Microbubbles Enhanced Synthetic Phorbol Ester Degradation by Ozonolysis

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Abstract—A phorbol-12-myristate-13-acetate (TPA) is a synthetic analogue of phorbol ester (PE), a natural toxic compound of Euphorbiaceae plant. The oil extracted from plants of this family is useful source for primarily biofuel. However this oil might also be used as a foodstuff due to its significant nutritional content. The limitations for utilizing the oil as a foodstuff are mainly due to a toxicity of PE. Currently, a majority of PE detoxification processes are expensive as include multi steps alcohol extraction sequence.

Ozone is considered as a strong oxidative agent. It reacts with PE by attacking the carbon-carbon double bond of PE. This modification of PE molecular structure yields a non toxic ester with high lipid content.

This report presents data on development of simple and cheap PE detoxification process with water application as a buffer and ozone as reactive component. The core of this new technique is an application for a new microscale plasma unit to ozone production and the technology permits ozone injection to the water-TPA mixture in form of microbubbles.

The efficacy of a heterogeneous process depends on the diffusion coefficient which can be controlled by contact time and interfacial area. The low velocity of rising microbubbles and high surface to volume ratio allow efficient mass transfer to be achieved during the process. Direct injection of ozone is the most efficient way to process with such highly reactive and short lived chemical.

Data on the plasma unit behavior are presented and the influence of gas oscillation technology on the microbubble production mechanism has been discussed. Data on overall process efficacy for TPA degradation is shown.

Keywords—Microbubble, ozonolysis, synthetic phorbol ester.

I. INTRODUCTION

The Euphorbiaceae family of plants consists of more than 7500 species. The Jatropha curcas Linn from this plant family has been known for a long time as an oil rich source comparable to corn, soybean and wheat. This plant has high ability to grow at poor agricultural conditions including acid soil, high temperature and low fertility soil [1], [2]. With 60% of oil content in a kernel the Jatropha was recognized as a valuable source for biofuel, therefore currently it is widely cultivated in the equatorial belt [3]-[5].

In addition to high oil content Jatropha has significant levels of protein and amino acids, up to 50-58%. This makes de-fatted Jatropha - Jatropha cake applicable as fertilizer and an additionally as a potential foodstuff.

There are two known types of Jatropha plants which differ in Phorbol ester (PE) content naturally produced in the Euphorbiaceae family [6], [7]. Chemically the PE is described as tigliane diterpenes where fatty acid is esterified by two hydroxyl groups connected to carbon atoms. Non toxic PE (type a) differs from the toxic carcinogenic PE (β) [8, 9] by position of “–OH” group in the molecular structure [10], [11].

The non-toxic, Mexican variety of Jatropha plant has a low level of PE that is below the edible threshold and found to be 0.27 mg/ml in the oil [11], [12]. However Jatropha curcas Linn from all other locations of the planet is toxic in all parts including leaves, bark and fruit [7], [13].

To meet the growing demand on food in developing countries Jatropha cake detoxification methods have been a focus over the last decade. From the best of our knowledge there is no well established low cost detoxification process. The combination of heat and chemical treatment [14], traditional oil refining approach [15], biodegradation [16] and various solvents extraction processes [8] were tested for this propose. They require high energy input or highly consumable on alcohol solvents.

The molecular structure of PE is very similar to phorbol-12-myristate-13-acetate [6] which called as synthetic phorbol ester. A phorbol-12-myristate-13-acetate (TPA) is commonly used as external standard in investigations targeting phorbol ester removal.

Similar to other compounds in esters group, TPA has a carbon double bond which is linked to an oxygen atom. This makes TPA a good candidate for treatment with ozone. Ozone is expected to attack the TPA carbon double bond which resulted in different molecular structure of formed non toxic esters. The reactions between ester and ozone are well characterized and known as Criegee mechanism [17].

Application of electric plasma discharge is one well known approach for ozone production. High energy and specific equipments required to perform ionization process in plasma. Conventionally, there are several types of plasma reactors applied at industrial scale for ozone production. A dielectric barrier discharge (DBD) is a well established basis. Typically a DBD reactor consists of two metal parallel electrodes with at least one covered by dielectric layer. Electrodes are separated with a small gap in between [18]. In order to ignite plasma, gases are passed through the electrodes and induced by electrical current. The process occurs in nanoseconds, hence resulting in low heat loss [19].

To mimic the oil production conditions, TPA...
decomposition has to be done in a liquid. In aqueous environment, there are two modes of ozone reaction which are direct and indirect kinetics reactions [20]. In the direct reaction, \(O_3\) molecules are predominant while in the indirect reaction, \(\cdot OH\) radicals control the reaction [21]. As mentioned in the literature, during the process, ozone breaks down into species such as \(\cdot OH\) radicals [22]. Ozone dissolves to a liquid from the gas-liquid interface. This process is mass transfer limited. To improve mass transfer the ozone should be introduced to the bulk of liquid in form of microbubbles with high surface to volume ratio [23]. The smaller size of bubble allows higher driving force in terms of mass transfer in the solution [24].

This paper presents the very first report on evaluation of the efficacy of TPA degradation process by microbubble enhanced ozonolysis.

II. MATERIALS AND METHODS

The sample of TPA containing distilled water (DIW) mixture was made in two steps. At first step a 1000\(\mu\)g of granulated TPA is dissolved in 2ml of Methanol. This step was taken to avoid problems with low solubility of TPA in water. Then on the second step, 8ml of Methanol mixture is diluted with DIW to produce 400ml of the TPA mixture. The mixture produced has TPA concentration of 12 ppm.

TPA was purchased from Acros Organics, New Jersey, USA. The supply code: 356150010 and CAS: 16561-29-8. DIW was HPLC grade purchased from Sigma Aldrich.

The experimentation was performed in set up presented on Fig. 1. There are three main parts: 2L stainless steel cylindrical reactor, the ceramic diffuser and the plasma microreactor.

The plasma DBD microreactor was located at the bottom part of reactor, Fig. 1. It has two parts with round copper electrodes diameter 28mm covered with dielectric layers made form 140\(\mu\)m thick glass in each. When reactor assembled the electrodes located face to face and spaced by 300\(\mu\)m gap in-between.

solution was stored in the freezer with controlled temperature (-20°C). The calibration solutions were prepared by diluting the stock solution with methanol in various ratio (in the range of 0-40 ppm).

III. RESULTS AND DISCUSSION

Fig. 2 presents the calibration curve of TPA. The calibration equation was determined by plotting peaks height versus TPA concentration.

Fig. 2 Calibration curve of TPA

Fig. 3 plots the HPLC chromatogram of TPA profile before ozonolysis. There were 2 major peaks detected at 232nm wavelength which are solvent peaks and the TPA peak. The solvent peaks appeared around 2 minutes while TPA retention time is 17.5min.

Fig. 3 Chromatogram of TPA before ozonolysis

It was found, Fig. 4, that after 5 minutes of ozonolysis the TPA peak position and shape were changed. There was significant reduction in TPA peak height and the retention time. This can be described by TPA decomposition and degradation with formation of other type esters. The TPA concentration was found equal to 2.63ppm.

Fig. 4 Chromatogram of TPA after 5 minutes of ozonolysis

The HPLC chromatogram of TPA profile after 10 minutes of ozonolysis is presented on Fig. 5. It was shown no peaks other than mobile phase. It was clear indication that the concentration of TPA is lower than detection limit of analytical device.

Fig. 5 Chromatogram of synthetic PE after 10 minutes ozonolysis

IV. CONCLUSION

We have presented the experimental results on TPA degradation process by microbubbles enhanced ozonolysis. This paper is the very first report on practical application of the new micro DBD plasma reactor coupled with diffuser to inject ozone directly to the bulk of liquid in form of microbubbles. It was shown that this method is very effective in facilitation of ozone driven reactions in aqueous environment. This can be described by fast ozone dissolving to the bulk of liquid due to high interface surface area supported by microbubbles. It was found that with the method presented a 12ppm of TPA in mixture with DIW can be fully decomposed within 10 minutes.

The results obtained are directly applicable to the case of PF decomposition and detoxification of Jatropha curcas Linn meal and oil down to the eatable level. Experimental data contribute to the plasma micro reactors development and industrial application for a food sector.

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