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Recent development of ozone treatment for agricultural soil sterilization and biomedical prevention

***Abstract**. We have studied gaseous ozone sterilization for soil and biomedical prevention for infectious diseases and for the protecting of citizens' health. Experimental results obtained using ozone generation apparatuses of various construction and ozone sterilization systems, following with AFM measurement were shown. Change of soil properties in the process of ozone treatment, plant growth, biological phenomena and DNA visualization were investigated. Application of this technique to environment protection was proposed.*

***Streszczenie**. Zbadano proces sterylizacji za pomocą ozonu jako jedną ze skutecznych metod prewencji w przypadku skażeń gleby lub epidemii chorób zakaźnych. Przedstawiono rezultaty otrzymane przy użyciu systemów sterylizacyjnych wykorzystujących ozonatory o różnej konstrukcji. Poddano dyskusji możliwość zastosowania proponowanych rozwiązań w inżynierii środowiska. (**Rozwój metody sterylizacji gleby za pomocą ozonu i wskazanie jej biomedycznych zastosowań**).*

Keywords: ozone, soil sterilization, biomedical prevention,
Słowa kluczowe: ozon, sterylizacja gleby, techniki prewencji w biologii i medycynie

Introduction

Ozone (O_3) is the strongest commercially available disinfectant and also is very effective at destroying bacteria, viruses and odors. It has a very short half-life in water and soil, and decomposes in simple diatomic oxygen or oxidizes other compounds.

We have studied the application of gaseous ozone to soil sterilization and the biological effect of gas phase ozone on the DNA genome of bacteria and viruses [1-10]. We have proposed the gaseous ozone sterilization system for agricultural soil which ensures a secure agricultural production. The reaction of the soil components with ozone causes complicated and rapid change in physical, chemical and biological properties.

The pH value defined by pH=-log10[H^+] is an informative indicator to estimate soil properties treated by ozone. In-situ measurement of pH value during ozone treatment showed that the pH linearly decreased with time at the initial process phase which is due to formation of H^+ ions (decomposition of OH- ions) [9,10].

Ozone sterilization system for disinfection of large area of agricultural land was developed. The dielectric barrier discharge generated in the coaxial type geometry of a screw type electrode provided high ozone concentration over 100g/m^3 in oxygen. Additionally, ozone diffusion length in the soil to estimate the appropriate arrangement for ozonized gas distribution system in the soil was studied [2,4].

The agricultural field trials for plant growth proved that the developed ozone sterilization system is effective to decontaminate the agricultural soil [8,10].

Atomic force microscopy (AFM) has become one of the most widely used technique of biochemical study in understanding of nano-size structure of the DNA samples. We employed the AFM technology to investigate the biochemical effect of ozone treatment on the DNA. AFM images showed that the molecular structure of the λ-E.Coli DNA collapsed completely using 5% ozone concentration in oxygen carrying gas [7-10].

Our experimental results of ozone soil sterilization can be used to develop a new environmental technology related to air purification at the shelters, temporary houses, and emergency medical treatment centers, for disposal of tsunami debris and for improvement of saline and radioactive soil.

Experimental

Ozone generation system [4-8]

The dielectric barrier discharge was used to produce highly concentrated ozone available for sterilization of bacteria, viruses and nematodes in the soil. A pulsed electric power was generated by high frequency oscillator (10-30kHz).

Ozone sterilization system [1-10]

Generated ozone was directly injected into the agricultural soil. We have developed an ozone injection system with 12 channel electrode injectors as shown in figure 1. Figure 2 shows the outline of ozone treatment system for large scale agricultural field (US Patent 5624635). The ozone supplier is moved over the field by a trailer or a vehicle. In-situ measurements of the pH and the electrical conductivity were carried out using pH/Nitric ion and pF/EC meter.

DNA visualization [5-10]

AFM (SPI3800N) was applied to observe nano-size structure of ozone treated DNA samples. Ozone was bubbled into the micro centrifuge tube containing the DNA solution. The ozone treated DNA solution was dropped on the substrate and was dried.

Fig.1. The 12 channel DBD electrode system

Results and Discussion

Figure 3 shows temporal change of pH value of the soil at various treatment conditions (tab.1). Most of pH values rapidly decreased in the initial phases. pH less than 6.5 or larger than 11 have been recommended to prevent highly contagious foot-and-mouth disease.

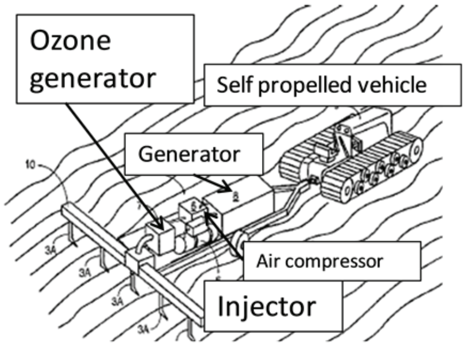

Fig.2. Field sterilization (US Pt. 5624635)

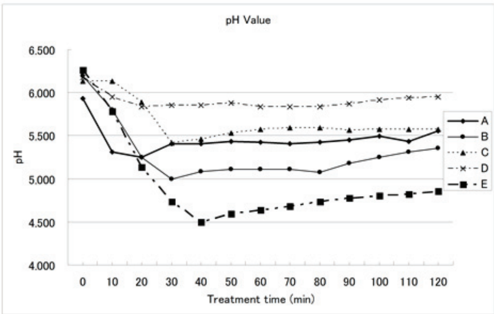

Fig.3. pH value as a function of time with sterilization conditions

Table 1. Soil treatment conditions

Sample number		A	B	C	D	E (2ch)
Ozone concentration	g/m^3	100	100	100	40	100
Treatment Time	min	60	60	60	60	60
Flow rate	lite/ min	2	2	3	2	2 x 2
Distance	mm	30	30	30	30	30
O_3 dosage	g	12	12	18	4,8	24

We reported that 80% bacteria in the soil were eliminated by 20 min ozone treatment with 20 gO_3/m^3.

Figure 4 shows the recovery of pH value of ozone treated soil. The agricultural soil with initial pH=5.19 was treated by 100 gO_3/m^3 during 30 min at 1 liter/min. Total ozone weight of 3 g was supplied to the soil of 150 g (ozone density in the soil is 0.02 gO_3/g of soil). After the treatment, pH quickly fell down to pH=2 and gradually increased and recovered toward initial state.

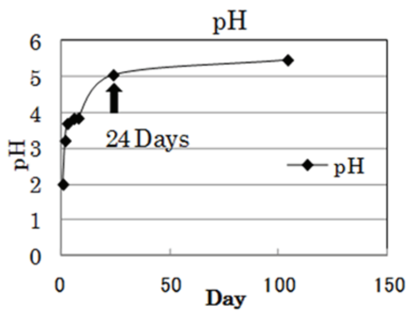

Fig.4. The recovery of pH value of ozone treated soil

We planted young seedlings 24 days after ozone treatment (arrow in figure 4). The stem height was measured. The result shows that the plant growth in ozone treated soil was suppressed (fig.5).

Major elements essential for plant growth are N, P, K, Ca, Mg and S. Plants take up nutrients from soil either as cations or anions. Many of the nutrients elements are cations including $NH4^+$, Ca^{++}, Mg^{++}, K^+, Fe^{++}, Mn^{++}, Zn^{++}, Cu^{++}. Other important cations are H^+ and Al^{+++} that lower soil's pH.

Cations are attracted to negatively charged surface of clays and organic particles (colloids). These cations held highly on absorption sites (clay and colloids) can move into soil's water solution, where they are available for roots' uptake. Cations are in balance with each other because an excess of one of cations can suppress the uptake of another. When ozone reacts with soil, the accompanying breakdown of the cation-balance occurs and highly unstable and complex processes take place resulting in lowering pH value due to increase of H^+ ions or decrease of OH^- ions. Lower pH value of soil decreases the availability of nutrient elements for plant uptake.

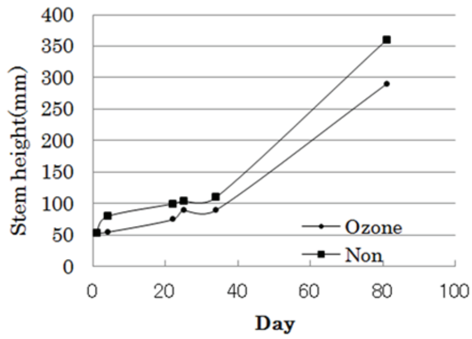

Fig.5. Plant growth of Chinese cabbage in ozone treated soil and non-treated soil. Treatment conditions are same as in figure 4.

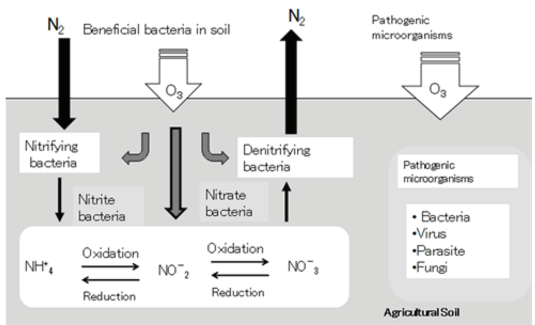

Fig.6. Simplified nitrogen cycle in soil and ozone soil sterilization

Ozone treatment has a critical influence on the nitrogen cycle in the soil. Nitrogen is present in variety of chemical forms including organic nitrogen, ammonium (NH_4^+), nitrate (NO_3^-) and nitrogen gas (N_2). Nitrogen cycle is the transformation which is carried out via both biological and non-biological processes. Important processes in the nitrogen cycle include nitrogen fixation ($N_2 \rightarrow NH_4^+$), mineralization (organic $N \rightarrow NH_4^+$), nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$), and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). Figure 6 shows a simplified diagram of nitrogen cycle in soil and ozone soil sterilization.

The nitrogen-fixing bacteria are able to combine gaseous nitrogen with hydrogen to produce ammonia. Nitrifying bacteria perform the conversion of ammonium (NH_4^+) to nitrate (NO_3^-). The primary state of nitrification is oxidation of ammonium by nitrite bacteria, which converts ammonia to nitrite (NO_2^-) and, then, nitrate bacteria species play the main role in oxidation of the nitrites into nitrates. Denitrification, which is conversion of nitrates (NO_3^-) to nitrogen (N_2) is performed by denitrifying bacteria species and then the nitrogen cycle is completed.

These bacteria in the nitrogen cycle are valuable and important microbes to maintain fertile state of agricultural soil.

Purpose of soil sterilization is to kill the pathogenic microorganisms such as bacteria, viruses, fungi and nematodes. Unfortunately, ozone soil sterilization eliminates or inactivates the beneficial microbes including nitrogen-fixing bacteria, nitrifying bacteria, nitrificating bacteria and denitrifiying bacteria as well. Our previous experiment showed that ozone treatment of conditions (20 gO_3/m^3, 20 min, 1 liter/min) decreased bacteria CFU/cc from $3.8 \cdot 10^7$ to $8.5 \cdot 10^6$ and fungi from $1.8 \cdot 10^5$ to $2.7 \cdot 10^4$ (CFU-colony forming unit). The measurement also showed that ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) increased by 16 times and 2 times, respectively. These results suggest that nitrifying bacteria and denitrifying bacteria were inactivated by ozone treatment. Excess nitrogen also causes injuries in plants.

The biological influence of ozone containing gas treatment on bacteria and viruses was investigated.

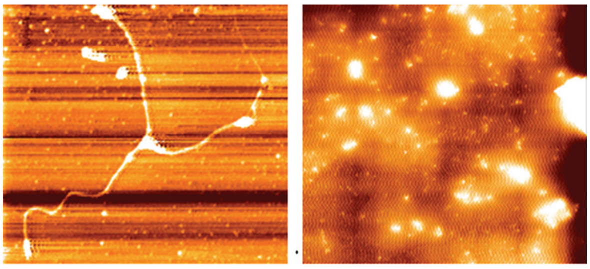

Fig.7. AFM images of DNA and antibody before (A) and after the treatment (B)

The reaction of DNA with ozone is essential to understand the sterilization in agricultural soil. A typical bacterium has a rigid cell wall containing a cell membrane and within the membrane, chromosomes made up of DNA. A virus consists of nucleic acid (DNA or RNA) surrounded by a protein coat called capsid.

We showed that λ-*E.Coli* DNA was destructed by the 5-20 min ozone treatment of 5% wt. ozone [10]. Figure 7 shows AFM image of DNA (white thread) conjugated with IgG antibody (white spot). The binding of antibody to DNA provides insight into high-sensitive medical diagnostics to identify infectious pathogenic organisms. Figure 7 indicates that the DNA structures are broken by ozone treatment (100 gO_3, 0.5 liter/min, 20 min). The DNA molecule is composed by two anti-parallel strands forming double helix and held together by hydrogen bonds between complementary base pairs. A hydrogen bond of DNA results from a dipole-force with a hydrogen atom bonded to nitrogen, oxygen or fluorine. The present ozone treatment for DNA is considered to break hydrogen bonding of nucleobases (thymine–adenine pair, cytosine-guanine pair) of the DNA.

An outbreak of the highly contagious foot–and–mouth (FMD) disease occurred and affected cattle, pigs, goats and other cloven-hoofed animals. The FMD virus comprises an icosahedrally symmetric protein capsid containing a single-strand positive polarity-RNA. Avian influenza (also known as bird flu or H5N1) is a highly infectious disease carried by birds. With the structure of single stranded RNA.

Results obtained in the ozone sterilization experiment as well as fundamentals of DNA destruction by ozone can help to develop biomedical technologies including sterilization and prevention against pathogenic microorganisms and infectious diseases at disaster areas. Recently Japan suffered great disasters: earthquakes, tsunami, floods, accidents of nuclear power stations. These disasters resulted in shortage of food, water, shelter, medicine and fuel for survivors. Many shelters were not sufficiently equipped. Urgent actions for recovery and reconstruction from these disasters including air purification and disinfection, emergency medical treatment, purification of drinking water, soil salinization, tsunami debris and radioactive waste materials decontamination should be promoted.

Conclusion

Ozone large scale agricultural sterilization system based on dielectric barrier discharge and consisting of multichannel injectors was proposed. Ozone DNA treatment was performed. Effect of ozone treatment on the soil was evaluated by pH measurement, bacteria survivability and plant growth. Nitrogen cycle in the soil was studied. Beneficial bacteria in the nitrogen cycle were inactivated by ozone treatment as well as pathogenic microorganisms. Suppression of plant growth in the ozone treated soil was due to low pH state based on increase of H^+ ions or decrease of OH^- ions.

These results can help to develop decontamination technologies for urgent medical and sanitary treatment at disaster area.

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