PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2007, 34(12): 1288~1295
www.pibb.ac.cn

Effects of Atomic Oxygen Radical Anion on *Escherichia coli* Cells *

WANG Lian[1)], SONG Chong-Fu[1)], SUN Jian-Qiu[1)], YOUSHIFUMI TORIMOTO[2)], MASAYOSHI SADAKATA[2)], LI Quan-Xin[1)**]

([1)]*Department of Chemical Physics, University of Science & Technology of China, Hefei* 230026, China;
[2)] *Department of Chemical System Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo* 113-8656, Japan)

Abstract The effects of atomic oxygen radical anion (O^-) on the inactivation and morphological changes of *Escherichia coli* (*E.coli*) on the surface of bio-indicator carrier were investigated. The O^- flux was generated from a novel developed O^- generator where the O^- storage-emission material of $[Ca_{24}Al_{28}O_{64}]^{4+}\cdot 4O^-$ (abbreviated as C12A7-O^-) was used as a pure O^- emitter. Present results showed that inactivation of *E.coli* was sensitive to the O^- intensity and the cell mortality was enhanced to more than 3-logarithm reduction with the exposure to 1.5 mA/cm^2 O^- flux for 120 min. Field emission scanning electron microscopy (FESEM) observations showed that O^- flux destroyed cellular structures. Lipid peroxidation reaction induced by atomic oxygen radical anion for *E. coli* cells was also observed using product of malondialdehyde (MDA) as an index. The concentration of MDA increased to 1.2 μmol/g(dry weight) of cells when *E. coli* suspension (5.6×10^7 cfu/ml) was treated by the O^- flux (1.5 μA/cm^2) for 15 min. The findings revealed that the atomic oxygen radical anions, with strong oxidation power, was effective in inactivating *E. coli* and caused lipid peroxidation reaction at the first time, which would be potential useful to develop a novel approach for the microbial decontamination and for the study on the interactions between microorganisms and O^-.

Key words atomic oxygen radical anion, *Escherichia coli*, inactivation, lipid peroxidation, malondialdehyde

Reactive oxygen species (ROS), such as the superoxide anion radical ($O_2^{\cdot-}$), the hydroxyl radical (•OH), and the singlet oxygen ($^1O_2$), are important chemical radicals in the biochemistry and biophysics. Due to high reactivity of ROS, all the cellular components, lipids, proteins, nucleic acids and carbohydrates may be damaged by their reactions with ROS, giving rise to metabolic and cellular disturbances or cell death [1~5]. Considering high reactivity of O^- radical anion in chemical reactions [6, 7], we therefore speculate that the active O^- would have significant effects on microorganisms' cells through chemical reactions. Kelly-Wintenberg *et al* [8]. have pointed out that ROS species including O^- play important roles in killing microorganisms by one atmosphere uniform glow discharge plasma.

Unsaturated fatty acids (polyunsaturated phospholipids) are the primary target of ROS [9, 10]. The increase in the lipid peroxidation has been considered as a common mechanism leading to the destruction of cell membrane due to the cytotoxic products generated during the lipid peroxidation. Oxidative deterioration of lipids leads the formation of malondialdehyde (MDA) during the last stage of the breakdown of endoperoxidases formed during intramolecular rearrangements in the structure of unsaturated fatty acids. Therefore, MDA is usually as an index to measure whether or not the existence of lipid peroxidation and to assess the degree of cell membrane damage.

* This work was supported by a grant from Hi-Tech Research and Development Program of China (2006AA05Z118) and The Century Program of The Chinese Academy of Sciences (2002).
**Corresponding author.
Tel: 86-551-3601118, E-mail: liqx@ustc.edu.cn
Received: March 30, 2007 Accepted: June 7, 2007

O^- can be generated from the process of glow discharge and some inorganic materials such as MgO surface, Cs surface, and the Au-or Ag-deposited Y_2O_3-stabilized ZrO_2 (YSZ) electrolyte surface [11 ~13]. However, it is very difficult to obtain high-purity O^- flux by conventional methods. Recently, we have developed a new technology to generate pure and sustainable O^- flux, where the O^- radical anions are emitted from the anionic storage-emission materials of $[Ca_{24}Al_{28}O_{64}]^{4+} \cdot 4O^-$(abbreviated as C12A7-$O^-$) [6, 14]. The microporous crystal of C12A7-O^-, prepared by the solid-state reaction of $CaCO_3$ and γ-Al_2O_3 under flowing dry O_2 environment at 1 350 ℃, can store and emit O^- [15]. O^- is a monovalent anion and also considered as a radical because it has an unpaired electron in its outmost orbit. The structure of C12A7-O^- is characterized by a positive charged lattice framework $[Ca_{24}Al_{28}O_{64}]^{4+}$ including 12 sub-nanometer sized cages with a free space of about 0.4 nm in diameter. The concentration of O^- stored in the cage of C12A7-O^- is about 6×10^{20} cm $^{-3}$ [6]. Furthermore, the O^- stored in the cages can be emitted into the gas phase and be formed the high pure O^- flux (>95%) by heating the sample (500～800℃) [15, 16]. The sustainable and relative pure O^- flux developed with using the above method has provided the basis for studying the effects of O^- on the inactivation of microorganisms[17]. The objective of this study was to evaluate the viability of *E. coli* cells influenced with different intensity of O^- flux and the kinetics of lipid peroxidation induced by the O^- radical anions.

1 Materials and methods

1.1 O^- flux preparation and experimental apparatus

The O^- flux was generated from an O^- generator (length:12 cm, width:12 cm, thickness:0.8 cm), which consisted of three parts: a ceramic support, a Fe-Cr alloy filament heater embedded in ceramic layer (220V, 750W), and a C12A7- O^--coated film ((50 ±5) μm) using a plasmas-spraying process. A photo image of the O^- generator was shown in the inset of Figure 1. Electron paramagnetic resonance (EPR) measurements were performed to investigate the characteristic of O^- in the C12A7-O^- material with a Bruker ER-200D spectrometer (Germany) at 77 K. The concentration of O^- stored in the C12A7-O^- was determined from the second integral of the EPR spectra using $CuSO_4 \cdot 5H_2O$ as a standard calibration [6]. We controlled the emission intensity of O^- by changing temperature of the O^- generator via adjusting the output power of O^-generator. The O^- flux was carried by a fast flowing inert gas (5 L/min argon) to decrease the loss of O^- magnitude. A time-of-flight (TOF) mass spectrometer was used for the analyses of anion species emitted from O^- generator. The experimental apparatus and detailed conditions in this contribution for studying the anions emission using TOF mass spectroscopy was the same as those described previously [15]. The current of O^- was detected via a Keithley model 6485 picoammeter (USA) together with an Au electrode (diameter: 1.6 cm).

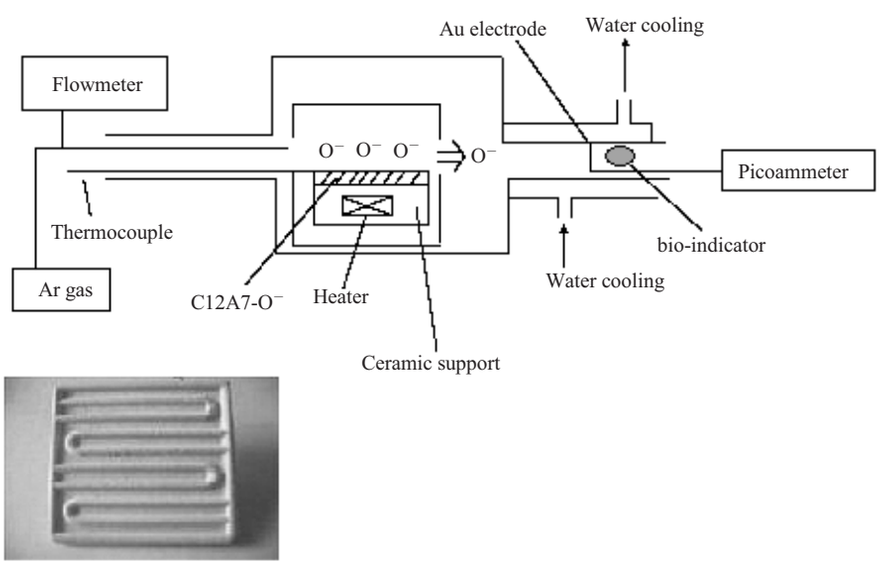

Fig. 1 A schematic diagram of experimental apparatus

The inset at the lower left corner is a photograph of O^- generator.

The experimental arrangement for investigating the effects of O^- on *E. coli* was made up of two parts: an O^- emission chamber (diameter: 20 cm, length: 18 cm) installed with the O^- generator and a reaction chamber (diameter: 2 cm; length: 28 cm) for positioning bio-indicator carrying *E. coli* (Figure 1). The reaction chamber was cooled by a water-cycled system to control the temperature of bio-indicator at 30℃. The distance from the O^- generator to the bio-indicator was 28 cm. The Au electrode and Keithley model 6485 picoammeter used to detect current intensity of O^- near bio-indicator was removed when the O^- began to react with *E. coli*.

1.2 Bio-indicator preparation

E. coli (AS 1.1018) was obtained from China General Microbiological Culture Collection Center (Beijing, China). *E. coli* was inoculated into 100 ml of nutrient broth (pH7.20) containing 1.0 g of beef extract, 1.0 g of peptone, and 0.5 g of sodium chloride (NaCl) with shaking for 24 h at 37℃. The cells were harvested by centrifugation and washed twice with a sterile 0.9% NaCl solution. The cell pellet was re-suspended in 0.9% NaCl solution to obtain a test suspension. In order to obtain a uniform cell concentration, the cell suspension was serially diluted through several 1∶10 dilutions, and the final dilution 0.1 ml was pipetted onto duplicate agar plates, which was evenly spread using a glass spreader. The plates were then incubated for 24 h at 37℃ to generate visible colonies, which were counted and used to determine the number of colony forming units per ml (cfu/ml) in the original suspension. A polypropylene test cap was used as bio-indicator carrier with a diameter of 1.6 cm (inoculation area: 2 cm^2). The bio-indicator carrier was sterilized at 120℃ for 20 min. Then, 0.1 ml of cell suspension was applied to each sterile polypropylene carrier. The bio-indicator was dried in vacuum sample oven at 35℃ for 6 h with fresh generated silica gel to absorb the vaporized moisture [18]. The survival population of cells on each bio-indicator was counted before being used according to the method mentioned below.

1.3 Evaluation of the survivors

Bio-indicator exposure to argon (Ar) gas flux (5 L/min) only was used as a control. The survivors from the bio-indicators exposed to O^- flux and the control ones were recovered in 10 ml of phosphate buffered (pH 7.0) physiological saline solution (0.9% NaCl) with 0.3 g/ml glass beads (diameter 0.6 cm) [18]. Test tubes with the bio-indicators were vortexed for 5 min at room temperature. After proper dilution, 0.1 ml of suspension was inoculated onto nutrient agar. The survivors were counted as colony forming units (cfu) per bio-indicator carrier after incubation at 37℃ for 24 h. All trials were repeated three times.

1.4 FESEM observation

For field emission scanning electron microscope (FESEM) measurements, *E. coli* suspensions were inoculated onto plain polypropylene sheet as described above, and subjected to 60 min of O^- or Ar (5 L/min) treatments. The preparation process of FESEM sample for *E. coli* was similar to that used before [19]. A thin, conductive film of gold was then deposited onto the surface, after which the control and treated samples were viewed at 5 kV accelerating voltage in a JSM－6700F field emission scanning electron microscope (JEOL, Tokyo, Japan).

1.5 Detection of lipid peroxidation

Formation of malondialdehyde (MDA) was used as an index to measure the existence of lipid peroxidation. Quantification of MDA was done following the methods described before [9, 10]. The method is actually based on the formation of pink pigment which is formed from the reaction of MDA with thiobarbituric acid (TBA). The pink pigment has an absorption maximum in acidic solution at 532 nm. We bubbled of the O^- flux (carried by 5 L/min Ar) and Ar (5 L/min) only (i.e. control) into 20 ml *E. coli* suspension of 5.6×10^7 cfu/ml for different duration at 30℃, respectively. After reaction, 1 ml of suspension was mixed with 2 ml of 10% (w/v) trichloroacetic acid (TCA) and the solid was completely removed via centrifugation at 4 000 r/min for 10 min. The aqueous supernatant was obtained and 3 ml of freshly prepared 0.67% (w/v) TBA solution was added. The mixture was placed in a boiling water bath for 15 min and the absorbance at 532 nm was measured with a UV-2401 spectrophotometer (Shimadzu Co., Japan) after cooling. The concentration of the MDA formed was calculated based on a standard curve of MDA prepared by acid hydrolysis of 1,1,3,3-tetraethoxypropane (Tokyo Kasei Kogyo Co. Ltd. Japan) [20,21]. Equal volumes of 1,1,3,3-tetraethoxypropane and 0.2 mol/L hydrochloric acid were placed in 95℃ water bath for 10 min. It was assumed that one molecule of 1,1,3,3-tetraethoxypropane yields one molecule of MDA plus four molecules of ethanol when acidified. The extent

of lipid peroxidation was expressed in nanomoles of MDA per mg (dry weight) of cells.

Degradation of MDA itself by the O^- was also carried out to determine the degradation pathways. We bubbled of the O^- flux into 20 ml of MDA (1.2×10^{-5} mol/L). After reaction for a given time, we evaluated the changes of MDA using MDA-TBA method under the same condition mentioned above.

1.6 Statistical analysis

Microbial counts were expressed as cfu per bio-indicator. The reported data are the mean values of three trials±standard deviation. Significant differences in plate counts were calculated at the 95% confidence interval using analysis of variance (one way) with origin software release 7.0 (OriginLab Corporation, USA).

2 Results

2.1 Characteristics of O^-

The O^- flux was generated from an O^- generator where C12A7- O^- was the O^- storage-emission material. To investigate the characteristic of O^- in the C12A7-O^-, electron paramagnetic resonance (EPR) measurement of C12A7-O^- powder used for preparing O^-generator was performed (Figure 2a). The *g*-values of the signal extracted from the spectral simulation were $g_{xx}=g_{yy}$ =2.041 and g_{zz}=1.997. These *g*-values agree well with those of the O^- radical in the literature[6, 22]. The O^- concentration stored in the C12A7-O^- was determined from the second integral of the EPR spectra using $CuSO_4\cdot5H_2O$ as a standard calibration[6]. The O^- concentration stored in the C12A7-O^- was as high as about 3.7×10^{20} cm^{-3}. Figure 2b shows a typical mass spectrum as the O^- generator ran at 650 W. The peak at the mass number of 16 was attributed to O^- emitted from the O^- generator. The emitted species from the generator surface were almost O^- radical anions (>95%) together with a bit of electrons (<5%). The inset part of Figure 2b was a schematic diagram to describe the emission process of O^- from the O^- storage-emission material of C12A7-O^-. The O^- radical anions stored in the C12A7-O^- bulk (cages) were migrated onto the material surface by thermal diffusion and then were desorbed into the space forming the gas-phase O^- radical anions. A Keithley model 6485 picoammeter together with an Au electrode (area: 2 cm^2) was used to detect the emission current of O^- near the bio-indicator. The emission intensity of O^- from the O^- generator strongly depended on the surface temperature of the O^- generator (Figure 2 c). When the temperature was below 600℃, emission current was very weak. The emission current of O^- increased rapidly from 0.3 μA to 3 μA when the temperature increased from 650 to 800℃. Therefore, the intensity of O^- flux can be controlled through changing surface temperature of O^- generator via adjusting the output power of O^- generator. The intensity of O^- flux varied from 0 to 1.5 $\mu A/cm^2$ was used in this study.

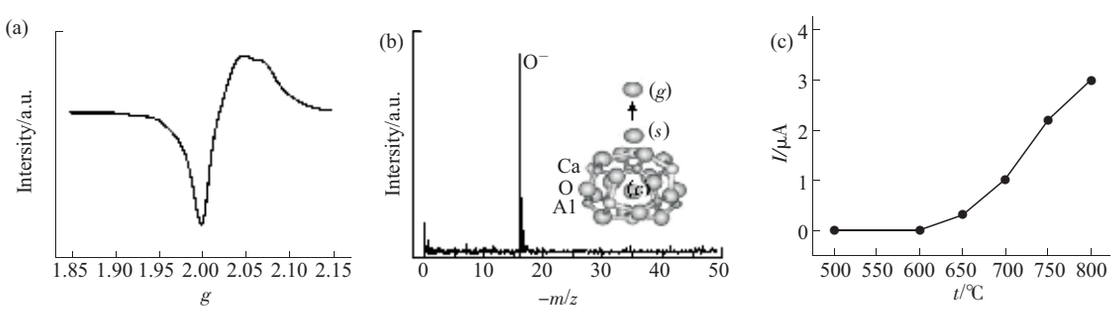

Fig. 2 Experimental EPR spectrum of C12A7-O^- material (a), TOF mass spectrum of O^- emitted from the O^- generator as the O^- generator ran at 650 W ,700 ℃ (b), and O^- emission current (μA) from the O^- generator versus the surface temperature of the O^- generator (c)

EPR spectrum was measured at 77 K. The inset part of Figure 2b is a schematic diagram to describe the emission mechanism of O^- from the O^- storage-emission material of C12A7-O^-, where the c, s, and g represent the O^- in the cage, on the sample surface, and in the gas phase, respectively.

2.2 Effects of O^- on survival of *E. coli* cells

Figure 3 shows the results of the *E. coli* decay (*i.e.*, the surviving populations versus exposure time) to determine the inactivation efficacy of O^- flux with two different intensities of 0.5 $\mu A/cm^2$ and 1.5 $\mu A/cm^2$, respectively. The initial population (N_0) of

E. coli on bio-indicator after dried was 2.5×10^7 cfu (t=0 min). The populations of *E. coli* slightly decreased by 0.20 and 0.55 lg cfu after exposed to Ar flux of 5 L/min (i.e. the control) at 30℃ for 60 and 120 min, respectively.

As the *E. coli* cells were exposed to the 0.5 μA/cm^2 O^- flux, however, significant reductions in cell viability with mean reductions of 0.84, 1.5, and 2.0 lg cfu were obtained for 30, 60, and 120 min, respectively. With increasing of the O^- flux intensity, the reductions in cell viability became more obvious. The population of *E. coli* had a remarkable reduction of more than 3 lg cfu with exposure to 1.5 μA/cm^2 O^- flux for 120 min at 30℃. As can be seen from Figure 3, the inactivation efficacy was very sensitive to the intensity of the O^- flux ranging from 0.5 to 1.5 μA/cm^2. Statistically significant difference was observed between the O^- flux and the argon flux ($P<0.05$). The above results clearly indicated that O^- flux could cause the *E. coli* inactivation.

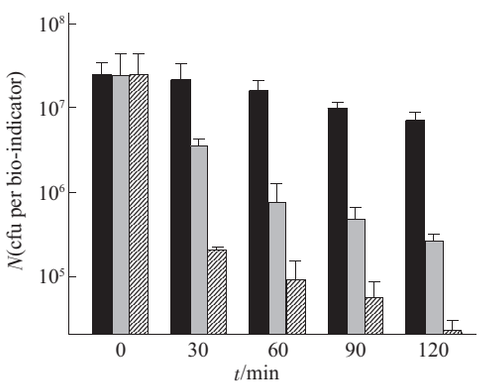

Fig. 3 Inactivation effects of the O^- flux and the argon flux on *E. coli* cells

N stands for the *E. coli* survivors on the bio-indicator exposed to argon flux or O^- flux. ■: Ar (5 L/min), ▨: O^-(0.5 μA/cm^2), ▨: O^- (1.5 μA/cm^2).

2.3 Effects of O^- on morphological changes of *E. coli* cells

To make clear the effects of the O^- flux on the *E. coli* cells, the alterations of *E. coli* cellular structures were investigated by field emission scanning electron microscopy (FESEM). As shown in Figure 4a, the cells in the control condition (i.e., exposed to an Ar flow of 5 L/min for one hour), appeared relatively smooth and regular shape. The cells in control condition had the similar bacilliform shape to those of the normal *E. coli* cells. However, after being exposed to O^- for 60 min (Figure 4b), the cell surface became some irregularities, and some internal matter appeared leaking from the cell wall. Thus, we can conclude that the outer membrane of cells would be destroyed by O^- radical anions. The similar phenomena were also observed for *E. coli* exposed to plasma containing active oxygen radical (i.e. O^-, •OH) [21]. Although FESEM observations of *E. coli* do not allow drawing conclusions about the mechanism for the inactivation processes, they do clearly show the alterations of *E. coli* cellular structure after being exposed to O^-.

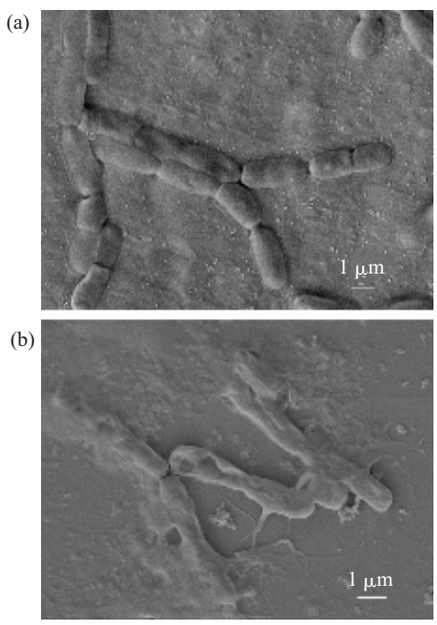

Fig. 4 Typical FESEM images of *E. coli* cells (a) exposed to the argon flux (5 L/min) and (b) exposed to 1.5 μA/cm^2 O^- flux for an hour at 30℃, respectively

2.4 Lipid peroxidation of *E. coli* induced by O^- flux

In order to clarify further the damage of cell membrane, lipid peroxidation was also measured with malondialdehyde (MDA) as an index. Figure 5 presents the formation kinetics of MDA due to the lipid peroxidation of *E. coli* cells induced by the O^- flux (1.5 μA/cm^2). The MDA concentration increased sharply for initial 15 min, reached a maximum value of 1.2 nmol per mg (dry weight of cells) for about 15 min, and then slightly decreased over 15 min. For the control condition (i.e., *E. coli* cells exposed to Ar flow of 5 L/min), the MDA concentration was lower than 0.4 nmol per mg (dry weight of cells), which might be formed in the analysis process of MDA-TBA in the air. The above results clearly show that lipid peroxidation of polyunsaturated phospholipids of *E. coli* occurs

through the O^- flux treatment.

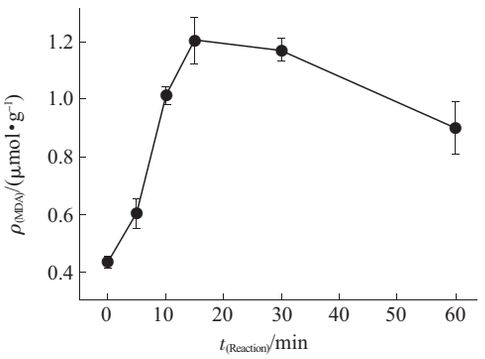

Fig. 5 Kinetics of the lipid peroxidation induced by the O^- flux (1.5 μA/cm^2) for *E. coli* cells

The MDA concentration was quantified by the TBA assay.

The concentration of MDA decreased after 15 min treatment would attribute to the reactions between the formed MDA and the O^- radical anions. This assumption was confirmed by the following experiment. We injected the O^- flux (1.5 μA/cm^2) into the MDA (1.2×10^{-5} mol/L) solution and then observed the reactions by measuring the residual amount of MDA by the MDA-TBA method. Figure 6 shows the residual MDA concentration measured as the function of reaction time. The MDA concentration lost nearly 42% after 60 min. In previous work, the reaction between O^- and aldehyde has been observed[23]. Thus, the subsequent decrease in the MDA concentration for longer treatment time shown in Figure 5 is attributed to degradation of MDA via the O^- radical anion, where the degradation rate exceeds the formation rate of MDA. Thus, it could be considered that O^- can attack polyunsaturated phospholipid in *E. coli* cell, subsequently leading to a breakdown of the cell membrane structure and the inactivation of the *E. coli* cells.

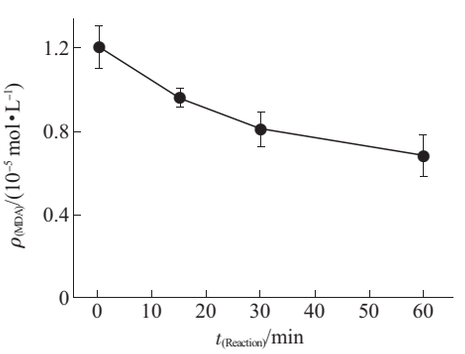

Fig. 6 Degradation of MDA via O^- flux (1.5 μA/cm^2) was measured as a function of the reaction time

The MDA was prepared by acid hydrolysis of 1,1,3,3-tetraethoxypropane.

3 Discussion

Radicals especially oxygen radical (reactive oxygen species) can induce damages to many kinds of cells *in vitro*. The radicals containing unpaired electron have high reactivity towards all the cellular components, lipids, proteins, nucleic acids and carbohydrates, giving rise to metabolic and cellular disturbances or cell death[1~5]. When radicals react with non-radical compounds, new radicals may be formed and induce chain reactions. Active radical anion species of O^-, with strong oxidation power, is high reactive radical in the anion chemistry, particularly in low-temperature oxidation of hydrocarbons [6, 7, 23 ~25]. Present method to produce O^- provided a new and convenience approach to generate pure and sustainable O^- radical anions. In our work, O^- radical anions showed as a devastator on *E. coli* cells through reactions between O^- and cells.

Cell death would be associated with either structural damage or physiological dysfunctions. Among structural damage, disruption of envelopes, DNA conformational changes, ribosome alternations or protein aggregation have been widely explored [26, 27]. The cell envelope of *E. coli* cell consists of three layers (starting from outward to inside): an outer membrane, a monolayer of peptidoglycan, and a cytoplasmic membrane. Polyunsaturated phospholipids are an integral component of cell membrane. The susceptibility of these compounds to be attacked by reactive oxygen species (ROS) has been wide investigated [28]. Malondialdehyde (MDA) is a lipid peroxidation products formed from the oxidation of *E. coli* membrane, i.e., phospatidylethanolamine [9]. Generally, initiation of lipid peroxidation is known to require some form of radical attack. As initiated, the reaction propagates by generating radical intermediate that undergoes peroxidation with another unsaturated lipid molecule. According to the investigations of lipid peroxidation, high active O^- species may first attack polyunsaturated phospholipid in *E. coli* cell, subsequently lead to a breakdown of the cell membrane structure and finally inactivate the *E. coli* cells.

It is concluded that the atomic oxygen radical anions possess high reactivity towards *E. coli* cells and damage the cellular structures through lipid peroxidation, which is potentially useful to study O^- on microorganisms. We suggested that present

approach would be extended into other fields such as the O^- effects on protein, DNA, enzyme. Work toward this goal is in progress. Moreover, it is also anticipated that the O^--induced microorganisms' inactivation may be practical for developing a new dry room-temperature sterilization method.

References

1 Randerath K, Reddy R, Danna T, *et al*. Formation of ribonucleotides in DNA modified by oxidative damage *in vitro* and *in vivo*-characterization by ^{32}P-postlabeling. Mutat Res, 1992, **275**(3～6): 355～366

2 Gu J, Zhang W, Tang H, *et al*. Studies on nano Ag_2O_2-PbO_2 modified electrode-induced cell membranes and DNA damage of *E. coli*. Chem J Chinese Universities，2005，**26** (12): 2214～2217

3 Aikens J, Dix T. Perhydroxyl radical (HOO •) initiated lipid peroxidation: the role of fatty acid hydroxides. J Biol Chem, 1991, **266** (23): 15091～15098

4 Hui H X, Zhao X N, Jin M, *et al*. Free radicals and Apoptosis. Prog Biochem Biophys, 1996, **23** (1): 12～16.

5 Cao C Z, Bu L S, Gao S, *et al*. The injured effect of hydrogen peroxide on cultured cardiac myocytes. Prog Biochem Biophys, 2000, **27** (6): 628～632

6 Hayashi K, Hirano M, Matsuishi S, *et al*. Microsporous crystal $12CaO \cdot 7Al_2O_3$ encaging abundant O^- radicals. J Am Chem Soc, 2002, **124** (5): 738～739

7 Aika K, Lunsford J H. Surface reactions of oxygen ions: dehydrogenation of alkanes by O^- on the MgO. J Phys Chem, 1977, **81** (14):1393～1398

8 Kelly-Wintenberg K, Hontie T, Hodge A, *et al*. Mechanism of killing of microorganisms by a one atmosphere uniform glow discharge plasma. IEEE International Conference, Monterey, USA, 1999

9 Maness P C, Smolinski S, Blake D M, *et al*. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl Env Microbiol, 1999, **65** (9): 4094～4098.

10 Sokmen M, Candan F, Sumer Z. Disinfection of *E. coli* by the Ag-TiO_2/UV system: lipidperoxidation. J Photochem Photobiol A Chem, 2001, **143** (2～3): 241～244

11 Tashiro T, Watanabe T, Kawasaki M, *et al*. Partial oxidation of methane with oxygen over magnesium oxide at low temperatures. J Chem Soc Faraday Trans, 1993, **89** (8): 1263～1269

12 Greber T, Grobecker R, Morgante A, *et al*. O^- escape during the oxidation of cesium. Phy Rev Lett, 1993, **70** (9): 1331～1334

13 Torimoto Y, Harano A, Suda T, *et al*. Emission of O^- radical anions from a solid electrolyte surface into the gas phase. Jpn J Appl Phys, 1997, **36** (2B): L238～L240

14 Lacerda M, Irvine J T S, Glasser F P, *et al*. High oxide ion conductivity in $Ca_{12}Al_{14}O_{33}$. Nature, 1988, **332** (6164): 525～526

15 Li Q X, Hosono H, Hirano M, *et al*. High-intensity atomic oxygen radical anion emission mechanism from $12CaO \cdot 7Al_2O_3$ crystal surface. Surf Sci, 2003, **527** (1～3): 100～112

16 Li Q X, Hayashi K, Nishioka M, *et al*. Absolute emission current density of O^- from $12CaO \cdot 7Al_2O_3$ crystal. Appl Phys Lett, 2002, **80** (22): 4259～4261

17 Li Q X, Totimoto Y, Sadakata M. O^- anion generator fabricated by sintering the cerium-doped oxidation material. Japan, Japan Patent, 160374.2004-05

18 Purevdorj D, Igura N, Hayakawa I, *et al*. Inactivation of *Escherichia coli* by microwave induced low temperature argon plasma treatments. J Food Eng, 2002, **53** (4): 341～346

19 Laroussi M, Alexeff I, Kang W L. Biological decontamination by nonthermal plasmas. IEEE Trans Plasma Sci, 2000, **28** (1): 184～188

20 Strauss R G. Malonaldehyde formation is not a suitable screening test to detect oxidation in human neutrophils. J Clin Pathol, 1981, **34** (7～12): 800～802

21 Kiwi J, Nadtochenko V. New evidence for TiO_2 photocatalysis during bilayer peroxidation. J Phys Chem B, 2004, **108** (45): 17675～17684

22 Hayashi K, Matsuishi S, Hirano M, *et al*. Formation of oxygen radicals in $12CaO \cdot 7Al_2O_3$: instability of extraframework oxide ions and uptake of oxygen gas. J Phys Chem B, 2004, **108** (26): 8920～8925

23 Lee J, Grabowski J J. Reactions of the atomic oxygen radical anion and the synthesis of organic reactive intermediates. Chem Rev, 1992, **92** (7): 1611～1847

24 Tashiro T, Watanabe T, Kawasaki M, *et al*. Partial oxidation of methane with oxygen over magnesium oxide at low temperatures. Chem Soc Faraday Trans, 1993, **89** (8): 1263～1269

25 Dong T, Li J, Huang F, *et al*. One-step synthesis of phenol by O^- an OH^- emission material. Chem Comm, 2005, (21): 2724～2726

26 Mackey B M, Forestiere K, Isaacs N S, *et al*. The effect of high hydrostatic pressure on *Salmonella thompson* and *Listeria monocytogenes* examined by electron microscopy. Lett Appl Microbiol, 1994, **19** (6): 429～432

27 Niven G W, Miles C A, Mackey B M. The effects of hydrostatic pressure on ribosome conformation in *Escherichia coli*: an in vivo study using differential scanning calorimetry. Microbiol Sgm, 1999, **145** (Part 2): 419～425

28 Kappus H. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance, In: Sies H ed. Oxidative Stress. London: Academic Press, 1985. 273

原子氧自由基阴离子对大肠杆菌细胞作用的研究 *

王　莲[1)]　宋崇富[1)]　孙剑秋[1)]　YOUSHIFUMI TORIMOTO[2)]
MASAYOSHI SADAKATA[2)]　李全新[1)**]

([1)]中国科学技术大学化学物理系，合肥 230026；
[2)] *Department of Chemical System Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo* 113-8656, Japan)

摘要　研究了原子氧自由基阴离子(O^-)对大肠杆菌的失活作用和形貌变化的影响．实验所用的 O^- 自由基由新研制的 O^- 发生器制备, 其中$[Ca_{24}Al_{28}O_{64}]^{4+}\cdot 4O^-$ (缩写为 C12A7-O^-)材料是 O^- 发生器中发射 O^- 的部分． 实验结果表明, 大肠杆菌的失活率随 O^- 强度的变化而变化，在 O^- 强度为 1.5 μA /cm² 时，细胞的死亡率大大加强，作用 120 min 后，细胞死亡率超过 3 个对数量级．通过场发射扫描电子显微镜观察发现 O^- 对细胞结构具有破坏作用．通过丙二醛 (MDA) 的形成证实了 O^- 诱导大肠杆菌发生脂质过氧化反应过程的存在，这可能是大肠杆菌死亡的潜在原因． 当 1.5 μA /cm² 的 O^- 流通入到大肠杆菌悬浊液后，丙二醛浓度开始升高，15min 后达到最高值 1.2 μmol/g，然后缓慢下降．结果显示，原子氧自由基阴离子能失活大肠杆菌，诱导脂质过氧化反应，这对发展一种新的净化微生物污染方法和研究微生物与原子氧自由基阴离子相互作用具有潜在的意义．

关键词　原子氧自由基阴离子，大肠杆菌，失活，脂质过氧化反应，丙二醛
学科分类号　Q937，Q93-334

---

* 国家高技术研究发展计划(863)(2006AA05Z118) 和中国科学院百人计划(2002)资助项目.
** 通讯联系人. Tel: 0551-3601118, E-mail: liqx@ustc.edu.cn
收稿日期：2007-03-30，接受日期：2007-06-07