Sterilization Techniques for Soil Remediation and Agriculture Based on Ozone and AOP

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Abstract:
The sterilizing and remediation effects of the non-equilibrium atmospheric pressure plasmas are known for decades. Low temperature atmospheric pressure plasmas are considered as a promising alternative to conventional sterilizing methods, which have numerous drawbacks. Influence of various parameters such as discharge regimes, reactor geometries, gases, etc., on formation and effectiveness of plasma were investigated by many research groups. This paper presents the review of recently developed, environmentally safe technologies applied for the agriculture and soil remediation. The results of ozone soil sterilization and ozone influence on the DNA structure of Escherichia coli are described. The results of oxidants' influence on humic acid are presented.

Introduction

The idea of plasma sterilization was already proposed in 60-ties (1) as a good, low toxicity method for environment and operating staff. In spite of fact that the number of research papers and devices related to this topic is constantly increasing, most of the solutions were not fully implemented, mostly because of the lack of system optimization, lack of comparability between the proposed reactors and methods, lack of matching between plasma properties and sterilized material, and because of the incomplete sterilization caused by the small penetration range. Therefore, in spite of existing commercial plasma sterilization devices (2) industrial plasma-based decontamination is still a great challenge.

Multiple methods of microorganisms inactivation using oxidative species have been developed for agricultural applications. Direct application of ozone within soil might be especially advantageous in extreme cases such as soil remediation after bioterrorist attack. On the other hand it was observed that oxidative species might drastically change the properties and micro flora of soil, thus low and moderate doses of ozone can be used for customization of soil properties for various kinds of crops. The growth and fruit formation process for some plants (such as tomatoes) was enhanced using ozone treatment process (3).

Additionally, ozone technology plays an important role in mass scale long time food preservation. Ozone is widely used in common refrigerators and as a deodorizing and antimicrobial agent.

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Ozone and oxidative species inactivate pathogens via:

- direct destruction, volatization and etching of the cells,
- decreasing of the biofilm adhesivity by the decomposition of polymer matrix,
- oxidative stress (due to the fact of formation various active agents (ozone, OH and O radicals, hydrogen peroxide, etc. during the electrical discharge),
- nitrogen stress (recent research results suggest cells' damage from reactive nitrogen intermediates as nitric oxide, peroxynitrite, nitrous acid, nitrogen trioxide, etc.(4)).

In the paper review of ozone involved sterilization technologies is presented emphasizing the soil sterilization possibilities.

General Conditions of Soil Decontamination

Pollutants might be distributed in soil in several ways (5):

- in soil matrix,
- in vapor phase,
- in non-aqueous phase,
- in groundwater.

Soil remediation is especially difficult procedure because it must proceed on site and cover relatively large surface area with various geological settings while keeping also penetration deepness.

The processing of soil depends on several factors including:

observed to be independent of the ozone gas pore velocity, indicating that the overall reaction rate is dominated by chemical reactions rather than by interfacial mass transfer (23).

To achieve complete purification, combining ozone remediation process with bioremediation via occulation with Pseudomonas alcaligenes PA-10 seems to be very beneficial (24, 25). Miller et al., reported that that Fenton's treatment of pendimethalin contaminated soil created favorable conditions for microorganisms (Pseudomonas) desirable for bioremediation (30).

In highly cultivated areas, the soil and ground-water contamination with herbicides and pesticides causes severe health problems (bioacumulation) and decreases the amount of crops. Decomposition of trifluralin (carcinogene of herbicidal properties) and aniline (used in pesticides and agriculture industry) was studied by Pierpoint et al. (26). Rapid degradation of 77–98% of aniline exposed to 0.6% O_3 at 200 ml/min after 4 min in the moist conditions was observed. Initial ozonation products included nitrosobenzene and nitrobenzene, while further oxidation led to simple CO_2. Trifluralin removal rates were slower, requiring 30 min to achieve removals of 70–97%.

Moreover, 80% effectiveness of new generation of peroxygen chemicals (calcium or magnesium peroxide and persulphate with or without addition of H_2O_2) for the chlorophenols contaminated soil remediation at natural soil pH was demonstrated by Goi et al. (31).

Bactericidal Decontamination of Soils Using Ozone and Oxidant's Influence on Growing Plants

The anti-microbial properties of plasmas in the case of decontamination of water, ambient air and surfaces were previously widely proven (26-30, 32-34). The contamination of soils in the public grounds with various microorganisms including the parasite eggs coming from pets, wild animals and severs in both: developing and developed countries rises the public concern. The contamination of samples with ascarid eggs can reach up to 92% and it is estimated that 1.4-billion people are infected worldwide (35-37).

Mun et al. reported 0.13 log inactivation of quite ozone-recalcitrant Ascaris lumbricoides eggs in 25 g of soil with 5.8 ± 0.7 mg/l of dissolved ozone dose for 30 min in a continuous diffusion reactor (38). Moisan et. al reported that at reduced pressure (10 torr) the UV photons dominated the inactivation process associating with the DNA destruction of the spores (39). Choi et.al showed that the dielectric barrier discharges at atmospheric pressure sterilized E.coli with 99.99% efficiency and that ozone molecules were the dominant germicidal species (40).Various aspects of high concentration ozone soil treatment and its further influence on plant growth, pH, temperature, biological phenomena were analyzed in previous works (3, 41-44).

Influence of ozone in air and soil on seeds' and plants' development was broadly investigated by numerous researchers (45-51). Results highly depended on the kind of plant and were sometimes contradictory. Chronic exposure to ozone significantly reduced the rate of CO_2 assimilation in sugar maple (45). On the other hand, there was no treatment effect on the freezing tolerance of roots, even though roots in the high-O_3 treatment accumulated higher concentrations of the cryoprotective oligosaccharides raffinose and stachyose than control roots. Ozone (alone or in mixtures with CO_2 to maintain its concentration within soil) injected in previously oversaturated soil was reported to be beneficial for growth of tomatoes or some kinds fungi (such as Trichoderma, spp.), which are parasitic to detrimental fungi such as fusarium (46).

AOTs (ozone, hydrogen peroxide, electric field, UV radiation, etc.) were widely used for the treatment of seeds and bulbs (47-51) causing 15–20%, improvement of immunity of cotton plants to diseases and improvement of morphobiological and technological parameters of cotton fiber (47); 12-15% higher grain yield with better quality of corn grain (49); over 60% improvement in bean seeds germination (50).

Experimental Apparatus

Research was performed using independently working set-ups.

The soil sterilization system using high concentration of ozone generated by dielectric barrier discharges was described in (41-43, 52, 53). The gaseous ozone injection field-scale system is presented in Figure 1(A-C). It consisted of 10 electrodes 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.

Figure 1(D) depicts next stage of research: the laboratory-scale set-up used for DNA treatment. Atomic Force Microscopy (AFM) was employed to investigate the biological effect of ozone treatment on the DNA (54).

0.46 μg/μl of λ-E.Coli DNA (Nippon Gene) was diluted with 10 mM Tris-HCL (pH 7.9) and 1.0 M EDTA. The DNA solution was further diluted with 0.5 ml distilled water in a microcentrifuge tube. A stream of oxygen containing 5% wt. ozone was bubbled into

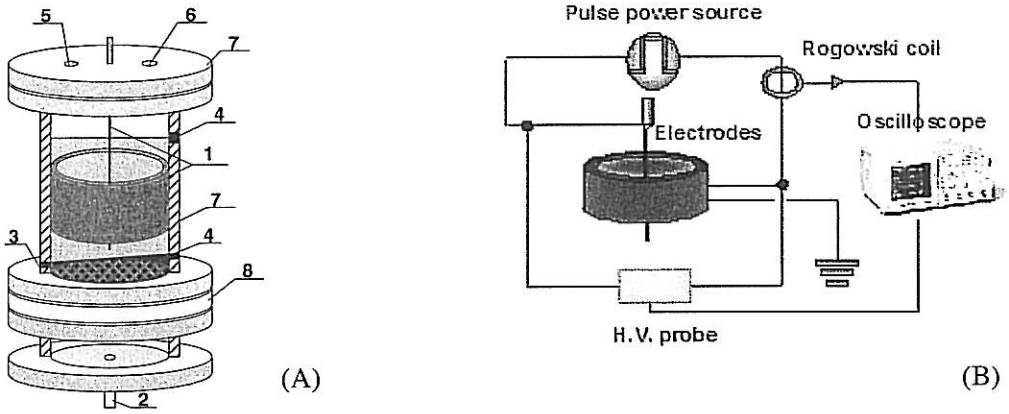

Figure 2. Cylindrical foaming column. 1- electrodes, 2- gas inlet, 3- water inlet, 4- overflow, 5- gas outlet, 6- sampling point, 7- polyacrylate housing, 8- ceramic diffuser (A). Schemata of electrical pulse power source (B).

Table 1. Soil sterilization by in-situ ozone treatment.

	Bacteria	Fusarium Oxysporum	
Untreated [cfu/cc]	1.8×10^5	5.7×10^6	
Gas flow rate	1 lit/min	3 lit/min	
Concentration	20 g/m^3	10 g/m^3	20 g/m^3
Duration	20 min	10 min	10 min
Ozone treated [cfu/cc]	2.7×10^4	1.4×10^5	1.7×10^2
Sterilization rate	86 %	97.5 %	99.9 %

Table 2. Soil temperature, EC and pH in the process of ozone treatment.

Stage	Temperature	EC	pH
Initial	26°C	48 mS/m	5.7
t=20min	63°C	65 mS/m	4.9
Final	32°C	82 mS/m	5.2

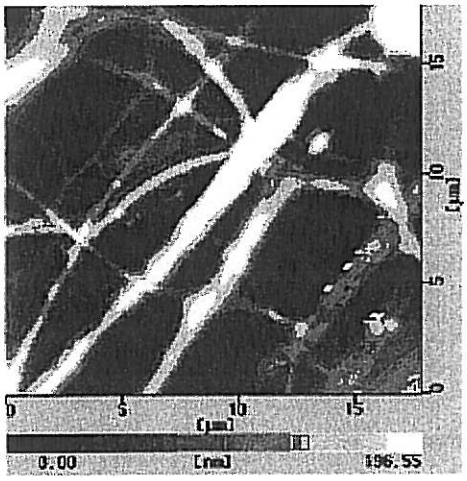

Figure 3. AFM image of DNA sample prepared on mica substrate.

acids originating from the oxidation of the soil organic matter.

The ozonation enhanced the dissolution of hydrophilic fraction of organic matter into the soil's water phase. According to Ohlenbusch et al. the decrease of the humic acid fraction and a reduction of its average molecular size could be recognized. In contrast to this, there was an increase of the building block fraction and the low molecular acid fraction (what implies possibility of easy biodegradation, if soil would be post-occulated) (59). Non-linear pH changes could be associated with consequent changes and oxidation of compounds containing nitrogen (60-62).

Samples prepared at various treatment time were analyzed by AFM. Figure 3 shows the image of DNA sample deposited on the mica substrate. It was found that there were many kinds of structures depending on the location and localized conditions (such as DNA condensation). The fine threads were considered to be double strand DNA and the bundle structures of the heavy threads were composed of multiple fine DNA threads with surface covered by diluted material remnant. The threads and the bundles are connected together to form mesh type structures. The molecular structure of DNA collapsed completely when high concentration of ozone was introduced into the DNA solution. Figure 4 shows the images of the DNA sample which was treated during 5 min. This image indicated that ozone process broke the E. coli DNA and split it into many fragments. The small rectangular pieces distributed on the surface were considered to be fragmented DNA (Figure 5). The typical length and width ranged 380-390 nm and 15 nm, respectively. These peculiar pieces have almost been not observed when the DNA samples were treated for 10 and 20 min.

Several possible paths of cell inactivation by oxidative species were presented in literature data (60, 63-73).

dosed solutions showed no significant change that is also confirmed by other literature data (59, 76-78).

Conclusions

Numerous research works prove that ozone and advanced oxidation processes can be successfully applied for the treatment of various fractions of soils. In this paper gaseous ozone sterilization of soil was studied in dependence on the treatment conditions.

The influence of ozone processing on soil properties and plant growth was investigated. 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.

Oxidizing of humic acid solution resulted in its decomposition while TOC showed no significant change. Humic acids play an important role in nutrient's transport in soil therefore further, complex investigation of AOP influence on soil conditioning is necessary.

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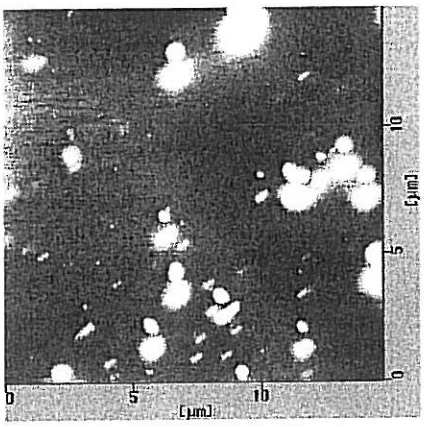

Figure 4. AFM image of O_3 treated DNA.

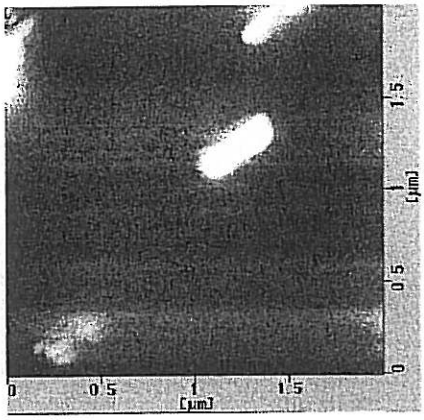

Figure 5. DNA fragments after O_3 process.

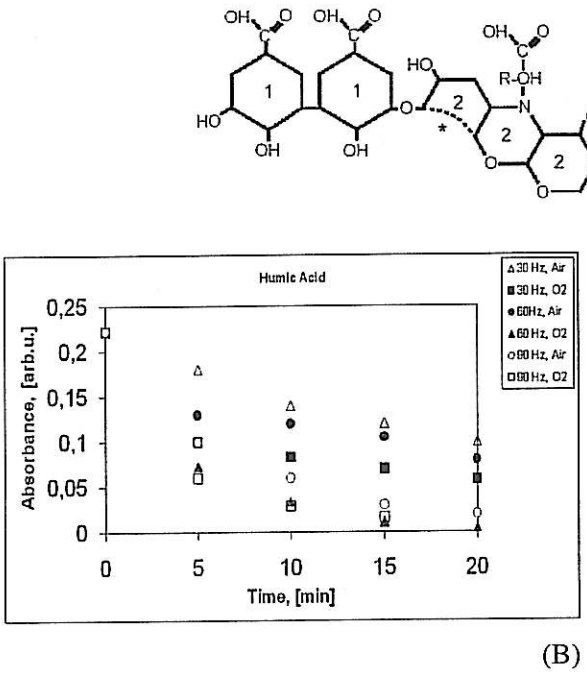

Figure 6. Humic acid: fragment of the molecular structure (A), the decomposition of HA in dependence on supplied gas and frequency (B).

Oxidation caused change in DNA structure (in all base and sugar moieties; resulting in strand breaks, DNA inter-strand cross-links and DNA-protein cross-links) as follows:

- change of dGMP, dCMP and dTMP bases, production of 8-oxoguanine, which leads to common gene mutations: G–A transversion (directly by ozone),
- DNA backbone cleavages, dAMP base and the deoxyribose ring destructions (indirectly, by secondary oxidants such as OH radicals or/and singlet oxygen).

Humic Acid under the Influence of Oxidative Species

Various active species as OH radicals, hydrogen peroxide (78 mg/l and 86 mg/l of H_2O_2 after 20 minutes of discharge operation at 90 Hz in foam formed with air and oxygen, respectively), dissolved ozone (2.01 mg/l (air) and 3.21 mg/l (oxygen)), and gaseous ozone were obtained during the discharge in foaming column (Figure 2).

Humic acids (HA, Figure 6(A)) are the organic compounds arising from the physical, chemical and microbiological transformation of biomolecules, which primarily can be found in manure, peat, lignite coal, and leonardite (74, 75). The HA act as the major buffering system, absorbent, and nutrient transporting medium.

The influence of combined oxidants produced during the quasi-arc discharge in foaming system on humic acid was investigated. 100 mg/l of HA suspended in 60 ml of pure water was dosed into the reactor. The characteristic UV absorption lines of HA (260 nm) were used to determine its concentration in the liquid using HACH spectrometer. After 20 minutes of the treatment the color of the solution became much weaker and the amount of suspended matter after the sedimentation significantly decreased (Figure 6(B)). The total organic carbon (TOC) content of ozone-

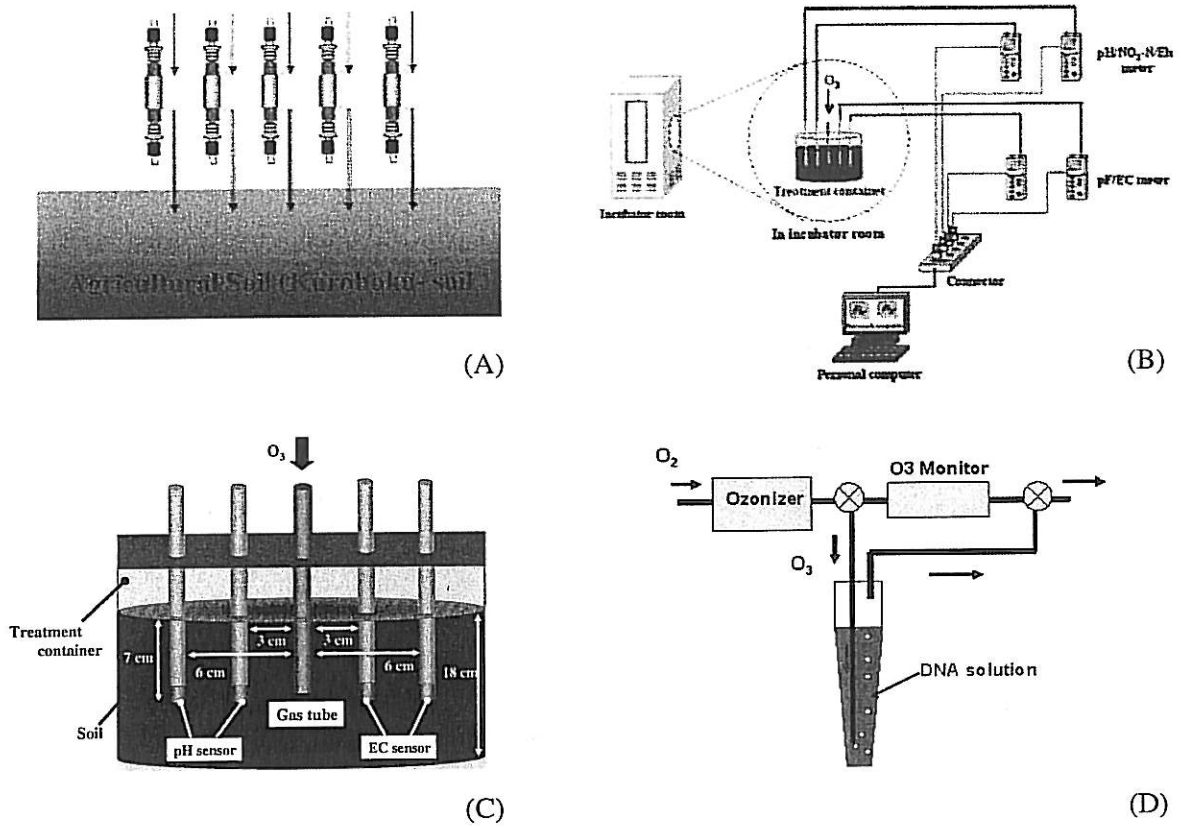

Figure 1. Experimental set-up for general soil treatment e.g. multi-electrode injection system (A), set-up overview (B), pH sensor and EC probe arrangement (C) and DNA treatment (D).

the microcentrifuge tube containing the DNA solution. 0.2-1 g of ozone of was supplied to 0.5 ml of DNA solution during 5-20 min of treatment at 0.5 l/min gas flow rate. In this case ozone was generated by the high-power TiO_2 based surface discharge ozonizer (OP-20W, Max.100g/m^3, Iwasaki (55) during the surface discharge.

Before and post-ozone treatment DNA samples were prepared on the mica substrates. The DNA solution of 10 µl was dropped on the mica substrate and was dried in the chamber at reduced pressure and room temperature.

Set up for investigation of oxidants on humic acid consisted of 50 mm diameter reactor shown in Figure 1(A). The central HV electrode of 1.5 mm diameter was placed inside the inner, grounded electrode (40 mm of diameter, 30 mm of height). The solution of humic acid was dosed to the reactor where foam was formed using gas flow flux through the ceramic diffuser. Quasi-arc electrical discharge occurred in foam. An electrical circuit is shown in Figure 1B. The pulse power source was operated at various frequencies. Discharge voltage and current were measured using the high voltage probe and the Rogowski coil, respectively with an oscilloscope (56, 57).

Results and Discussion

Influence of Ozonation on DNA

A conventional biological method of the CFU (colony forming unit) counting showed that bacteria and Fusarium oxysporum (fungus causing plants' diseases) in the soil were almost eliminated by ozone treatment with the concentration over 20 gO_3/m^3 achieving sterilization rate of 86% and 99.9%, respectively. The decontamination results are summarized in Table 1.

The reaction with high concentration ozone caused the remarkable change in physical and chemical properties of the soil.

Rapid temperature increase up to 70 °C observed in ozone treatment was considered to be due to the chemical exothermic reaction between the soil and the gas-phase reaction.

In-situ measurements showed the rapid decrease of the pH value and gradual increase of the electrical conductivity. The physicochemical properties of treated soil are presented in Table 2.

Obtained data were in a good accordance with literature data (58), where pH values of the soil extracts were found to decrease with longer ozonation times, and this was due to the formation of carboxylic

- type of soil (content of water, organic compounds, consistence, structure),
- type of pollutant,
- treatment technique,
- geological and atmospheric circumstances.

Many techniques of soil remediation such as heating, flushing with chemical additives (surfactants), irradiation, irradiation with catalyst, soil vapor extraction, landfilling, incineration, aeration, oxidation and bioremediation were tested alone or in combinations.

Oxidation techniques involved ozone, hydrogen peroxide, chlorine dioxide, and potassium permanganate. Trials employing usage of ozone alone or combined with Advanced Oxidation Processes (AOPs) were performed for the treatment of soil by international research groups (6). Because of its relatively good solubility in aqueous phase, ozone generated during electrical discharges seems to be especially potential for the soil treatment because it can be applied in both: gaseous and aqueous phase. Ozone reacts with pollutants directly or decomposes via catalytic reaction with other species (such as metal oxides) present in the environment to form the secondary oxidizing agents: omnipotent hydroxyl radicals (7), ozonide radicals, hydrogen peroxide, perhydroxyl, superoxide, and singlet oxygen (8-10). There are numerous works covering application of Fenton's Reagent to the soil treatment. Higher H_2O_2 concentration provided faster reaction times but less efficient oxidants use (11-13). Watts reported using of H_2O_2 for cleaning soils mentioning following main factors influencing efficiency:

- availability of iron (crystalline soils are easier to treat than amorphous ones)
- buffering capacity (Fenton reactions perform best at pH 2-4 and carbonate ions are usually strong free radical scavengers (retarding of reaction))
- natural humus content (competitive oxidative reactions between background and contaminant).

Organic compounds from badly shielded landfills, leaky storage-tanks, old gasoline stands, refineries and accidental spills are considered as a main source of soil pollution (14). However, one cannot omit presence of heavy metals and bactericidal pollution.

Removal of Organic Pollutants from Soil

The contaminated soil may contain variety of organic pollutants including simple hydrocarbons, alkanes (making up to 80% of diesel fuel), volatile organic compounds, chlorinated organic compounds, PAHS (Polycyclic Aromatic Hydrocarbons), BTEX (benzene, toluene, ethyl benzene, and xylenes) found in petroleum derivatives, pesticides and herbicides.

Ozone remediation process can be divided onto 2 phases (15):

- instantaneous ozone demand phase, when the rapid interactions with soil organic matter and metal oxides occur and most of pollutants removal process takes place,
- relatively slow decay stage.

AOT results in ring cleavage of poorly soluble aromatic compounds, and insertion of oxygen, which increases their water solubility, thereby facilitating their degradation in the natural environment (11).

Yu et al. reported 94% removal of diesel range organics (DRO) over 14 h of continuous ozone injection. Ozone oxidation demonstrated effective removal of non-volatile DRO in the range of C_{12}–C_{24}. Each alkane compound displayed comparable degradation kinetics. An estimated ozone demand was 32 mg O_3/mg DRO (16). The ozone treatment supported acetic acid flushing reduced the remediation time more than 29% in the case of trichloroethylene (17).

PAHs are a group of hydrophobic compounds that consist of two or more fused benzene rings. They are widely distributed in the environment, being present in coal tars, diesel fuel, oil and gasoline, as the by-products of incomplete combustion or pyrolysis. PAHs tend to be absorbed on organic fraction of solid (18) and are highly recalcitrant. Numbers of PAHs are catalogued as carcinogens by the international agencies (19). The extensive studies of PAHs treatment with ozone were reported by various researchers (20-29) concluding that ozone oxidation can be successfully applied to their degradation.

The decomposition goes via direct reaction with molecular ozone. It was found that soil's pH and moisture content impacted the effectiveness of PAHs oxidation in unsaturated soils. In air-dried soils, as pH increased, removal increased, such that pyrene removal efficiencies at pH 6 and pH 8 reached 95–97% at a dose of 2.22 mg O_3/mg pyrene (20). The removal of pyrene was slower in moisturized soils, with the efficiency decreasing as the moisture content increased. Greater than 95% removal of phenanthrene was achieved with an ozonation time of 2.3 h at an ozone flux of 250 mg/h. More hydrophobic PAHs tended to react more slowly than would be expected on the basis of their reactivity with ozone, suggesting that partitioning of the contaminant into soil organic matter may reduce the reactivity of the compound (21). Rivas et al. (22), reported 50, 70, 60 and 100% of conversion for acenaphthene, phenanthrene, anthracene and fluoranthene, respectively. In this case, the influence of the gas flow rate on the efficiency was not noticed. The rate of reaction for ozone with the soil matrix was