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Free radicals induced in aqueous solution by non-contact atmospheric-pressure cold plasma

Atsushi Tani,^{1,a)} Yusuke Ono,² Satoshi Fukui,¹ Satoshi Ikawa,³ and Katsuhisa Kitano² ¹Department of Earth and Space Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan ²Center for Atomic and Molecular Technologies, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan ³Technology Research Institute of Osaka Prefecture, Izumi, Osaka 594-1157, Japan

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To understand plasma-induced chemical processing in liquids, we investigated the formation of free radicals in aqueous solution exposed to different types of non-contact atmospheric-pressure helium plasma using the spin-trapping technique. Both hydroxyl radical (OH·) and superoxide anion radical $(O_2^{-} \cdot)$ adducts were observed when neutral oxygen gas was additionally supplied to the plasma. In particular, $O_2^{-} \cdot$ can be dominantly induced in the solution via oxygen flow into the afterglow gas of helium plasma. This type of plasma treatment can potentially be used in medical applications to control infectious diseases, because the $O_2^{-} \cdot$ is crucial for sterilization of liquids via atmospheric-pressure plasma. @ 2012 American Institute of Physics. [http://dx.doi.org/10.1063/1.4729889]

Low-frequency (LF) plasma jets, which comprise atmospheric-pressure plasma at low gas temperatures, have considerable potential for use in plasma applications involving soft materials and liquids.^{1–3} Once the plasma is exposed to the liquid, advanced reaction fields will be induced not only on the liquid surface but also inside the liquid itself, because some of the plasma-induced active species such as ions, molecules, and radicals can easily diffuse into the liquid. This feature of LF plasma processing makes it significantly different from the ordinary plasma processing of solids where the reaction fields are restricted only to the surface.

For medical applications that involve the control of infectious diseases, the inactivation of bacteria in liquids is extremely important. High bactericidal activity using the LF plasma jets has been achieved in liquids via the reduced pH method where the solution is sufficiently acidic.^{4–6} It is interesting to note that there is a critical pH value of \sim 4.7 for bactericidal effects to occur. Bacteria in aqueous solution are efficiently inactivated below the critical pH value, whereas they are hardly affected by plasma treatment above this pH value. It has been also found that the presence of superoxide anion radical (O_2^{-}) in water is necessary for this method.⁴ The critical pH value may be associated with the acid dissociation constant, pK_a , between O_2^{-} and the hydroperoxy radical (HOO·); this value is known to be approximately 4.8.^{7,8} This means that O_2^{-} in acidic aqueous solutions (lower than a pH value of 4.8) tends to capture a proton (H^+) to form HOO, which shows considerably stronger bactericidal activity.⁹ These results indicate that plasma-induced radicals are crucial for plasma processing in liquids.

Recently, Kitano *et al.* reported that the afterglow gas of helium LF plasma (not the plasma itself) can lead to high bactericidal activity, and this afterglow gas can be used for sterilization.^{10,11} This suggests that the afterglow gas may

also induce active species in an aqueous solution, even if the plasma does not come into contact with the surface of water. In the light of this discussion, in our study, we investigated the free radicals induced in an aqueous solution via noncontact atmospheric-pressure helium plasma, using different types of plasma sources.

We used four different types of experimental setups, as shown in Fig. 1. The main differences among these setups are summarized in Table I. The type I setup in Fig. 1(a) involved the demonstration of a general experiment that used an LF jet with an atmosphere-controllable chamber where oxygen gas was supplied from one of the side ports to the chamber. The helium plasma jet was exposed directly to the aqueous sample in the glass, and the tip end of the plasma was in contact with the surface of the sample. The distance from the electrode to the nozzle of the tube was 60 mm and the length of the tube inside the container was 5 mm.

In the type II (Fig. 1(b)) and the type III setups (Fig. 1(c)), the afterglow gas of the helium plasma (instead of the plasma itself) was made to flow into the chamber. This type of plasma is sometimes called as remote plasma. The discharge area was limited within the glass tube between two grounded electrodes above and below the central electrode that was connected to the high-voltage supply. The interval between the electrodes was 20 mm. The primary difference in these setups was in the method of supplying neutral oxygen gas into the system; in type II, oxygen gas was supplied from one of the side ports, similar to the method in type I, whereas in type III, oxygen gas was mixed with the afterglow gas of the helium plasma using a tee tube. The location of the tee joint was 100 mm apart from the nozzle of the tube.

The type IV setup in Fig. 1(d) was a unique plasma system, where two electrodes were set beyond the joint of the tee tube (the interval between the electrodes was 15 mm). In this setup, once oxygen gas is made to flow directly into the discharge area, the gas flow near the joint becomes a

^{a)}Author to whom correspondence should be addressed. Electronic mail: atani@ess.sci.osaka-u.ac.jp.



FIG. 1. Schematic illustrations of four different experimental setups used for plasma generation. (a) Type I: the plasma jet is in direct contact with the solution surface, (b) type II: the afterglow gas of helium plasma is mixed with oxygen gas in the chamber, (c) type III: the afterglow gas of helium plasma is mixed with oxygen gas in the glass tube, and (d) type IV: oxygen gas is in contact with helium plasma in the tube.

multiphase flow and most of the discharge occurs only in the section with the pure helium, as shown in Fig. 2. The distinctive feature of the type IV setup is that oxygen gas is in direct contact with the helium plasma in the multiphase flow. The tip end of the plasma did not come into contact with the surface of the solution, as in types II and III. The distance from the lower ground electrode to the nozzle of the tube was 200 mm and the length of the tube inside the container was 15 mm in type II, III, and IV.

We used a low-frequency, high-voltage power supply $(\sim 10 \text{ kHz} \text{ and } \sim 10 \text{ kV}, \text{ respectively})$ in all the experiments. The total dissipated power in each type was $\sim 3 \text{ W}$. After purging the gas in the chamber with helium (2 slm) and oxygen (0.5 slm, if not mentioned) for 1 min, the plasma was generated and maintained for 3 min in each case. The flow rates were controlled by mass flow controllers (Kofloc, 8500MC).

In general, free radicals are unstable and cannot be easily observed, particularly in liquids. To solve this problem, we used the spin-trapping technique;¹² in this technique, spin trapping compounds react with unstable radicals (with short lifetimes) in liquids to form spin adducts (having longer lifetimes) that can be measured using a conventional electron spin resonance (ESR) spectrometer. CYPMPO, 5-(2, 2-dimethyl-1, 3-propoxycyclophosphoryl)-5-methyl-1-pyrroline N-oxide), was used as a spin-trapping compound because it has the unique property of being able to distinguish between $O_2^{-} \cdot$ and hydroxyl radical (OH·) among reactive oxygen species via the ESR spectrum.¹³ The aqueous solution of CYPMPO (5 mmol/l) was prepared with ultrapure water. The reagent of CYPMPO (commercially available with Radical Research) was used without any further purification.

TABLE I. Summary of characteristics and results for four different plasma sources.

	Type I	Type II	Type III	Type IV
Plasma type	Contact	Non-contact	Non-contact	Non-contact
Plasma gas	He plasma	He plasma	He plasma	(He+O ₂) plasma
O ₂ supply	To chamber	To chamber	To afterglow	To plasma
	Intense OH·	OH∙	Less OH-	OH·
Induced radical	O_2^- ·	O_2^- ·	O_2^- ·	Less O_2^- .

Although no obvious signal was detected in the solution before plasma processing, both of O_2^{-} and OH adducts were recognized in the solution samples after plasma processing for all the setups (Fig. 3). Their intensities were clearly different; the signal for OH was very weak for the type III setup, whereas that for the type I setup showed significant intensity. The ESR spectra results are also summarized in Table I.

In the type I setup, where the plasma was in direct contact with the aqueous solution, OH· were formed from water dissociation as shown in Fig. 3(a). This result agrees well with our previous result and other recent reports on the diagnosis of OH· formation in water by the use of atmosphericpressure cold plasma jets.^{14–16} The O₂⁻· adduct was also observed because of the abovementioned characteristic of CYPMPO. This result implies that oxygen gas flowing into the chamber partially changes to O₂⁻·.

The results for the type II setup in Fig. 3(b), where the afterglow gas of helium plasma was mixed with oxygen gas in the chamber, clearly show that the afterglow gas can be used for plasma processing in liquids, because active species like O_2^{-1} and OH are induced in the aqueous solution. Additional experiments in the type II setup without the use of



FIG. 2. Image of plasma at tee joint in the type IV setup shown in Fig. 1(d).

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FIG. 3. Electron spin resonance (ESR) spectra of sample solution after plasma processing at room temperature. (a), (b), (c), and (d) correspond to types I, II, III, and IV, respectively. Most of the observed signals are attributed to the two spin adducts of O_2^{-} and OH. These signals can be distinguished by the peaks in the central part; the solid triangles indicate the O_2^{-} adduct and the solid diamonds indicate the OH adduct.¹³ Microwave power was 4 mW in all measurements.

oxygen flow showed that only OH· and atomic hydrogen (H·) were induced in the solution; no O_2^{-} · was observed. Formation of atomic hydrogen was also confirmed using another spin-trapping compound of PBN, phenyl-*tert*-butyl nitrone. These results reveal that O_2^{-} · may have formed from oxygen gas in the gas phase and subsequently dissolved into the aqueous solution. Moreover, energetic helium (the so-called "meta-stable helium") may play an important role in the production of such radicals on the water surface as well as in the gas phase. Meta-stable helium is one of the key species in plasma processing of this type.

In the type III setup, where the afterglow gas of helium plasma was mixed with oxygen gas in the tee tube, a relatively small amount of OH· was formed as shown in Fig. 3(c). In particular, the signal of the OH· adduct was not clearly observed under an oxygen flow rate of 1.5 slm (Fig. 4(b)) even though this flow rate was three times larger than that for the results shown in Fig. 3 (Fig. 4(a)). This may be



caused by the exhausting of most of the meta-stable helium in the tube through reaction with neutral oxygen gas. This result suggests that this type of plasma can be used for supplying O_2^{-} selectively to liquid systems without causing OH· formation.

The ESR spectrum for the type IV setup in Fig. 3(d), where oxygen gas was in contact with helium plasma in the tube, shows a relatively small amount of O_2^{-} adduct. Given that the amount of OH adduct decreases with distance from the plasma discharge to the aqueous solution, the meta-stable helium or other active oxygen species such as atomic oxygen may partially reach the water surface and induce the formation of OH in the aqueous sample. Further, O_2^{-} may be formed in the mixture of two gases and transferred to the solution.

These results indicate that the plasma-induced free radicals strongly depend on the type of plasma sources used (such as the method of oxygen supply and contact/non-contact plasma). Remarkably, O_2^{-} can be selectively supplied to the liquid without any dissociation of water using the type III setup, where the helium-plasma afterglow gas mixes with oxygen gas in the tee tube. Since the presence of the O_2^{-} is crucial to the inactivation of bacteria in liquids via the use of the reduced pH method, we believe that our findings can be significantly useful for the sterilization of liquids. The resultant reduced OH formation can be potentially useful for medical or dental applications that use plasma because OH formation usually affects healthy cells.

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