Abstract

PLASMA INDUCED MULTIGENERATION EFFECTS ON PLANT GROWTH AND CROP YIELD
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There is an urgent need to increase agricultural efficiency to resolve food crisis [1,2]. Can new technology play a part in helping us produce more food with less space? Plasma is such a new technology.

Direct plasma irradiation to plants normally suppresses plant growth because of deleterious effects of ROS and RNS provided by plasma, whereas plasma irradiation to plant seeds have various influences on growth of the plants depending on the irradiation duration or “plasma dose” [3-10]. Low dose plasma irradiation has no influences on plant growth. Middle dose plasma irradiation enhances plant growth by 10-150%. High dose plasma irradiation suppresses plant growth. Three questions arise. Do plants have memory of plasma irradiation in storage? Do plants have memory of plasma irradiation in their former generation? Are there any methods to enhance further plant growth? To answer these questions, here we report multigeneration study of effects of plasma irradiation to seeds. In the first generation, we compared plant growth using two kinds of seeds: seeds with and without plasma irradiation. Under appropriate irradiation conditions, growth rate, from their germination to growth saturation, for the plants with plasma irradiation is 1.25 times faster growth than that without plasma. The average period from the cultivation to the first seed production for plants with plasma is 6.6 days shorter than that without plasma. The plasma irradiation also improves crop yield [10]. The total weight of seeds and their number increase 56 % and 39 %, respectively. The seeds with plasma irradiation keep this growth enhancement ability more than a year! The irradiation speeds up the cell cycle so the plant and seeds grow faster overall, with reactive oxygen species playing a key role in the effects. Plants from plasma-irradiated seeds contain more glucose. This may bring about more tasty crops and higher yield of bioethanol. The gene oncology analysis has shown that the oxidative stress is an important kinetics for crop yield improvement. Moreover, the multi-generation experiments of plasma irradiation to seeds suggest that long-term memory of plasma irradiation is engraved in seeds the next generation with little gene mutation.

Last, but not least, researchers in this field should make effective domestic and international regulations on plasma agriculture on their full cooperation.

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DEVELOPMENT OF PLASMA SOURCES AND APPLICATIONS TO PLANT GROWTH, MICROBIAL ACTIVATION, AGRICULTURE AND FOOD TREATMENT

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Nonthermal biocompatible plasma (NBP) sources and their characteristics operating at atmospheric pressure have been introduced and overviewed for plant growth, microbial activation, agriculture and food treatment, especially used in Plasma Bioscience Research Center (PBRC), Korea. The electron temperatures and plasma densities are measured and characterized to be 1.1 ~ 2.3 eV and 1×10¹⁴ ~ 2×10¹⁵ cm⁻³ for the NBP sources, in which plasma jet, dielectric barrier discharge (DBD) plasmas, glass tube DBD plasma and high voltage nanosecond pulsed plasma are currently employed in PBRC. Herein, we have also introduced the applications of these NBP sources to plant growth, microbial activation, agriculture and food treatment.

High voltage nanosecond pulsed plasma and DBD plasma devices have been applied to spinach (Spinacia oleracea L.) and coriander (Coriander sativum). Germination and dry weight of seedlings were increased after high voltage pulse shots applied to spinach seeds. However seeds treated with many shots (10 shots) showed a decrease in germination rate and seedling growth. Spinach seeds treated with air DBD plasma exhibited slightly higher germination and subsequent seedling growth than those treated with N₂ plasma. Seed germination of coriander was shown to be more rapidly increased over time compared to control one after micro-DBD N₂ plasma treatment. Germination and seedling growth were also elevated by nitric oxide generated from microwave plasma torch, where we observed a threshold concentration of nitric oxide activating coriander development.

We applied the micro-DBD plasma to promote the ability of the effective microorganisms. It was shown that the growth activity of beneficial bacteria on rice was more promoted by air than N₂ plasma treatment. The levels of ROS and RNS from plasma, and transferred discharge energy were different from each other depending on the gases supplied to DBD plasma, which might work as critical factors in activating the microorganisms. Effective bacteria activated by plasma seem to be increased in the density and fixability within rice plant compared to untreated control. Attachment and colonization of effective bacteria after plasma treatment in rice plant will be analyzed in the future study.

This work was supported by a grant from the National Research Foundation of Korea (NRF-2010-0027963) funded by the Ministry of Science, ICT and Future Planning (MSIP) of Korean Government and R&D Program of ‘Plasma Advanced Technology for Agriculture and Food (Plasma Farming)’ through the National Fusion Research Institute of Korea (NFRI) funded by the Government. This work has been also partially supported by Korea Food Research Institute in 2015.
DECONTAMINATION AND PRESERVATION OF PERISHABLE FOOD WITH ATMOSPHERIC PRESSURE PLASMAS

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Contamination by microorganisms is a critical problem in many sectors of the food industry. Pathogenic microorganisms such as Escherichia coli (e.g. EHEC), listeria, salmonella, and mold and the spread of multi-resistant strains pose a substantial health risk to the consumer. 

Currently used disinfection or sanitation methods for fresh fruits and vegetables often lack antimicrobial effectiveness. Further they are high in costs, water consumption or use of chemicals. Non-thermal atmospheric pressure plasma could be one alternative to be applied. Different technologies such as jet-plasmas, dielectric barrier discharges or microwave plasmas etc. can be used for this purpose. 

Especially for fresh and fresh-cut produce with a limited shelf life of several days allowing only a regional distribution of that produce plasma could prolong the shelf life. At all stages in the value chain such as production, processing, transport, deployment and even before preparing, microbial contamination can occur leading to losses of fresh produce and therewith limiting the shelf life. Current losses of fresh produce have a remarkable economic impact on the food industry. 

The properties of plasma and its generated cocktail of physical and chemical compounds leads to a high microbial inactivation on various specimens and offers a wide range of possible applications. 

Non-thermal atmospheric pressure plasmas offer a low-cost possibility to inactivate microorganisms on fresh produce surfaces. Since plasma's microbicidal effects are caused by a multitude of components, it is effective against a broad spectrum of microorganisms and, as opposed to pure chemical and thermal processes, products are treated gently. They can be used in dry and wet environments and allow a treatment of fresh food and virtually every conceivable surface in batch or inline processes along the whole value chain of food production. Especially they can serve as an alternative to ozone or chlorine dioxide treatment. Accordingly developed and investigated plasma sources are in use in medical, pharmaceutical, and food industries for quick disinfection of materials and packaging.

The contribution intends to give an overview about own activities in the field of food sanitation by means of various plasma sources, setting the focus on microwave plasmas, which generate plasma processed air (PPA) or plasma processed water (PPW), containing manifold reactive nitrogen species-based chemical and antimicrobial compounds.


Civilization is founded on agriculture - the science and practice of cultivating crops and the rearing of animals to provide food, wool, and other products - remaining as important today as when it was born 10,000 years ago. Agriculture powers the economies of most developing countries and in industrialized countries, exports of agricultural products are worth billions of dollars annually. Since the middle of the 20th century agricultural productivity in developed countries has increased significantly (doubled in the U.S.), enabling farmers to feed more people with less land and labor. Economic analyses indicate that agricultural productivity (total output per unit of aggregate input) is driven by innovations in on-farm tasks, changes in the organization and structure of the farm sector, and research & development aimed at improvements in farm production. In the short-term, measured agricultural productivity can also be affected by random events like weather (i.e. climate change).

The development of novel technologies and applications like cold- or non-thermal plasma technologies (PT) promises suitable applications for agricultural commodities and products, thus enhancing further agricultural productivity. We contend that these technologies, once proven viable to large-scale agricultural scenarios, will likely be more economically suitable/justifiable to intensive rather than extensive agricultural crops and enterprises. High-value, intensively managed horticultural crops and products (fruit, vegetables, ornamentals, spices, etc.) could absorb the costs associated with the use and operation of PT compared to conventional agronomic crops (large cultivated areas with low profits/unit area), except in instances when yielding a value-added product (e.g. hybrid, certified seed lots).

There are already reports of PT applied to agricultural (fresh, minimally-processed and fresh-cut fruits and vegetables) and food products to disinfect/inactivate plant and human pathogenic microorganisms, and to promote seed germination. The relevance of these findings is quite significant considering that estimates of the total post-harvest losses of food grains and horticultural products approach 25% and 50%, respectively.

The treatment of large volumes of liquids, specifically flowing water, with plasma for disinfection purposes should prove to be a phenomenal solution for the treatment and reuse of agricultural drainage and runoff effluents from intensive horticultural operations (greenhouses, nurseries, hydroponic plant factories), water used in facilities processing fresh, minimally-processed and fresh-cut fruits and vegetables, and even municipal reclaimed water intended for irrigation of public parks and landscapes. Equally exciting are the reports that plasma treatment of water generates significant levels of nitrate-nitrogen without additional energy inputs, potentially representing a major contribution to the fertilization of nutrient-demanding hydroponic and greenhouse crops.

While PT offers the potential to address significant issues and needs in agriculture, promising further enhancement to its productivity and efficiency (and sustainability), we should not lose track of addressing the scientific and technological challenges to overcome for commercial application to farming operations.
GROWTH CONTROL OF BUDDING YEAST CELLS THROUGH ATOMIC OXYGEN DOSE
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Recently, atmospheric-pressure plasmas are expected to be employed on various applications in medical and agricultural fields, and so on. In the agricultural field, plasma are mainly applied to the sterilization of microorganisms or fungal spores and the enhancement of production. However, the each effect of species in the plasmas on microorganisms has not been clarified yet because various factors such as ultraviolet, electrons, ions, and neutral radicals exist in the plasmas. We have focused on neutral radicals in the plasmas.

To investigate the effects of neutral oxygen radicals on the inactivation of microorganisms, we quantitatively inactivated Penicillium digitatum spores using an oxygen radical source that supplies only neutral oxygen species on the basis of the measurement of the densities of O(3P), O2(1Δg), and O3 using vacuum ultraviolet absorption spectroscopy (VUVAS) and ultraviolet absorption spectroscopy (UVAS). We showed that the inactivation rate corresponds to the density of O(3P), but not that of O2(1Δg) or O3, and concluded that O(3P) is an effective species for inactivating P. digitatum spores. Moreover, we elucidated the multiple inactivation process that neutral oxygen radicals affected P. digitatum spores on the basis of O(3P) dose under the flux of 2.3×1017 cm−2 s−1. These results demonstrate the importance of discussing the effects of individual ROS based on its dose.1

On the other hand, we found a growth promotion effect of budding yeast cells in PBS treated with oxygen radicals using an atmospheric-pressure oxygen radical source. We treated the suspensions of yeast cells, varying the fluxes of O(3P) from 1.3×1016 to 2.3×1017 cm−2 s−1. Proliferation was promoted at doses of O(3P) ranging from 6×1016 to 23×1017 cm−3, and suppressed at doses ranging from 3×1017 to 1×1018 cm−3; cells were inactivated by O(3P) doses exceeding 1×1018 cm−3, even when the flux was varied over the above flux range. These results showed that the growth of cells was regulated primarily in response to the total dose of O(3P). However, the main factors for the promotion and the inactivation of budding yeast cells in PBS solutions treated with oxygen radicals have not been elucidated.2

In this study, budding yeasts were treated with neutral oxygen radicals as functions of treatment distance, sample volume and kinds of suspensions and so on. The activation and inactivation effects of neutral oxygen radicals on yeast cells were investigated using a cell count and a colony count method, respectively. Based on the measurements of free residual chloride and hydrogen peroxide concentrations in the solutions treated with oxygen radicals, we have investigated their effects on the growth. From these results, we have concluded that the main factor for the inactivation is due to the hypochlorous acid generated in the PBS solutions irradiated with oxygen radicals. On the other hand, we have found that the main factor for the promotion is not the hypochlorous acid but other radicals.

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References
R&D STATUS OF PLASMA APPLICATIONS TO AGRICULTURE IN NATIONAL FUSION RESEARCH INSTITUTE (NFRI)

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The traditional agriculture was innovatively replaced by the chemical agriculture with much higher productivity owing to the invention of agricultural pesticides and chemical fertilizers. Due to the pesticide residue, however, the chemical agriculture has been increasingly replaced by the inorganic agriculture, which has even the disadvantage of lower productivity. Even though the inorganic agricultural products are free of the pesticide residue, they are sometimes exposed to deadly infection of harmful microorganisms bred well in the eco-friendly cultivation environment. The recent agricultural issues of the food safety and productivity can be innovatively overcome by adopting the plasma technology.

Low temperature plasmas have the characteristics such as activation, sterilization, and catalyzer, that can be applied to the whole agricultural cyclic phases: The cultivation, post-harvest, and securing safety of agricultural products and foods. We named the whole agricultural cyclic phases with the plasma treatments as ‘Plasma Farming’ which includes plasma cultivation for productivity promotion, plasma post-harvest for freshness preservation, and plasma safety for securing safety of agricultural products and foods.

Korean government has financially supported the project of the plasma farming since 2014. In order to perform the project in systematically, we organized a consortium. Based on the strong collaboration of the consortium, we have been able to accelerate the R&D of the plasma applications to agriculture and produced many of interesting results that will be introduced in this paper.
Plasma-liquid interactions and implications for biological applications
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Cold atmospheric plasma (CAP) interactions with liquids are common to many applications involving CAP-living system interactions. The fundamental principles governing interactions at the ionized gas - liquid electrolyte phase boundary are poorly understood at present. In this talk, I will address several key questions, especially about the phase boundary, and present experimental and modeling results that address these issues. One important question regards charge-transfer dynamics at the interface. Modeling results coupling plasma with liquid electrolyte highlight the role of electrons as they enter the liquid from the plasma to become hydrated in solution. Calculations suggest that there are at least three spatial scales involved with reactive species entering the liquid from the plasma: charged species like hydrated electrons are lost within a few tens of nm; highly reactive neutrals like OH are lost within microns; and more stable neutrals like H$_2$O$_2$ and NO$_2^-$ survive mm to cm into the liquid. Experimental studies of liquid dye reacting with air plasma-generated species support the idea that near-surface reactions can dominate the effects of CAP interaction with liquids. Implications of these processes on plasma-biological interactions will be discussed.
ATMOSPHERIC PRESSURE PLASMA TREATMENT OF SEEDS: EVALUATION OF PLASMA COMPONENT EFFECTS

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The quality of seeds not only includes their capacity of rapid germination and fruitful growth, but also their protection against, at least, major well know diseases that can threaten their development (for example diseases provoked by Tilletia caries or Ustilago tritici (smut) in the case of wheat). Taking into account the fact that, in the coming years, the new regulations will lead to the impossibility to use the up to now frequently used chemicals, seed producers will have to find new physical treatments if they don’t want to face problems when putting their production on the market. They are then facing another problem: physical treatment must cure seed from pathogens, but, at the same time, they must be totally safe for seed germination and grow. Also, the longer term effects on plants and potential gene modifications should be considered. Due to seed characteristics, especially design to survive in aggressive environments during the latency period, this represents a huge challenge not easy to overcome. Low Temperature Non Thermal Plasmas (LTNTP) appear as a very interesting potential treatment in that domain, especially for very high added value seeds, and can open new routes in the seed processing.

Due the complex nature of plasmas produced at atmospheric pressure in air, preliminary experiments must be conducted to allow determination of plasma components (at least RONS, UV and Electric field) main action trends. Experiments, dedicated to that, have been conducted on various seeds and discharge systems (including jet, multijets, volumic plasmas, low pressure plasmas) and will be presented with emphasis on growth regulation and potential toxicity.
MICROWAVE AND LOW-FREQUENCY PLASMAS APPLIED TO ENHANCED DECONTAMINATION, RESIDUAL-PESTICIDE TREATMENTS, AND GERMINATION CONTROL IN AGRICULTURAL PRODUCTS

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Plasma devices driven by microwave or low frequency have demonstrated effective elimination of residual pesticides in mountain ginseng roots below regulatory limit and effective decontamination of microbes. The decontamination capability of various bacteria was very different between realistic situations (foods) and lab environment (Petri dish). The germination enhancement by microwave plasmas were often observed. Sometimes germination and growth by microwave plasmas were enhanced by an order of magnitude compared with that with low-freq. plasma devices. The physiological preconditioning was found to play a vital role.

The characteristics from our modelings of atmospheric pressure plasma devices driven by microwave will be presented. At or above 0.1 GHz, the microwave discharges are similar after the sharp transitions at high pd region, where the Paschen breakdown near or below 100 volts might be feasible for He plasma. The discharges driven by stronger electric fields before the transition show fully kinetic characteristics with less population of energetic electrons whereas the discharges driven by weaker electric fields after the transition show more abundant energetic electrons with fluid-continuum ones. These energetic electrons with energies exceeding 4-5 eV can break up the molecular bonds or generate many radicals that are responsible for the observed biological effects.
IN-PACKAGE INACTIVATION OF HUMAN PATHOGENIC BACTERIA AND VIRUSES ON LEAFY GREENS USING ATMOSPHERIC COLD PLASMA AS A TERMINAL PROCESSING STEP

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Atmospheric cold plasma (ACP) treatment is a novel, promising antimicrobial method. Dielectric barrier discharge forms of ACP are of particular interest, due to their potential for in-package decontamination. The objectives of this work were to quantify ACP inactivation of E. coli O157:H7, Salmonella, L. monocytogenes, and Tulane virus (TV, a surrogate for human norovirus) inoculated on Romaine lettuce, and to evaluate combining ACP with moisture vaporization, modified atmospheric packaging (MAP), and post-treatment storage on the inhibition as methods to enhance antimicrobial efficacy. Romaine lettuce was inoculated with E. coli O157:H7, Salmonella, L. monocytogenes (~6 log CFU/g lettuce), or TV (~2 log PFU/g) and packaged in a petri dish (150 mm diameter) or a polyethylene pouch (152 x 254 mm). Leaves were packaged with and without moisture vaporization inside package. For MAP studies, pouch-packaged leaves were flushed with MAP composed of O2 at 5\% or 10\%, with the balance being N2. Samples were treated by ACP at 47.6 kV for 5 min. Treated packages were analyzed for the inhibition of microorganisms, either immediately or following post-treatment storage for 24 h at 4 °C. ACP treatment inhibited E. coli O157:H7, Salmonella, L. monocytogenes, and TV by 1.1±0.4, 0.4±0.3, 1.0±0.5 CFU/g, and 1.3±0.1 PFU/g, respectively, without environment modification with moisture and gas in packages. Lettuce packaging and moisture vaporization did not significantly influence the inactivation of bacteria (P>0.05). MAP using the N2-O2 mixtures reduced the inhibition rates of E. coli O157:H7 and TV. Following storage, L. monocytogenes declined by an additional 0.4 log CFU/g, suggesting sublethal injury as a mode of action. ACP in air effectively inactivated E. coli O157:H7, Salmonella, L. monocytogenes, and TV in lettuce, with Salmonella being the least sensitive. L. monocytogenes continued to decline in post-treatment cold storage. Reduced-oxygen MAP gas composition reduced the effectiveness of ACP. Both rigid and flexible conventional plastic packages were suitable for in-package decontamination of lettuce using ACP. E. coli O157:H7, Salmonella, L. monocytogenes, and TV were effectively reduced by ACP.
Abstract

Non-Equilibrium Gliding Arc Discharge Plasma-Activated Water in Plasma Agriculture: Pathogen Control, Plant Growth Enhancement, and Reduction of Irrigation Water Consumption

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In the last 10+ years, the emerging field of Plasma Medicine produced startling new advancements in development of new medically-relevant therapeutic treatments, ranging from sterilization of medical instruments to treatment of cancers in human clinical trials. Every day new scientists are joining this ever-expanding new field of plasma chemistry and plasma physics and, no doubt, will create many new valuable medical solutions. However, the advances in Plasma Medicine have also led to some interesting developments in the closely-related fields, creating new satellite technological solutions based on atmospheric plasmas. One such example we want to highlight is the recent developments in the field of plasmas in agriculture. Agricultural applications, similar to medical applications, involve interaction of non-equilibrium discharges with living plant cells and tissues and with soil. Developments of plasma treatment of liquids for medical applications initiated this field, leading to the use of plasma-treated water for seed germination, plant growth enhancement, and others. Significant breakthrough in this direction happened during the last 3-5 years with introduction of transitional non-equilibrium plasmas (like the non-equilibrium gliding arc discharges and the non-equilibrium pulsed power plasma systems). This opened the possibility of generating cold atmospheric plasmas at relatively high power levels, sufficient to treat large volume of liquid. This, in turn, triggered possibility of large-scale applications of plasma-activated water (of sufficiently-high water volume) for sterilization, washing, seed germination, plant growth stimulation, etc, which has an important impact in food safety and agricultural applications.
Plasma treatment of seeds and plant calli

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If we consider all options for using plasma in agriculture the most and the least effective at the same time seems to be treatment of seeds. The largest overall effect is when you treat seed and affect how the plant turns out in the end. One mostly considers the improved germination probability as an intended target. Yet, the most valuable and frequent crops have germination probability above 90% so adding a costly, complex stage in production seems like a futile exercise. The situation changes considerable when we consider rare, endangered and some specialized medicinal plants. Plasma treatment improves the germination efficiency considerably from sometimes around 10% up to 70% or 80%. Thus plasma improved germination becomes a viable technology from the standpoint of both economy and the need for preservation of the rare species. We have used several seeds in our studies most importantly seeds of Paulownia tomentosa that is known to have a complex kinetics of initiation of germination triggered both by NO molecules and photons of a special wavelength.

Treatment of seeds of mass produced crops, unfortunately may become an option once one needs to remove or at least reduce the fungi, spores or some form of infection including accumulated toxins. While plasma reduced the infection and toxins, one should be weary of the increased damage to the surface topography that may harbor infection in due course.

Our experience with plant calli is the most promising. Even a slight treatment provides a dramatic increase of the number of cells and the size by a large factor (typically 2.5). Other aspects of plant development such as dormancy are also improved. This line of application is particularly promising for bioreactors.
Nitrogen fixation by plasma – new technology for future?

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Conventionally nitrogen is fixed with the Haber-Bosch process, which fixes nitrogen in the form of ammonia by the reaction of nitrogen with hydrogen at high pressure and temperature. The Haber-Bosch process, consumes almost 1-2% of the world’s total energy production and ~ 2% of the total natural gas output and emits more than 300 million metric tons of carbon dioxide\textsuperscript{[1]}. Another industrial scale nitrogen-fixation process by thermal plasma, Birkeland-Eyde process\textsuperscript{[2]}, was eventually abandoned by the industry because of the poor energy efficiency as compared to the H-B process. Less than 3% of the supplied energy was utilized for the reaction, while rest of the supplied energy (97%) was wasted in establishing conditions suitable for the reaction to take place. Nitrogen fixation by non-equilibrium plasma presents one of the alternative way for a greener and less energy consumption nitrogen fixation process which aims to convert the renewable power to chemicals which is easier and better for renewable energy storage. The realization of this new process will open a new operation window in chemical engineering. However the low energy efficiency of non-thermal plasma nitrogen fixation process is the first challenge to be solved for such ambition. It is not only the plasma reactor which influence the energy consumption, but also the whole process which allow for a systematic heat integration may improve the energy efficiency. In this research, both DBD and GA reactors\textsuperscript{[3]} were studied with/without catalyst, the energy consumption and energy efficiency will be considered in process design for optimization and followed by life cycle assessment for environmental profile study.

FUNGAL DISEASE CONTROL AND PLANT DEVELOPMENT BY PLASMA
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In this study, atmospheric non-thermal plasma was applied to sterilize rice seeds infected with Fusarium fujikuroi (causing rice bakanae disease), and water or phosphate buffer treated with gas (mostly nitric oxide) generated from microwave plasma torch were used for enhancing the development of spinach. Arc plasma discharged in water effectively disinfected the fungal contaminated rice seeds. About 80-90% of infected rice seeds was sterilized by arc discharged plasma in a condition of 12 frequency time per second at 10 kV. The combined treatment of ozone and ultrasonication has also disinfected rice seeds effectively. We analyzed the real time level of nitric oxide in water and phosphate buffer treated with plasma generated nitric oxide (PGNO). Level of nitric oxide in water and phosphate buffer was increased to about 100 µM after treatment with PGNO for 50 min. Results on plant development will be discussed. Our work was supported by the National Research Foundation of Korea (NRF) grant (No. 2010-0027963), Rural Development Administration (RDA) grant (No. PJ009891) and National Fusion Research Institute (NFRI) grant.
Plants and fungi have always been a primary source for high value substances that are needed especially for pharmaceuticals. The emergence of bacteria that are resistant against many established therapeutic drugs has now emphasized the need to find new sources and extract new antimicrobial metabolites that can be exploited accordingly. In this search microalgae have been identified as a promising resource [1].

The challenge for the commercial use of algae is an efficient extraction of bioactive compounds with high yield and without destroying the desired ingredients. In this respect non-thermal processes are of interest. One method that is already investigated is the exposure to pulsed electric fields (PEF), although with the main objective to produce biofuels [2]. Pulsed electric fields are known to be highly effective for the disruption of cell membranes by inducing irreversible electroporation. However, algae and other plant cells are also protected by a robust cell wall that hinders and even prevents the extraction of cell contents. Unfortunately, the cell wall does not seem to be affected by PEF-treatments. We have therefore explored the possibility to break up cell walls of algae by different plasma treatments. As target, we chose *Chlorella vulgaris* (*Chlorellaceae*) which is a thoroughly studied model organism rich in proteins, unsaturated fatty acids, carotenoids and other compounds. The algae is further known for its robustness, including a strong cell wall structure. Algal suspensions were treated with a dc plasma jet that was immersed into the suspension, a volume dielectric barrier discharge that was in contact with the suspension, a pulsed corona discharge that was generated directly in the suspension and a submerged pulsed spark discharge. The different plasma technologies provide different reaction mechanisms, such as shockwaves and pulsed electric fields, as well as different chemistries in air-water mixtures or water only. All physical interaction mechanisms and reaction chemistries can be expected to affect cell walls, although in different ways. We further compared the plasma-treatments with pulsed electric field treatments and with some standard extraction methods, such as ultrasound, Soxhlet-extraction and microwave extraction. After treatment, algae were investigated by scanning electron microscopy and the total protein content was quantified by a photometric protein assay. The highest yield was obtained for microwave extraction but treatment with spark discharges resulted in a very similar yield. A major difference between both treatments is, however, the strong temperature increase for microwave exposures close to 100ºC while the temperature of the suspension did not rise above ~25ºC with spark discharges. Some further, but preliminary studies on spark discharge extraction have shown that the electrical excitation scheme for instigating a spark and the energy that is delivered, determine the extraction yield.


IMPROVED GROWTH OF GARLIC BY PLASMA TREATMENT OF CLOVES

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Recently plasma technologies have gained much attention in the field of agriculture, as it was shown that plasma treatment has a positive effect on the growth and germination of seeds, suppresses development of aggressive phytopathogens that cause diseases in plants and can increase the plant's resistance to infection. In our study the influence of plasma treatment of garlic (Allium sativum L.), specifically the Slovenian autochthonous species, which is listed as agriculture plant species in danger of being lost from farming, was studied. Different plasma treatment conditions were used for treatment of garlic cloves and the influence of treatment on their surface properties and growth were studied. Changes in surface properties of garlic cloves were analyzed by measuring the change in surface wettability by water contact angle measurements, while changes in surface morphology were studied from images taken by atomic force microscopy (AFM) and scanning electron microscopy (SEM). As plasma treatment can induce changes in the chemical composition on the surface, the changes in surface chemical composition of garlic husks were studied by X-ray photoelectron spectroscopy (XPS). The germination of garlic cloves was studied in the laboratory by measuring the root length and time of sprouting at controlled moist and temperature. Furthermore, the seeding growth of garlic was studied after planting the garlic clove in the field to observe changes in length of roots and leaves as well as the change in the size of the bulb and its weight. The results of our studies show that plasma treatment of garlic cloves is a prospective approach to increase garlic germination and increase its yields. At appropriate plasma conditions the garlic bulbs are in the average almost 50% heavier and have about 13% larger bulb's surface area. Moreover, the initial state of germination of plasma treated garlic cloves exhibits faster sprouting as well as growth of roots, which is important for its initial resistance to infections.
THE EFFECT OF PRE-SOWING PLASMA SEEDS TREATMENT ON GERMINATION, PLANTS RESISTANCE TO PATHOGENS AND CROP CAPACITY


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The effect of pre-sowing plasma seed treatments of some important agricultural plants (Triticum aestivum L., Lupinus angustifolius and Zea mays L.) and perennial species, well known for ornamental and medicinal use (Morus nigra L. (MN) and Rhododendron smirnowii Trautv. (RS)), on seed germination and plant resistance to common diseases during vegetation is studied. The early stages and longer-term observations of seedling development after seed treatment by physical stressor were investigated in order to estimate the sustainability of the observed effects. Optimal conditions for plasma treatment ensuring its maximum biological efficiency for different species are established in laboratory and field tests. The seed coat surface structure and seed EPR signal are investigated and some important factors of plasma treatment are discussed.

Treatments was carried out in a planar geometry capacitively coupled 5.28 MHz plasma reactor consisting of two plane-parallel water-cooled copper electrodes placed in a stainless steel vacuum chamber with the inner volume of 0.053 m³. All treatments were performed in air at a pressure of 60–100 Pa and a specific power density was varied in the range of 0.68–0.1 W/cm². The duration of plasma exposure was 2 – 7 min.

Plasma treatment stimulated germination of annual seeds by 5–15%. The greatest stimulation effect in the field tests has been achieved on Triticum aestivum (10–12%). The effects on perennial plants were stronger: plasma treatment increased germination of MN seeds by 27% and stimulated further seedling development, but practically non-affected RS seed germination. Vacuum exerted no effect on MN, but significantly improved germination rate and yield (by 72%, from 25 to 43% of germinated seeds) of RS seeds.

A drastic reduction in the total infection level of blue lupine seeds has been observed as a result of 5 min-treatment, which decreased up to 23% (100% in the control). Plasma treatment inhibited Fusarium by 90% that causes the most harmful root disease of spring wheat and blue lupine, suppressed the anthracnose spreading on blue lupine and spring wheat during the vegetative phase up to the flowering stage. This allowed minimizing the potential negative impacts on environment by avoiding the application of fungicides in the plant vegetation period. Plasma treatment leaded also to a significant reduction (up to 40%) in boil smut infection (caused by the fungus Ustilago maydis (DC.) Corda) developed on maize seedlings. Plasma seeds treatment owing to its stimulatory and fungicidal effects increased crop yield of spring wheat by 4-6%, maize – by 1.5-2.0%, lupine – by 20-40% as compared to control.

EPR spectra were measured immediately after treatment and repeated in few following days. It was revealed a substantial burst of stable paramagnetic species, whose relative concentrations and dynamics during the first days after the treatment depended on plant species, duration of exposure and seed dormancy status (fresh or after ripened).

Longer-term observations of perennial plants seedling revealed that the effects persisted for more than a year: plants grown from treated seeds performed better (developed more leaves and had greater total leaf surface area) than the control plants. Moreover, the most negative stressor effects on seed germination are followed by the most rapid leaf growth during later stages of plant development. These findings imply that commonly used stressor-effects estimates, such as germination rate or seedling morphology are not sufficient for qualifying stress response.
INTERACTION OF NITROGEN-CONTAINING SURFACE MICRODISCHARGE (SMD) AND RF ATMOSPHERIC PRESSURE PLASMA JET (APPJ) WITH SURFACES OF BIOMOLECULES AND MODEL POLYMERS: THE INFLUENCE OF DISCHARGE COMPOSITION ON NITROGEN UPTAKE

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A number of different cold atmospheric pressure plasma (CAPP) sources are currently being investigated for applications ranging from agriculture to plasma medicine. These sources can strongly differ in their operating conditions, and consequently this affects the incident reactive particle fluxes to which the surfaces are exposed. In this work we compare differences in surface-interaction mechanisms of a time modulated MHz powered atmospheric pressure plasma jet (APPJ) operated using Ar/N₂/O₂ gas mixtures with a kHz powered surface microdischarge source (SMD) using a N₂/O₂ chemistry. Both CAPP sources were housed in well-controlled gaseous environments to study surface interaction mechanisms with a set of model polymers and biomolecules. After CAPP treatment, the surface nitrogen content was determined by x-ray photoelectron spectroscopy. We find that the surface nitrogen uptake varies strongly with both CAPP source type, operating conditions, and substrate material, which allows conclusions as to systematic differences in the reactive particle fluxes from these sources and surface interaction mechanisms.
Abstract

With climate change and increasing world population, the competition for water available for irrigation of crops has increased. Current means to address this problem are addition of fertilizers and genetic engineering. However, the short and long-term impact of these techniques on health and environment are major concerns. The study presented here demonstrates that the challenge can instead be met by Non-Thermal Plasma Technology (NTP) for treatment of water. Plasma produces a wide variety of metastable radicals, predominantly reactive oxygen and nitrogen species (ROS, RNS) that has been previously demonstrated to activate plant defense response and accelerate plant growth. We used NTP treated deionized water to irrigate Arabidopsis thaliana plants for 6 weeks. Plasma treatment decreased overall water consumption for irrigation, simultaneously enhancing plant growth and yield. We propose that the plasma generated NO3-Ns are responsible for the increased fecundity of plants.
Abstract

**DBD PLASMA EFFECT ON THE PHYSICAL - CHEMICAL PROPERTIES OF THE SEED COAT AND SEED GERMINATION UMBU (SPONDIAS TUBEROSE ARR CAMARA.)**

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Umbu (Spondias tuberosa Arr. Camara) is an exotic tropical fruit species that is important both as an alternative economic and subsistence resource for rural communities in the semi–arid, northeastern regions of Brazil. Plants adapted to semiarid conditions tend to have dormancy (resistance to germination) due to the short period and irregular rainfall. In order to eliminate or reduce these limitations, plasma produced by dielectric barrier discharge (DBD) was used. Plasma jet produced by helium gas discharge in flux of 0.03 l/s was applied to each seed at a distance of 13 mm during 60 s. Under these conditions, voltage 10 kV, frequency 750 Hz and power of 150 W were required. Seed treated and untreated were characterized with respect to mechanism and capacity of water absorption, as well as germination rate. It was found that the distal region has an important role in the absorption of water. Observing the vertical drag in treated and untreated seeds placed in a dye solution, it was found that besides higher absorption rate of the water it also was observed color dye more intense in the treated seeds.
PLASMA TREATMENT OF SEEDS – FROM LABORATORY EXPERIMENTS TO HARVEST YIELD ENHANCEMENT

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In the past several years we have intensively studied the influence of plasma on the seed germination and health improvement in laboratory conditions. Tailed laboratory investigation of the plasma treatment effect on the seed was performed both in the laboratory and field experiments. Both the low-pressure and atmospheric pressure discharge were used. The low-pressure discharge was excited by a microwave power source, the atmospheric pressure plasma was generated by a gliding-arc plasma torch.

The influence of seeds treatment was evaluated in the laboratory bioassay. The seeds from each variant were placed on the wet filter paper in germination chamber (10x10 seeds). The evaluation of seeds germination was observed 3rd and 7th days of experiments. The index of root development and germ development was marked in each seed and the mean of variant were calculated. The seeds germination in each variant was from 95 to 100%. Seed health was of good quality and comparable in all variants. The roots and germ development of barley seeds treated by pesticide was slower than in all variant. Plasma treated seeds evidenced better development more than 4 % in compare with control seeds.

In 2015, the seeds of each variant were sown in the field on 38 750 sq ft. The yield of rapeseed oil was comparable with the average yield of the crop in Czech Republic. LPMD treatment showed positive effect on the yield of rapeseed oil, similarly like in the field experiments performed in 2014. The yield from plasma treated seeds was more than 28% higher than yield obtained from variant where seeds were treated by pesticide.

Interesting results were achieved also for barley seeds. An yield increase was observed for the treated seeds, ranging from 1.42% to 9.54% in comparison with the control sample. A statistically significant effect of the seed treatment on the yield at full maturity of spring barley has been observed. Recently, repetitive experiments in field conditions are performed in order to optimize the process parameters for various kind of seed – rape, barley, wheal and seed of some vegetables.

In both the laboratory and field experiments we have also tested combinations of the plasma discharge and the biological protection - dual treatment method. In the first step of the dual treatment the surface of seeds is sterilized from fungal infection by plasma discharge. Consequently, the surface is coated by a fungi which prevents from re-settlement of the surface by pathogens and which poses no risk for the seeds. This approach represents a more ecologic alternative to the classical methods of chemical seed dressing. This dual treatment of seeds showed better results in the roots and germ development in compare with untreated seeds. The positive effect of the dual treatment was observed also both in the laboratory and field experiments.

Preliminary model economic calculation made for the rape oil will also be presented.
STIMULATION OF METABOLITE PRODUCTION IN MEDICAL FUNGI BY ATMOSPHERIC PRESSURE PLASMAS

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Seeking out new solutions for old problems – testing the applicability of non-thermal atmospheric pressure plasmas in agriculture and related research areas is gaining increasing interest. Encouraging results from numerous research groups may indeed indicate the unique potential of plasma in this application driven field.

Organisms used in biotechnical processes are characterized by a high production rate of compounds of interest. These high performance strains are generated by time consuming cultivator techniques. Beside direct genetic manipulation, irradiation and chemical noxae are typically applied. Non-thermal plasma generates both irradiation and chemical entities, its parameters can be tuned in a wide range and it can be implemented into almost every process by engineering. This seem to make plasma an ideal candidate as an alternative means in biotechnological cultivation procedures.

To test this hypothesis in a eukaryotic environment, two different model organism were used: The medicinal mushroom *Ganoderma lucidum* was chosen for its wide spread use in Asian traditional and current medicine and substantial literature background. On the other hand, micro algae like *Scenedesmus sp.* are current candidates for CO₂-fixation and biofuel production. In both cases, an optimization of cultivation processes along with an increased production of metabolites is desirable. Plasma treatment was achieved by direct or indirect treatment of the mycelium or the cells using either a surface barrier discharge or a volume surface barrier discharge in air, and an argon based plasma jet (kinpen). Depending on treatment intensity production of secondary metabolites by *G. lucidum* was modified: lipophilic compounds and the desired triterpenes increased while no effect was found on steroid production. Also the amount of detected of immune stimulating β-glucans increased due to the plasma treatment. At the same time, biomass production was only slightly increased indicating an increased expression level of enzymes from secondary metabolism processes. To verify this, liquid chromatography coupled with high resolution mass spectrometry was applied. Results show, that while general overlap in expressed proteins is high strong differences in expression levels exist. First analysis of the data reveals an increase in metabolic activity and cellular remodeling. Of note, the carbohydrate metabolism is increased correlating with the increased presence of glucans. These results show that the underlying mechanisms are coupled to changes in cell physiology rather than chemical modification of small molecules. In the micro algae *Scenedesmus sp.*, first results showed an increase in biomass production and colony size after a direct treatment with the plasma jet. Further experiments will clarify the production rate of primary metabolites (e.g. lipids) after the challenge.

In conclusion, these two examples show the applicability of non-thermal plasma to modify and improve the production of primary or secondary metabolites in biotechnologically important eukaryotes. Future work is scheduled to broaden this view and to further substantialize the mechanisms behind this interesting plasma application.


PHOTODYNAMIC PROCESSES TO IMPROVE THE SAFETY OF WASH WATER USED IN THE FRESH PRODUCE INDUSTRY

ABSTRACT TITLE

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Post-harvest spoilage of fresh produce is a critical challenge. Despite advances in hurdle technologies, over 30% of the fresh produce in North America is wasted, largely due to microbial spoilage that results from limitations of the current washing and sanitation procedures in achieving a significant inactivation of spoilage microorganisms, cross-contamination, and adaptability of microflora to grow under refrigerated and modified storage conditions. The problem is further exacerbated by limited efficacy of existing chlorine based sanitizers. To address these challenges we evaluate a synergistic combination of novel, food-grade photosensitizers and UV light to improve the microbial (bacterial and viral) inactivation rate in wash water and fresh produce. These compounds, upon exposure to light produce reactive oxygen species that aid in microbial inactivation. We will discuss several food-grade photosensitizers, their efficacy and limitations.
PLASMA DECONTAMINATION OF NATURAL TOXINS

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The intense growth of population increases the food demand and for this reason, there is an urgent need to develop new, more sustainable and effective approaches to agriculture and food production, transport, and storage [1]. The aim is to get the highest possible output and keep the produced food unspoiled or preserved has been present since the beginning of agriculture. Therefore, many preventive measures are already carried out or being developed. However, the problem of food contamination still persists, and use of chemicals is not desired. Even more, all preservation methods typically affect the organoleptic and nutrition properties of the food products [2].

Very concerning is recently recorded growing trend of food contamination with mycotoxins, toxic secondary metabolites of various fungi species. It is estimated that around 25 % of the cereals are unsuitable for consumption because of the mycotoxin contamination. One of the main reasons behind undesirable fungal growth and mycotoxin production is incorrect agricultural and harvesting practices as well as the poor effectiveness of prevention methods [3]. Due to this, many countries worldwide adopted strict legislation to control the mycotoxin presence in the food and reduce potential danger to human health [4]. Despite tight legislation and control, the trend of mycotoxins food contamination is still growing. Various studies revealed that this is almost unavoidable since there is a relation between increased presence of mycotoxins and recent climate changes. [5]. Moreover, the climate changes are present also in global economy, where food is transported long distances from producer to consumer. These issues include changes of local climate, transport and long storage times. All of them contribute to increased food contamination [1].

Considering this, agriculture and food industry require a new universal approach to deal with mycotoxin contamination of food. Plasma technology shows a lot of promise as a new non-thermal decontamination method. It was already demonstrated that it can efficiently eliminate microorganisms and other hazardous agents from various sensitive substrates including food products [6]. However, not much work has been done on plasma degradation and detoxification of mycotoxins. Therefore, the aim of this talk is to represent our work in the field of plasma decontamination of mycotoxins and to show the impact of the investigation and potential implementations and drawbacks of such treatments.

Plasma based degradation of mycotoxins

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The prevention of fungal contamination of agronomical and further products in the food chain is essential. The growth of a vast variety of different fungi leads to high contents of toxic secondary fungal metabolites, so called mycotoxins. These toxins have led to spoilage of entire maize crops and still are one of the main reasons of import bans on certain commodities when entering European markets. These mycotoxins possess high potential to cause gastro-intestinal ailments as well as carcinogenic, mutagenic and teratogenic effects leading to food refusal, growth retardation and productive disorders in livestock. Fungal infestation raises huge financial damage in the agricultural economy every year. Therefore, particular attention is paid to toxin content levels in food and feedstuff, which are regulated within e.g. the enactment (EG) No. 1881/2006. Different methods have been studied and developed over the last decades, ranging from chemical approaches, like application of oxidizing agents, chlorinating, ammonia to biological methods like enzymatic metabolisation or genetic enhancement of certain strains leading to suppression of constitution of various toxic metabolites.

After addressing fungal spores and hyphae directly1, as well as first experiments on the treatment behavior of seeds2, the focus of the subsequent studies went directly to the toxic fungal metabolites. To demonstrate an approach for the degradation of different mycotoxins we present our findings on the efficacy of a dielectric barrier discharge (DBD) working with ambient air at atmospheric pressure, excited by a kHz-pulsed AC-power supply. The main task of the presented experiments is the evaluation of the general behavior of mycotoxins exposed to a gas discharge as well as the dose dependency of the degradation effects. The presented studies initially considered mixtures of extracts containing several mycotoxins, which were produced by fungal species in liquid media. These studies were followed by investigation of different pure mycotoxin standards of Fusaria and Aspergilli-mycotoxins in an in-vitro setup. The results show a significant degradation of all toxins under investigation and indicate a dependency of degradation effectivity on structural properties of the specific toxin.

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Effects of Chemical Species in Atmospheric Pressure Wet-Air Plasma Effluent on Strawberry Pathogen Conidia

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Abstract

Reactive oxygen and nitrogen species (RONS) such as OH, NO\textsubscript{x}, are known to be important in living organism and also known to be rich in atmospheric pressure air plasma. Recently, agriculture applications of plasma-generated reactive species have been suggested [1] for sterilization and growth promotion, and etc. The atmospheric pressure air plasma device exhausts plasma-generated RONS toward the plants, which allow us to treat the large area of farming field with the plasma-generated RONS as shown in Fig.1. The control of RONS composition in wet-air plasma effluent has been experimentally studied with nanosecond pulse discharges, conventional low frequency discharges, and combination of those discharges [2], which shows the capability on control of the RONS composition in the plasma effluent at farming field with air and water for pathogen control. The species measured in the plasma effluent are O\textsubscript{3}, OH, NO, NO\textsubscript{2}, N\textsubscript{2}O, N\textsubscript{2}O\textsubscript{5}, HNO\textsubscript{2}, HNO\textsubscript{3}.

In this work, we focus on strawberry pathogen control with plasma effluent. Conidia of Colletotrichum gloeosporioides (C.glo) is a pathogenic conidia for strawberry, which water droplet may carry. The effect of the plasma effluent exposure to the C.glo conidia in 5 \(\mu\)L water droplet has been experimentally investigated [3]. The plasma-generated RONS exposure is found to be effective to suppress germination of the C.glo conidia within two minutes as shown in Fig. 2. Higher density fraction of the water molecule in plasma enhanced the germination suppression effect. Figure 3 shows the RONS concentration in 5 \(\mu\)L water droplet without C.glo conidia after 90 sec exposure to the plasma effluent. Higher water injection flow rate results in significantly higher density of HNO\textsubscript{3}, though HNO\textsubscript{3} at the density in Fig.3 has no significant effect on the conidia germination. This indicates that the species related to HNO\textsubscript{3} generation in the plasma effluent would be a key to suppress the germination suppression. The mechanism of the generation of the effective species for C.glo germination suppression will be discussed.

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Investigation of plasma-water discharges and their agricultural applications

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Abstract: In recent years, non-thermal plasma in and with liquids has attracted considerable interests in several research areas including biomedical applications and agricultural applications due to its attractive physical and chemical activities. Here, various stable diffuse under water discharges with a liquid as ground electrode are presented. The under water discharges by using microplasma jet array in water solution can result in a rapid inactivation of various harmful virus. The inactivation efficiency is strongly dependent on the feed gases used, the plasma treatment time, and the discharge power. Optical diagnostic measurements show that bountiful chemically and biologically reactive oxygen species (ROS) such as OH, O, H₂O₂, O₃, etc, which are beneficial for effective inactivation in some areas, are produced. The inactivation efficiencies can be well described by using the chemical reaction rate model, where reactive oxygen species play a crucial role in the inactivation process. With TiO₂ suspended in aqueous solution, the inactivation efficiency can be effectively improved due to the enhancement of ROS generation. These under water discharges can also provide reactive nitrogen species (RNS) as well as ROS, which can provide nutrient for seed germination and crops growth. To conclude, the under water discharges show their potentials for irrigation water inactivation, seed germination, crops growth and plant disease management.
MEASUREMENT OF REACTIVE SPECIES
IN VARIOUS GAS PLASMA BUBBLED-UP WATER FOR HYDROPONIC CULTURE

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A sanitation control of water is important in hydroponic culture. Using plasma for sanitation control of water, it is necessary to introduce reactive species which have high oxidant potential such as hydroxyl radical (HO·), singlet oxygen (¹O₂) into water efficiently. Additionally, the production cost is also important, for reduction of the cost it is desired that reactive species are generated selectively from low cost gases for example generating NO₂⁻ from ambient nitrogen and oxygen. Cold atmospheric plasma can introduce reactive species from air without changing the water temperature which is important parameter on hydroponic culture. However, in conventional researches cold atmospheric plasmas are generated with He or Ar gas, and reactive species are generated by mixing other gases or ambient air. Therefore the species and amount of generated reactive species were limited. In our lab a multi-gas plasma jet (PCT-DMJ02, Plasma Concept Tokyo, 9 kV, 16 kHz) which can generate stable plasmas with various gases such as He, Ar, O₂, N₂ and their mixed gases was developed [1]. In this study, reactive species introduced into water by various gas plasmas were measured, and the bactericidal effect was investigated.

In this experiment plasma was introduced into water with bubbling method. Plasma source is connected to the bottom of a glass container. In this method reactive species produced by each plasma gas species are transported into liquid without interacting with atmospheric air. Additionally, since liquid is stirred by plasma introduction from underneath, whole of liquid is processed evenly.

In this study amount of HO·, ¹O₂, NO²⁻, H₂O₂, O₃, NO⁻₂ and NO₃⁻ were measured. Plasmas were generated with Ar, O₂, N₂, CO₂ and mock air (80% N₂ + 20% O₂) at 3 L/min gas flow rate for 30 sec. HO·, ¹O₂ and NO⁻ in 3 mL normal saline were measured by Electron Spin Resonance method with each reagent. As the results, the highest concentration of HO·, ¹O₂, NO⁻ was detected with N₂ plasma, O₂ plasma, mock air plasma as 70 µM, 420 µM, 5.9 µM. Then H₂O₂, O₃, NO²⁻ and NO₃⁻ in 200 mL normal saline were measured by absorption spectrophotometry method with each reagent. As the results, the highest concentration of H₂O₂, O₃ was detected with O₂ plasma as 20 µM, 15µM, and NO²⁻, NO₃⁻ was detected with mock air plasma as 70 µM, 86 µM.

To verify bactericidal effect of plasma-bubbling, the effects on E. coli, P. aeruginosa, S. aureus and E. faecalis were investigated. Bacteria in 200 mL normal saline (initial bacterial concentration; 10⁶ CFU/ml) were bubbled by Ar, O₂, N₂, CO₂ and mock air plasma at 3 L/min gas flow rate respectively. As shown in Fig. 1, by O₂, CO₂, N₂ and mock air plasma bubbling, E. coli was decreased 6-digit within 30 sec, 1, 3 and 10 min, respectively. This trend was showed on other bacteria. This difference of bactericidal effect of plasma gas species attributes the difference of the quantity or the kinds of generated reactive species by each plasma gas species. However CO₂ plasma which did not generate high concentration of each reactive species shows also high bactericidal effect on each bacterium. It is considered that bactericidal effect of CO₂ plasma is affected by other reactive species. Then we investigated reactive species generated by CO₂ plasma in detail. These results also will be presented.

INFLUENCE OF DIFFERENT PLASMA TREATMENTS IN THE GERMINATION PROCESS OF INDIAN CRESS (TROPAEOLUM MAJUS) SEEDS

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In this work we present preliminary research results about the influence of plasma treatments with different regimes in the germination process of Indian Cress seeds. Particularly, the effects of atmospheric pressure plasmas (diffuse and filamentary discharges) as well as low-pressure plasma discharge have been studied as a function of treatment time. Surface treatments with Dielectric Barrier Discharge (DBD) at atmospheric pressure conditions have been carried out with a mixture of helium and air gases applying low power values for treatment times lower than 10min. In the case of the low pressure plasma treatment, a radiofrequency parallel plates reactor has been used with air gas at a low fixed value of autobias polarization voltage for treatment times lower than 2min. Surface chemical composition has been studied by means of FTIR and XPS analysis. Changes on the surface morphology of the Indian Cress seeds have been analyzed by means of scanning electron microscopy. Water contact angle measurements have been implemented to determine changes on wettability. Additionally, water vapor adsorption and absorption studies have been performed for simulating real processes during seed germination. Finally, germination rate of the plasma treated seeds have been evaluated as a function of the different experimental conditions.
INVESTIGATION OF HYDROPONIC CULTURE USING PLASMA BUBBLING

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In recent years, efforts of indoor vegetable plant using hydroponic culture with LED are increasing, because it can culture vegetable without soil and agricultural chemicals and raise production efficiency with 24 h of the artificial light irradiation. However, the culture method has the potential to cause plant disease and harmful influence on food sanitation by bacterial contamination from the atmosphere and grower, thus, scrupulous attention to sanitary control and more short-term cultivation are required.

Application of atmospheric pressure plasma has attracted much attention in agriculture field as a novel method, because it causes the bacterial inactivation and plant growth with several benefits, such as low toxicity, high reactivity and low cost. It is considered that reactive species generated by plasma such as ROSs cause bacterial inactivation and NO\textsubscript{2} and NO\textsubscript{3} in liquid have a deep relationship with plant growth. However, most of conventional plasma sources have limitation in gas species, and they could only generate air plasma and argon plasma; argon is easy to generate plasma. Therefore, the reaction process, production amount of reactive species and the effect of gas composition on the plasma are not well studied.

Our group succeeded in development of multi-gas plasma jet, which can generate plasma with various gas species. It can generate stable plasma with helium, argon, oxygen, nitrogen, carbon dioxide, air and their mixture gases under atmospheric pressure. Using this plasma source, the effects by reactive species can be investigated in detail, since they can be generated selectively by supplied gas species.

In this study, bacterial inactivation in hydroponic culture and influence on cultivation by generated reactive species were investigated using the plasma source. As setup of hydroponic culture, 20 L liquid fertilizer was added in 450 x 300 x 254 mm of container, and leaf lettuce was cultured under 20 W of white LED like shown in Fig. 1. And the liquid fertilizer was bubbled by various plasma gases for 10 min/week. After treatment, bacteria tests of leaf and liquid fertilizer were performed on the day 30 of cultivation. The result of untreated and oxygen plasma treated sample were shown in Table 1. From the result, the number of common bacteria was unchanged even when oxygen plasma was treated, but it founds that oxygen plasma treatment has potential to prevent contamination or multiplication of coliform group.

In this presentation, the details of the plasma source, result of the experiments using various gas plasmas will be presented.

![Fig 1. Setup of hydroponic culture](image)

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<th>Table 1. Surviving bacteria in hydroponic culture</th>
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Effect of gliding arc plasma on plant nutrient content and enzyme activity

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In treating vegetables and fruits by Gliding Arc (GA) plasma, most efforts are usually directed toward elimination of common pathogens. In this work we consider mainly the effect of GA plasma on valorisation of nutritional values in fresh produce agro products.

GA used in this work operates at DC mode and consists of two knife-shaped stainless steel electrodes. Nominal values of operation are 3 kV and 2 Amps. The discharge occurs at atmospheric pressure under argon gas flow injected through a nozzle placed above the 3-mm gap at the neck between the two electrodes. During treatment, specimens are placed 13 cm from electrodes' extreme tips in the afterglow region where the value of the temperature is of reasonable value compared to that in the arc region. So, specimens don't suffer from excessive heating while taking advantages from impacts of highly reactive species, ozone and UV radiations coming from plasma.

We consider here the results of gliding arc plasma applications on enzymes and nutrients in fresh produce vegetables and fruits. We analyse the effect of plasma on polyphenoloxidase (PPO) enzyme contained in plant cells of Golden Delicious apples. PPO enzyme is considered to be the main cause of apple browning. Apple slices, few millimeters thick, are exposed to GA argon plasma at atmospheric pressure for different time intervals. PPO enzyme contents in control and plasma treated samples are extracted and analyzed. PPO contents in treated apple slices and its residual activities are found to decrease with plasma treatment time as compared to control one. During plasma treatment, PPO enzyme which is a form of protein, will be subjected to actions of reactive species produced in plasma as ROS, atomic oxygen and OH radicals. This may lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation and lowering of PPO enzyme by plasma. Reducing the activity of this enzyme by plasma treatment could be profitable regarding preservations of apple nutritional values at postharvest period during storing and industrial food processing.

We consider also the effect of GA plasma on nutrients as carbohydrates in potato as well as polysaccharides in apples and sugar cane during plasma treatment applied for 240 seconds. Carbohydrate contents in apple and in potato show gradual increases with plasma treatment time as compared to control untreated samples. Also, total polysaccharides contents in apples and sugarcanes show gradual increases with plasma treatment time as compared to control untreated samples.

Explanation of this effect may resides in considering effects of chemical species, reactive molecules, excited-state neutral molecules and radicals produced during exposure of fresh produce agro products by GA plasma causing cells membranes disintegration. This leads to an increase in permeability of plant cell walls and subsequent raise of total carbohydrates and total polysaccharides due to diffusion of inner plant cell components from intracellular to extracellular medium. GA plasma is also applied to sugar cane cells. It has been found that plasma affects cell sizes and shapes as observed on SEM photos for different treatment times. As plasma treatment time increases, cellular shapes become more irregular and may even rupture and collapse for large exposure time. This may explain the increase of polysaccharides measured in sugar cane plasma treated samples.
PLANT GROWTH ENHANCEMENT OF KOMATSUNA (BRASSICA RAPA VAR. PERVIRIDIS) BY OZONATED WATER SUPPLIED INTERMITTENTLY TO THE UNDERGROUND ROOTS

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Ozone is well known as the most damaging air pollutant to plants. Ground-level ozone causes more damage to the plants than all other air pollutants combined. Ozone enters leaves through stomata during normal gas exchange. As a strong oxidant, ozone causes several types of symptoms including chlorosis and necrosis.

On the other hand, plants grown in elevated atmospheric ozone are known to undergo several biochemical changes before any actual damage can be detected. These reactions include increases in the activities of enzymes associated with general plant defense mechanisms. Ozone exposure often causes a surge in the production of the plant hormone ethylene, as well as changes in polyamine metabolism and increases in the activities of several phenylpropanoid and flavonoid pathway enzymes.

The object of this study is to investigate the effect of ozone on the activation of plants due to triggering the initial defense system described above and a resultant abrupt growth [1]. For the plant growth, an absorption of nutrients from the roots is quite important as well as photosynthetic reaction in the leaves. For this reason, we here focus on the activation of the underground roots of plants by feeding ozonated water under the ground. Here, ozone was produced by an atmospheric barrier discharge with a rotary electrode, and was mixed with tap water to produce ozonated water of ozone concentration of 1 ppm.

As an example of the plants we here cultivate Komatsuna (Brassica rapa var. perviridis), Japanese mustard spinach, which is a leaf vegetable. Through the cultivation experiment, the effect of ozonated water on the growth of Komatsuna was examined. The ozonated water was supplied intermittently twice a day, except rainy and cloudy days, to the underground roots of Komatsuna by changing the feeding time interval. Being apart from the feeding position, the weight of Komatsuna increased by 2.4 times for 5 s treatment and 2.9 times for 78 s treatment, compared to those of the control. Intermittent supply of suitable amount of ozonated water to the underground roots was found to give an enhancement on the plant growth.

ATMOSPHERIC PRESSURE DOUBLE DIELECTRIC BARIER DISCHARGE TREATMENT OF WATER AND SEEDS FOR STIMULATION OF GERMINATION AND PLANTS GROWTH

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Non-thermal plasma (NTP) has been proven as an efficient technology for pollutant degradation and organic decontamination, surface modification, disinfection and ozone generation, etc. More recently, it is demonstrated that the NTP could be used for agricultural applications. Indeed, the plasma-activated water (PAW) has shown significant impacts on plant growth and also reduced the amount of water usage. Additionally, NTP assisted pre-sowing seeds treatment was shown to be ecologically safe and effective method for the improvement of seed germination and their resistance to diseases.

In this study, we focus specifically on plasma treatment of seeds and water to improve the seeds germination and the plant growth. A double dielectric barrier discharge (d-DBD) reactor driven by sub-microsecond high voltage pulses (> 25 kV) was used to produce the reactive species in gas phase and then bubbled through the deionized water (DIW).

The activated water was then applied to Radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), and sweet Spain pepper (*Capsicum annum*).

The amounts of reactive species was controlled by the plasma input energy (<10 mJ/pulse) at constant air flow rate (1 L/min) and HV pulse frequency (400 Hz). The gas phase was analyzed and quantified online using FTIR spectrometer, multi-gas and ozone analyzers.

In addition, changes in water chemistry; i.e. the electric conductivity, pH, nitrate (NO$_3^-$) and nitrite (NO$_2^-$) ions, hydrogen peroxide (H$_2$O$_2$), and temperature; following the plasma treatment were analyzed. The total concentration of nitrate, nitrite, and hydrogen peroxide were measured using digital photometer and test strips.
AGRICULTURAL APPLICATIONS OF ATMOSPHERIC-PRESSURE PLASMA USING PULSED POWER TECHNOLOGY
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Atmospheric-pressure plasma based on pulsed power technologies were utilized for agriculture, fishery and food processing applications. Repetitively operated compact pulsed power generators with a moderate peak power were developed for the applications in several stages of agriculture and fishery. Types of pulsed power that have biological effects are caused with gas discharges, water discharges, and high-electric fields. The discharges yield free radicals, intense electric field, and shock waves. Biologically based applications of pulsed power are performed by selecting the type that gives the target objects the adequate result from among these agents. For instance, intense electric fields form pores on the cell membrane, which is called electroporation, or influence the nuclei. The radicals in water react with cell membrane of bacteria. The electrostatic force drives airborne particulates and mushroom hypha. These applications are mainly based on biological effects and can be categorized as germination of plant seeds; promoting growth of the vegetables and fruits such as Brassica rapa var. perviridis, Fragaria × ananassa, Spinacia oleracea and Raphanus sativus var. sativus as shown in Fig. 1 [1]; improvement of yielding rate of mushroom as shown in Fig. 2 [2]; keeping freshness for a relatively longer period of perishables such as fish and shellfish [3]; decontamination of air and liquid to inhibit degradation of agricultural products [4]. These applications can contribute a food supply chain in Japan and the world.

![Fig. 1: Photographs of Brassica rapa var. perviridis cultivated for 28 days at (a) w/o plasma and with (b) 10 min. or (c) 20 min. treatment per day.](image1)

![Fig. 2: Typical photograph of the cultured L. edodes with and without electrical stimulation.](image2)

References:
Plasma-induced germination of Arabidopsis thaliana using 3 low temperature plasma devices

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Compared to the plasma medicine field, there are a small number of investigations devoted to the use of the plasmas in plant biology in the case of more particularly plasma-induced seed germination and growth. Literature works involve many kinds of seeds (maize, oat, wheat, radish, tomato, bean, lentil, safflower, honey clover and soy, etc.) using different plasma setups at low or atmospheric pressures for various gas compositions (Air, Ar, He, O₂, etc.) and power supply or devices (RF discharges, microwave discharges, corona, gliding discharges, surface discharges, etc.). These literature investigations have often underlined interesting improvements of germination and plant growth. However, it is obvious that better understandings of the plasma species responsible of such effects and the corresponding mechanisms require further research works.

The present work is a first step to better understanding the effect of the plasma species on the early events of germination of Arabidopsis thaliana seeds chosen because their genome is completely sequenced thus allowing us future investigations on the plasma-induced mechanisms. The following three low temperature plasma setups (already detailed elsewhere) are considered to get a wider diversity of active plasma species:

- a DBD setup generating a pulsed plasma jet using He carrier gas at atmospheric pressure [1]
- a double DBD setup generating a pulsed plasma jet using Ar carrier gas at atmospheric pressure [2]
- a microwave afterglow device at low pressure (a few Torr) using N₂ and O₂ mixtures [3].

Furthermore, 5 mg of dry seeds of A. thaliana (corresponding to about 200 seeds) have been used per treatment for different plasma exposure times (5min to 20 min). Each treatment is duplicated. We used ELISA plate for DBD plasma jets (15 min) and 5-cm Petri dishes for low pressure afterglow (20 min). Seeds were sown in 5-cm Petri dishes containing half concentrated MS medium in 0.8 % agar with 1.5 % sucrose. Seeds were stratified in darkness at 4°C for 2 days and grown at 24°C in continuous light conditions. Testa rupture (TR) and endosperm rupture (ER) were quantified 24h and 30 h after transfer to light.

Fig 1: A. thaliana early steps germination following seeds imbibition. TR: testa rupture and ER: endosperm rupture (taken from [4]). NB: the seed size is about 0.5 mm length.

Fig 2: Effect of the different cold plasmas on A. thaliana seed germination rate. Germination was evaluated after 30h the treatment with DBD He, DBD argon (left side) and N₂ afterglow without and with O₂ proportions (5 and 8%) on the right side.

The two atmospheric pressure plasma jets have shown quasi-similar interesting efficiency on seed germination. The low pressure afterglow plasma has shown the most interesting results in the case of the N₂/8%O₂ mixture containing the higher O concentration while pure N₂ has no effect on germination. This means that oxygen is certainly one specific species that contribute to improve of the germination. Next step is to extend the study towards the post-germination stage during the plant growth.

PERFORMANCE TWO DIFFERENT DBD PLASMA ATMOSPHERES IN INACTIVATING FUNGI ASPERGILLUS

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Mulungu (Erythrina velutina Willd.) is a tree of great drought resistance, native of the Brazilian semi-arid, with hardiness and rapid growth and can be used for recovery of degraded areas. Because the seeds are infected surface or internally by pathogens, germination in large areas may be unfeasible. In this study, the main fungi present in the seeds have been identified and partially or completely inactivated. We used the plasma produced by dielectric barrier discharge (DBD) in helium jet or static air (coplanar configuration) atmospheres with duration of application of 3, 6 and 9 minutes. It was found that when plasma applied directly to the seed, the fungi present were more resistant than when applied to fungal isolates. Is attributed to this lower effectiveness to the fact that fungi are present internally in the seed, reducing the action of the plasma. For fungal isolates, it was found that the plasma condition with air was more effective than helium jet where the application for 9 minutes had higher efficacy.
Effect of cold plasma on the enzymatic activity in germinating mung beans (*Vigna radiate*)

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**Abstract**

The aim of this work is to study the ability of the cold plasma in the enhancement of mung bean seeds germination. The effect of two plasma powers and three exposure times were used to investigate the changes in the seeds. Results showed that, cold plasma significantly increased the germination % by 36.2%, radical root length by 20% and 102% in conductivity of seed membrane when compared to the untreated sample. Cold plasma treated mung beans achieved equilibrium at 5th hour of soaking, and is considered as optimum soaking time. We also observed that there is increase in the activity of hydrolytic enzymes like amylase, protease and phytase after the treatment. There is increase by 25.27% in the amylase activity compared to untreated sample. Likewise, we also observed an increase of protease enzyme activity by 37%. The trypsin inhibitor activity and phytic acid content in 60W 20min plasma treated sample was decrease by 39.23% and 52% respectively, compared to the control sample after 24 h of germination. The decrease in antinutritional properties like trypsin inhibition activity and phytic acid showed a positive effect of cold plasma treatment. The plasma treatment dramatically decreased the apparent contact angle and increased the surface energy in all the samples compared to the untreated. The contact angle decreased by a maximum of 57.02% (*p* < 0.05) and surface energy increased by 210.13% (*p* < 0.05), in the 60W 20min treated sample compared with the untreated sample. Thus, cold plasma application can significantly benefit the seed germination during drought conditions.
INACTIVATION MECHANISM OF *P. DIGITATUM* SPORES USING AN ATMOSPHERIC PRESSURE OXYGEN RADICAL SOURCE

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To establish the plasma applications for inactivation of microorganisms, it is important to discuss the effects of individual active species. We have focused on the effects of neutral oxygen species on the inactivation of microorganisms. We measured the densities of oxygen radicals such as ground-state atomic oxygen (O(3P)), singlet oxygen molecule (O2(5Δg)) and ozone using an atmospheric-pressure oxygen radical source, which produce only neutral oxygen species, by vacuum ultraviolet absorption spectroscopy, and showed that O(3P) was the dominant species responsible for the inactivation of *Penicillium digitatum* spores quantitatively.[1][2]

Moreover, we have elucidated an inactivation mechanism of *P. digitatum* spores with oxygen radicals using a confocal-laser fluorescent microscopy. We showed that oxygen radicals caused a functional inhibition of cell membranes of the spores and oxidized the intracellular organelles. On the other hand, the major morphology of spores treated with oxygen radicals was almost same with control spores by the observation of scanning electron microscopy. [3]

Figure 1 shows the correlation of oxidation degree and inactivation efficiency as a function of the dose of O(3P). Spores were stained with diphenyl-1-pyrenylphosphine (DPPP), which proved lipid peroxide. The oxidation degree in the spore was quantified by DPPP intensity. Besides, we can determine the dose of O(3P) from the treatment time and O(3P) flux, which is estimated by the density of O(3P) and a flow velocity. The spores hardly oxidized at O(3P) dose less than 2.1×10^19 cm^-2. Then, the oxidation degree steadily increased with the treatment of O(3P) dose from 2.1×10^19 to 9.8×10^19 cm^-2. The increase turned to be saturated at a dose over 9.8×10^19 cm^-2. On the other hand, the number of surviving spores rapidly decreased to be less than 1/1000 with the treatment of O(3P) from 2.1×10^19 to 9.8×10^19 cm^-2. These results suggested that the oxidation degree in the spore highly influences the inactivation. [4]

From these results, we propose the inactivation mechanism of *P. digitatum* spores treated with oxygen radicals on the basis of O(3P) dose: First, oxygen radicals affect the cell wall and cell membrane of *P. digitatum* spores and inhibit their functions without causing major superficial morphological changes with the treatment at a low dose of O(3P). Next, intracellular organelles are also oxidized and degraded dose-dependently up to a medium dose, in which most spores are inactivated. Finally, high dose of O(3P) completely decomposes intracellular organelles including the membrane structure.

![Figure 1](image-url)

**Fig. 1** Correlation of oxidation degree and inactivation efficiency as a function of O(3P) dose.

References

COMPACT ELECTRODELESS HID SOURCES FOR PLANT GROWTH

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D2D Scientific, LLC

Metal-halide lamps have been a part of the general lighting and horticultural source mix for decades. The bane of HID sources in general and metal-halide in particular is electrode degradation as the lamp operates. This degradation results in a steady decline in the output of the lamp over the course of its life. Electrodeless lamps have been imagined and tested as far back as the end of the 19th century (Tesla), but little progress was made with commercial systems due in part to a lack of low-cost, reliable high-frequency power supplies. With the advent of low-cost solid-state microwave power amplifiers, the feasibility of commercial electrodeless lighting systems has been greatly enhanced. Over the past decade or so, a number of companies have been formed to commercialize electrodeless lamps and have found a potential market with horticultural applications. An electrodeless metal-halide lamp has a number of unique features that will benefit horticultural applications making use of this source type. Because there are no electrodes these sources exhibit very steady light output, eliminating the need to change out lamps on a regular basis. Electrodeless bulbs are physically smaller than their electroded counterparts, allowing for more compact and efficient reflector designs. They can be continuously dimmed, which produces a potentially useful dynamic between the red and blue parts of the spectrum. Microwave excitation produces unique spectral distributions, which can be crafted to suit specific requirements. So for example, a chemical combination can be derived that produces a solar-like spectrum or one that emphasizes the red and blue spectral regions. There are certainly more technical challenges to be overcome, but the commercialization of this technology has provided one more tool to the burgeoning horticulture industry.
EFFECT OF SURFACE ROUGHNESS ON MICROBIAL INACTIVATION USING COLD ATMOSPHERIC PRESSURE PLASMA (CAPP) AND PLASMA ACTIVATED WATER (PAW)

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Centers for Disease Control and Prevention (CDC) reported over 35 major outbreaks in the US in the last decade from bacterial contamination of fresh produce. It has become necessary to develop new and effective processing techniques to achieve efficient pathogen inactivation on fresh produce before it reaches the consumer.

Non-thermal plasma (NTP) is a novel non-thermal technology and has shown promising results for inactivation of food borne pathogens on fruits, vegetables, and meat. Detailed and rigorous studies need to be conducted to explore the potential of NTP technique to achieve food safety and to understand the effect of different factors such as plasma composition and substrate properties including surface roughness on the microbial inactivation efficacy.

The aim of this study was to evaluate the effect of surface roughness on the microbial inactivation efficacy of gaseous Cold Atmospheric Pressure Plasma (CAPP) and Plasma Activated Water (PAW). Inactivation studies for Enterobacter aerogenes for surfaces with different roughness (Pq) values were conducted using model systems (sand papers) and real systems (fruits). It was observed that increasing Pq values of 6.12±2.88 μm, 13.07±5.26 μm, 16.98±6.74 μm for apples, oranges and cantaloupes respectively, resulted in reduced inactivation of 1.86 ± 1.27 log CFU/fruit, 0.77 ± 0.86 log CFU/fruit and 0.61 ± 0.78 log CFU/fruit, respectively. Both real and model systems showed lower microbial inactivation with increasing surface roughness, although model system with similar roughness values as real fruits showed higher inactivation, indicating higher non-uniformity in roughness in a real system.

The second stage of the study focused on evaluating the microbial inactivation efficacy of Plasma Activated Water (PAW) on Enterobacter aerogenes. To isolate the effect of pH from the effect of reactive species, a buffer system at lower pH similar to the pH of PAW was studied. A 2 log microbial reduction from the initial 9 log population was obtained with PAW, however no reduction was observed for the buffer system. This confirmed that the inactivation was due to reactive species in PAW. However, for Plasma Activated Buffer (PAB), a synergistic effect between acidic environment (pH) and the presence of reactive species was observed to give as high as 5 log reduction in Enterobacter aerogenes population. Further studies are being conducted to understand the effect of roughness for microbial inactivation in PAW and PAB systems.
PLASMA DECONTAMINATION OF SPROUT SEEDS IN AN ATMOSPHERIC PRESSURE DIELECTRIC BARRIER DISCHARGE

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Fresh produce is increasingly demanded by consumers worldwide as it is associated with a healthy lifestyle and considered an important source of nutrients, vitamins and fibers. However, there is also a significantly growing number of produce-associated foodborne disease outbreaks, like the EHEC outbreak in central Europe in 2011, caused by sprouts contaminated with \textit{E. coli} O104:H4. Clearly, there is a need to decontaminate granular food particles like sprout seeds from microorganisms, and plasma treatment is a promising approach.

In our research project, we focus on the plasma inactivation of microorganisms on granular food particles (e.g. wheat grains, plant seeds) in reduced and atmospheric pressure systems. Here, we report on our findings from the decontamination of sprout seeds from their negative microflora or an artificially applied \textit{E. coli} contamination (as a surrogate for Gram-negative pathogens). Experiments have been conducted in a dielectric barrier discharge, which in principle is scalable do industrial dimensions. While the lab scale setup was mounted on a horizontally vibrating table, which keeps particles in motion to support homogenous treatment, it can also be inclined to generate a directed particle transport by vibrating conveying for continuous processing.

In a previous study (Butscher et al., Food Control, 60:636-645, 2016) we could show that bacterial endospores could be efficiently inactivated on polymeric model substrates in this DBD, while the decontamination of wheat grains turned out the be considerably more challenging – an effect which was attributed to the complex surface structure of native food products, where microorganisms are sheltered by the rough surface or hidden in crevices. Similar results were obtained for the inactivation of artificially applied \textit{E. coli} on different sprout seeds (onion, radish, alfalfa, cress) where the plasma decontamination efficiency of DBD treatment decreased with increasing surface complexity.

As a further important parameter related to the substrate, the role of moisture content was studied. The inactivation of the native microflora on alfalfa seeds was maximized at a moisture content of 17 % (wet based), and this optimum can be explained by two competing effects: First, moisture from the substrate, which also evaporates to the gas phase, enhances the formation of reactive oxygen species, which can efficiently inactivate microorganisms. But then, moisture also quenches the discharge since energy is transferred to the excitation of vibrational and rotational energy levels in the water molecules and lost for plasma ionization.

Besides the optimization of the moisture content, the treatment efficiency can also be increased by tuning the parameters of the plasma power supply. Elevating voltage and frequency enhances the power density and the formation of reactive species in the discharge, finally improving microbial inactivation. At the same time, however, the gas temperature in the discharge is increased.

Closer investigations of the predominant inactivation mechanisms in our experiments demonstrated, that thermal stress did not affect the viability of microorganisms and neither did the mechanical stress from the vibrating table and electrical stress from the electromagnetic field. As a consequence, the sterilizing effect could be clearly attributed to the action of plasma-generated reactive species and chemical sputtering was assumed to be the predominant mechanisms.

Besides the decontamination of seeds it is of central importance not to deteriorate their functional properties. Thus, the influence of plasma treatment on the germination ability of alfalfa seeds was tested. While harsh treatments (high power input, long treatment times) considerably reduces the germination probability, mild treatment could even increase germination. In conclusion, the results from this study inspire confidence that adequate plasma treatment conditions may be established that efficiently reduce microbial contamination on sprout seeds and at the same time even improve their germination and growth potential.
IMPROVEMENT OF FUNCTIONALITY OF WATER BY PLASMA DISCHARGE

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Abstract

Plasma in water has been applied for water treatments, sanitation and medical treatments with UV emission, shock waves, electrical charges and reactive chemical species, such as hydroxyl radical, atomic oxygen, and hydrogen peroxide, which are effective agents against biological/chemical pollutants [1]. There are three types of discharges in water: a primary streamer, a secondary streamer and a spark for a positive polarity. Streamers in water are pre-breakdown phenomena, observed as filamentary luminescence accompanying generation of gas channels due to plasma generation from the tip of a needle electrode. When streamers get to the opposite electrode, they are transferred to a spark which flows a larger amount of electrical current than those of streamers. In addition, spark discharges in water generate heat due to Joule heating. Thus, in general, streamers in water are used for low-temperature treatments such as biological and medical applications and spark and arc discharges are used for material processes and decomposition of persistent substances.

Secondary streamers are characterized by a filamentary structure having a few branches with intense light and the propagation velocity of about 25 - 30 km/s and accompany shock waves with an amplitude of 2-3 GPa. A local electric field above 20 MV/cm is required to generate secondary streamer propagation. Therefore, the streamers have potential to improve quality of water even under low-temperature treatments [2, 3].

The aim of the present study is to clarify the interaction mechanism between the discharge characteristics and the water quality change by using ultrapure water and the plasma in water induced by pulsed high-voltage discharge focusing on the electrode polarity. In this study, such interaction mechanism was clarified by using a plasma in water focusing on the electrode polarity. The positive electrode polarity is more suitable for producing hydrogen gas and hydrogen peroxide than the negative electrode polarity, though the energy consumption during the discharge period is slightly lower in the positive case than in the negative case. The positive discharge in water significantly decreased the ORP of ultrapure water down although the pH value was stable [4].

This study was partly supported by the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research, and by a Collaborative Research Project of the Institute of Fluid Science, Tohoku University. We wish to thank Tomoki Nakajima, Tohoku University, Japan for his technical support.

References

SPARK PLASMA PROCESSING OF ANAEROBIC DIGESTION EFFLUENT FOR IMPROVED PROCESS CONDITION MANAGEMENT

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Anaerobic digestion (AD) is a process in which bacteria is used to decompose manure to yield biogas and is in practice worldwide to sustainably produce bioenergy from organic waste. The produced biogas can be used as fuel for purposes on site such as electric generation or heating, making this process particularly of interest for dairy and poultry farms. As AD is a complex microbial process that requires careful maintenance of process conditions such as nitrogen (N) nutrient management, microbial presence, and foam control. During the AD process, a liquid effluent is generated in the biogas byproduct known as digestate. The resulting liquid effluent is often further processed to generate a concentrated fertilizer for field or greenhouse crops. Manure from dairy and poultry farms has generally been land applied on site as fertilizer and has consequently resulted in issues from N nutrient imbalance such as ammonia toxicity and eutrophication as well as an increased risk of bacterial contamination. Therefore, the removal and monitor of biological ammonia and coliform growth in AD liquid effluent is crucial in order for safe onsite reuse. A spark plasma test bench shown in Figure 1 was used to investigate the ability of a spark discharge pulsed directly in liquid effluent could address any of the current concerns with effluent processing.

For nutrient management, current commercial units utilize heated aeration processes which releases the supersaturated carbon dioxide (CO₂) produced during AD processing in order to shift chemical equilibriums to favor generation of hydroxide (OH⁻) and release of dissolved ammonia. This, however, does not affect the remaining nitrogen species present in the liquid effluent. Preliminary results show that when spark plasma treatment can increase the amount of ammoniacal nitrogen (NH₃-N) by up to 20% without disrupting the supersaturated CO₂ concentration while utilizing requiring minimal energy costs of less than 0.01kWh/gal. This has potential benefits for processes that already utilize a heated aeration process by converting additional nitrogen compounds (i.e. organic, nitrate-nitrogen) to free ammonia for removal through a cost-efficient method. Spark plasma treatment also has potential operational benefits with its ability to degrade organic molecules at certain pulse parameters. Preliminary results have demonstrated this characteristic to show potential benefits in two operational concerns: coliform growth and excessive foaming. Excessive foaming is a commonly problematic for AD systems because at high levels, foam can interfere with pipe flow and cause overflow in tanks. In addition, the ability to eliminate coliform growth in liquid effluent would broaden the scope of applications for its reuse. A sample pulse characterization in which energy calculations are based on is shown in Figure 2 in which current and voltage measurements are taken with a Pearson 411 and Tektronix P6015A, respectively, and determined for the experimental flow rate of effluent and pulse rate.
Nitrogen Plasma-Enhanced Biomass Gasification Char for Advanced Nano-Fertilizer Applications

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ABSTRACT

Development and demonstration of a system for processing biomass-derived char utilizing a nano-scale nitrogen delivery system via plasma nitrogen adsorption is discussed. This value-added feature would provide another pathway to further improve upon other potential benefits of char residue as an agricultural soil amendment and carbon sequestering material. Developmental objectives that support this goal include: identifying and characterizing gasified biomass char suitable for plasma nitrogen enhancement, demonstrating a plasma, nano-char processing method, documenting nitrogen retention as a function of processing parameters, developing a model for plant uptake mechanisms of the nitrogen-enhanced bio char, and planning a program for uptake demonstration via plant growth testing. A discussion will be presented by which Phoenix Solutions Co will utilize its plasma hardware experience and nano-materials processing expertise to demonstrate and quantify how biomass gasification char can be fortified by monatomic nitrogen.

It is postulated that nitrogen-doped, nano-scale char particles will be utilized by naturally-occurring, nitrogen-fixing micro organisms. It is hypothesized that N-doped char will provide a more efficient, less energy intensive form of nitrogen for microbes to fix into organic nitrogen for plant uptake. Development of N-doped carbon nanoparticles produced from plasma generated, biomass-derived char may address industry priorities for improved economic and environmental sustainability of agriculture, including: soil conditioning, mineral recapturing, carbon sequestering and biomass-to-energy sustainability.

As biomass waste-to-energy gasification installations contribute to a world-wide commitment to reduce fossil fuel consumption and to move toward a more carbon-neutral economy, the thermal plasma generated, gasification char, transformed to an advanced fertilizer product from post-gasification, plasma nitrogen fortification, may provide support for global agricultural resource stewardship.
Improving Organic Fertilizer and Nitrogen Use Efficiency via Air Plasma and Distributed Renewable Energy

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Abstract

Synthesis of reactive forms of nitrogen such as ammonia is one key to modern agricultural productivity but present agricultural technology utilizes reactive nitrogen inefficiently, leading to numerous and growing environmental problems. Animal, human and food waste all contain significant quantities of organic nitrogen that are transformed into ammonia (NH₃) by bacterial degradation of the organic waste. If not captured, this volatile form of reactive nitrogen is lost to the environment, thus reducing the N-content and therefore the agricultural value of the organic waste. Furthermore, ammonia loss to the environment initiates a cascade of environmental problems. Non-equilibrium air plasma technology creates reactive nitrogen that can be readily converted to dilute aqueous nitric acid solutions. If mixed with decaying organic waste, NH₃ loss is greatly reduced via the formation of involatile ammonium nitrate, a potent nitrogen fertilizer. Air plasma technology for fixed nitrogen manufacture is currently limited only by the availability of electricity and the energy efficiency of the process. The price of electricity via distributed, renewable routes such as solar photovoltaic or wind turbines is rapidly dropping. Increasingly inexpensive wind and solar power sources, coupled with recent advances in air plasma energy efficiency, suggest this technology could play a significant role in improving nitrogen utilization efficiency and reducing the environmental and other threats associated with the current system.
Abstract

It has been recently reported that plasma can enhance agricultural productivity because it increases plant germination and protects seed from fungi and bacteria. In order to understand these, the chemical composition and characterization of plasma-treated water (or solutions) need to be thoroughly studied. We intend to report our studies on plasma chemistries in solutions and chemical species that are generated in water or solutions treated with plasma. A variety of water-containing solutions with various organic compounds, including sugar, amino acids, lipids, and pure water have been tested. We also report the determination of the chemicals in water treated by dielectric barrier discharge (DBD) plasma technique by a variety of methods, including UV spectroscopy, \(^1\)H NMR. Raman spectroscopy, Electron Spin Resonance, and Mass spectroscopy experiments.
Delivery of Non-Thermal Plasma Activated Mist and its Applications
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Non-thermal plasma (NTP) has been shown to be an effective agent in killing a number of vegetative bacterial species as well as spores of Bacillus and Clostridium species. We have developed a method to deliver NTP antimicrobial elements via plasma activated mist (PAM). The reactive oxygen species (ROS) synthesized in NTP can be transferred to water droplets in fractions of a second. The antimicrobial properties of this plasma activated mist (PAM) paired with the xerographic characteristics of the charged droplets result in uniform coverage independent of droplet size with a 5-log kill of microbes.

Our delivery device is comprised of three stages: (1) an atomizer vaporizes liquid water into a fine mist, (2) the droplets are then infused with antimicrobial ROS as they pass through a dielectric barrier discharge (DBD) plasma, and (3) the resultant PAM is evenly deposited onto the targeted surface. The device was originally envisioned and developed for hand sanitization in the hospital setting to address shortcomings of the currently available options: soap and water (time consuming, potential for skin irritation) or alcohol gels (failure to kill spores of Clostridium difficile). The rough prototype presented at ICPM 5 demonstrated satisfactory proof of concept as an effective delivery system for PAM, but further refinements are being contemplated. The device could be adapted to service a broad array of applications requiring pathogen control, including food sanitation where the microbes of concern are essentially the same as those posing a challenge for hand hygiene. Indirect (medium-based) NTP offers a centralized reaction chamber to synthesize PAM by bringing each droplet to its floating potential in a single location. This localized central plasma source can reduce costs by minimizing the surface area of plasma treatment in comparison to direct NTP systems. NTP treated mist, on the other hand, can be utilized dynamically in a variety of deposition setups, offering a modular alternative to direct plasma treatment systems. Although promising, there are a number of remaining challenges presented by this technology, including delivery efficacy of the PAM to the deposition components, maximizing atomization volume, and avoiding premature condensation of the PAM.

**Keywords:** non-thermal plasma; agriculture; surface decontamination; plant growth