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On-site ozone treatment for agricultural soil and related applications

Abstract. *In this paper, ozone sterilization of agricultural soil, ozone treatment of water and nano-scale biological phenomena of DNA genome treated by gas-phase high ozone concentration are reported. High concentration ozone generation system was developed using a pulsed electric power from the high frequency oscillator operating at 1kHz-30kHz, pulsed rise time of 5μs, and Vp-p 15kV. Ozone concentration over 100 g/m^3 with energy efficiency of 50 g/kWh was obtained. The multi-channel electrode system for the dielectric barrier discharge was developed to inject the gas-phase ozone into the agricultural soil. The chemical and biological properties of the soil were studied and the plant growth experiments for several popular vegetables were tried.*

Streszczenie. *Praca przedstawia wyniki dotyczące sterylizacji gleb uprawnych, oczyszczania wody oraz wpływu wysokich stężeń ozonu na bakteryjne DNA. Dla potrzeb stanowiska badawczo-pomiarowego zainstalowano ozonator wykorzystujący zasilacz impulsowy o wysokiej częstotliwości. Otrzymano ponad 100 g/m^3 ozonu przy wydajności energetycznej 50 g/kWh. Gaz roboczy był wprowadzony do gleby przy użyciu systemu wielokanałowych elektrod. Zbadano fizykochemiczne własności gleby oraz wpływ ozonu na wzrost popularnych gatunków warzyw. (**Miejscowa obróbka gleb uprawnych ozonem i pokrewne zastosowania).***

Keywords: ozone, soil properties, soil sterilization.
Słowa kluczowe: ozon, własności gleby, sterylizacja gleby.

1. Introduction

Development of new technologies is required to solve global environmental issues including worldwide-spreading infectious diseases caused by pathogenic microorganisms, such as bacteria, viruses, parasite or fungi (swine influenza, foot-and–mouth disease, etc), air/water/spoil pollution, environmental degradation and the destruction of ecosystems. Application of ozone (O_3) to air, water, soil and organic matter is the most promising environmental technology for decontamination and destruction of bacteria, viruses, fungi and mold. Ozone possesses unique properties, which can be used in agriculture, food processing, medicine, semiconductor industry, etc [1-6]. In certain conditions, this strong oxidant can destroy all known pathogens as well as eliminate odors.

When ozone comes into contact with bacteria or a virus, direct reaction with molecular ozone or indirect reaction with the radical species formed by ozone decomposition results in the disruption of the cell membrane of bacteria, the protein structure of virus capsid or nucleic acid of a virus.
We have developed the gaseous ozone sterilization system for agricultural soil which ensures a secure agricultural production [1, 4, 7-14].

The infectious diseases in human population lead to disability (or even death), and social and economic disruption in the contaminated area. Foot-and-mouth disease (FMD) is a typical contagious disease of even-toed animals (cattle, swine, sheep, goats) caused by a virus that is quite difficult to control with existing vaccines and results in the high loses in farm animal population.

The FMD virus belongs to the picornavirus family and comprises an icosahedrally symmetric protein capsid containing a single-strand positive polarity -RNA genome.

Considering the present status of infectious diseases, the biological effect of gas-phase ozone on the DNA genome contained in bacteria and viruses was studied. We observed that highly concentrated ozone could react with DNA causing its fragmentation [13, 14].

The soil suitable to agriculture should consist of 40% of solids, 30 % of liquids and 30% of gases. Special attention should be paid to the effect of ozonation on the liquid (water). When ozone reacts with water, highly unstable and rapid decomposition processes take place.
The elementary reactions are as follows:

(1) $OH^- + O_3 \rightarrow HO_2 + O_2^-$
(2) $HO_2 \rightarrow H^+ + O_2^-$
(3) $O_2^- + O_3 \rightarrow O_2 + O_3^-$
(4) $O_3^- + H^+ \rightarrow HO_3$
(5) $HO_3 \rightarrow O_2 + OH\cdot$
(6) $O_3 + OH\cdot \rightarrow HO_2 + O_2$

The pH value defined by $pH = -\log_{10}[H^+]$ is dependent on hydrogen ion $[H^+]$ or hydroxide ion $[OH^-]$. A half-life time of ozone in aqueous solution was measured to be in the range from 2 min to 165 min depending on the conditions such as temperature, pH value and gas dynamics [9,15]. We measured the pH value of the drinking water and purified distilled water, which were treated by ozone. The results showed that the contamination (for instance minerals in water) significantly effects on pH value.

Additionally, nano-size biological phenomena occurring in DNA strands during ozonation were visualized. The reaction of ozone with DNA is essential to understand the sterilization in agricultural soil. The structure images of the DNA samples were observed by non-contacting mode measurement with the atomic force microscopy (AFM). It was shown that highly concentrated ozone broke hydrogen bonding of nuclei bases of DNA in a very short time.

2. Experimental procedures

2. 1 Ozone sterilization of agricultural soil

Our system of ozone sterilization was composed of ozone generation apparatus, ozone injection system, and soil properties measurement system. The electric power circuit of the ozone generator and ozone injection system are presented in Fig.1 and Fig.2, respectively.

The dielectric barrier discharge (DBD) in the coaxial type geometry was developed to produce highly concentrated ozone to achieve the ability of sterilization of bacteria, virus and nematodes in the soil. A pulsed electric power was generated by the high frequency oscillator which operated at the continuous mode and the interval mode (frequency range: 1kHz-30kHz, pulse rise time: 5μs, Vp-p:15kV, 2kW and 4kW, PHF-2K,4K, HAIDEN). The dielectric barrier discharge was formed between a screw-type cylindrical electrode (outer diameter - 10mm) and the gold electrode coated on the quartz tube (inner diameter 12mm, axial length of 250mm). Electrode was water-cooled in order to suppress the dissociation of ozone. The multi-

channel electrode system consisting of the 10 sub-electrode elements could produce large volume of highly concentrated ozone. Oxygen gas was introduced to the electrodes at various flow rates. Generated ozone (over 20g/m^3) was directly injected into the agricultural soil (Kuroboku) at a depth of about 70 mm~150 mm.

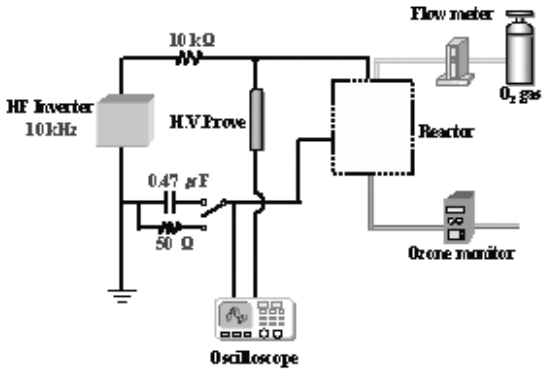

Fig.1. Electric power circuit for ozone generator.

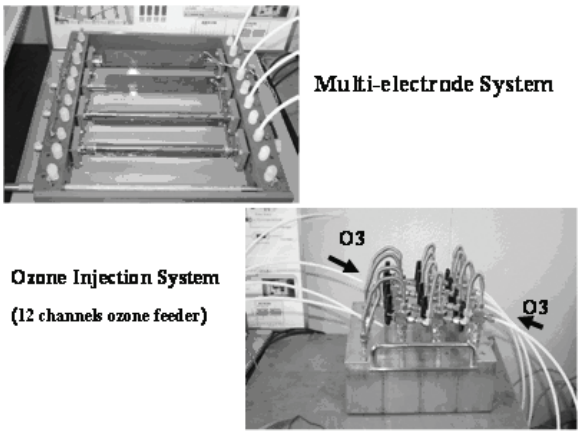

Fig.2. Multi-electrode ozone injection system.

In-situ measurements of the pH and the electrical conductivity of the soil were carried out using pH/Nitricion and pF/EC meters (PRN-41 and PFC-42, Fujiwara Scientific Co.,LTD) during and after ozone treatment.

2.2. Plant growth observation

Ozone sterilization experiments were carried out in the green house and at the outdoor field. The Kuroboku soil was filled in the drain bed container (1200x900x500mm). Gaseous oxidant was injected into the soil at half area of the drain bed. Temperatures of ambient air and soil surface, humidity, insolation, solar energy spectrum, and wind velocity were measured and automatically recorded. Time resolved meteorological data and plant pictures were remotely sent to the main computer server.

2.3. Ozone treatment of water

Ozone was bubbled into the glass beaker containing drinking water or purified distilled water. In this experiment, ozone of 5% wt. concentration was generated by the ozonizer (OP-20W,Max.100g/m^3, Iwasaki) using the dielectric ceramic surface discharge. The time change of the pH value was measured during the treatment. We also studied the effect of minerals in the ozonated water.

2.4. DNA collapse due to ozone treatment

Basic experiment to understand biological phenomena attributed to ozone sterilization was performed (Fig. 3). DNA used here was Lambda DNA (Nippon Gene) λ-E.Coli of 0.46μg/μliter concentration diluted with 10mM Tris-HCL(pH7.9) and 1.0M EDTA. The molecular weight of 3.15×10^7 Daltons (48,502 bp) corresponds to about 16μm (1bp~0.34nm) length. DNA solution was diluted with 0.5 milliliter of distilled water in a micro centrifuge tube. 0.5 liter /min of oxygen containing 5% wt. of ozone was introduced into the micro centrifuge tube containing mentioned DNA solution. Approximately, 0.2-1 gram O_3 was supplied to 0.5 milliliter of DNA solution during 5-20 min of the treatment.

DNA samples were prepared on the mica and glass substrates before and after ozone treatment. The DNA solution of 10μliter was dropped on the substrate and was dried in the chamber at reduced pressure and at the room temperature.

Nanosize structures of the DNA samples were observed by non-contacting mode measurement of the atomic force microscopy (SPI3800N, resolution: 0.2nm XY, 0.01nm Z, Seiko Instruments).

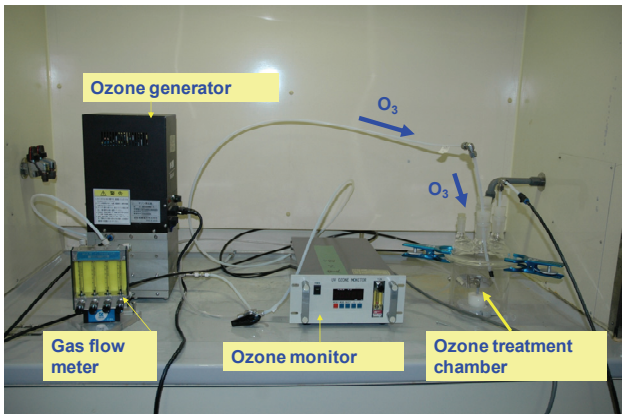

Fig.3. Ozone treatment system for DNA.

3. Results and discussion

Agricultural soil is a complex substance consisting of mud, sand, organics, minerals, water and some kinds of gases. The reaction of the soil components with ozone causes complicated and rapid change in physical, chemical and biological properties.

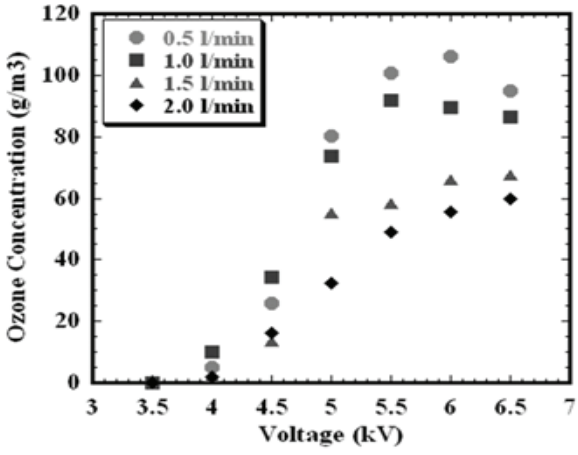

Fig.4. Ozone concentration as a function of applied voltage.

Ozone concentration as a function of applied voltage is depicted in Fig. 4. The maximum concentration of 106 g/m^3 was obtained at 0.5 literO_2/min (generation efficiency

20gO_3/kWh). The ozone generation efficiency showed a maximum value of 55gO_3/kWh at 50-60 gO_3/m^3.
Ozone diffusion dynamics in the soil was studied to establish the proper way of injection pipes arrangement in the agricultural soil using colored silica gel. 1 gO_3/m^3 ozone concentration could diffuse and decolorize silica gel to a diameter of 80mm during 60min [8, 10]. Thus, pH and temperature sensors were placed 30 mm away from the outlet of the injection pipe embedded in the soil at 150mm depth.

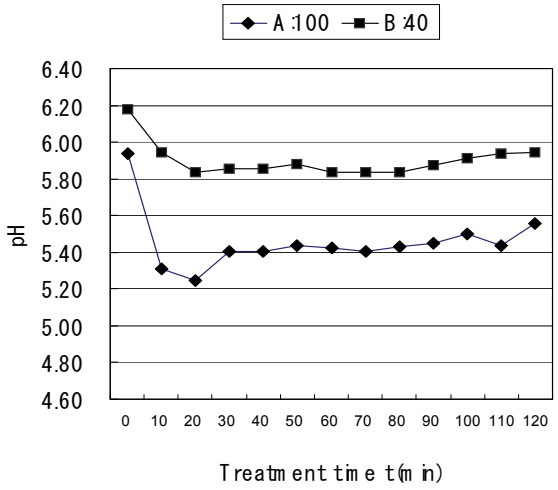

Fig.5. pH value as a function of treatment time(t). A:100g/m^3; B:40g/m^3.

Fig.5 shows typical characteristics of the pH value as a function of treatment time (t). Ozone was supplied at concentration of 100 gO_3/m^3 (A curve) and 40gO_3/m^3 (B curve) during 60 min. The pH curve for the 100g/m3 concentration indicated abrupt decrease in the initial phase till 20min and recovered to pH~5.95. Using the definition of pH value and the reaction equation (1)-(6), first order kinetics with respect to both ozone and hydroxide ion [OH^-] is given by the equation (7).

$$pH=-c\cdot Kw\cdot k[O_3]t + pH_0 \quad (7)$$

where c is constant and pH_0 is the pH before ozone treatment (t=0sec). pH value is inversely proportional to treatment time (t) at a given ozone concentration.

The experimental result of initial drop of pH in Fig.5 could be explained by the reaction of ozone with water in the soil. The curve B indicates that ozone treatment at lower concentrations gives only small change of pH value.
Sterilization conditions were studied using a conventional method of the CFU (colony forming unit) counting (Table 1). Fusarium oxysporum was almost eliminated by ozone with 20 gO_3/m^3. Table 1 shows that 80% bacteria were inactivated after 20min of the treatment (20gO_3).

The influence of ozone soil treatment on the plant growth of several vegetables was reported [13, 14].

Table 1. Ozone sterilization conditions of agricultural soil.

	Bacteria	*Fusarium oxysporum*	
Untreated (CFU/cm^3)	$1.8x10^5$	$5.7x10^6$	
Gas flow rate	1 liter/min	3 liter/min	
Concentration	20 g/m^3	10 g/m^3	20g/m^3
Duration	20 min	10 min	10 min
O3 treated (CFU/cm3)	$2.7x10^4$	$1.4x10^5$	$1.7x10^2$
Sterilization rate	86%	97.5%	99.9%

CFU: colony forming unit

Results of plant growth after the 20 min. of soil decontamination with 100gO_3/m^3 are depicted in Fig. 6. The pH value of the ozone treated soil changed after the procedure as presented in Fig.7.

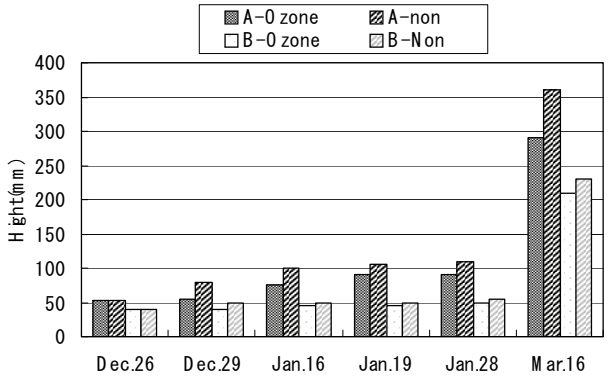

Fig.6. Plant growth experiment.

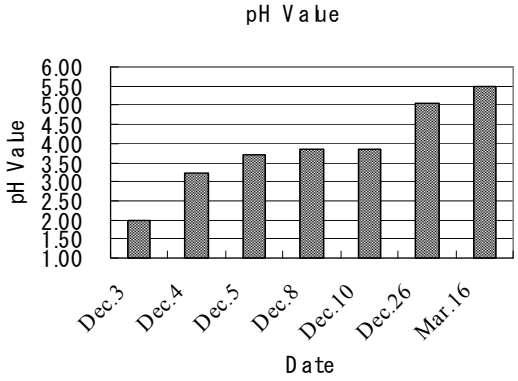

Fig.7. pH value in the plant growth experiment.

Young seedlings of Chinese cabbage and crown daisy were planted in separated pots with ozone treated soil (after 23 days, when pH value recovered to pH=5) and non-treated soil. Growth of the plants was strongly influenced by the ozone treatment. In the case of cabbage, it was suppressed by 24% after 79 days in treated soil due to low pH value and parallel elimination of microorganisms useful for soil enrichment.

It is desirable for agricultural soil to have about 45% soil mineral particles, 5% of organic matter, 20-30% of water and 20-30% of air. Water in the soil is essential for plant growth as a carrier of minerals, carbon, hydrogen nitrogen, oxygen, etc. The effect of gaseous ozone treatment on water was studied. Three samples of water were treated by 5% wt. ozone as shown in Table 2.

Table 2. pH value of distilled and drinking water treated by ozone.

Samples	Before treatment(pH)	After treatment(pH)	Conditions
Purified distilled water(A)	7.00	6.2	100 gO_3/m^3, 1 L/min, 7min,200cc
Exposed distilled water(B)	5.84	4.91	100 gO_3/m^3, 1 L/min, 30min,100cc
Drinking water(C)	7.18	8.05	100 gO_3/m^3, 1 L/min, 10min, 100cc

A: purified distilled water,
B: distilled water exposed in air,
C: drinking water

Distilled water (A) had initial pH value of 7.00 (15Mohm-cm), however, its exposure to air for long time caused acidification (pH=5.27) due to CO_2 diffusion. Drinking water (pH=7.18) contained mineral elements such as Ca, Mg, S, K and Na. The results presented in Table 2 show that the pH value decreased for two samples of the distilled water, and on the contrary, it increased for the drinking water. It can be explained by the fact that the formation of H^+ ions (or decomposition of OH^- ions) in the distilled water and the reaction with minerals in the drinking water were dominant in the ozone treatment process.

Fig.8 presents pH value of the distilled ozonated water (B) when soil was gradually added. The 50 g water sample of initial pH=4.91 showed abrupt change of pH caused by addition of saturated soil of pH~7.

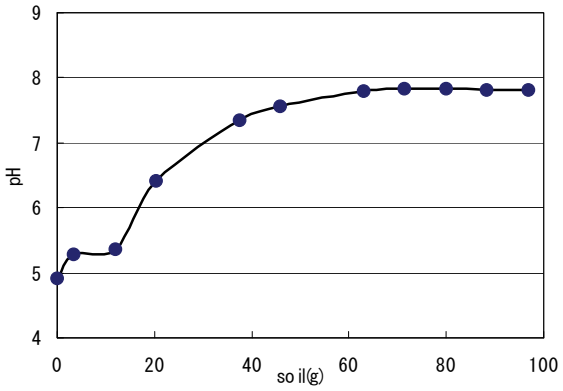

Fig.8. Change of pH value of distilled water by adding soil.

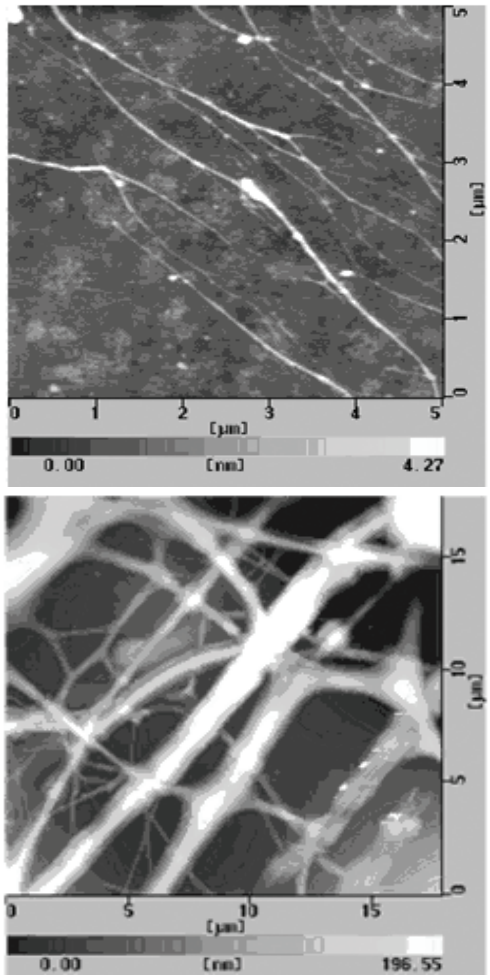

Fig.9. AFM images of DNA prepared on mica (upper) and glass (lower) substrates.

The soil sterilization aims at destroying or eliminating microorganisms without significantly altering the chemical and physical characteristics of the agricultural soil. Bacteria are microscopically small, single cell creatures of primitive structure, which is sealed by solid-cell membrane, which can be broken by the sufficient amount of ozone. In the case of viruses, for example, the foot-and-mouth disease virus, ozone diffuses through the protein coat (capsid) into the nucleic acid core, leading to the damage or destruction of the RNA. RNA (Ribonucleic acid) is very similar to DNA and usually single-stranded. RNA nucleotides contain ribose and have the base uracil (U) rather than thymine(T).

The DNA molecule is composed by two anti-parallel strands forming double helix and held together by hydrogen bonds between complementary base pairs. Gaseous ozone DNA treatment was chosen because the reaction of ozone with DNA is essential to understand the sterilization in agricultural soil. DNA samples prepared at various conditions were analyzed by AFM. Fig 9 depicts the images of DNA samples deposited on glass (lower) and mica (upper) substrates. It is obvious that there are many kinds of morphological structures depending on the location and localized conditions such as DNA condensation.

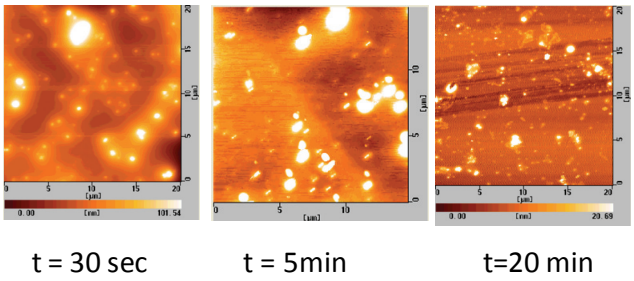

Fig.10. AMF images of DNA collapse at various treatment time.

The fine threads were considered to be double strand DNA and the bundle structures of the heavy threads were composed of high amount of fine DNA threads with the surface coated by diluted material remnant. The fine threads and the bundles were connected together to form mesh type structures.

AFM images of treated DNA after the different treatment times are presented in Fig. 10. Many remains of collapsed DNA could be observed in dependence of treatment time. It seemed that for the 30 sec treatment, week interactions between the fragments were still maintained. The DNA structures collapsed completely after 5min and 20 min treatment time.

A hydrogen bond of DNA results from a dipole-force with a hydrogen atom bonded to nitrogen, oxygen or fluorine. The thymine – adenine pair interacts through two hydrogen bonds and the cytosine-guanine pair has three hydrogen bonds. Highly concentrated ozone treatment could break hydrogen bonding of nucleobases of DNA in a very short time. There is the report that thymine and guanine have high ozone reaction rate constant due to OH radical formation [15].

4. Conclusion

Ozone sterilization system for agricultural soil and related applications was developed. Changes in bio-chemical properties caused by ozone injection were studied.

(1) Our sterilization system provided high ozone concentration of $100gO_3/m^3$ that is beyond the sterilization threshold value of $20gO_3/m^3$.

(2) The field trials for plant growth proved that the proposed system is effective to sterilize the agricultural soil.

The growth suppression in plant growth was considered to be due to low pH value and elimination of microorganisms.

(3) In-situ measurement of pH value during ozone treatment showed that the pH linearly decreased with time at the initial process phase. The phenomena was related to the formation of H^+ ions (or decomposition of OH^- ions).

(4) The AFM measurement revealed that the DNA was destructed after 5-20min treatment by 5%wt. ozone.

The ozone sterilization technology developed for soil and related applications will be useful to practical prevention of infectious diseases and food safety.

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