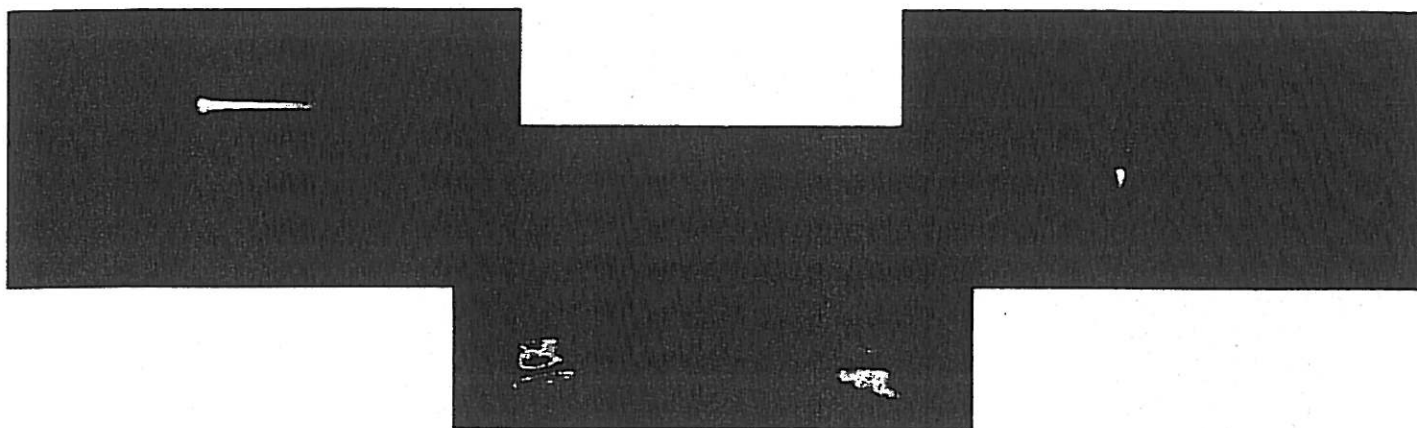


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Ozone soil conditioning and decontamination

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Résumé

This work introduces an overview on the bactericidal properties of plasma assisted ozone treatment for the soil remediation. Developed electrode system provided ozone concentration beyond 20 g/m^3 , which is the critical value of soil sterilization. The gaseous ozone injection system consisted of 10 injectors and the treatment container, which was developed to sterilize a large volume of agricultural soil. A stream of oxygen containing 5% by weight of ozone was bubbled into the microcentrifuge tube containing the λ -E.Coli DNA solution. 0.2-1 gram of ozone was supplied to 0.5 milliliter of DNA solution during 5-20 min of treatment with 0.5 liter/min causing complete collapse of the DNA structure.

Introduction

Sterilizing techniques employing active species such as ozone, hydrogen peroxide, OH radicals, oxygen singlets are especially beneficial for persistent microbial pollutions caused by the aggregations of microorganisms called biofilms, which are widely present on various surfaces and within soil. Anti-microbial properties of plasmas in the case of decontamination of water, ambient air and surfaces were previously widely proven [1-4]. Pollutants might be distributed in soil in several ways: in soil matrix, vapor phase, non-aqueous phase or groundwater [5]. Ozone based techniques are good alternative to traditional techniques like heating, flushing with chemical additives, landfilling, incineration, etc. Benefits of ozone applications in agriculture might be summarized as follows: use of ozone in soil treatment will not result in the build-up of any environmentally persistent or toxic compounds but ozone itself, and O_3 is immediately consumed in the soil treatment process; ozone is manufactured on site so it cannot be stored and its sudden release into the atmosphere is not possible like it could occur with compressed methyl bromide or other persistent, toxic gases or chemicals used for soil sterilization; thus, it assures minimum human severe and toxicity.

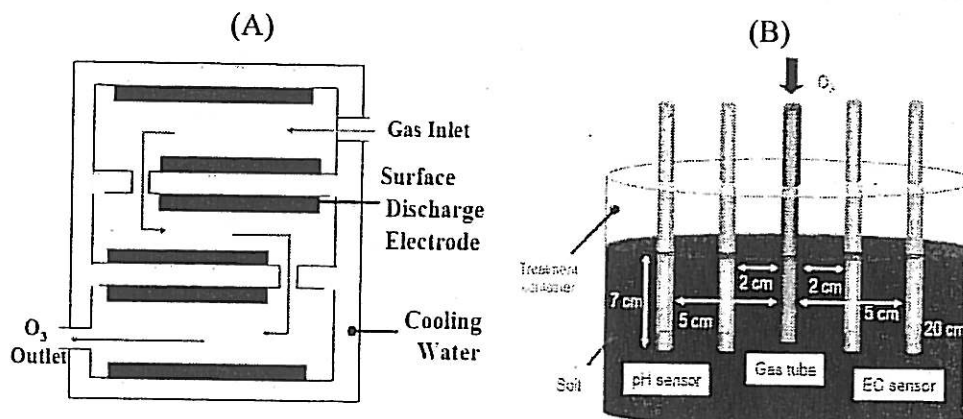


Fig. 1: OP-20W ozonizer (A), Multi-electrode injection system (B).

Experimental set-up

The main element of soil sterilization system was TiO_2 based surface discharge commercial OP-20W Iwasaki ozonizer, which is presented in Fig. 1(A). The gaseous ozone injection field-scale system is shown in Fig. 1(B). It consisted of 10 injectors and the treatment container, which was developed for sterilizing and monitoring of agricultural soil in large volume. The pH value, electrical conductivity and temperature of the soil were observed to investigate the effect of ozone treatment on soil properties.

Results and discussion

Conventional biological method of the CFU (colony forming unit) counting showed that bacteria and *Fusarium oxysporum* in the soil were almost eliminated by ozone treatment with the concentration over $20 \text{ gO}_3/\text{m}^3$ achieving sterilization rate up to 99.9%. The dependence of sterilization rate on the amount of ozone introduced to the soil is presented in Fig. 2(A).

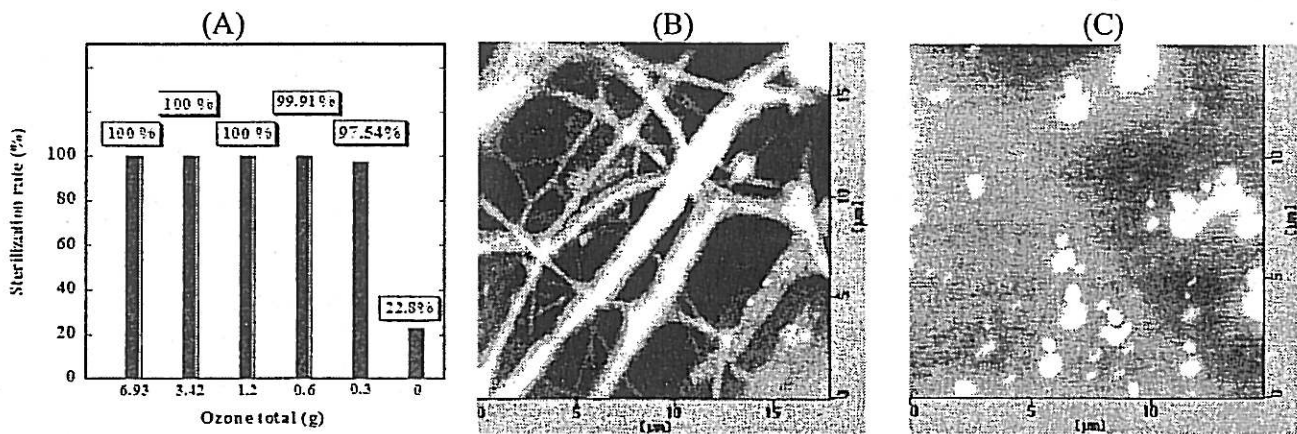


Fig. 2: Sterilization rate vs. ozone concentration (A), Sample of DNA *E. coli* (AFM, 48,502 base pair, $16\mu\text{m}$) before ozonation (B), Ozone treated DNA (C).

λ -*E. coli* DNA (Nippon Gene) was diluted with Tris-HCL and EDTA. The DNA solution was further diluted with distilled water in a microcentrifuge tube. A stream of oxygen containing 5% wt. ozone was bubbled into the microcentrifuge tube containing the DNA solution. 0.2-1 g of O_3 and was supplied to 0.5 ml of DNA solution during 5-20 min of treatment at 0.5 l/min gas flow rate. Fig. 2(B) depicts the image of DNA sample deposited on the mica substrate. The molecular structure of DNA collapsed completely when high concentration of ozone was introduced into the DNA solution. Treated DNA sample is presented in Fig. 2(C). It indicated that ozonation process broke the *E. coli* DNA and split it into many fragments of typical length and width of 380-390 nm and 15 nm, respectively. These peculiar pieces have almost been not observed when the DNA samples were treated for longer time, when DNA was decomposed completely.

Conclusions

The set up for agricultural microbial ozone sterilization purposes was developed. Gaseous ozone sterilization was proven to be satisfactory for treatment of soil infected by *Fusarium oxysporum*. Up to 99.9% sterilization efficiency was achieved using ozone dosage over $20 \text{ gO}_3/\text{m}^3$.

The fundamental experiments on biological reaction between the λ -*E. coli* DNA and ozone exposure suggested that the molecular structure of the DNA collapsed completely using 5% wt. ozone concentration.

The ozone can be successfully used for the soil remediation and removal of biological agents present in plasmonic and biofilm forms.

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