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Atmospheric pressure plasma jet for bacterial decontamination and property improvement of fruit and vegetable processing wastewater

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Abstract

An atmospheric pressure plasma jet was tested for decontaminating and improving the characteristics of wastewater derived from blackberry, date palm, tomato and beetroot processing industries. The jet was generated by blowing argon gas through a cylindrical alumina tube while a high voltage was applied between two electrodes surrounding the tube. Oxygen gas was mixed with argon at the rate of 0.2% and the argon mass flow was fixed at 4.5 slm. Images show that the generated plasma jet penetrated the treated wastewater samples. Plasma emission spectra show the presence of O and OH radicals as well as excited molecular nitrogen and argon. Complete decontamination of wastewater derived from date palm and tomato processing was achieved after 120 and 150 s exposure to the plasma jet, respectively. The bacterial count of wastewater from blackberry and beetroot was reduced by 0.41 and 2.24 $\log_{10}$ colony-forming units (CFU) per ml, respectively, after 180 s. *Escherichia coli* was the most susceptible bacterial species to the cold plasma while *Shigella boydii* had the minimum susceptibility, recording 1.30 and 3.34 $\log_{10}$ CFU ml^{-1}, respectively, as compared to the 7.00 $\log_{10}$ initial count. The chemical oxygen demands of wastewater were improved by 57.5–93.3% after 180 s exposure to the plasma jet being tested. The endotoxins in the wastewater were reduced by up to 90.22%. The variation in plasma effectiveness is probably related to the antioxidant concentration of the different investigated wastewaters.

Keywords: cold plasma jet, wastewater, bacterial decontamination, antioxidants, endotoxins

(Some figures may appear in colour only in the online journal)

1. Introduction

The food-processing industry consumes water to meet its individual daily needs. Most of the water is used in the fruit and vegetable sector for washing and rinsing. During these processes, water is contaminated with a high load of saprophytic and pathogenic organisms as well as hazardous and persistent compounds (Shaw and Schudram 2000). Wastewaters with high quantitative and qualitative pathogenic levels must be decontaminated prior to discharge. Typically, chlorine is used to decontaminate the wastewaters. Ultraviolet (UV) radiation, ozone, and other non-traditional decontamination processes have achieved some acceptable results due to stricter rules on the amount of residual chlorine levels in discharged wastewaters. However, industries are in search of disinfectants that are more efficacious against emerging and common pathogens as well as safe usage in many particular applications of food processing. A non-thermal plasma could represent a potential alternative technique for the disinfection of wastewater compared to the conventional methods.

Plasma is an ionized gas containing ions, electrons and reactive species such as atoms, molecules and radicals (OH, O, NO) as well as energetic photons (e.g. UV) (Moisan *et al* 2001). Plasma has been shown to be a sterilizing agent to inactivate a wide range of microorganisms (Deng *et al* 2005, Laroussi *et al* 2006, Kolb *et al* 2008, Park *et al* 2012, Hao *et al* 2014, El-Sayed *et al* 2015, Ouf *et al* 2015, 2016). Several researchers have demonstrated the application of non-thermal plasma in the food industry. Representative contributions include the application of plasma in the decontamination of grain and legumes infected by fungi (Selcuk *et al* 2008), inactivation of surface-borne microorganisms of *cicer arietinum* seeds (Mitra *et al* 2014), disinfection of packaged liquid food products (Misra *et al* 2012), treatment of ready-to-eat meat (Rød *et al* 2012), decontamination of almonds infected by *Escherichia coli* (Deng *et al* 2005), treatment of contaminated fresh fruit and vegetable slices (Wang *et al* 2012), and decontamination of the fruit pericarps from spoilage and pathogenic microorganisms (Perni *et al* 2008, Mohamed *et al* 2014). Many types of cold atmospheric pressure plasma sources have been established to provide reasonable reactivity with a low gas temperature. These plasmas are easy to set up because they do not require a vacuum system and are capable of running with numerous feeding gases under a wide range of driving powers and frequencies. However, the plasma operating conditions have to be optimized to induce maximum inactivation of the microorganisms and at the same time minimize the damage to the exposed materials (Anderson 1989, Lerouge *et al* 2000).

Atmospheric pressure plasma is not expensive compared to low pressure plasmas used for food processing due to the elimination of a vacuum system. It is feasible for use in food decontamination and water purification since it has the advantage of utilizing non-toxic gas and has no dangerous radiation. The reactive chemical species that are the main plasma antimicrobial effective agents are generated from the operating gas (such as He, N_2, Ar, O_2, Ne) and even from air. Atmospheric pressure plasma is accessible and flexible enough to be used for different shapes, varieties of environments and in continuous decontamination chain processes.

The objective of the present study is to evaluate the possibility of using atmospheric pressure plasma to improve water quality by reducing the chemical oxygen demands and to decontaminate the wastewater of its bacterial load.

2. Materials and method

2.1. Sample collection

Thirty-two samples of wastewater were collected from three sites, Jeddah, Yanbu and Al-Madinah Al-Munawwarah, Saudi Arabia, during May 2012. The samples were collected from plant processing industries of blackberry, date palm, tomato and beetroot. The wastewater samples were collected in pre-sterilized 500 ml amber polypropylene high-density bottles equipped with Teflon-lined polypropylene caps. The samples were transferred into the laboratory immediately after collection to avoid unpredictable changes, then they were stored in a refrigerator at 2 °C until analyzed. The temperature recorded during the sample collection months ranged between 32 and 38 °C. Control samples of water before being used in processing did not contain more than 10^3 CFU ml^{-1} bacteria.

2.2. Physical and chemical characteristics

2.2.1. pH. The pH value of each sample of wastewater, before and after cold plasma treatment, was determined using a pH meter (Ion 510, Bench pH/Ion/mV Meter, Oakton, USA) set directly into each solution. The pH meter, accurate to 0.1, was first calibrated according to the manufacturer's instructions, using buffer standards of pH 4 and pH 7. Twenty milliliters of each wastewater sample were placed in a 150 ml beaker, the pH meter electrode was immersed in the wastewater and the reading was recorded (Cavalcanti *et al* 2008). Inbetween readings, the electrode was washed in distilled water to make sure that no cross-contamination occurred. Each analysis was made in triplicate.

2.2.2. Total suspended solids (TSS). The TSS concentration was measured using a Hach 2100P Turbidimeter (Hach Company, Loveland, CO). The method is based on the theory that light scattering increases with the concentration of particles (Sadar 1998). The data were reported in NTU (nephelometric turbidity units).

2.2.3. Total sugars and proteins. The total proteins in the wastewater samples were determined using the method of Lowry *et al* (Lowry *et al* 1951). Standards of bovine serum albumin ranging in concentration from 0.01 to 1.0 mg ml^{-1} were running simultaneously with the examined samples. The content of total sugars was estimated based on the phenol-sulphuric acid reaction of sugars (Saha and Brewer 1994). Glucose standards ranging in concentration from 0.01 to 0.1 mg ml^{-1} were also tracked using the same method.

2.2.4. Chemical oxygen demands (COD). The examined sample was oxidized under reflux using a certain amount of potassium dichromate in strong sulphuric acid with silver sulphate as a catalyst. Organic matter reduced part of the dichromate and the residual part was estimated by titration with iron (II) ammonium sulphate using ferroin as indicator. Interference from chloride was inhibited by the addition of mercuric sulphate to the reaction mixture. The chemical COD was expressed in mg of oxygen absorbed from standard dichromate per liter of sample (APHA 1992).

2.2.5. Antioxidant determination. The antioxidants were assayed according to the method of ferric reducing antioxidant power (FRAP) described by Benzie and Strain (1999) using an Agilent 8453 Diode Array UV/VIS spectrophotometer, Model G1103A. The method is based on the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at low pH. This reduction is measured using the absorption change at 593 nm. To conduct the assay, a 2.95 ml aliquot of a FRAP reagent, a mixture of 0.1 mol l^{-1} acetate buffer, 10 mmol l^{-1} 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mmol l^{-1} ferric chloride (10:1:1 v/v/v), were combined with 50 μl of the sample. The absorbance was recorded after 30 min incubation at 37 °C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mmol of Fe^{2+} equivalents per liter of sample.

2.3. Bacterial count

A bacterial count in the wastewater was performed, before and after plasma treatment, using the spread plate method. The exposure times of the cold plasma were 30, 60, 90, 120 and 150 s. To count the total number of CFU, 0.1 ml of wastewater samples diluted according to the expected number of colonies was spread on nutrient agar (Oxoid, UK) medium plates. The plates were incubated at 28 °C. The total number of bacterial colonies were counted after 48 h of incubation. Measurements were carried out in triplicate.

A preliminary examination of the investigated wastewater using different specific media showed that the most common bacterial species were *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Campylobacter jejuni* and *Shigella boydii*. The specific solid culture media employed for bacterial recognition were violet red bile lactose agar (Oxoid) for *E. coli*, cetrimide agar (Oxoid) for *P. aeruginosa*, bile esulin azide agar (Difco) for *E. faecalis*, campylobacter blood free selective agar base (modified CCDA-Preston) (Oxoid) for *C. jejuni* and SS agar (Oxoid) for *S. boydii*. The isolated bacterial species were identified by the Oxoid biochemical identification system.

To test the effect of atmospheric pressure cold plasma on the isolated bacterial species, suspensions of each individual bacterial species containing about 10^7 cells ml^{-1} in saline solution were prepared from colonies previously grown on the defined medium for 48 h. An aliquot (2 ml) of the bacterial suspension was transferred to a 2.5 ml capacity Eppendorf tube (Sigma) and exposed to cold plasma for 30, 60, 90, 120 and 150 s. The number of CFU in the bacterial suspension before and after cold plasma treatment was determined by standard plate counting as a reference method and using the spectrophotometric enumeration method (Shin *et al* 2007).

2.4. Endotoxins activity assay

Endotoxin activity was analyzed according to the method previously described by Tsuji *et al* (1984) using chromogenic limulus (*Tachypleus tridentatus*) amebocyte lysate (LAL) endpoint assay kits (Xiamen Houshiji Ltd, China). The wastewater samples were diluted in pyrogen-free diluents based on the expected endotoxin concentrations. The diluted samples were mixed with LAL, incubated for 8 min at 37 °C, mixed with the chromogenic substrate, and incubated for an additional 6 min. The reaction was stopped with 20% acetic acid. The absorbance of the developed yellow color was read at 405 nm in a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The endotoxin activity was obtained from the standard curve using endotoxin standards from the *E. coli* O111:B4 strain.

2.5. Plasma jet setup

The atmospheric pressure cold plasma jet system consisted of a cylindrical alumina (Al_2O_3) insulator tube with an outer diameter of 4.8 mm and 152 mm length. The tube had a capillary with hole diameter of 3 mm. A high voltage 25 kV AC sinusoidal wave, with ~25 kHz frequency, was applied to a copper ring electrode surrounding the alumina tube. The high voltage electrode was 7 mm width and its lower end far from the jet nozzle at 20 mm. One ml of the sample was inserted into the capacity Eppendorf tube surrounded by a second identical copper ring acting as ground electrode as shown in figure 1. The bottom end of the ground electrode was located 22 mm away from the jet nozzle. A high voltage electric probe (Tektronix P6015) and Person current monitor probe (model 6585) were used to measure the voltage and current waveforms, respectively. The probes were connected to a 1 GHz, 5 GS s^{-1} digital phosphor oscilloscope (Tektronix DPO 4104B). In this work, the current and the high voltage waveform signals were measured at the high voltage electrode. The jet was formed by blowing argon gas through the alumina tube when a high voltage signal was applied between the two electrodes. Oxygen gas was mixed with argon at the rate of 0.2% from argon and the argon mass flow was fixed at 4.5 slm as shown in figure 1. The gas flow rates were measured using ALICAT MC-5SLPM-D/5 and ALICAT MC-20SLPM-D/5 mass flow meters for oxygen and argon, respectively. The temperature of the treated water sample was measured immediately after treatment using a thermocouple probe.

2.6. Power consumption

A Lissajous figure, charge–voltage (Q–V) characteristics, was estimated using a capacitance C means of 15 nF as explained previously by Eliasson and Kogelschatz (1991). The energy dissipated during one period of the applied voltage corresponds

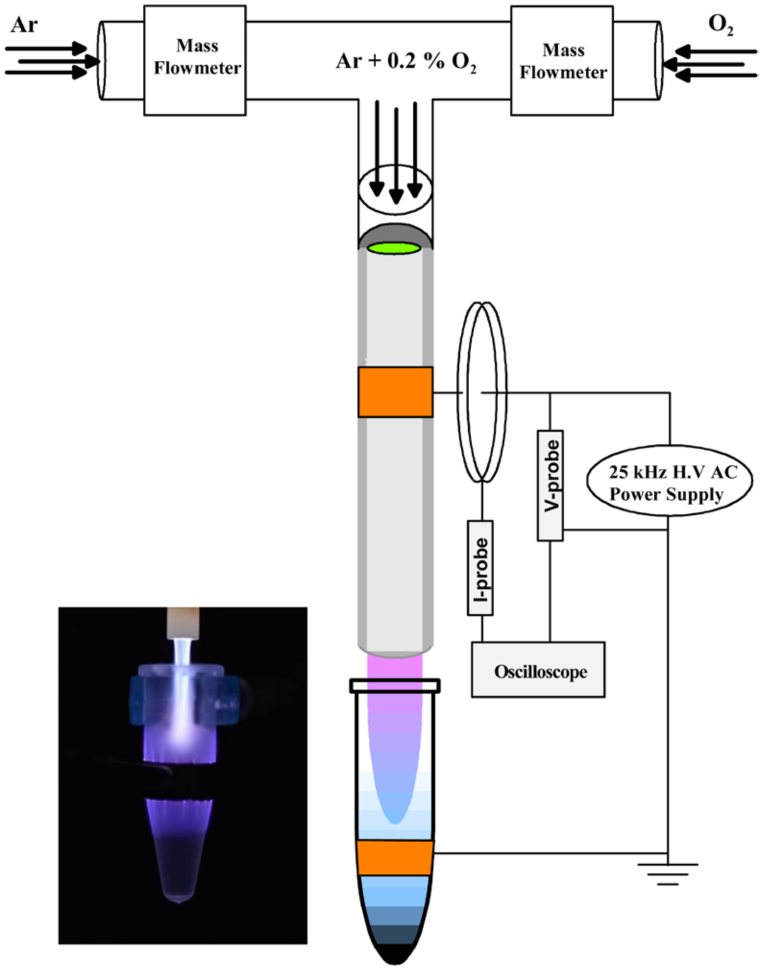

Figure 1. Atmospheric pressure argon plasma experimental setup (right) and a picture of the plasma interacting with a treated wastewater sample (left).

to the enclosed area in the Lissajous figure according to the following equation:

$$P = \frac{1}{T}\int_0^T u(t)i(t)\,\mathrm{d}t.$$

The power consumption is estimated by multiplying the consumed energy by the operating frequency. The power consumption was estimated at 12.5 kV of peak to peak applied voltage and 27 kHz of plasma operating parameters.

2.7. Emission spectra measurements

The emission spectra of the generated atmospheric pressure Ar/O_2 plasma jet in air were investigated by optical emission spectroscopy using an Acton SP-2356 imaging spectrograph. This system is a 0.5 m focal length spectrograph that has three gratings: 3600 gr mm^{-1}, 1800 gr mm^{-1}, and 150 gr mm^{-1} blazed at 240 nm, 500 nm, and 500 nm, respectively. The spectrograph is equipped with a built-in photomultiplier detector with high sensitivity ranging from 190 to 900 nm (model ARC-P2, Princeton instrument). A UV–VIS optical fiber bundle was used to couple the spectra to the spectrograph. Through this work, the fiber bundle was oriented perpendicular to the long axis of the jet far from the nozzle at a distance of 2 mm.

2.8. Statistical analysis

A statistical analysis (linear regression or ANOVA with statistical significance level fixed at $p < 0.05$) was carried out using Microsoft Excel (Microsoft, Roselle, IL).

3. Results and discussion

3.1. Plasma electric characteristics

The current and voltage waveforms of 4.5 l min^{-1} argon mixed with 0.009 l min^{-1} (0.2%) oxygen are shown in figure 2. A sinusoidal waveform of 12.5 kV was applied to the high voltage electrode to produce about 36 mA peak to peak current. Wastewater from date palm fruits was used as a representative sample. The average current waveform revealed that the generated plasma consists of multi streamers, while fewer streamers, tending to be homogenous, were observed when pure argon was used as the operating gas. Moreover, an applied voltage instability was noticed for all the wastewater samples, particularly in the case of that derived from the blackberry processing industry (Tang *et al* 2010). The waveforms illustrate that the discharges ignite at ~4 kV for the positive and the negative half cycle of the applied voltage waveforms.

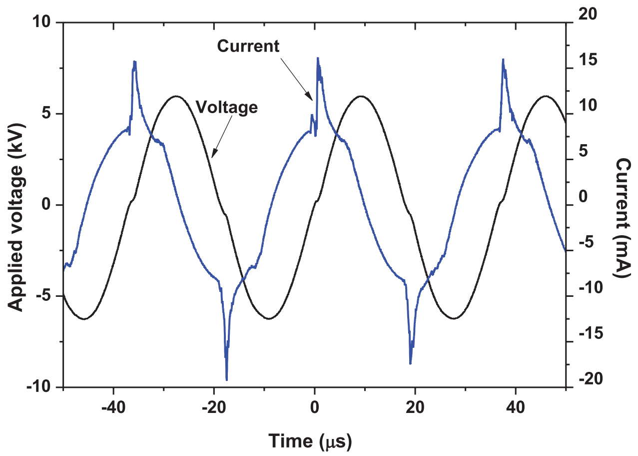

Figure 2. Voltage–current waveforms of an atmospheric pressure plasma of argon mixed with 0.2% oxygen interacting with wastewater derived from date palm fruit processing as a representative sample.

Table 1. Consumed power and energy for different wastewater samples necessary to achieve elimination or maximum reduction in bacterial count[a].

Source of wastewater	Consumed power (W) Ar	Consumed power (W) Ar + 0.2% O_2	Energy density (kWh l^{-1})
Date palm	9.04 ± 1.03	9.02 ± 1.04	0.15
Beetroot	9.13 ± 1.19	8.60 ± 1.03	0.21
Blackberry	16.77 ± 2.55	15.95 ± 1.88	0.40
Tomato	9.44 ± 1.70	8.14 ± 1.07	0.17

[a] The consumed energy was calculated by multiplying the consumed power by the time required for elimination or maximum reduction in bacterial count, which is different from sample to sample. Then the energy per unit volume was calculated by dividing the consumed energy by the volume of the treated wastewater sample (2 ml).

3.2. Plasma power consumption

The discharge consumed power in the case of pure argon plasma used in treating different wastewater samples was generally higher than the corresponding values in the case of argon mixed with 0.2% oxygen plasma (table 1). The average consumed power for the plasma interacting with different investigated samples ranged from 8.14 W (figure 3(a)) in the case of wastewater from tomato processing treated with argon mixed with 0.2% oxygen plasma, to 16.77 W (figure 3(b)) in the case of wastewater from blackberry processing treated with pure argon plasma.

The consumed power is higher than that for a similar plasma in air without water for a discharge frequency of 20 kHz under a discharge of 8 kV in pure Ar plasma (Ying *et al* 2013). This higher value for the power consumption in this work is due to the use of water inside the discharge zone and the higher operating voltage (12.5 kV). As a comparison, Leverenz *et al* (2006) indicated that the energy usage of ultraviolet light used for the disinfection of wastewater effluent with biological oxygen demands and total suspended solids less than 30 mg l^{-1} at a flow rate up to 3 gal min^{-1} was 35 W, whereas it was 23 W for the disinfection of drinking water.

3.3. Plasma emission spectra

The emission spectra of pure argon and argon mixed with 0.2% O_2 plasma were compared in the ranges 200–450 nm and 450–850 nm (figure 4). Very strong argon lines in the range 650–850 nm and in the N_2 second positive band system ($C^3\Pi_u \rightarrow B^3\Pi_u$) in both UV and visible ranges were recorded. The N_2 band is present due to the interaction of the argon plasma jet with air, since it operates at atmospheric pressure in open air and the spectra is measured 2 mm below the jet nozzle. The emission spectra indicate the generation of reactive radicals, mainly OH ($A^2\Sigma^+ \rightarrow X^2\Pi_{3/2}$), in the range 305–312 nm, and O radicals at 777.4 nm.

The most expected radicals that participated in microbial decontamination are OH ($A^2\Sigma \rightarrow X^2\Pi$) and O (777.4 nm). The spectra of OH and O radicals differ according to the nature of the wastewater and the quantitative ratio of the operating gases, whether pure argon or argon mixed with oxygen. In preliminary tests, the optimum concentration of oxygen mixed with argon to achieve maximum decontamination was 0.2%. Figure 5 shows that the O (777.4 nm) emission spectrum is highly promoted on using argon mixed with 0.2% oxygen. This promotion enhances the plasma sterilization efficiency for wastewater treated samples. The maximum increase in the O (777.4 nm) spectrum was 608.4% recorded when plasma interacted with beetroot processing wastewater as compared to a relatively smaller increase in the case of blackberry processing wastewater (75.4%) as shown in table 2. On the other hand, the relative intensity of OH radicals generated from argon mixed with 0.2% oxygen showed little increase, ranging from 24.1% to 80.8% in the case of plasma interacting with wastewater of the different tested samples, except blackberry, which induced a reduction in OH radicals (−21.2%). The variation in the O and OH radical intensities are probably responsible for the differential efficacy of plasma as a decontamination agent.

The generated plasma free electron mean energy in the discharge decreased due to oxygen addition, which is mainly related to the electron attachment. Adding oxygen will capture a lot of free electrons, forming negative oxygen molecules (O_2^-); this process is enhanced when the plasma is operated in humid air (Penetrante *et al* 1997, Wang *et al* 2004) as follows:

$$e + O_2 \rightarrow O_2^-$$

$$e + O_2 + \ M \rightarrow O_2^- + M \quad 3.2 \times 10^{-31}\,cm^3\,s^{-1}.$$

The oxygen molecule dissociates and a lot of [O] and [O] (1D) are generated and produce OH on interaction with H_2O in humid air as follows (Yang *et al* 2008):

$$e + O_2 \rightarrow O^- + O \quad 1.2 \times 10^{-12}\,cm^3\,s^{-1}$$

$$e + O_2 \rightarrow e + O + O(1D) \quad 3.2 \times 10^{-11}\,cm^3\,s^{-1}$$

$$e + O_2 \rightarrow O^+ + O + 2e \quad 4.6 \times 10^{-16}\,cm^3\,s^{-1}$$

$$O(1D) + H_2O \rightarrow OH + OH \quad 2.3 \times 10^{-10}\,cm^3\,s^{-1}.$$

However, OH radicals may be also produced through the dissociative reactions of water cluster ions (Sigmond 1984).

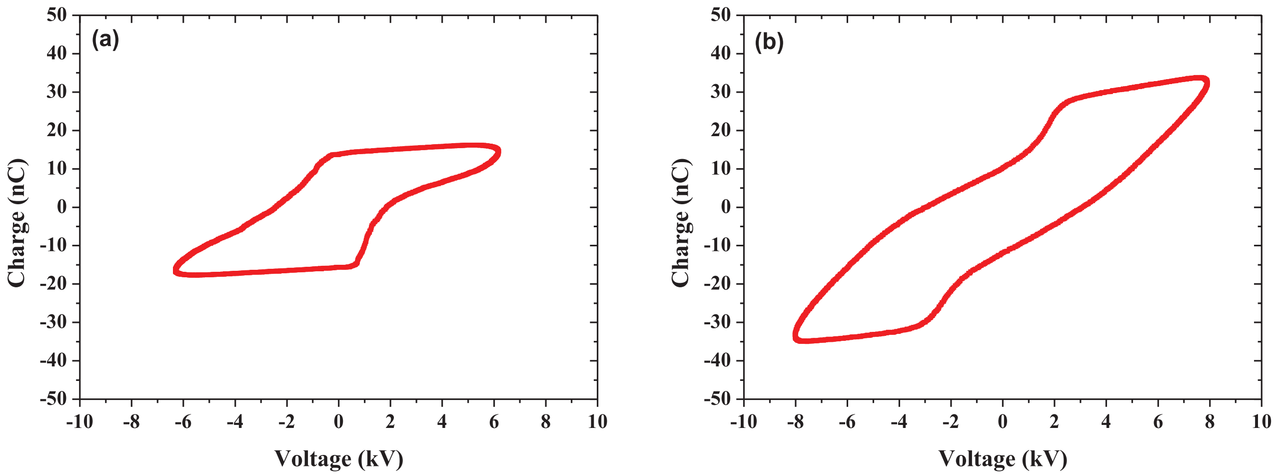

Figure 3. Examples of Lissajous figures, charge–voltage (Q–V) characteristics in the case of (a) wastewater from tomato processing treated with argon mixed with 0.2% oxygen plasma, 8.14 W, (b) wastewater from blackberry processing treated with pure argon plasma, 16.77 W.

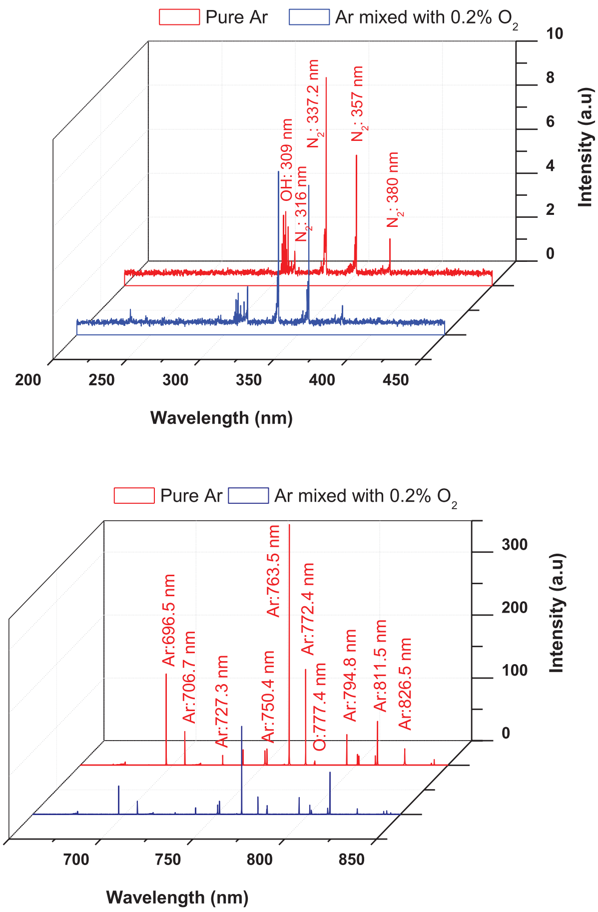

Figure 4. A comparison between emission spectra of atmospheric pressure plasma jets of argon and argon mixed with 0.2% oxygen interacting with blackberry processing wastewater in the ranges 250–450 nm (above) and 650–850 nm (below).

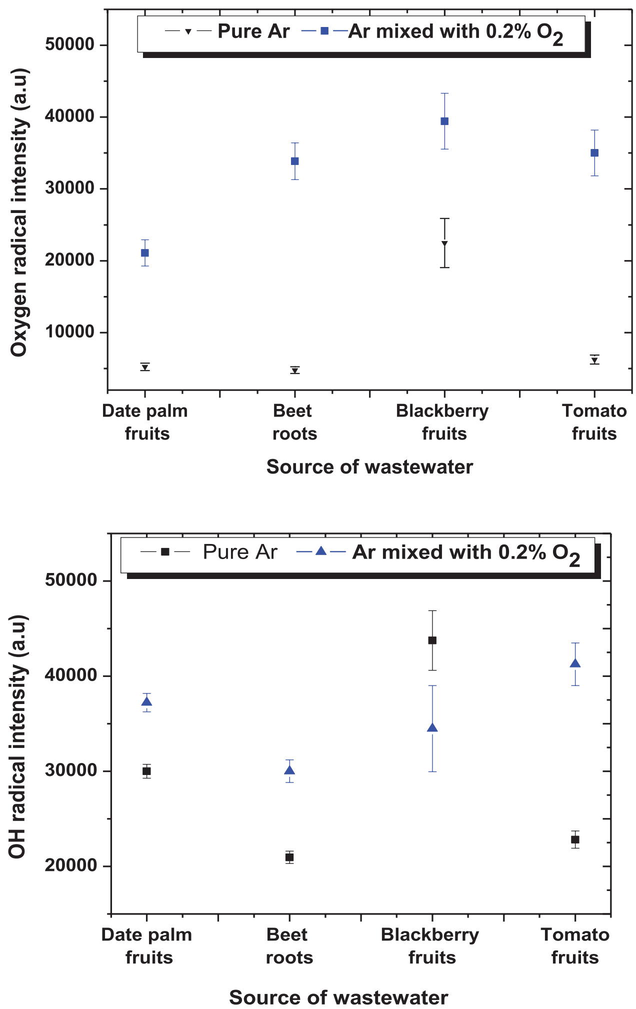

Figure 5. Comparison of the emission spectra of O radicals, 777.4 nm (above), and OH radicals, central band 309 nm (below), from argon and argon mixed with 0.2% oxygen plasma interacting with different fruit and vegetable processing wastewaters.

The above reactions show that the free electrons are depleted on mixing argon with 0.2% O_2. The process leads to an increase in the plasma electric instability and the tendency to form streamer plasma.

On the interaction of plasma with water, many chemical reactions may occur. These reactions could generate some chemical species inside the water such as nitrite and nitrate ions (NO_2^-, NO_3^-), hydrogen peroxide (H_2O_2), ozone (O_3) and hydrogen molecule H_2 in addition to the formation of some

Table 2. Percentage change in OH and O (777.4 nm) radical emission spectra on applying an argon mixed with 0.2% oxygen plasma in relation to pure argon plasma on treating fruit and vegetable processing wastewater samples.

	Percentage change	
Source of wastewater	O	OH
Blackberry	+75.4	−21.2
Date palm	+302.5	+24.1
Tomato	+460.5	+80.8
Beetroot	+608.4	+43.3

radicals (OH•, H•, O•) (Ruma *et al* 2014). These reactive chemical species possibly participate in the inactivation of the microorganisms existing in the water (Oehmigen *et al* 2010, 2011). Moreover, the reactive nitrogen species could be formed on the interaction of atmospheric pressure plasma with water, depending on the physical processes induced by the plasma. These processes are controlled by many variable parameters such as the water conductivity, plasma electron density, temperature and the operating gas. The main reactions involved in the production of free radicals and their termination on the reaction of the plasma with water have been summarized previously (Malik *et al* 2001, 2010). The thermocouple measurements show an increase in the treated sample temperature above its original room temperature (20 °C) by 3–5 °C, which indicates that the temperature does not have a role in sterilization and the decontamination is mostly related to the free radicals. The maximum increase in sample temperature was recorded in the case of wastewater from blackberry processing.

3.4. Plasma treatment

3.4.1. Physical and chemical characteristics. The wastewater produced from blackberry processing had higher carbohydrate and protein content and COD, while that produced from tomato processing showed the lowest values of these materials (table 3). The variation in wastewater characteristics is probably due to the type of plant material used, manufacture, nature of product, different additives, flavors, and the processes involved in the production including washing, peeling, blanching, slicing (or dicing), canning and retorting. The wastewater from blackberry processing has the highest antioxidant capacity (table 4), which is largely attributed to the presence of anthocyanins, phenolics and other flavonoids (Wang and Lin 2000, Siriwoharn *et al* 2004, Ortiz *et al* 2013). In their investigation on berries as natural antioxidants, Huang *et al* (2012) showed that blackberry fruits contain a range of phenolic acids and flavonoids (flavone, flavonols, flavanols, anthocyanidins) that have a remarkably high scavenging activity toward chemically generated radicals.

A significant reduction in total solids, total organic carbon and total nitrogen content, and pH values of the investigated wastewater samples was observed after 180 s exposure to the cold plasma (table 3). The lowest reduction was observed in the case of wastewater from blackberry processing where the pH dropped to only 5.5 compared to 5.9 recorded before treatment. It is possible that the reaction of the generated reactive species, including N, O, OH and ozone, with the wastewater components, such as organic acids and proteins, are mainly responsible for the change in pH to be more acidic (Fernandez and Thompson 2012). The change of the media pH towards the acidic range enhances plasma bactericidal efficacy (Liu *et al* 2010).

The efficiency of cold atmospheric argon plasma in reducing COD in the test samples after 180 s exposure time reached 93.3 and 84.4% in the case of wastewater from date palm and tomato processing, respectively. The lowest reduction in COD was recorded in the case of wastewater from blackberry processing (57.5%). The removal of COD seems to depend on the nature of the wastewater. Spectroscopy shows that the plasma content includes chemical reactive species such as O and OH radicals as well as excited molecular nitrogen and argon. The hydroxyl radicals have been shown by several investigators to aggressively attack a broad range of organic contaminants and micro pollutants (Aleboyeh *et al* 2008, Wu and Linden 2008, Yuan *et al* 2009, Olmez-Hanci *et al* 2011). Grundmann *et al* (2007) reported that the produced oxidizing reactive species can decompose pollutant molecules, organic particulate matter or soot, resulting in a reduction of COD. One of the main limitations of this process, however, is that the treatment efficiency is significantly influenced by the water quality, such as the presence of natural organic matter, and/or alkalinity (He *et al* 2012).

The relatively high reduction in COD in the case of date palm and tomato processing wastewater may be due to the relatively lower antioxidant capacity that results in a longer life span of the produced reactive species as compared with the depletion of the reactive species resulting from the higher antioxidants in the case of wastewater from blackberry processing. Wang and Jiao (2000) stated that the blackberry has the highest scavenger capacity against superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen. Moreover, spectral analysis shows instability in the produced plasma in the wastewater of blackberry processing, probably due to the high antioxidant power that decreases the strength of the reactive species. The reactions between the oxygen ions and chemicals present in the wastewater will generally produce inert gases and/or precipitates with little or no negative impact.

3.4.2. Bacterial count. The wastewater from fruit and vegetable processing operations varied in bacterial load according to the processed material (figure 6). Wastewater from blackberry processing had the highest bacterial population at 6.85 $\log_{10}$ CFU ml^{-1}, followed by that from date palm, beetroot and tomato processing, reaching 5.91, 5.72 and 5.37 $\log_{10}$ CFU ml^{-1}, respectively. The most common bacterial species were identified as *E. coli*, *P. aeruginosa*, *E. faecalis*, *C. jejuni* and *S. boydii* (figure 7). Initially, fruits and vegetables are commonly exposed to microbial contamination through contact with soil composted with organic manure, water and dust, and through handling at harvest or during postharvest processing. Contamination may also be due to storage conditions and how long the wastewater was kept before it

Table 3. Physical and chemical characteristics of fruit and vegetable processing wastewater before (BT) and after (AT) atmospheric pressure plasma jet treatment of argon mixed with 0.2% oxygen applied for 180 s.

	pH		Total solids (TS)		Total organic carbon ($mg\ l^{-1}$)		Total nitrogen ($mg\ l^{-1}$)		COD ($mg\ l^{-1}$)m	
Source of wastewater	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
Blackberry	5.9 ± 0.2	5.5 ± 0.2	840 ± 26	629 ± 22	260 ± 12	155 ± 11	92 ± 8	58 ± 6	930 ± 28	395 ± 13
Date palm	6.1 ± 0.3	5.3 ± 0.3	471 ± 19	261 ± 14	110 ± 8	31 ± 7	37 ± 5	12 ± 3	410 ± 15	27 ± 4
Tomato	5.3 ± 0.3	4.6 ± 0.2	322 ± 15	143 ± 14	88 ± 7	26 ± 5	21 ± 4	9 ± 2	294 ± 16	45 ± 6
Beetroot	6.2 ± 0.4	5.6 ± 0.3	630 ± 18	312 ± 16	170 ± 10	44 ± 8	43 ± 5	19 ± 4	501 ± 19	141 ± 10

Table 4. Ferric reducing antioxidant power (mmol $Fe^{2+}\ l^{-1}$) of fruit and vegetable processing wastewater samples.

Source of wastewater	Antioxidant power (mmol $Fe^{2+}\ l^{-1}$)
Blackberry	14.82
Date palm	1.03
Tomato	3.34
Beetroot	5.70

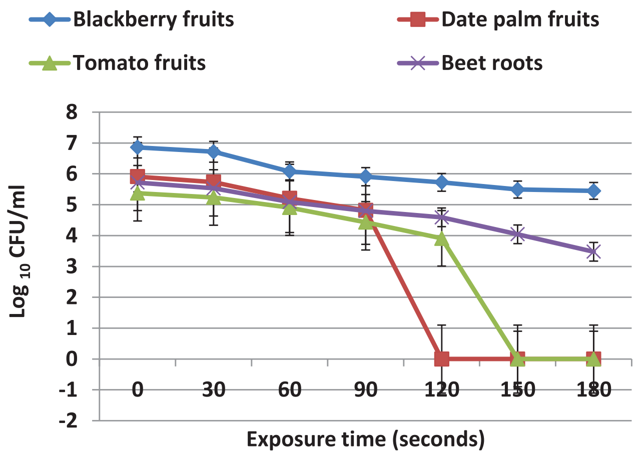

Figure 6. Effect of atmospheric pressure plasma of argon mixed with 0.2% oxygen on bacterial count ($\log_{10}$ CFU ml^{-1}) of fruit and vegetable processing wastewaters.

was obtained for sampling. The wastewater probably contains different fermentable sugars, a broad spectrum of waste soluble organic materials, and essential elements originating in processing. These nutrients facilitate colonization of bacterial isolates and most likely account for the high microbial counts. More significantly, bacteria on the produce may multiply over time depending on the storage conditions, especially those that are psychrotrophic (Abadias *et al* 2008, Montville and Matthews 2008).

A number of experiments were carried out with different configurations of the atmospheric pressure argon cold plasma to study the best processing conditions that achieve the highest microbial inactivation. The effect of the atmospheric cold plasma treatment on the reduction of total count of bacteria in different wastewater samples is shown in figure 6. Plasma treatment for 150 s induced complete decontamination of the wastewater derived from tomato processing, while 120 s was sufficient for decontamination of wastewater from date palm processing. Meanwhile, plasma treatment induced a significant reduction in the bacterial count by 1.41 and 2.43 $\log_{10}$ CFU ml^{-1} in the case of wastewater from blackberry and beetroot processing, respectively. The degree of inactivation probably depends on the type of microorganism, the inactivation medium, number of cells, operating gas mixture, gas flow and physiological state of cells (Yu *et al* 2006, Song *et al* 2009). The difference in effectiveness of reactive species produced by the atmospheric plasma is mostly due to the variation in the antioxidant capacity of the wastewater. Critzer *et al* (2007) reported the ability of atmospheric plasma to reduce inoculated microbial populations on fresh produce surfaces. Fernandez *et al* (2013) stated that cold atmospheric gas plasma achieved 2.72, 1.76 and 0.94 log reductions of *Salmonella typhimurium* on lettuce, strawberry and potato, respectively, in 15 min of plasma treatment time.

Considering the effect of the cold plasma on isolated bacterial species in saline (figure 7), *E. coli* was the most susceptible bacterial species, and its count dropped to 1.4 $\log_{10}$ CFU ml^{-1} after 90 s exposure to cold plasma, compared to 7.0 $\log_{10}$ CFU ml^{-1} for the initial untreated sample. *S. boydii*, on the other hand, had the minimum susceptibility, recording 3.34 $\log_{10}$ CFU ml^{-1} as compared to the same initial count. All isolated species failed to grow on extension of the exposure time to 120 s. It is suggested that charged particles of plasma can inactivate bacterial cells through the rupture of the cytoplasmic membrane of bacterial cells, where the electrostatic force caused by charge accumulation on the outer surface of the cell membrane overcomes the tensile strength of the membrane, thereby causing its rupture (Mendis *et al* 2000, Laroussi *et al* 2003). Surowsky *et al* (2014) suggested that the load of *Citrobacter freundii* in apple juice was reduced by about 5 log cycles after plasma exposure of 480 s using argon and 0.1% oxygen followed by a subsequent storage time of 24 h. The results indicate that direct contact between bacterial cells and cold plasma is not necessary for achieving successful inactivation. They also showed that the plasma generated compounds in the liquid, such as H_2O_2 and most likely hydroperoxy radicals, are particularly responsible for microbial inactivation. van Gils *et al* (2013) used a radio-frequency atmospheric pressure argon plasma jet to inactivate *P. aeruginosa* in solutions, and showed that HNO_2, $ONOO^-$ and H_2O_2 detected in the liquid phase were the responsible species for inactivation. The effectiveness of the cold plasma on bacterial species contained in saline appears more efficacious than that recorded for the bacteria occurring in wastewater, undoubtedly due to the absence of antioxidant. Surowsky *et al* (2014) stated that the reactive species scavenging role of the

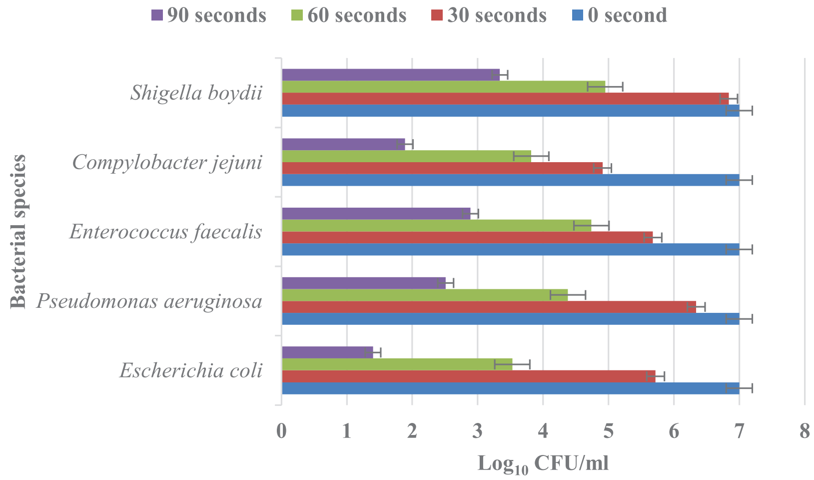

Figure 7. Effect of atmospheric pressure plasma of argon mixed with 0.2% oxygen on count ($\log_{10}$ CFU ml^{-1}) of the common bacterial species isolated from fruit and vegetable processing wastewater.

Table 5. Occurrence of endotoxins in fruit and vegetable processing wastewater samples treated with atmospheric pressure cold plasma jet of argon mixed with 0.2% oxygen for 180 s.

	Endotoxins (ng ml^{-1})		
Source of wastewater	Before treatment	After treatment	% reduction
Blackberry	530.5 ± 32	239.6 ± 12	54.84
Date palm	272.1 ± 22	26.6 ± 4	90.22
Tomato	136.6 ± 14	33.0 ± 6	75.84
Beetroot	359.0 ± 26	108.3 ± 11	69.83

antioxidants in the apple juice might play an important role in the attenuation of reactive species. The percentage change in OH and O radicals of the emission spectra (table 2) shows that the role of antioxidant is mostly related to OH radicals rather than O radicals, where the OH radicals are negatively changed in wastewater from blackberry processing (−43.3%). Although the cold plasma differentially promoted the O radicals of all tested samples, wastewater from blackberry processing showed the lowest increase of these radicals, probably due to the high antioxidant level.

3.4.3. Toxin inactivation. The bacterial endotoxins varied in different test wastewater samples. Wastewater from blackberry processing contains the highest concentration (530.5 ng ml^{-1}) and that of tomato processing has the lowest concentration (136.6 ng ml^{-1}), as indicated in table 5. The occurrence of endotoxin in wastewater from fruit and vegetable processing is expected and reflects the occurrence of the high level of Gram negative bacteria including *E. coli*, *P. aeruginosa*, *C. jejuni* and *S. boydii*. The acceptable endotoxin level that the body can tolerate without adverse effect ranges from 0.5 to 0.02 ng kg^{-1} of body weight per hour (US pharmacopeial Convention Inc. 2004). The effect of endotoxins on *in vitro* cell growth and function depends on their levels and cell type. Endotoxin levels as low as 1.0 ng ml^{-1} reduces pregnancy success rates in *in vito* fertilization (Snyman and Van der Merwe 1986), while a higher level of 100 ng ml^{-1} induces proliferation of monocytes (Mattern *et al* 1994).

It is supposed that a considerable endotoxin increase in the medium of the cold plasma treated samples results from accumulation of the released lipopolysaccharide components in the cell walls of destroyed Gram-negative bacteria. However, the treatment of wastewater samples with cold atmospheric argon plasma for 180 s induced a reduction in the endotoxins ranging from 90.22% (in the case of date palm) to 54.84% (in case of blackberry). Extension of the exposure time to 360 s induced complete elimination of endotoxins (unrecorded data). He *et al* (2012) indicated that cyanobacterial toxin cylindrospermopsin, at an initial concentration of 1 μM, was significantly degraded from 75% to 100% by means of OH radicals and sulfate radicals using UV-254 nm activation of H_2O_2, persulfate and peroxymonosulfate. They also stated that certain radical scavengers in tap water samples inhibited the destruction of cylindrospermopsin by the OH radicals. It is suggested that concentrations of antioxidants in different wastewaters significantly affect the oxidation potentialities of reactive species and consequently their efficiency in toxin breakdown.

4. Conclusions

The wastewater produced from fruits or vegetables processing industries are a problem for both the environment and drainage systems, although it contains relatively small quantitative amounts of microorganisms of saprophytic and sometimes pathogenic nature. To allow for a more reliably safe environment, the recycling of this type of wastewater is essential and is easier to decontaminate than other hazardous wastewater. A cold atmospheric pressure plasma jet with argon or a mixture of argon and 0.2% oxygen as operating gases was generated and characterized. The jet was generated by blowing the gas through an alumina tube powered by a 25 kHz, 25 kV power supply. The plasma transfers from a single homogenous jet, when argon was used individually, to multi-streamers on its mixing with 0.2% oxygen. The generated plasma jet was employed to improve the physical and microbiological characteristics of wastewater derived from processing industries of blackberry, date palm, tomato and beetroot.

The plasma interacting with the wastewater samples was run in unstable mode and obviously penetrates the investigated samples. Regardless of the OH radicals that were reduced in case of treated blackberry fruits, the addition of 0.2% oxygen to the argon steadily induced an increase in the O and OH radical emission spectra in the case of wastewater from the all treated samples. The results indicate significant efficiencies of the cold plasma in improving the physical and chemical characteristics of the tested wastewater. The maximum COD reduction of wastewater samples was counted in the case of date palm (93.3%) and the minimum one was recorded for that of blackberry (57.5%). Although the bacterial load of wastewater from blackberry and beetroot processing was significantly reduced after 180 s, the cold plasma was able to completely decontaminate the wastewater from date palm and tomato processing after 120 and 150 s, respectively. The effectiveness of the cold plasma on the isolated bacterial species *E. coli*, *P. aeruginosa*, *C. jejuni*, *S. boydii* and *E. faecalis* contained in saline appears more efficacious than that recorded for the bacteria occurring in wastewater, undoubtedly due to the absence of antioxidants. It is interesting to note that the variation in cold plasma effectiveness was related to the antioxidant activity of each type of wastewater. The higher the antioxidant power, the lower the efficacy of the cold plasma. The plasma efficiency, particularly on using a mixture of argon and 0.2% oxygen, as a decontamination agent was reduced by antioxidant constituents in wastewater that act as scavengers for the active free radicals produced by plasma and recorded by spectral data. The reduction of endotoxins in water ranged from 54.84, in the case of wastewater from blackberry processing, to 90.22 in the case of wastewater from date palm processing. This indicates the ability of cold plasma to degrade the bacterial toxins, and possibly eliminate them completely on extension of exposure time.

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References

Abadias M, Usall J, Anguera A, Solsona C and Viñas I 2008 Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments *Int. J. Food Microbiol.* **123** 121–9

Aleboyeh A, Olya M E and Aleboyeh H 2008 Electrical energy determination for an azodye decolorization and mineralization by UV/H_2O_2 advanced oxidation process *Chem. Eng. J.* **137** 518–24

Anderson D C 1989 Gas plasma sterilization: innovation in practice *Navy Med.* **80** 9–10

APHA 1992 *Standard Methods for Water and Wastewater Examination* 18th edn (Washington, DC: American Public Health Association)

Benzie I F F and Strain J J 1999 Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration *Methods Enzymol.* **299** 15–27

Cavalcanti A L, Fernandes L V, Barbosa A S and Vieira F F 2008 pH, titratable acidity and total soluble solid content of pediatric antitussive medicines *Acta Stomatol. Croat.* **42** 164–70

Critzer F J, Kelly-Winterberg K, South S L and Golden D A 2007 Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces *J. Food Prot.* **70** 2290–6

Deng X T, Shi J J, Shama G and Kong M G 2005 Photon localization in resonant media *Appl. Phys. Lett.* **87** 153901

Eliasson B and Kogelschatz U 1991 Modeling and applications of silent discharge plasmas *IEEE Trans. Plasma Sci.* **19** 309–23

El-Sayed W S, Ouf S A and Mohamed A-A H 2015 Deterioration to extinction of wastewater bacteria by non-thermal atmospheric pressure air plasma as assessed by 16S rDNA-DGGE fingerprinting *Front. Microbiol.* **6** 1098

Fernandez A and Thompson A 2012 The inactivation of *Salmonella* by cold atmospheric plasma treatment *Food Res. Int.* **45** 678–84

Fernandez A, Noriega E and Thompson A 2013 Inactivation of *Salmonella enterica* serovar *Typhimurium* on fresh produce by cold atmospheric gas plasma technology Food *Microbiol.* **33** 24–9

Grundmann J, Müller S and Zahn R-J 2007 Extraction of ions from dielectric barrier discharge configurations *Plasma Proc. Polym.* **4** S1004–8

Hao X, Mattson A M, Edelblute C M, Malik M A, Heller L C and Kolb J F 2014 Nitric oxide generation with an air operated non-thermal plasma jet and associated microbial inactivation mechanisms *Plasma Process. Polym.* **11** 1044–56

He X, Pelaez M, Westrick J A, O'Shea K E, Hiskia A, Triantis T, Kaloudis T, Stefan M I, De la Cruz A A and Dionysiou D D 2012 Efficient removal of microcystin-LR by UV-C/H_2O_2 in synthetic and natural water samples *Water Res.* **46** 1501–10

Huang W Y, Zhang H C, Liu W X and Li C Y 2012 Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing *J. Zhejiang Univ. Sci.* B **13** 94–102

Kolb J F, Mohamed A A H, Price R O, Swanson R J, Bowman A M, Chiavarini R L, Stacey M and Schoenbach K H 2008 Cold atmospheric pressure air plasma jet for medical applications *Appl. Phys. Lett.* **92** 241501

Laroussi M, Lu X and Malott C 2003 A non-equilibrium diffuse discharge in atmospheric pressure air *Plasma Sources Sci. Technol.* **12** 53–6

Laroussi M, Tendero C, Lu X, Alla S and Hynes W L 2006 Inactivation of bacteria by the plasma pencil *Plasma Proc. Polym.* **3** 470–3

Lerouge S, Guignot C, Tabrizian M, Ferrier D, Yagoubi N and Yahia L H 2000 Plasma-based sterilization: effect on surface and bulk properties, and hydrolytic stability of reprocessed polyurethane electrophysiology catheters *J. Biomed. Mater. Res.* **52** 774–82

Leverenz H, Darby J and Tchobanoglous G 2006 Evaluation of disinfection units for onsite wastewater treatment systems *Report No. 2006-1* University of California, Davis

Liu F *et al* 2010 Inactivation of bacteria in an aqueous environment by a direct-current, cold atmospheric pressure air plasma microjet *Plasma Process. Polym.* **7** 231–6

Lowry O H, Rosenbrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin-phenol reagent *J. Biol. Chem.* **193** 265–75

Malik M A 2010 Water purification by plasmas: which reactors are most energy efficient? *Plasma Chem. Plasma Process.* **30** 21–31

Malik M A, Ghaffar A and Malik S A 2001 Water purification by electrical discharges *Plasma Sources Sci. Technol.* **10** 82–91

Mattern T *et al* 1994 Endotoxin and lipid A stimulate proliferation of human T cells in the presence of autologous monocytes *J. Immunol.* **153** 2996–3004

Mendis D A, Rosenberg M and Azam F 2000 A note on the possible electrostatic disruption of bacteria *IEEE Trans. Plasma Sci.* **28** 1304–6

Misra N N, Ziuzina D, Cullen P J and Keener K M 2012 Characterization of a novel cold atmospheric air plasma system for treatment of packaged liquid food products *American Society of Agricultural and Biological Engineers (Dallas, TX, 29 July–1 August 2012)* p 121337629

Mitra A, Li Y-F, Klämpfl T G, Shimizu T, Jeon J, Morfill E and Zimmermann J L 2014 Inactivation of surface-borne microorganisms and increased germination of seed specimen by cold atmospheric plasma *Food Bioprocess Technol.* **7** 645–53

Moisan M, Barbeau B, Moreau S, Pelletier J, Tabrizian M and Yahia L H 2001 Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanism *Int. J. Pharm.* **226** 1–21

Mohamed A H, Al-Mashraqi A A, Shariff S M, Benghanem M, Basher A H and Ouf S A 2014 Generation of large volume atmospheric pressure air plasma *IEEE Trans. Plasma Sci.* **42** 2488–9

Montville T J and Matthews R 2008 *Food Microbiology: an Introduction* 2nd edn (Washington, DC: ASM Press)

Oehmigen K, Hähnel M, Brandenburg R, Wilke C, Weltmann K-D and von Woedtke Th 2010 The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids *Plasma Process. Polym.* **7** 250–7

Oehmigen K, Winter J, Hähnel M, Wilke C, Brandenburg R, Weltmann K-D and von Woedtke Th 2011 Estimation of possible mechanisms of *E. coli* inactivation by plasma treated sodium chloride solution *Plasma Process. Polym.* **8** 904–13

Olmez-Hanci T, Arslan-Alaton I and Basar G 2011 Multivariate analysis of anionic, cationic and nonionic textile surfactant degradation with the H_2O_2/UV-C process by using the capabilities of response surface methodology *J. Hazard Mater.* **185** 193–203

Ortiz J, Marín-Arroyo M R, Noriega-Domínguez M J, Navarro M and Arozarena I 2013 Color, phenolics, and antioxidant activity of blackberry (*Rubus glaucus* Benth.), blueberry (*Vaccinium floribundum* Kunth), and apple wines from Ecuador *J. Food Sci.* **78** C985–93

Ouf S A, Basher A and Mohamed A-A H 2015 Inhibitory effect of double atmospheric pressure argon cold plasma on spores and mycotoxin production of *Aspergillus niger* contaminating date palm fruits *J. Sci. Food Agric.* **95** 3204–10

Ouf S A, Mohamed A-A H and El-Sayed W S 2016 Fungal decontamination of fleshy fruit water washes by double atmospheric pressure cold plasma *CLEAN - Soil, Air, Water* **44** 134–42

Park G Y, Park S J, Choi M Y, Koo I G, Byun J H, Hong J W, Sim J Y, Collins G J and Lee J K 2012 Atmospheric-pressure plasma sources for biomedical applications *Plasma Sources Sci.* **21** 043001

Penetrante B M, Bardsley J N and Hsiao M C 1997 Kinetics analysis of nonthermal plasmas used for pollution control *J. Appl. Phys.* **36** 5007–17

Perni S, Liu D W, Shama G and Kong M 2008 Cold atmospheric plasma decontamination of the pericarps of fruit *J. Food Prot.* **71** 302–8

Rød S K, Hansen F, Leipold F and Knøchel S 2012 Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality *Food Microbiol.* **30** 233–8

Ruma S H R, Hosseini H, Yoshihara K, Akiyama M, Sakugawa T, Lukeš P and Akiyama H 2014 Properties of water surface discharge at different pulse repetition rates *J. Appl. Phys.* **116** 123304

Sadar M J 1998 Turbidity science *Technical Information Series* Booklet no. 11 (Loveland, CO: Hach)

Saha S K and Brewer C F 1994 Total sugar protein estimation *Carbohydrate Res.* **329** 254–7

Selcuk M, Oksuz L and Basaran P 2008 Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillum* spp. by cold plasma treatment *Bioresour. Technol.* **99** 5104–9

Seo Y S, Mohamed A H, Woo K C, Lee H W, Lee J K and Kim K T 2010 Comparative studies of atmospheric pressure plasma characteristics between He and Ar working gases for sterilization *IEEE Trans. Plasma Sci.* **38** 2954–63

Shaw G and Schudram J F 2000 Congenital malformation and birth weight in areas with potential environmental contamination *Arch. Environ. Health* **47** 147–54

Shin S J, Han J H, Manning E J B and Collins M T 2007 Rapid and reliable method for quantification of *Mycobacterium paratuberculosis* by use of the BACTEC MGIT 960 system *J. Clin. Microbiol.* **45** 1941–8

Sigmond R S 1984 The residual streamer channel: return strokes and secondary streamers *J. Appl. Phys.* **56** 1355–70

Siriwoharn T, Wrolstad R E, Finn C E and Pereira C B 2004 Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties *J. Agric. Food Chem.* **52** 8021–30

Snyman E and Van der Merwe J V 1986 Endotoxin-polluted medium in a human *in vitro* fertilization program *Fertil. Steril.* **46** 273–6

Song S H, Park K U, Lee J H, Kim E C, Kim J Q and Song J 2009 Electrospray ionization-tandem mass spectrometry analysis of the mycolic acid profiles for the identification of common clinical isolates of mycobacterial species *J. Microbiol. Methods* **7** 165–77

Surowsky B, Fröhling A, Gottschalk N, Schlüter O and Knorr D 2014 Impact of cold plasma on *Citrobacter freundii* in apple juice: inactivation kinetics and mechanisms *Int. J. Food Microbiol.* **17** 63–71

Tang J, Duan Y and Zhao W 2010 Characterization and mechanism studies of dielectric barrier discharges generated at atmospheric pressure *Appl. Phys. Lett.* **96** 191503–6

Tsuji K, Martin P A and Bussey D M 1984 Automation of chromogenic substrate *Limulus* amebocyte lysate assay method for endotoxin by robotic system *Appl. Environ. Microbiol.* **48** 550–5

US Pharmacopeial Convention Inc. 2004 *United States Pharmacopeia 27-National Formulary 22* (Rockville, MD: US Pharmacopeial Convention, Inc.)

van Gils C A J, Hofmann S, Boekema B K H L, Brandenburg R and Bruggeman P J 2013 Mechanisms of bacterial inactivation in the liquid phase induced by a remote RF cold atmospheric pressure plasma jet *J. Phys. D: Appl. Phys.* **46** 175203

Wang S Y and Jiao H J 2000 Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen *J. Agri. Food Chem.* **48** 5677–84

Wang S Y and Lin H-S 2000 Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage *J. Agric. Food Chem.* **48** 140–6

Wang W C, Liu F, Zhang J L, Liu Y and Wang Y N 2004 Study on density distribution of high energy electrons in pulsed corona discharge *Vacuum* **73** 333–9

Wang R X, Nian W F, Wu H Y, Feng H Q, Zhang K, Zhang J, Zhu W D, Becker K H and Fang J 2012 Atmospheric pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation *Eur. Phys. J. D* **66** 276

Wu C and Linden K G 2008 Degradation and byproduct formation of parathion in aqueous solutions by UV and UV/H_2O_2 treatment *Water Res.* **42** 4780–90

Yang G, Wang W, Liu F and Zheng S 2008 Experimental research of OH and spatially resolved spectra in a needle-plate pulsed streamer discharge *Plasma Chem. Plasma Process.* **28** 317–30

Ying J, Chunsheng R, Liang Y, Jialiang Z and Dezhen W 2013 Atmospheric pressure plasma jet in Ar and O_2/Ar mixtures: properties and high performance for surface cleaning *Plasma Sci. Technol.* **15** 1203–8

Yu H, Perni S, Shi J J, Wang D Z, Kong M G and Shama G 2006 Effects of cell surface loading and phase of growth in cold atmospheric gas plasma inactivation of *Escherichia coli* K12 *J. Appl. Microbiol.* **101** 1323–30

Yuan F, Hu C, Hu X, Qu J and Yang M 2009 Degradation of selected pharmaceuticals in aqueous solution with UV and UV/H_2O_2 *Water Res.* **43** 1766–74