such as ethanol can rapidly reacts with both ${}^{\circ}OH$ and SO₄ ${}^{\bullet}$, but alcohol without an α hydrogen such as TBA only reacts rapidly with ${}^{\circ}OH$. Therefore, ethanol was also used in Na₂SO₄ electrolyte to examine the generation of SO₄ ${}^{\bullet}$.⁴

The possible formation of $\bullet O_2^-$ was tested using NBT salt (NBT²⁺⁾ which can be reduced by $\bullet O_2^$ and form a purple monoformazan product.¹ Preliminary experiments confirmed that negligible changes in absorbance occurred without current applied (data not shown). Greater absorbance was detected in all electrolytes which further increased with time (Figure S5). However, it has to be pointed out that NBT may also be reduced electrochemically.⁵ In order to further confirm whether $\bullet O_2^-$ was generated, both TBA and NBT were added into different electrolytes. The purpose of TBA addition was to inhibit the generation of $\bullet OH$ and consequently the $\bullet O_2^$ formation (Table S2). As shown in Figure S5, similar or even higher absorbance were observed with the TBA addition. These results imply that the NBT reduction should be mainly caused by electrochemical reduction and $\bullet O_2^-$ generation was insignificant (if any) under the experimental conditions here. In addition, singlet oxygen (1O_2) may be formed via several chemical formation pathways, although some may have not been unequivocally proven scientifically (Eq. 8~11 in Table S2). Generation of 1O_2 was examined during electrolysis experiments with FFA as the probe compound. NBT and NaN₃ were also added along with FFA, serving as the $\bullet O_2^-$ (precursors for ${}^{1}O_{2}$ formation, Table S2) and ${}^{1}O_{2}$ scavengers respectively in selected runs. As shown in Figure S6, reduction rates of FFA with addition of different probe compounds were similar, which implies that like ${}^{\bullet}O_{2}^{-}$ the formation of ${}^{1}O_{2}$ does not seem to be significant in this study.

B-spizizenii experiments

B-spizizenii stock culture was prepared by inseminating a Tryptic Soy Agar with *B-spizizenii* (ATCC 6633), which was then incubated at 37°C for 24 hours. 0.5 mL of sterile single strength Lauryl Tryptose Broth (LTB-HACH 216315) was dissolved into 49.5 ml of sterile phosphate buffer saline to prepare 1% solution of LTB. To prepare *B-spizizenii* stock solution, a small amount of *B-spizizenii* growth from the stock culture was added to the 50 mL 1% LTB solution and aerated at 37°C for 24 hours. This stock solution should contain approximately 10⁸-10⁹ *B-spizizenii* cells per mL. In order to prepare the working solution, 20µL of the stock solution normally contained 10⁴-10⁵ cells/mL. During the disinfection experiments, the working solution was continuously mixed and pumped through the reactor at a flow rate of 100 mL/min and samples were taken at different time intervals and measured for oxidizing species as free chlorine, and *B-spizizenii* using standard spread plating techniques.

Electrode	<i>E.coli</i> log reduction	Experimental conditions	Note	Reference
BDD	≤3	0.2 M KH ₂ PO ₄ , 33 mA/cm ² , 120 min electrolysis		6
Pt	~1	0.2 M KH ₂ PO ₄ , 100 mA/cm ² , 180 min electrolysis		7
Ti/TiO ₂ -RuO ₂	insignificant	Different electrolytes: [0.01 M Na ₂ SO ₄ - 0.1 M NaNO ₃], > 30 mA/cm ² , 33 s electrolysis	Short-lived intermediate electrochemical products were believed to be the major killing function; persulfate production did not appear to have any major role	8
BDD	< 0.5	0.2 M Na ₂ SO ₄ , 5 mA/cm ² , 20 min electrolysis	Inactivation rate increased with increasing Na ₂ SO ₄	9
	~2.5	0.2 M Na ₂ SO ₄ , 5 mA/cm ² , 120 min electrolysis	concentrations and faster in Na_2SO_4 than in NaH_2PO_4 or $NaNO_3$	

Table S1. Comparison of *E. coli* reduction in inert electrolytes using different anodes

Reaction No.	Reaction	Reference
1	$\bullet OH \rightarrow \bullet O + H^+ + e^-$	6
2	$\bullet O + O_2 \rightarrow O_3$	6
3	$\bullet \mathrm{OH} + \bullet \mathrm{OH} \to \mathrm{H}_2\mathrm{O}_2$	6
4	$\bullet \mathrm{OH} + \mathrm{O}_3 \to \mathrm{HO}_2 \bullet + \mathrm{O}_2$	6
5	$\bullet OH + H_2O_2 \rightarrow HO_2 \bullet + H_2O$	6
6	$\mathrm{HO}_{2}\bullet \leftrightarrow \bullet\mathrm{O}_{2}^{-} + \mathrm{H}^{+}$	6
7	$\mathrm{H}_{2}\mathrm{O}_{2} + \mathrm{HO}_{2}^{-} \rightarrow {}^{1}\mathrm{O}_{2} + \mathrm{H}_{2}\mathrm{O} + \mathrm{OH}^{-}$	10
8	$\bullet O_2^- + \bullet O_2^- + 2H^+ \rightarrow {}^1O_2 + H_2O_2$	10
9	$\bullet \mathrm{OH} + \bullet \mathrm{O_2}^- \to {}^1\mathrm{O_2} + \mathrm{OH}^-$	10
10	$H_2O_2 + \bullet O_2^- \rightarrow {}^1O_2 + \bullet OH + OH^-$	10
11	$\mathrm{H_2O_2} + \mathrm{OCl}^- \rightarrow {}^1\mathrm{O_2} + \mathrm{H_2O} + \mathrm{Cl}^-$	11

Table S2. Summary of radical reactions

Parameters	WTP intake	Treated surface water before disinfection	Secondary effluent before disinfection
Turbidity (NTU)	2.9	0.0	1.0
рН	7.3	6.5	7.1
DO (mg/L)	9.6	9.2	8.2
Conductivity (µs/cm)	206	234	561
TDS (mg/L)	103	113	281
$PO_4^{3-}(mg/L)$	0.3	0.1	11.1
NH ₃ -N (mg/L)	0.09	0.1	0.15
Cl ⁻ (mg/L)	11.2	11.7	50.9
SO_4^{2-} (mg/L)	13.5	19.4	16.6
NO ₃ - (mg/L)	4.5	4.1	4.9
COD (mg/L)	8	0	9
NPOC ^a (mg/L)	1.7	1.1	3.2
E.coli (CFU/100mL)	205	N.A. ^b	1300~1500

Table S3. Characteristics of the real water samples

^a Non-Purgeable Organic Carbon. ^b No *E.coli* was detected in the sampled water possibly because chlorine was added before coagulation in the treatment plant.

Effect of applied	Current density (mA/cm ²)	Energy Consumption (kWh/m ³)	
current density	0.66	0.110	
(1.5 mM NaCl)	1.32	0.246	
· · · · ·	2.63	0.549	
Effect of electrolyte	NaCl Concentration (mM)	Energy Consumption (kWh/m ³)	
concentration	0.3	0.109	
(0.66 mA/cm^2)	1.5	0.11	
· · · ·	3	0.118	
	Type of Electrolyte	Energy Consumption (kWh/m ³)	
Effect of different	NaCl	0.549	
electrolytes	Na_2SO_4	0.597	
(Concentrating of	NaH ₂ PO ₄	0.608	
2.63 mA/cm^2	NaNO ₃	0.684	
	NaHCO ₃	0.618	

Table S4. Comparison of energy consumption under different conditions ^a

^a All electrolytes prepared in buffer solution: 2.5 mM $KH_2PO_4 + 2.5$ mM K_2HPO_4 ; electrolysis time: 20 min (see Figure 4 & 5 for log reduction).

Energy consumption $\left(\frac{kWh}{m^3}\right) = \frac{Current(A) \times Voltage(v) \times Time(h)}{Volume(m^3) \times 1000}$



Figure S1. Effect of washing media on *E.coli* inactivation [Electrolyte concentration: 1.5 mM NaCl; current density: 0.66 mA/cm²; saline solution concentration: 9 g/L NaCl].



Figure S2. Schematic diagram of the experiment set-up (A); and Photos of electrodes (B). *Reactor not to scale for illustration purpose, actual effective volume ~20 mL.



Figure S3. XPS spectra of Ti2p and O1s before cathodization (A& B) and after cathodization (C&D).



Figure S4. •OH formation detected by *p*CBA reduction. [Electrolyte concentration: 1.5 mM; pCBA: 0.25 mM; current density: 2.63 mA/cm²].



Figure S5. $\bullet O_2^-$ formation detected by NBT reduction. [Electrolyte concentration: 1.5 mM; NBT: 0.2 mM; TBA: 30 mM; current density: 2.63 mA/cm²].



Figure S6. ¹O₂ formation detected by FFA reduction. [Electrolyte concentration: 1.5 mM; FFA: 0.2 mM; NBT: 0.2 mM; NaN₃: 10 mM; current density: 2.63 mA/cm²].



Figure S7. Voltage ranges at different current densities at constant NaCl concentration (1.5 mM NaCl) (A); Different NaCl concentrations under constant current density (0.66 mA/cm²) (B); Different electrolytes (1.5 mM) under constant current density (2.63 mA/cm²) (C); Different real water matrices under constant current density (0.88 mA/cm²) (D).

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