

Sterilization of Food Contacting Surfaces via Non-Thermal Plasma Treatment: A Model Study with *Escherichia coli*-Contaminated Stainless Steel and Polyethylene Surfaces

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Abstract This study focuses on the utilization of glow discharge technique for the reduction of microorganisms on food contacting surfaces to determine whether non-thermal, low-pressure plasma could provide an effective alternative to current sterilization methods. Radio frequency (13.6 MHz) plasma environment was developed and tested for the inactivation of *E. coli* K12. Different plasma parameters (discharge power 0–100 W, exposure time 0–30 min) and selected gases (nitrogen, oxygen, air, water vapor) were tested. Following plasma treatment, survival curves and *D* values were determined. Contact angle measurements were performed to state the change of surface hydrophilicity. Determinations of structural changes on microorganisms were accomplished by atomic force microscopy (AFM) and transmission electron microscopy (TEM). Improved bacterial inactivation efficiency was achieved when air was used instead of pure oxygen or nitrogen gases. Water vapor was found to be the most effective (approximately 7 log₁₀ reduction) agent in destruction of the microorganisms. The results showed that surface topography and hydrophilicity also have an effect on the efficiency of plasma treatment. In this study, the *E. coli* inoculated on polyethylene surfaces showed more resistance to plasma treatment. Fragmentation of bacterial cell wall and leakage of cytoplasmic matter were observed following plasma experiments. This

study demonstrates that plasma is a promising technology for sterilization of food contacting surfaces, because of its safety, easy handling, capability of processing at low-temperature (<44 °C), relatively rapid sterilization.

Keywords Non-thermal sterilization · Low pressure plasma · Survival curve of *E. coli* · Food contacting surfaces · Water vapor plasma

Introduction

Sterilization is a physical or chemical process that eliminates or kills all forms of life especially microorganisms present on a surface or contained in a fluid such as biological culture media (Moisan et al. 2002). It is a key process for food processing and medical industries. Harmful microorganisms can cause detrimental effects such as spoilage or disease, consequently leading to economic losses. Inactivation of these microorganisms can be accomplished by conventional techniques such as heat, steam, chemical solutions or gases, radiation as well as recently developed techniques such as pulsed electric field, high hydrostatic pressure, ultrasound, pulsed light etc. However, most of these sterilization methods can cause damages to the material or limit complete sterilization (Park et al. 2007). This opens new research areas for development of alternative sterilization methods (Lee et al. 2006). Plasma sterilization is considered to be a promising alternative to conventional sterilization methods. It is a versatile, fast and efficient method that protects and conserves especially polymeric food packaging materials such as films, bottles and lids from microorganisms without affecting their bulk properties (Sen et al. 2011a, b).

Plasma, a quasi-neutral gas, is referred to as the fourth state of matter. It contains a wide variety of active particles, such as electrons, ions, radicals, metastable excited species, and vacuum ultraviolet radiation that have sufficient energy

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to break covalent bonds and initiate some reactions and form volatile compounds (Şen et al. 2012). Practically, active species disappear immediately once the plasma power is turned off; therefore plasma sterilization is environmentally safe and can be fulfilling all ecological standards (Lerouge et al. 2000; Şen et al. 2012). There are also some limitations of plasma sterilization technique such as weak penetrating power of plasma species and vacuum costs of pump. However, modifying this technology to the industrial applications can solve these limitations. For example, generating plasma inside a package containing the material to be sterilized and using atmospheric type plasma systems (Lerouge et al. 2001).

Cell inactivation factors using plasma for sterilization include UV radiation and a lot of reactive species. UV radiation induces the formation of thymine dimers in the DNA with the possible cause of inhibition of bacteria's replication ability (Laroussi et al. 2000). Microorganism inactivation by means of chemically reactive species generated in plasmas has been discussed in many studies (Moreau et al. 2000; Moisan et al. 2001; Philip et al. 2002). Reactive oxygen and nitrogen-based species (ROS and RNS) can be produced during non-thermal plasmas. They have strong oxidative effects (especially OH^- radicals) and are capable of damaging many organic molecules including nucleic acids, lipids and amino acids and cause erosion of the outer structures of cells leading to death (Meichsner et al. 2008). Charged particles also have significant role with the etching effect on the outer membrane of cells. Charge accumulation on the outer surface of the membranes generates electrostatic forces that disrupt the cell membrane (Mendis et al. 2000).

Pelletier (1993) assumed that microorganisms are biological macromolecules consisting of C, H, O, and N elements such as synthetic polymers and their surface can be degraded by forming volatile compounds. Carbon is not a self-associating element and therefore is not capable of forming volatile molecules. It requires other atoms for such a process, so that it can detach from surfaces. The volatile by-products having carbon in their structure include CO_2 , CH_4 , C_2N_2 , H_2O , CH_x . The main mechanism of plasma sterilization involves attacking the microorganisms' cell wall, and then disposing the remains through gas pumping (Moisan et al. 2002).

In addition to capability of killing bacteria, spores and viruses, it can be used to inactivate infectious agents such as the prion protein responsible for bovine spongiform encephalopathy (BSE), also known as "mad cow disease" (Moisan et al. 2000). NASA also investigated applicability of plasma for sterilization of life-searching probes of spacecrafts on planetary missions (Cooper et al. 2007).

Plasma sterilization is performed by using non-toxic gases or gases that already had biocidal/germicidal effect before plasma state starts. Commercial plasma assisted sterilization systems use strong oxidizers such as hydrogen peroxide and peracetic acid. These chemicals may induce surface

oxidation and leave potentially toxic residues or reaction products on surfaces. In the plasma-based sterilization systems the gas or gases involved have no biocidal effects unless they are activated by the electric field of the discharge. These types of systems are very appropriate for using in food industry (Lerouge et al. 2002).

In the present study, non-toxic gases (N_2 , O_2 , air, water vapor) have been used for determining the efficiency of sterilization. To investigate plasma sterilization under practical conditions, polyethylene (PE) and stainless steel type 316 (SS-316) were taken as substrates and *Escherichia coli* K 12 as a model microorganism. The efficiency of plasma treatment on test microorganism was expressed in terms of *D* value. Surface characteristics of test surfaces such as hydrophilicity, which influence the cell-surface interaction, were tested by contact angle measurements. The sterilization results were supported by AFM and TEM images.

Materials and Methods

Materials and Reagents

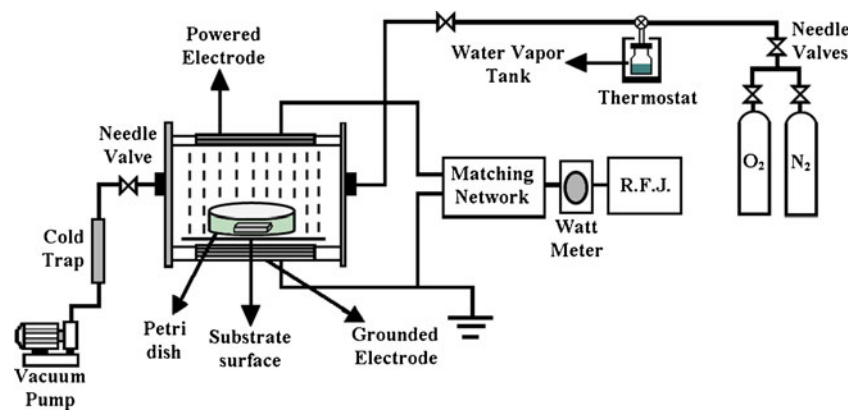
Substrate Surfaces, Gas Feed Composition, and Microorganisms

Polyethylene foil samples with a thickness of 22 μm , a relevant polymer for food packaging, and SS-316 type stainless steel sheets with a thickness of 2 mm, a relevant metal for industrial equipments, were chosen as test samples. They were cut into rectangular shapes with sizes of 10×20 mm for plasma experiments. First, the coupons were put through an ultrasonic bath (Elma, 182 Ultrasonic LC 30H, Germany) with acetone following ethanol (70 % *v/v*) and finally deionized water sequentially. Each incubation process was 5 min. After the cleaning cycle, sanitized coupons were dried in an incubator (Memmert 854 Schwabach W, Type 540,187 Germany) at 80 °C for 1 h under sterile condition. Commercial oxygen, nitrogen, air (Oksan Kollektif Co., Ankara, Turkey), and sterile distilled water vapor were used for plasma generation, separately. *E. coli* K 12 used in the current study was obtained from Refik Saydam National Public Health Agency (RSKK NO: 330 Ankara, Turkey).

Sterilization Apparatus

The RF plasma system shown in Fig. 1 was used for sterilization of test samples. PICO-type RF plasma equipment was supplied from Diener Electronics GmbH + Co. (Germany). The plasma chamber was stainless steel (150 mm radius and 320 mm length). A 13.6-MHz radio frequency generator (power range, 0–100 W) was used to sustain the plasma in the reactor. Plasma processes were

Fig. 1 Low pressure plasma system apparatus



generated in the fully enclosed and semi-automatic system. At plasma generation, low pressure was created in a recipient by means of a vacuum pump (Trivac, Germany).

Preparation of Microorganisms and Contamination of Surfaces (Adhesion Studies)

E. coli were grown on Brain Heart Infusion (BHI) broth medium (Merck, Germany) with suspension of 10^9 cfu/ml at 37 °C for 24 h. Microorganisms were inoculated on sterile SS-316 and PE sheets (spot contamination) that were in placed on a glass Petri plates and then dried under appropriate conditions (37 °C for 3 h) that did not affect cell viability.

Plasma Treatment

After drying procedure, the microorganism-inoculated test samples were exposed directly to the plasma. In order to test vacuum effect on bacteria in plasma chamber, microorganisms were kept in chamber without plasma generation and there was no effect observed. At a pressure of approximately 0.1 mbar, sterilization gases (air, O₂, N₂) were fed into the chamber, separately, and were allowed to flow at a specific rate (allowed pressure difference was 0.1–0.3 mbar). When the water vapor (H₂O) is used in plasma sterilization procedure, degassing is performed. For this purpose, 20 ml of sterile distilled water containing flask was attached to the monomer inlet. Monomer flask was chilled with liquid nitrogen to degas the monomer flask until the systems reached to its initial pressure (0.1 mbar). During the degassing process, the system was kept under vacuum for 10 min. After defrosting, water boiled to produce water vapor. Valve was opened and water vapor was allowed to flow at a 0.25-mbar pressure into the chamber until the relative humidity was ≥ 95 %. Then, RF power and exposed time were adjusted, and the microorganism-contaminated surfaces were exposed to glow discharge at 25, 75, and 100 W power for 10, 15, 20, 25, and 30 min. At the end of the process, RF generator was turned off automatically, and gas inlet was closed manually. The plasma system was fed with argon gas for 10 min to quench the potential radicals,

which might be formed during plasma. Finally, it was subjected to 0.1-mbar vacuum pressure for 15 min. Argon feeding and vacuum conditions were applied to deactivate remaining free radicals in the plasma atmosphere. A watt-meter is employed to indicate the power transmitted to the gas while an impedance matching network enables the operator to minimize the power reflected, toward the power generator, from the RF field applicator. The reflected power was minimized as 0–1 W for all experiments. The distance between the powered electrode and the top of the substrate surface was 4 cm. Temperature and humidity measurements were carried out by using a digital hygro-thermometer (TH-01 type, COTRONIC TECH., China).

Evaluation of the Survivors

Following plasma treatment, each test plates were placed into tubes for evaluating of the survivor microorganisms. Microorganisms prepared with the same initial microbial load but not exposed to plasma were used as control of the initial load of microorganisms. The control microorganisms were kept at 4 °C in a cooler to inhibit their growth, while experimental microorganisms had been exposed to plasma treatment. Survivors of the plasma exposed and control microorganisms were recovered by plunging the samples in 10 ml of 0.1 % (v/v) Tween 80 (Sigma, Germany) and 0.9 % (w/v) sodium chloride (Merck, Germany). Then the tubes were vortexed for 4 min at room temperature and 1 ml of the suspension was inoculated to Brain Heart Infusion (BHI) agar (Merck, Germany) from the appropriate dilutions and incubated at 37 °C for 24 h. At the end of the incubation period, all resulting colonies were counted as colony forming units (cfu). Figure 2 shows illustration of the experiments schematically in detail. Based on a dilution series of the resulting microorganism suspension, the number of colony forming units per test sample (cfu/sample) was estimated and the detection limit of this method was 10 cfu/sample. All tests were carried out at room temperature and kinetics of destruction for each was determined on at least three independently grown cultures. The data presented are the means of decimal logarithmic values \pm standard deviations.

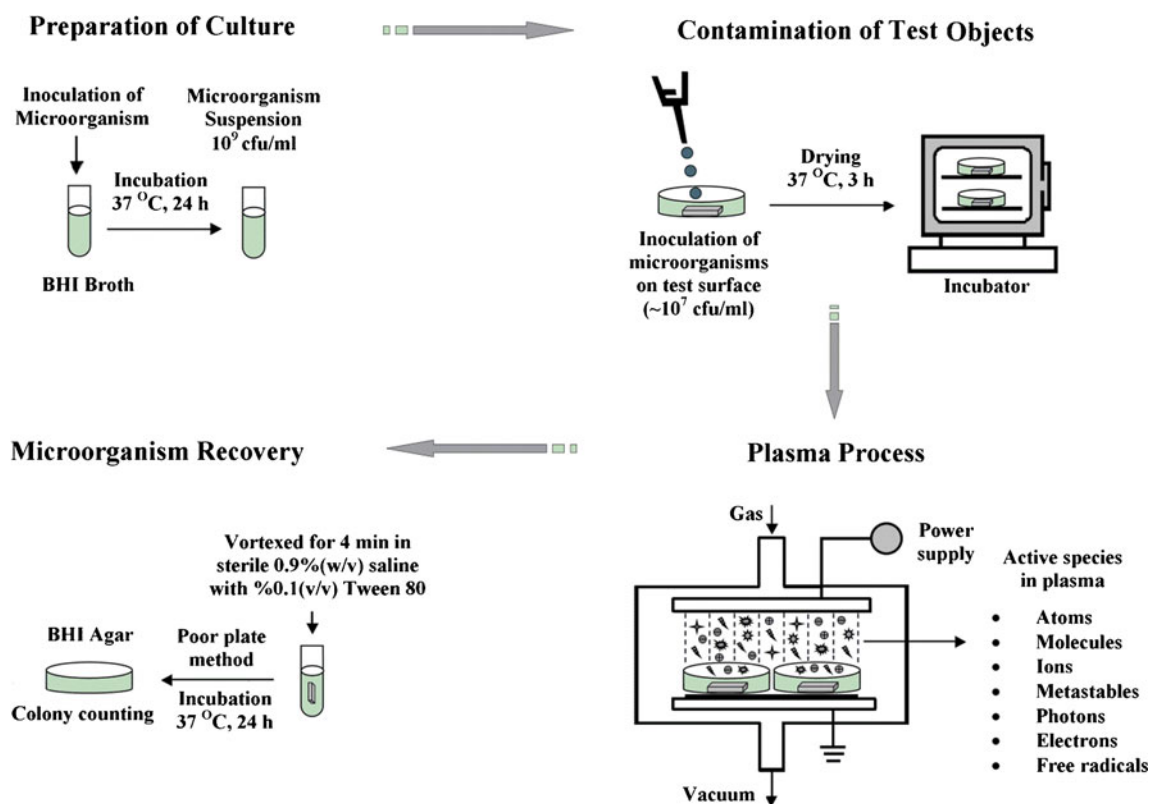


Fig. 2 Schematic illustration of the experiments

Determining of Survival Curves, Calculation of *D* Values

Survival curves were determined by plotting of the number of colony forming units per unit sample (cfu/sample) versus treatment time. The *D* value for *E. coli* K12 was obtained by averaging the numbers obtained at various initial concentrations of cells. All experiments were repeated three times and data are presented as the mean log (cfu/sample) value.

Contact Angle Measurements

In this study, the sessile drop technique was used for contact angle measurements. A dedicated microscope (QX3 computer microscope, 60X, Intel, Santa Clara, CA) and software (Wettability Pro Classic, version 2.0.0, Prague, Czech Republic) were used to measure the contact angles. The inner contact angles between the curvature surface of water drop and the solid surface were measured.

Atomic Force Microscopy

AFM studies were performed (Ambios-Quesant Q-Scope Universal SPM with AFM attachment, California, USA) in tapping mode due to its three-dimensional imaging capabilities. Preparation of surface for AFM imaging protocol is adapted from (Peng et al. 2004).

Transmission Electron Microscopy (TEM)

Transmission electron microscopy was used as a complementary technique to the AFM. It can be easy to examine sections of the treated bacteria. TEM imaging of the microorganisms was performed using (Jeol JEM 1200 EX, Tokyo, Japan). TEM imaging protocol used in this study is described in details elsewhere (Aldur et al. 2002).

Statistical Analysis

The results are presented as the mean (\pm SD) of experimental data obtained from three independently grown cultures. Statistical analysis was performed with SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) for analysis of variance. Differences in mean values among treatments were determined by one-way ANOVA followed by Duncan's test and confidence level of 0.05 was considered significant.

Results and Discussion

The goal of our work was to specify the decontamination conditions of low-pressure plasma for the bioindicator *E. coli* on test surfaces (PE and SS-316). The standard pour

plate method was used to evaluate the inactivation effect of various operating conditions such as working gas, plasma power and exposition time.

Temperature Distribution

Each gas discharge plasmas induced from different working gases in low pressure showed a low temperature distribution. Although the temperature distribution depended on the composition of the gas feed and applying power, there were no significant differences ($\pm 5\text{ }^{\circ}\text{C}$) observed among the temperatures.

Initial temperatures of plasma chamber were increased and monitored to simulate unwanted environmental temperature conditions. The highest temperature just below $45\text{ }^{\circ}\text{C}$ was found for $33\text{ }^{\circ}\text{C}$ initial temperature with 100 W applied

power. Increasing the temperature gradually provides protection of heat sensitive materials from sudden surface temperature changes.

Survival Curves and *D* Values

In general, there are three phases and three basic mechanisms that play a role in plasma sterilization: Destruction of DNA by UV irradiation, erosion of the microorganism through intrinsic photo-desorption, and etching. All plasma treatment combinations in this study significantly reduced the surviving microorganisms, relative to the untreated control. In general, higher plasma power resulted in greater inactivation at shorter times ($p \leq 0.05$). For the lowest plasma power, each successively longer treatment yielded significant additional increases in pathogen inactivation. Although

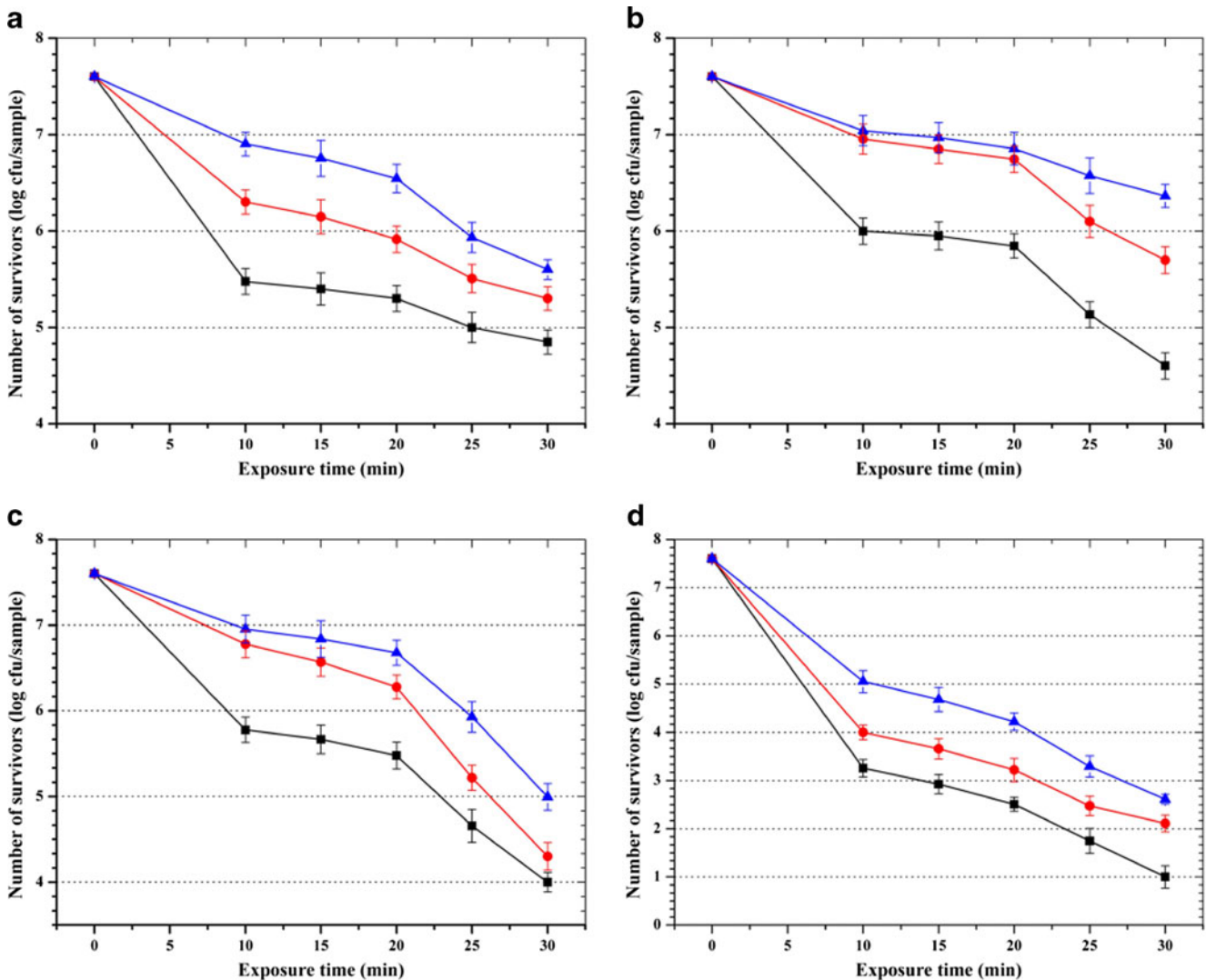


Fig. 3 Survival curves of *E. coli* K12 cells ($\sim 10^7$ cfu/ml) seeded on PE samples and exposed to plasma: **a** N₂, **b** O₂, **c** air, and **d** water vapor. (—■— 100 W, —●— 75 W, —▲— 25 W)

there was a difference at D values of SS-316 and PE surfaces at 100 W plasma parameters, these differences were not statistically significant ($p > 0.05$).

Figure 3 and 4, shows that survival curves of microorganisms do not form a complete linear curve, but instead comprises three linear segments. To characterize the slope of each segment, which is referred as inactivation phases, we use the D values. Survival curves shown in Fig. 3 and 4 are particularly noteworthy. D_2 and D_3 values were calculated from interpolated trend-lines between 10th–15th–20th minutes and 20th–25th–30th minutes data by using linear least-square method. (Laroussi 2005); Moisan et al. (2002, 2001)) also reported the same three phasic mechanisms. It can be clearly seen that pure N_2 and O_2 caused approximately 3 \log_{10} , air 4 \log_{10} and water vapor 7 \log_{10} reductions. The highest bacterial mortality was obtained at 100 W—30 min plasma parameters when water vapor was used as a carrier gas; where no cultivable cells could be detected ($p \leq 0.05$).

As already seen from Table 1, the first step of inactivation kinetics generally has the smallest D values (fastest kinetics) and second ones have the largest (slowest kinetics). Air and water vapor have small D_1 and D_3 values because they have a potential to produce radicals especially hydroxyl radicals. Water vapor is the most effective gas to inactivate *E. coli* cells. Probability of producing hydroxyl free radicals, which have a strong sporicidal effect and a relatively long life, was very high when water vapor was used. Purevdorj et al. (2003), Muranyi et al. (2008), and Volkova et al. (1984) mentioned that moistening the atmosphere enhances inactivation efficiency.

Water molecules present in the plasma chamber are generally broken down into two excited radicals, H^{\cdot} and OH^{\cdot} :



However, if there is not enough energy to excite all molecules in the plasma chamber, some molecules, especially

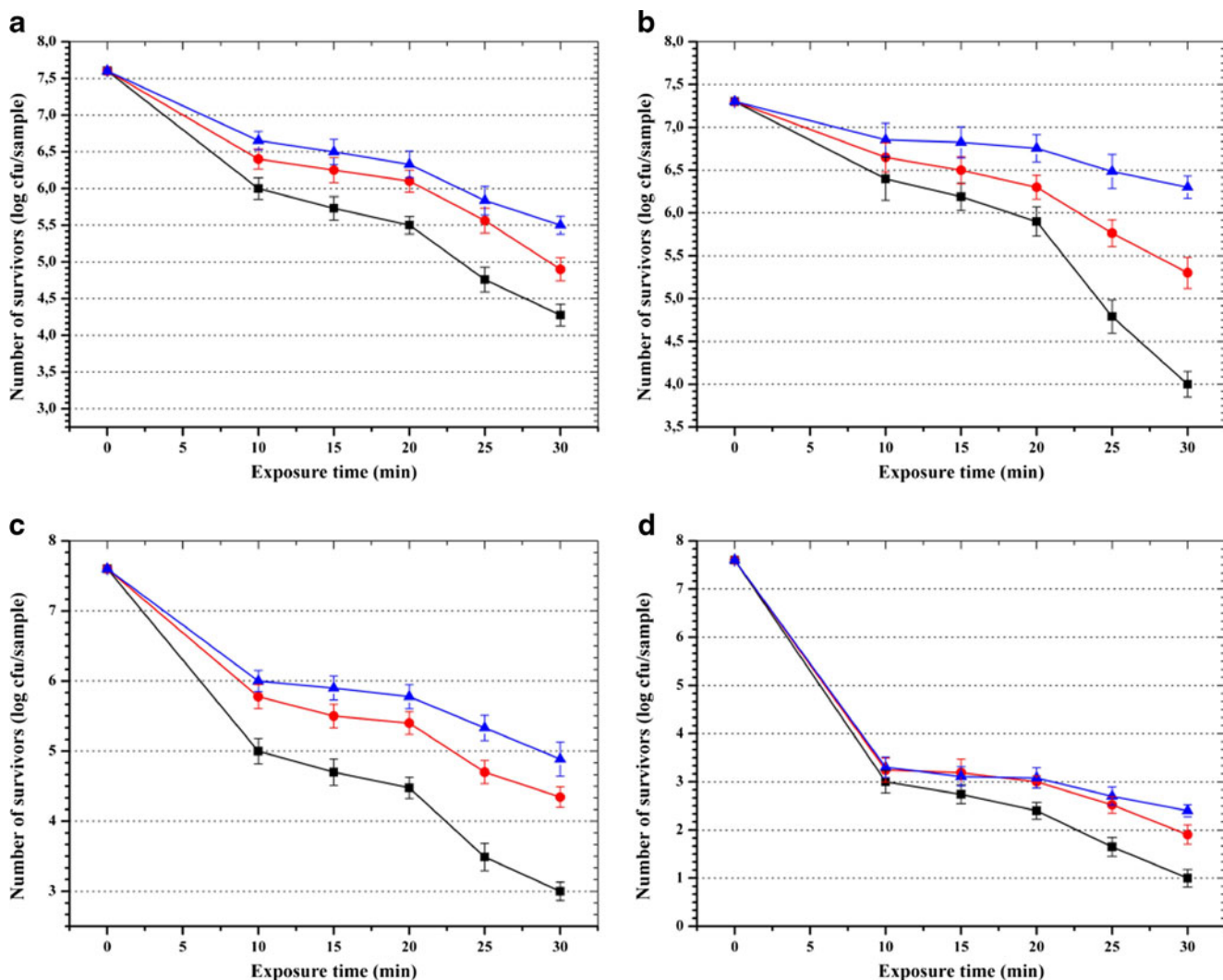


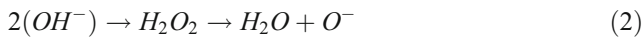
Fig. 4 Survival curves of *E. coli* K12 cells ($\sim 10^7$ cfu/ml) seeded on SS-316 samples and exposed to plasma: **a** N_2 , **b** O_2 , **c** air, and **d** water vapor. (—■— 100 W, —●— 75 W, —▲— 25 W)

Table 1 D values of *E. coli* under different plasma parameters^a

Inactivation gas			Oxygen (O ₂)			Nitrogen (N ₂)			Air (O ₂ + N ₂)			Water-Vapor (H ₂ O)		
D-value (min)			D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃
PE samples	Power (Watt)	25	105.9 ±36.2	291.1 ±149.6	107.1 ±98.0	85.0 ±19.4	149.1 ±73.5	52.4 ±11.0	91.7 ±29.6	194.4 ±85.7	30.5 ±5.4	23.3 ±2.5	39.4 ±19.7	19.4 ±2.8
		75	91.7 ±26.1	256.4 ±129.7	49.1 ±12.9	45.7 ±5.2	123.6 ±62.1	71.6 ±36.1	72.1 ±17.9	104.5 ±53.4	23.9 ±3.3	16.5 ±0.8	32.6 ±15.6	18.6 ±7.2
		100	37.1 ±3.4	290.8 ±150.6	34.9 ±6.8	27.9 ±2.5	229.1 ±115.1	85.3 ±43.5	32.5 ±3.6	143.3 ±72.1	27.1 ±4.7	13.7 ±0.7	27.2 ±13.2	9.5 ±1.8
SS-316 samples	Power (Watt)	25	126.9 ±44.2	532.8 ±121.4	113.8 ±68.9	62.6 ±10.5	157.6 ±75.4	57.6 ±25.4	37.1 ±4.5	201.9 ±101.4	48.3 ±27.5	13.8 ±0.7	92.2 ±48.1	27.3 ±13.8
		75	91.0 ±9.7	146.7 ±19.7	47.6 ±11.9	49.5 ±6.9	160.9 ±81.5	38.4 ±9.6	32.6 ±3.5	113.4 ±57.1	36.9 ±10.7	13.6 ±0.9	83.9 ±42.3	16.6 ±4.2
		100	62.8 ±23.6	97.6 ±49.3	22.9 ±3.3	37.1 ±4.2	90.1 ±46.6	32.8 ±6.7	22.8 ±1.8	68.6 ±34.8	20.7 ±3.3	12.9 ±0.7	30.1 ±15.2	9.5 ±1.7

^a Average and standard deviation of three independent experiments

radicals, cannot be excited and Eq. 1 never occurs. So; hydrogen peroxide molecules (H₂O₂) and atomic oxygen can be produced from unexcited OH⁻ radicals (Eq. 2):



This mechanism was also reported by Purevdorj et al. (2003) and Muranyi et al. (2008). From these experimental outcomes, the observed phases are attributed the following roles.

Phases 1 and 3 have generally the same D value and these are shorter than the D₂ value. The D₂ time shortens (Fig. 2) when nitrogen is added to the oxygen gas (pure N₂ and air has a long D₂ time). Phase 2 is very important because kinetics of this phase is limited due to erosion processes, which directly affect the efficiency of phase 3. In phase 2; various materials (organic materials, coats, debris, and dead microorganisms) and still living microorganisms covering the surface are cleaned from surface by means of erosion. Therefore, in phase 3, UV photons finally kill the remaining microorganisms. There is a germicidal wavelength range (220 to 280 nm), which UV is capable of inactivating cells. To obtain such a wavelength range, application dose should be adequate. In order to destruct the DNA material by UV photons, operation pressure must be low enough to reduce photon absorption by emitting gas. Kelly-Winterberg et al., (1998) demonstrated that microorganisms could not significantly be influenced by UV photons in atmospheric pressure plasmas. Because photons emitted in atmospheric pressure are reabsorbed by air, only a few of them affect cells (Moisan et al. 2001).

Vacuum effect on microorganisms was also checked. There was no change on microbial load during applied vacuum in the plasma chamber without generating plasma inside.

Contact Angle Measurements

According to the contact angle values, PE surfaces were determined to be more hydrophobic than SS-316 surfaces. Water vapor and also other inactivation gases used in this work are generally used for hydrophilization of surfaces. Therefore, the decrease in contact angle values was expected after plasma application (Table 2). However PE samples became more hydrophilic following plasma treatment.

Whatever the type of plasma gases used, it can change surface properties of materials and this influence effectiveness of plasma on microorganisms. PE and SS-316 were chosen because they mimic equipment or processing surfaces with different finishes or levels of wear and at the same time represent a good model system for investigating the influence of surface properties on plasma effectiveness in general.

Monitoring the Efficacy of Plasma on Microorganisms

To gain insight into the direct effect of plasma on the morphology of *E. coli* cells, AFM and TEM were used.

Table 2 Contact angle values of plain and plasma processed test surfaces^a

	SS-316	PE
Before plasma treatment	77.3±7.7	85±5.7
100 W—30 min plasma treatment	40±9.8	35±6.2

^a Average and standard deviation of five independent experiments (after 100 W, 30 min water-vapor plasma application)

TEM was not capable of imaging bacteria on SS-316 surfaces. So AFM was used for determining the micro and nano-ranged pits and cracks and morphological changes of bacteria on SS-316 surfaces.

Atomic Force Microscopy Images of *E. coli*

Stacked, clumped, or wrapped microorganisms (Fig. 5a) could affect the penetration of active plasma particles' diffusion. Although SS-316 surfaces used in industrial applications can have a lot of micro-scale cracks where microorganisms can find convenient areas to attach, Fig. 5b showed that there was no problem in sterilizing such kind of surfaces.

Transmission Electron Photomicrographs of *E. coli*

Transmission electron microscopy was used for monitoring the physical damage of plasma-exposed cells. The photomicrographs of plasma exposed *E. coli* cells are shown in Fig. 6. Figure 6a shows normal control cells with no plasma exposure, while Fig. 6b and c shows the effect of 30 min of plasma exposure on the *E. coli* cells. Integrity of bacterial membrane was lost and fragmentation of bacterial cell wall and leakage of cytoplasmic matter to the surrounding medium were observed.

Effects of Plasma on *E. coli* Inoculated PE and SS-316 Surfaces: General Overview

The effectiveness of plasma treatment on *E. coli* K12 that was inoculated on PE and SS-316 surfaces as a function of plasma parameters and applied gases were investigated. Results were graphically displayed and Fig. 7 shows comparison of bacterial mortality using different working gases. The highest

bacterial mortality was obtained at 100 W plasma parameter (Figs. 3 and 4; $p \leq 0.05$). Therefore comparison of bacterial mortality can be easily investigated at this power.

According to the results, that air plasma showed greater bacterial reduction compared to pure N_2 and O_2 plasma. Xu et al., (2007) reported that air-simulated N_2 – O_2 mixture gas discharge plasma more feasible than pure O_2 or pure N_2 discharge plasmas. This enhanced bacterial reduction maybe due to the presence of atomic oxygen and other reactive oxygen and nitrogen species. On the other hand, water-vapor plasma has the best reduction efficiency and kills all the microorganisms on the surfaces. It can be clearly observed that the inactivation of *E. coli* was accomplished with increasing gas humidity (water plasma). This indicates that the bacterial mortality increases approximately up to 3 \log_{10} by increasing the moisture level of air. Consequently, the reactive oxygen species, especially the ones including hydroxyl radicals (OH^\cdot) that were presented in the plasma, is believed to play the dominant role for such bacterial deactivation (Purevdorj et al. 2003). On the other hand, the addition of water to air decreases the value of D_2 by approximately 50 % for SS-316 samples and 80 % for PE samples. In addition to possibility to produce radicals, water vapor also increases the efficiency of etching effect of low-pressure plasma.

Microorganisms inoculated on PE surfaces cannot be inactivated easily compare to SS-316 surfaces. As indicated above, there were two main factors influencing the effectiveness of plasma apart from gas types and power. The first one is surface roughness and cracks leading to the attachment of microorganisms, which hides them from sterilizing agents. Another factor is surface hydrophilicity, which effects the attachment of microorganisms and their aqueous solutions, arranging the distribution of cells (Woodling and Moraru 2005).

Plasma has more effect on microorganisms inoculating on SS-316 surfaces. Due to the high surface roughness and

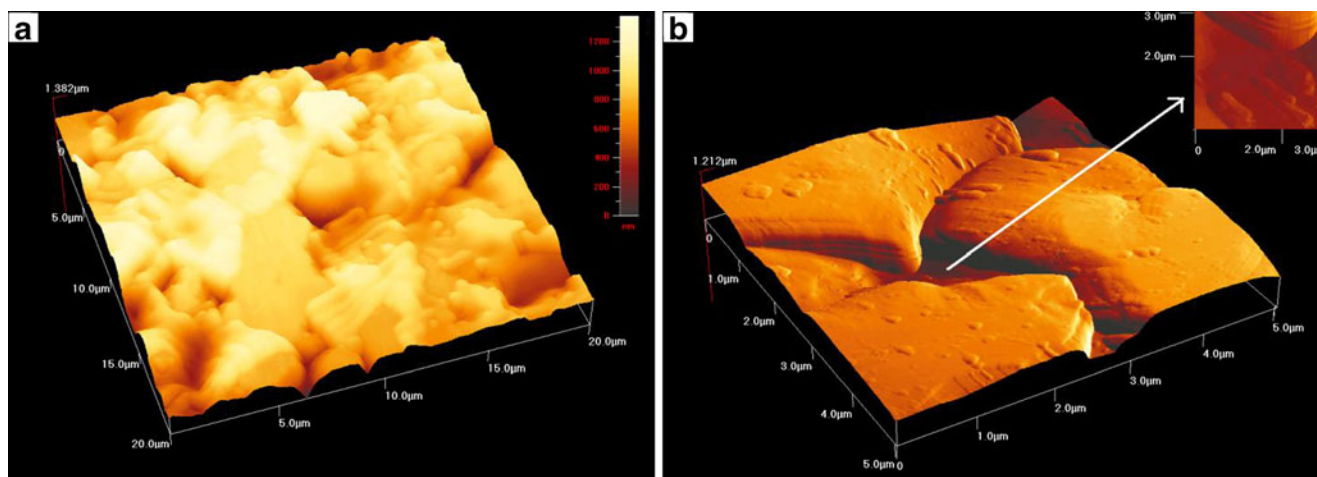


Fig. 5 AFM images of microorganisms: **a** untreated and **b** 100 W—30 min water-vapor plasma treated

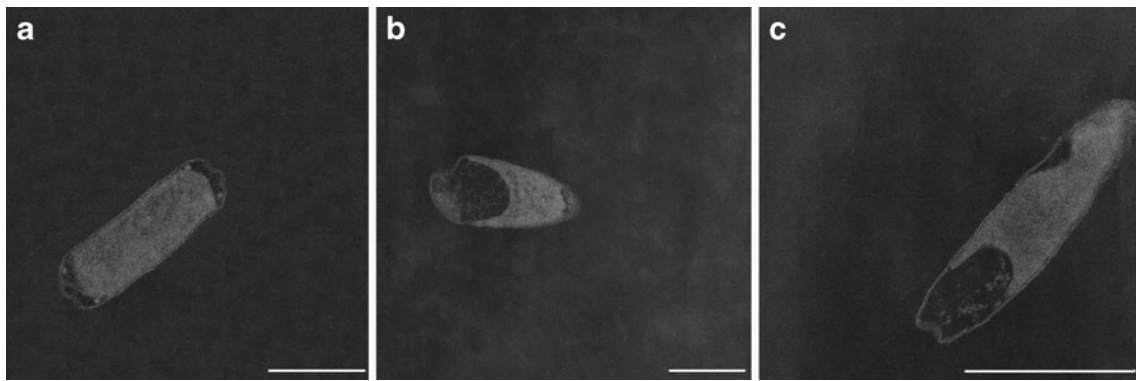


Fig. 6 TEM images of microorganisms: **a** control cells with no plasma exposure; **b, c** 100 W—30 min water-vapor plasma treated *E. coli* cells (*Bar*=1 μm)

cracks, SS-316 samples allows for a relatively uniform 2-dimensional distribution of the cells on the surface favoring homogenous treatment. Because of the smooth and hydrophobic nature of PE, it leads to cell clustering. Woodling and Moraru (2005) also reported that surface topography effected inactivation of microorganisms on stainless steel surfaces via pulsed light application. This results in slower diffusion rates of active species making the penetration difficult to inner layers. Therefore the microorganisms found in the inner part are not easily effected by the sterilization process. In comparison with PE surfaces, hydrophobicity could not effect sterilization process on SS-316 samples due to their low surface contact angles (Table 2).

No deformations or visible material damages of the samples were observed after plasma exposure. Our lab-scale plasma system is generally used in several material processing applications such as surface functionalization. In our previous study, we used this system for preparing bacteria antifouling surfaces to minimize the biofilm formation problem in food industry (Şen et al. 2012). In this study, we demonstrated this kind of commercial plasma systems could

be used as a sterilization apparatus even when non-toxic, natural gases are used. More experiments have to be conducted to evaluate the hypothesis described above, including investigations with langmuir probe, optical emission spectroscopy etc. The sterilization and decontamination studies concerning atmospheric systems are still under investigation by our group and many others worldwide.

Conclusions

The present experimental investigation could clearly show that cold plasma is suitable to inactivate and to eliminate microorganisms from the microstructure of food contacting surfaces at acceptable temperatures (<45 °C) and without affecting the bulk properties of these materials. The “water vapor” as precursor in plasma sterilization of the surfaces has given the most promising results on *E. coli*, which is the most studied food pathogen. When the scale up of the sterilization process for larger equipments of food industry is considered, the economics of the process is pointing out

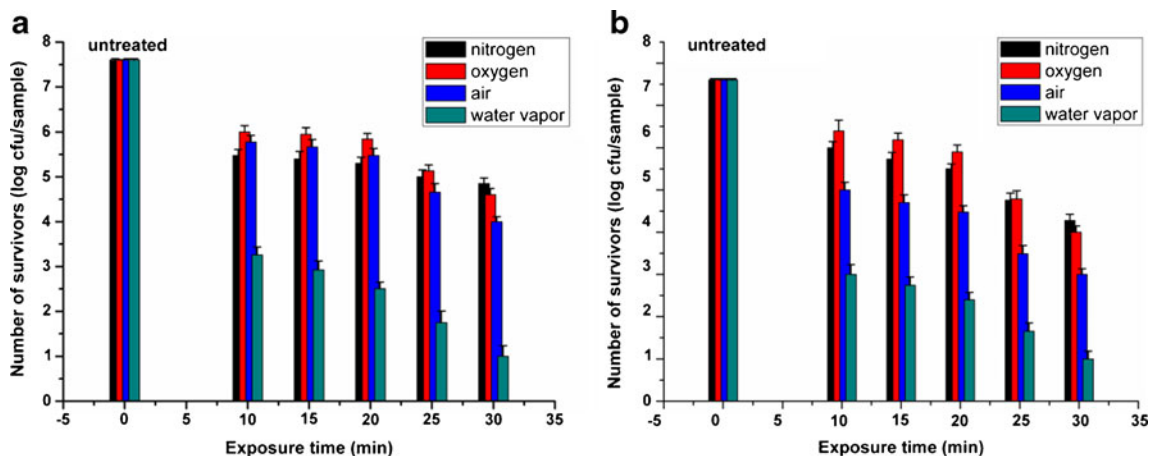


Fig. 7 Comparison of bacterial mortality in (■) nitrogen, (■) oxygen, (■) air, and (■) water-vapor plasmas, after 10, 20, and 30 min of exposure (*P*=100 W, *p*=0.14 mbar); **a** PE surfaces, **b** SS-316 surfaces

the atmospheric pressure plasma systems. Regardless of the type of plasma generating system, plasma techniques can be competitive to other methods.

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