Plasma application for detoxification of Jatropha phorbol esters

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Abstract. Atmospheric pressure non-thermal dielectric barrier discharge (DBD) plasma generated by helium gas at high voltage and input power of about 50 W was first applied to detoxification of Jatropha curcas phorbol esters (J. PEs) as well as standard phorbol ester (4β-12-O-tetradecanoyl phorbol-13-acetate, TPA) in water and methanol. Plasma irradiation on the solution sample was conducted for 15 min. In aqueous solution, only 16% of TPA was degraded and complete degradation of J. PEs was observed. On the contrary, complete degradation of both TPA and J. PEs in methanol was achieved by the same plasma irradiation condition. Hydroxyl radical (•OH) generated by plasma irradiation of the solution is expected as the main radical inducing the degradation of PEs.

1. Introduction
Plasma is a promising technology which is useful for various applications such as a tool for green chemistry, environmental analysis and waste management [1]. Beside hot (thermal) plasma, cold (non-thermal) plasma is currently an interesting approach in which the reactive chemical species can be produced at low gas temperature [2]. Non-thermal plasma is known as the mixture of electrons, highly excited atoms, molecules, ions, radicals, photons and so on [3]. Even though many kinds of non-thermal plasma generation devices have been known, dielectric barrier discharge (DBD) get much interest nowadays due to its simple configuration [4]. The type of highly excited atoms, molecules, ions, radicals in the plasma depends on applied gas species [5]. When high-energy electron, highly excited atoms entering into water, hydroxyl radical (•OH), hydrogen atom (•H) and electron (e) are formed [6]. The degradation of organic pollutants in aqueous solution by non-thermal plasma is mostly based on oxidation reaction between •OH and the pollutants [7]. Therefore, non-thermal plasma technology is currently classified as one of promising methods used in Advance Oxidation Process (AOP) [8].

Jatropha phorbol esters (J. PEs) were toxic compounds found in toxic-genotype jatropha such as Jatropha curcas (J. curcas) [9]. Phorbol esters are known as tumor promoters [10]. For eliminating the risk to the workers contacting to such toxic oil, methanol is normally used as solvent for extracting J. PEs from J. curcas oil before biodiesel production [11]. J. PEs in the oil was possibly degraded to undetectable level by natural sunlight exposure at least 6 days [12]. Stripping or deodorization at 260 °C under low pressure of about 3 mbar and 1% steam injection can completely degraded J. PEs [13]. Under optimized solid state fermentation conditions, P. aeruginosa PseA can completely degrade J.
PEs in *J. curcas* seed cake in nine days [14]. Approximately 71.35% of *J.* PEs in *J. curcas* seed cake were successfully removed by $\gamma$-irradiation at absorbed dose of 50 kGy [15]. Such methods need long treatment time, high temperature as well as large facility and high dose from $\gamma$-irradiation.

The objective of this present work is to apply the dielectric barrier discharge (DBD) non-thermal plasma to degrade/detoxify *J.* PEs in aqueous or methanolic solution. It is expected that the radicals entering into the solution or generated by interaction between plasma species and solvent molecule would be able to degrade the phorbol esters.

2. Materials and methods

2.1. Plasma system
The schematic drawing of plasma system used in this experiment is shown in Figure 1. The plasma source used in the present work is a small and simple one with a low-frequency power supply (Loggy Electronics Ltd, LHV-13AC) and a quartz tube as dielectric barrier [16-17]. In this study, plasma production condition is set as follows: helium gas with a flow-rate of 6 L/min supplied through the quartz tube (inner diameter of 5 mm) from upper inlet to the lower outlet, and the high voltage of 10 kV applied to the electrodes that create barrier discharge between electrodes, and the input power of about 50 W. Under the condition, a plasma plume, 5 cm in length, is ejected from the nozzle into the surrounding air before reaching the surface of solution.

![Figure 1 Schematic drawing of DBD plasma configuration used in present study](image)

2.2. Sample preparation, plasma irradiation and analysis
TPA in solid form in ampoule (Wako Pure Chemicals, Tokyo, Japan) was used to prepare stock solution of 1,000 mg/L of TPA by dissolving with methanol (MeOH). This TPA stock solution was further diluted with MeOH to be 100 mg/L of standard solution. *J.* PEs was obtained in our laboratory by methanolic extraction and the extract was then purified by silica-gel column chromatography [18]. The fraction containing *J.* PEs was then analyzed by High Pressure Liquid Chromatography (HPLC) to determine their concentrations. The concentration of each component is evaluated to be as equivalent as that of TPA. The molecular structures of TPA and *J.* PEs are shown in Figure 2.
A hundred micro liter of TPA stock solution (1,000 mg/L) was diluted with water (Milli-Q water) to obtain a 10 μg/mL sample solution in 10-mL-volumetric flask. The methanolic sample solution of TPA was prepared in the same way.

After purification, the 5,758 mg/L of J. PEs stock solution was prepared in methanol. The concentration was the total amount of phorbol esters contained in the solution. The sample solutions of both water and methanol were prepared by the same manner as in TPA.

For plasma irradiation, one milliliter of the solution was loaded onto a 1.5-mL-beaker, and then irradiated by the plasma for 15 min. The concentrations of phorbol esters were measured by HPLC equipped with a photodiode array detector (PDA) and an automatic sample injector (SHIMADZU Corporation, Japan). An analytical column was Shim-pack XR-ODSII (2.0 mm i.d × 75 mm).

Analytical conditions was as follows: mobile phase was a mixture of water (A) and acetonitrile (B) (Wako Pure Chemicals, Tokyo, Japan) and the run was started at initial mixing ratio of 50:50 (A:B) for 5 min, and in gradient mode to 75:25 for 10 min, and finally to 100:0 for 5 min and hold for 2 min. The column temperature was 35 °C and the injection volume was 5 μL. The phorbol esters were detected in a UV region at the wavelength of 280 nm [13].

3. Results and discussion

Figure 3(a) and 3(b) showed HPLC/UV chromatograms of TPA and J. PEs in the aqueous solution respectively. After plasma irradiation, approximately 16% reduction of TPA was observed as shown in Figure 3(a). On the other hand, before plasma treatment four peaks of phorbol esters in aqueous solution are observed but disappeared after plasma irradiation as shown in Figure 3(b).

During plasma irradiation, the oxidative degradation of phorbol esters in the solution by hydroxyl radicals (•OH) may occur through two way. Interaction between high-energy electron (e*) in the
plasma plume and the O\(_2\) and moisture (H\(_2\)O) would generated Ozone (O\(_3\)) and •OH then entering into the solution via air-solution interface [20].

\[
\begin{align}
O_2 + e^* & \rightarrow 2O^* + H_2O \rightarrow 2\cdot OH + e \\
O_2 + O^* & \rightarrow O_3
\end{align}
\]

(1) (2)

Entering into aqueous solution (Eq. 4), ozone (O\(_3\)) dissociates to O\(_2\) and O\(^*\) (Eq. 5) and the O\(^*\) react with H\(^+\) in the presence of e to form •OH radical [21]. Enhanced by ozone (Eq. 5-6), UV light from plasma discharge would also play a key role on producing •OH radicals [22].

\[
\begin{align}
O_3 & \rightarrow O_2 + O^* \\
O^* + H^+ + e & \rightarrow \cdot OH \\
O_3 + h\nu + H_2O & \rightarrow H_2O_2 + O_2 \\
H_2O_2 + h\nu & \rightarrow 2\cdot OH
\end{align}
\]

(3) (4) (5) (6)

When the plasma electron reaches the surface of aqueous solution (Eq.7), •OH radical is also generated [23].

\[
H_2O + e \rightarrow \cdot H, \cdot OH, e, etc.
\]

(7)

The generated •OH radicals (6.35 ± 0.77 × 10\(^{-7}\) moles) in water after 15 min plasma treatment was detected by KI-colorimetric method. This observation is not contradicted with the result found in literature [24].

On the contrary, It has been reported that •OH radical is formed under plasma irradiation of methanol [24]. Large amount of •OH radical may be generated that completely oxidized all phorbol esters molecules in the methanol solution. So we tried similar experiment as Figure 3 with methanol as solvent. In Figure 4, the disappearance of the peaks of TPA as well as J. PEs after plasma irradiation was confirmed.

\[
\begin{align}
CH_3OH + e & \rightarrow \cdot CH_3, \cdot OH, e, etc.
\end{align}
\]

(8)

The decomposition process of PEs depends not only on the kind of PE but also on solvent. It is expected that J.PEs was oxidized by •OH radicals through electrophilic reaction. For simplicity, a typical decomposition of TPA is shown in Figure 5.

**Figure 3** HPLC/UV chromatograms of (a) TPA and (b) 4 peaks of J. PEs in aqueous solution before and after plasma irradiation for 15 min.
Figure 4 HPLC/UV chromatograms of TPA (a) and 4 peaks of J. PEs (b) in methanolic solution before and after plasma irradiation for 15 min.

The •OH radicals attack to the double bonds of ring A and/or B (indicated in Figure 5 as the symbol “A” and “B”) in the structure of TPA leading to bond cleavage and ring destruction [26]. These intermediates would follow further oxidation with more •OH radicals producing carboxylic acids, carbon dioxide and water [27].

Figure 5 TPA degradation pathways by •OH radical attack.

4. Conclusion
We have firstly demonstrated that atmospheric non-thermal plasma would be usefully applied for degrading phorbol ester (TPA or J. PEs) in aqueous or methanolic solution, even though the exact mechanism on the degradation of phorbol ester should be further investigated in detail. After 15-minutes-plasma treatment, 16% and complete degradation of TPA were found in aqueous and methanolic solutions, respectively. Complete degradation of J. PEs was achieved in both aqueous and methanolic solutions. The generation of •OH radicals (6.35 ± 0.77 × 10⁻⁷ moles) in water after the same
plasma treatment condition were confirmed. The degradation of phorbol esters in methanolic solution is more efficient than in aqueous solution. Since •OH radical was not measured directly in our experiment, so more comprehensive study would be necessary. Parameter optimization on the plasma treatment and discharge condition is needed in future study.

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