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Nonequilibrium Plasma Decontamination of Corn Steep Liquor for Ethanol Production: SO_2 Removal and Disinfection

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ABSTRACT: The objective of this study is to investigate the effect of four plasma systems on corn steep liquor using (1) direct current spark discharge in liquid, (2) pulsed dielectric barrier discharge (DBD) with ultrafine misting, (3) reverse vortex gliding arc (RVGA), and (4) forward vortex gliding arc (FVGA) with droplet atomizer. The results show a notable reduction in bacteria using FVGA and RVGA, with a lower degree of reduction using spark discharge and DBD. DBD appears to accumulate the effect on SO_2 but only up to a certain threshold. DBD treatment reduces SO_2 level by 30% after 600 s. On the other hand, for spark discharge, the effect did not accumulate, although the treatment had the lowest reduction in SO_2 levels among the tested methods. RGVA gives the best results with regard to SO_2 levels. We found 60% SO_2 was left in water treated by FVGA, and 20% SO_2 was left in water treated by RVGA.

KEY WORDS: corn steep liquor, SO_2 removal, disinfection

I. INTRODUCTION

Corn steep liquor (CSL), a by-product of the wet corn milling process, contains a mixture of several proteins, amino acids, carbohydrates, vitamins, microorganisms, and leftover dissolved SO_2.[1] It is desirable to reduce the level of bacterial load and the amount of SO_2 before additional processing and treatment. CSL is used for several applications, such as penicillin production and animal food production.[2] The ultimate goal of this study is to use plasma-treated steep water to produce alcohol; thus, we need to reduce or remove SO_2 and lactic acid bacteria (LAB). LAB competes with yeast during fermentation and SO_2 affects ethanol quality. It is known that several plasma systems including spark discharge, dielectric barrier discharge (DBD), reverse vortex gliding arc (RVGA), and forward vortex gliding arc (FVGA) are able to inactivate bacteria and create chemical changes in water properties.[3–5] Here, we investigate how these four atmospheric pressure plasma systems can disinfect and remove SO_2 from steep water.

II. MATERIALS AND METHODS

Steep water is treated using four plasma systems including direct current (DC) spark discharge directly in liquid, DBD with an ultrafine misting generator, RVGA, and FVGA

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with a droplet atomizer. Treated water is then tested for bacteria and titrated for SO_2 concentration.

A. DC Spark Discharge in Liquid

The spark discharge system consists of a DC power supply, five capacitors (602 K, 30 kV, 44 s), two stainless steel electrodes, and a test tube. The electrodes are 0.3 cm in diameter, with one serially connected to capacitors and high voltage and one connected to a ground. The bottom of the high-voltage electrode is connected to a thin, round, stainless steel disk that is 2 cm in diameter. Electrodes are assembled in a wood cork that can fit into a test tube (Fig. 1). The spark frequency is ~10 Hz. We treated 25 mL of steep water in a test tube for 5, 60, 135, 180, and 600 s.

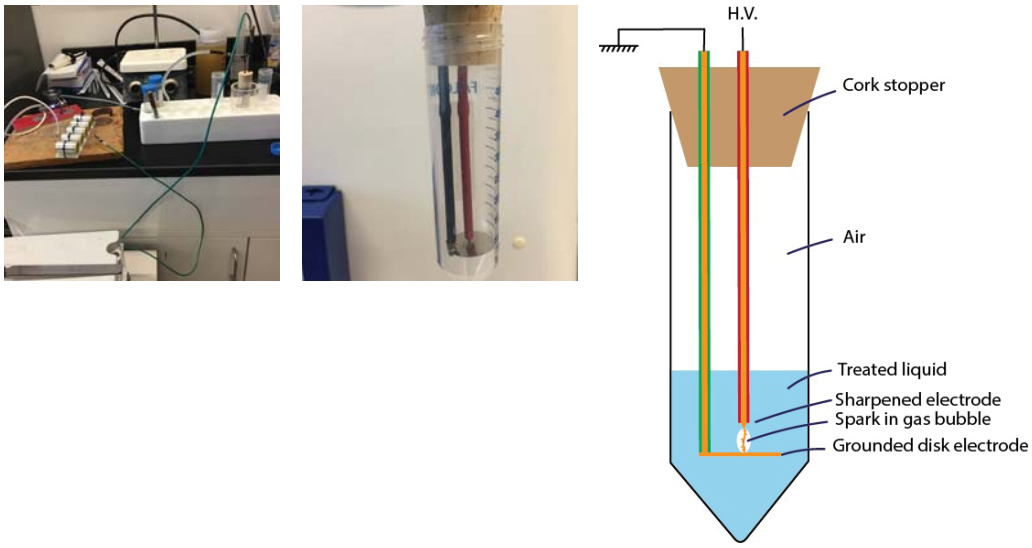

FIG. 1: DC spark discharge water treatment system. (Left) Setup; (center) electrode in the test tube, without water; (right) setup schematic. HV, high voltage

B. DBD with an Ultrafine Misting Generator

DBD is generated between two cylindrical electrodes, both covered by quartz dielectric with a thickness of 1 mm. The inner electrode is 5 mm in diameter with a 1.25-mm gap between electrodes. The mist generator is made from three ultrasonic nebulizers (similar to 241PG from Sonaer Ultrasonics, Farmingdale, New York, purchased via www.amazon.com/gp/product/B00N4OV8PU/) and connected to a 30-V DC power supply. Compressed air flows through the mist generator at 1.5 slpm. The output of the DBD is submerged into CSL liquid and allowed to bubble through. Figure 2 shows a photograph and schematic of the system. We treated 15 ml of the CSL liquid for 120, 240, 360, 480, and 600 s.

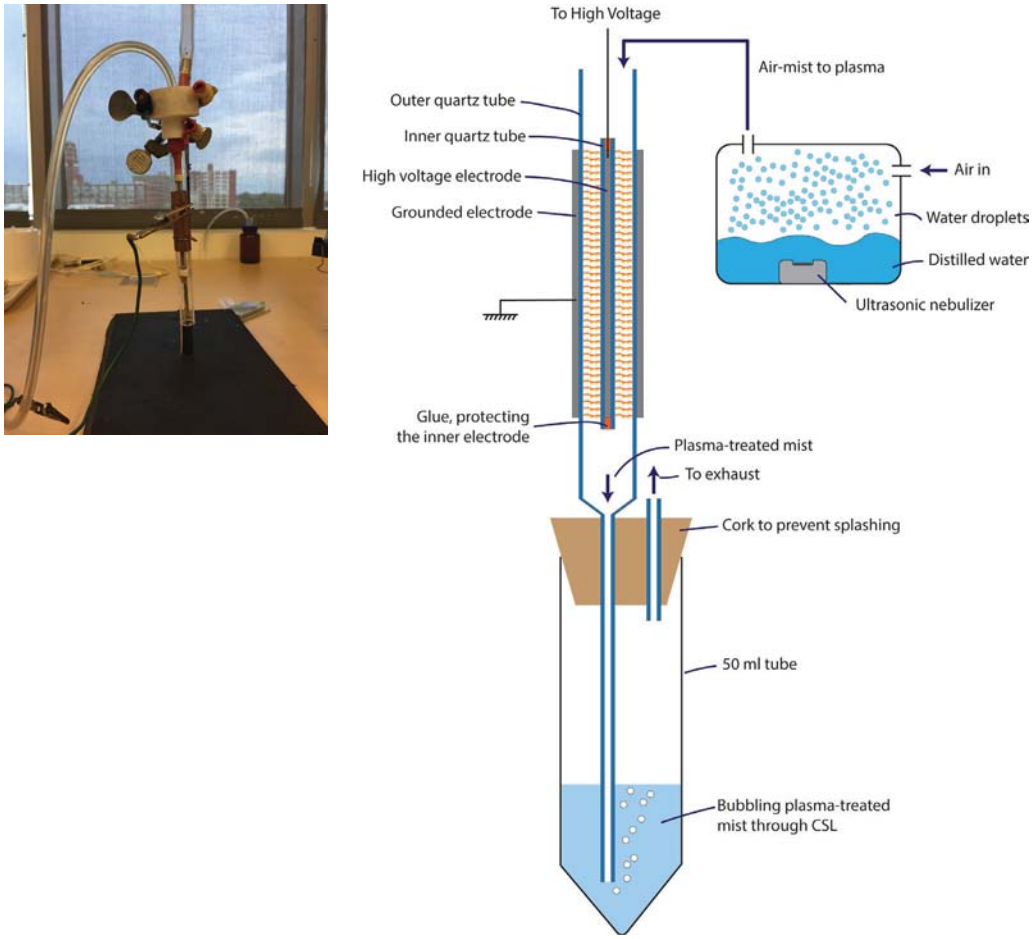

FIG. 2: DDB system with mist generator. (Left) setup; (right) setup schematic. CSL, Corn steep liquor

C. RVGA

The experimental setup is shown in Fig. 3. Water is pumped to the RVGA at a rate of 0.58 mL/s and mixed into the plasma zone with an air or N_2 flow at 60 slpm. Water is cooled down by a counter current double pipe heat exchanger and then collected at the bottom into a cup.

D. FVGA with Droplet Atomizer and Additives

Water is sprayed into the plasma using a droplet atomizer with ~10-slpm air flow. The treated water is cooled down by a shell and tube heat exchanger and collected at the bottom of the heat exchanger (Fig. 4). We add the following chemicals into the steep water

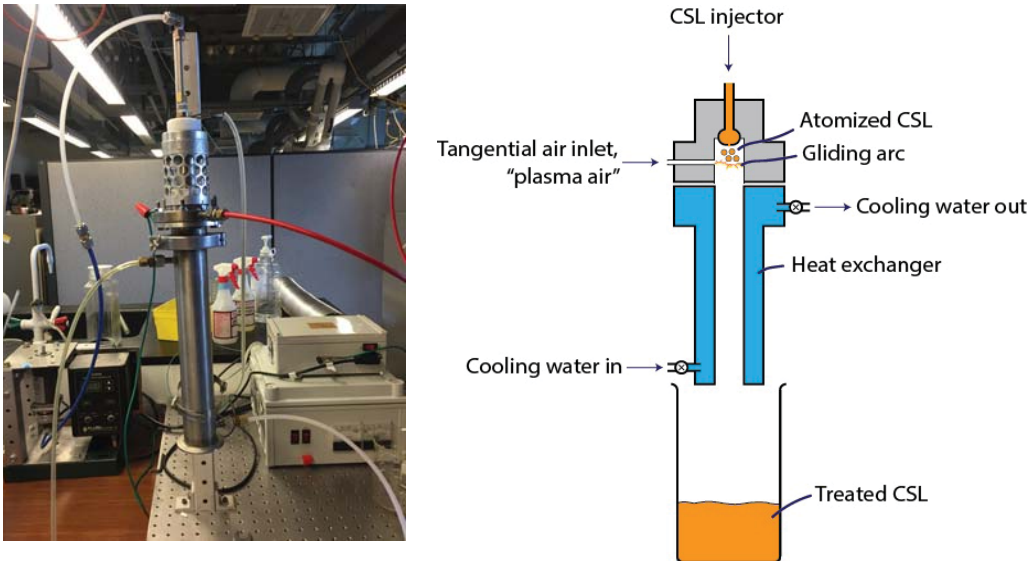

FIG. 3: RVGA system. (Left) Setup; (right) setup schematic. CSL, Corn steep liquor

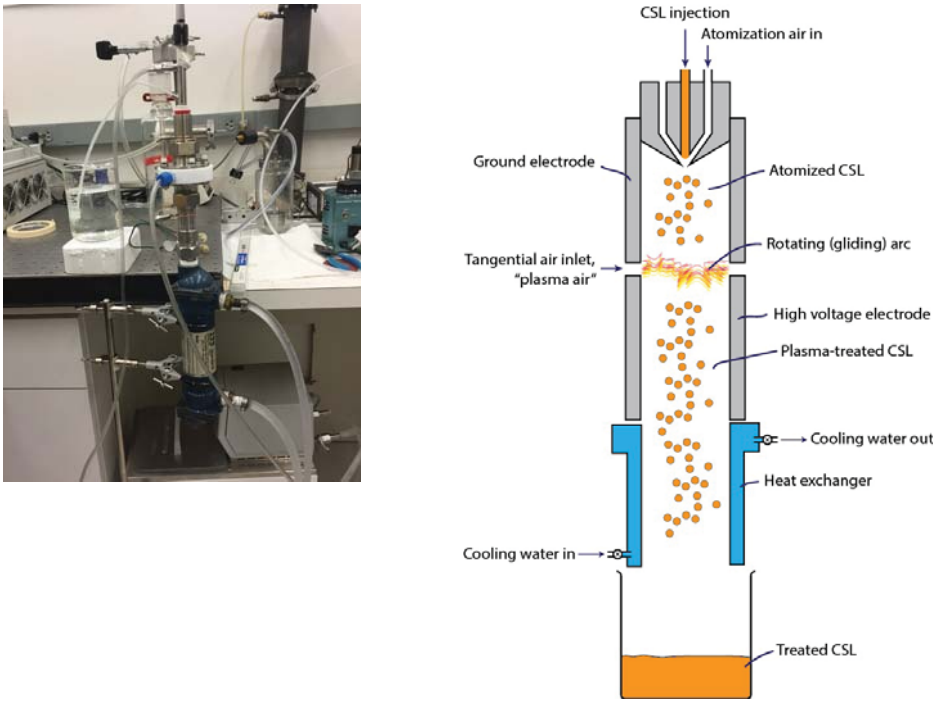

FIG. 4: FVGA system. (Left) Setup; (right) setup schematic. CSL, Corn steep liquor

before plasma treatment to enhance the desired effect: *N*-acetylcysteine (NAC), H_2O_2, and ethanol. We prepare 0.1% by volume of the NAC steep water solution; 0.3%, 1%, 3%, 7%, and 10% H_2O_2 steep water solution (using 30% H_2O_2; thus, 10% H_2O_2 refers to the addition of 10 mL of 30% H_2O_2, unstabilized, to 90 mL of CSL liquid); and 10% ethanol steep water solution. For multiple passes of the experiment, treated water is passed through FVGA, collected, and passed through again.

E. Bacteria Testing Procedures

We pipette 1 mL of treated CSL liquid into a clean tryptic soy agar Petri dish and spread the liquid to cover the entire surface. We allow the dish to dry under a hood for 30 min and then incubate for 24 h at 37ºC. There are two dishes for each sample. The original untreated steep water is used as the control sample.

F. SO_2 Titration Procedures

Materials needed for titration are 0.1 N standard iodine solution, starch solution, and deionized water. The titrant is made by diluting the iodine solution from a 0.1-N to a 0.01-N solution. As expected, and the SO_2 concentration is <1000 ppm, we pipette 2 mL of steep water into a 150-mL beaker, add 100 mL of deionized water and 3 mL of starch solution, and titrate until the blue color persists for a few seconds. If the expected SO_2 concentration is >1000 ppm, we use 1 mL of steep water.

G. Optical Characterization of Plasma

The optical emission spectrum is measured using an AvaSpec-ULS2048L-EVO (Avantes, Broomfield, CO). The cable is composed of a 600-μm fiber and is 2-m long with a COL-UV/VIS (collimating lens for ultraviolet/visible light range; Avantes, Broomfield, CO). The lens is placed at the bottom of plasma source and close enough so that it can pick up better signals, and measurement is recorded by Avasoft8 software. Chemical components are marked on the spectrum using Spectrum Analyzer 1.7 (Department of Physical Electronics of Faculty of Science of Masaryk University in Brno, Czech Republic, http://www.physics.muni.cz/~zdenek/span/). Figures 5-7 show the optical emission spectrum measured from DBD, RVGA, and FVGA.

H. Electrical Characterization of Plasma

The plasma power was characterized by digitally capturing voltage and current and then integrating it, similarly to the process described by the authors.[6] Three measurements were taken, averaged, and reported.

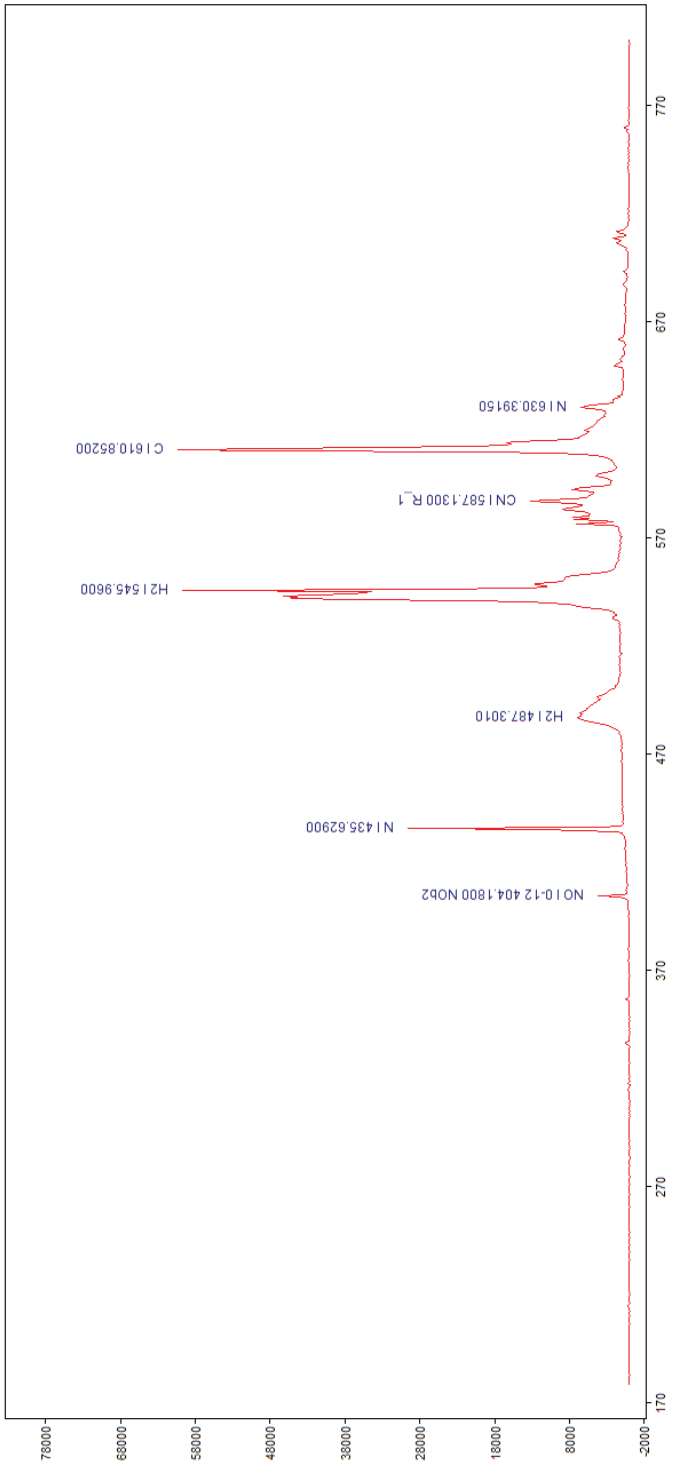

FIG. 5: Optical emission spectra of DBD

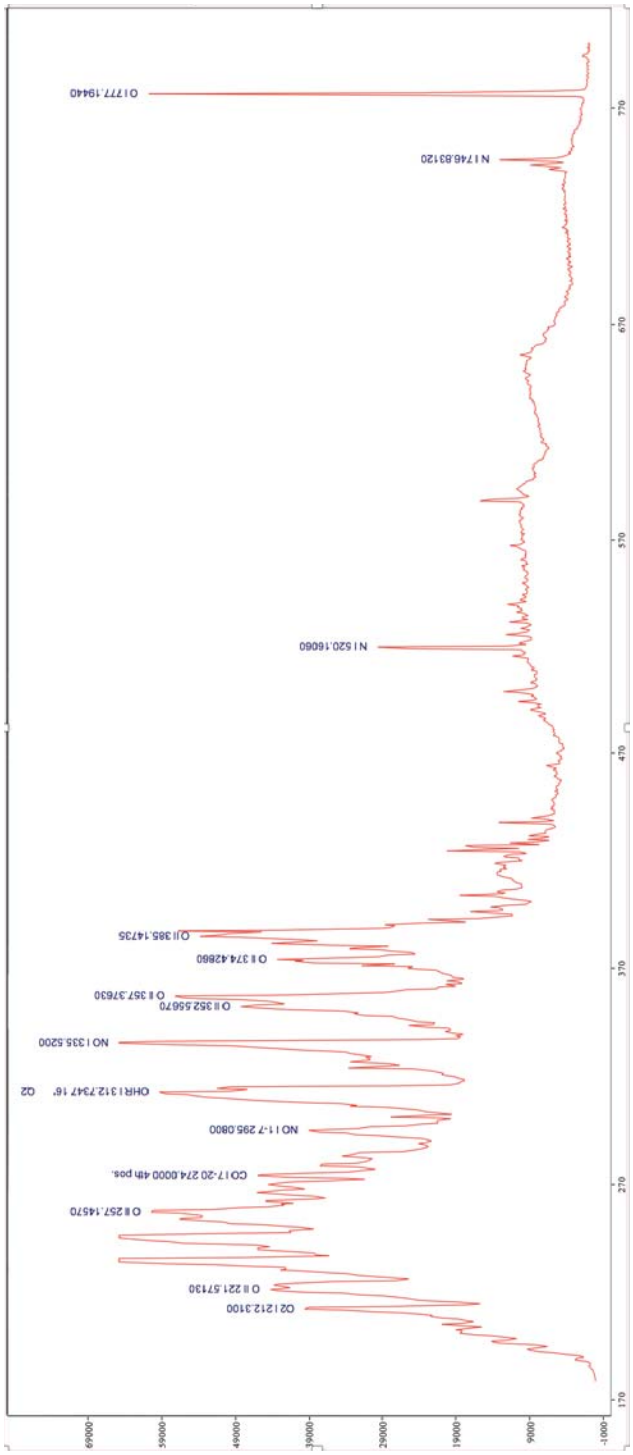

FIG. 6: Optical emission spectra of RVGA

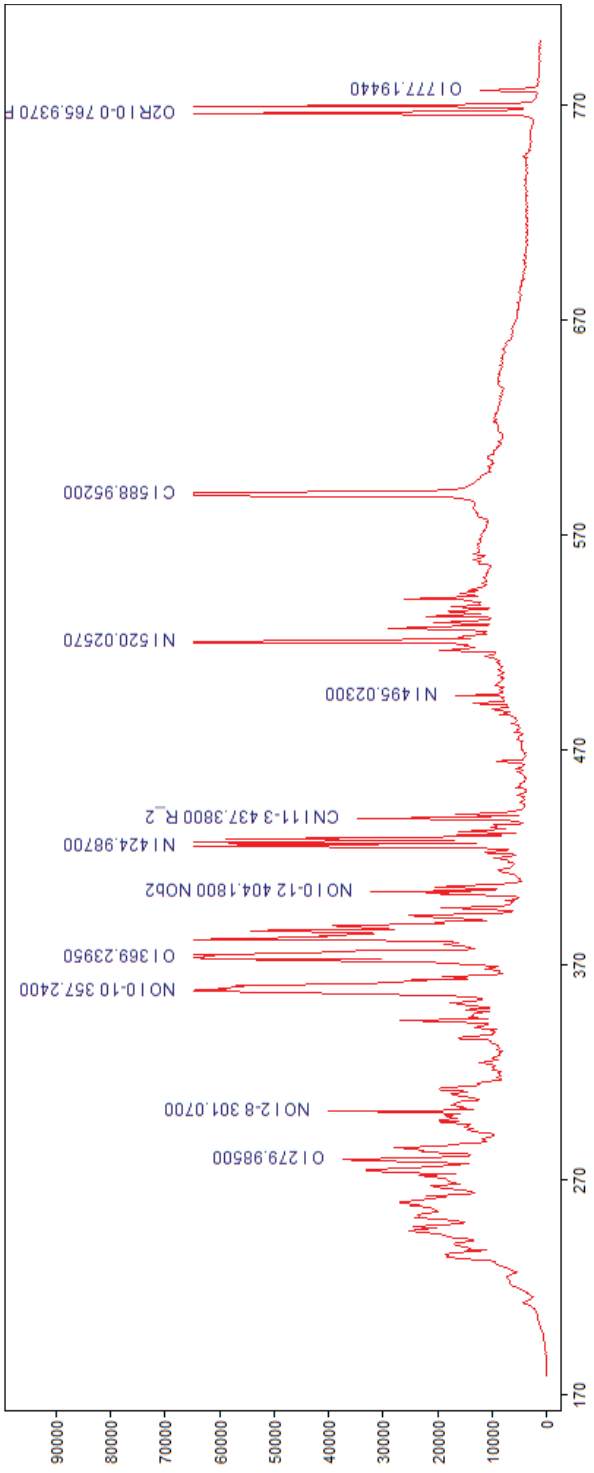

FIG. 7: Optical emission spectra of FVGA

III. RESULTS

A. Spark Discharge

Spark discharge is found to be least effective among the four plasma systems. Spark discharge does not show the effect during a long treatment time; the results obtained after 5 s of treatment are similar to results after 600 s of treatment. Figure 8 shows the percentage of SO_2 concentration remaining in the steep water.

As is evident from Fig. 9, practically no disinfection effect on steep water is found, even after a 600-s treatment. The first row shows nondiluted samples of untreated and treated steep water at different time periods, and no sign of sterilization is found in these treated samples. Therefore, we dilute samples 100× to count colonies (Fig. 10). We then observe ~20% bacterial inactivation in this treatment (which is, arguably, within the error range of the method).

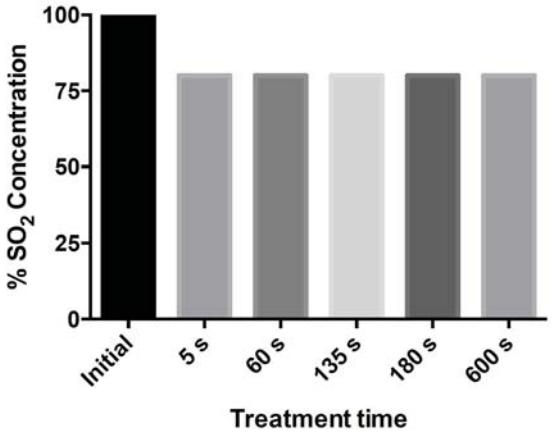

FIG. 8: Percent of SO_2 concentration remaining in steep water over treatment time

B. DBD

In a similar manor to spark discharge, DBD shows very little effect on SO_2 levels and disinfection. However, DBD shows a decrease in SO_2 level over time (Fig. 11). Although it decreases with time, the effect takes a long time to reach significant levels; after 600 s, 70% of SO_2 remains in steep water. Figure 12 shows bacteria results of samples treated. There is no clear difference between treated and untreated samples.

C. RVGA

RVGA results in a significant reduction in SO_2, probably due to the thermal nature of the discharge (Fig. 13). Compared to DBD and spark discharge, there is an obvious dif-

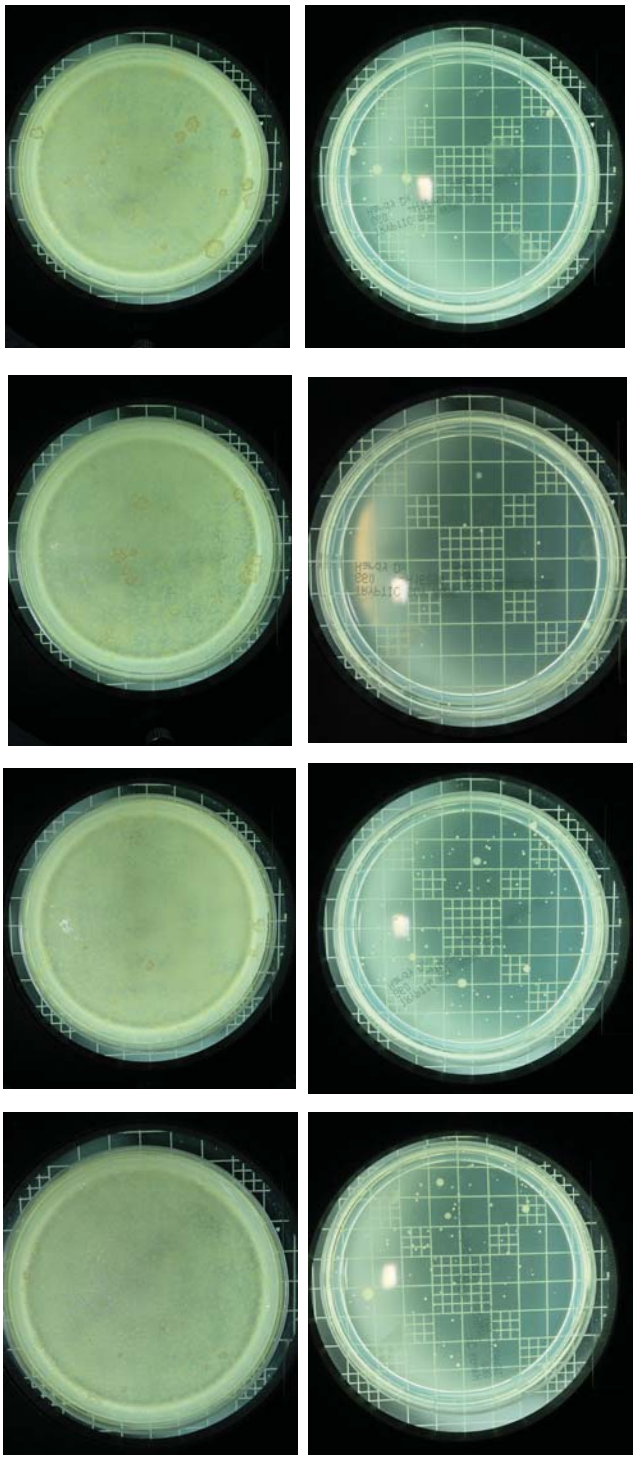

FIG. 9: Disinfection results using spark discharge. (Left to right) Control, 60-, 180-, and 600-s treatment time. (Top) Original samples; (bottom) samples diluted 100× by sterile water

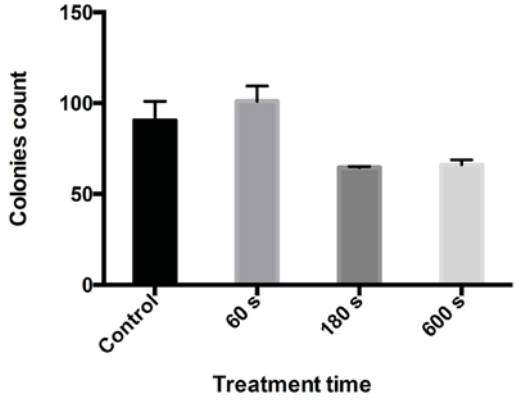

FIG. 10: Colony count in 100X diluted steep water treated by spark discharge

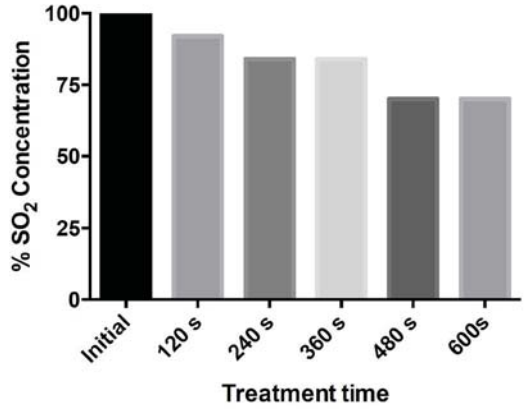

FIG. 11: Percent of SO_2 concentration remaining in steep water treated by DBD

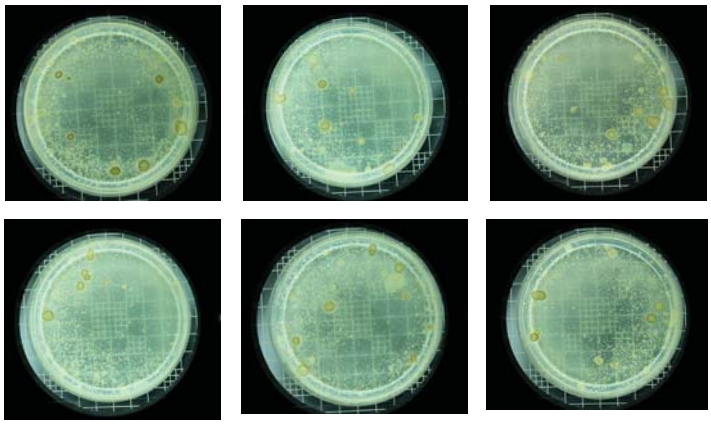

FIG. 12: Disinfection result of treated steep water by DBD over time. (Left to right) Control, 120-, 240-, 360-, 480-, and 600-s treatment time

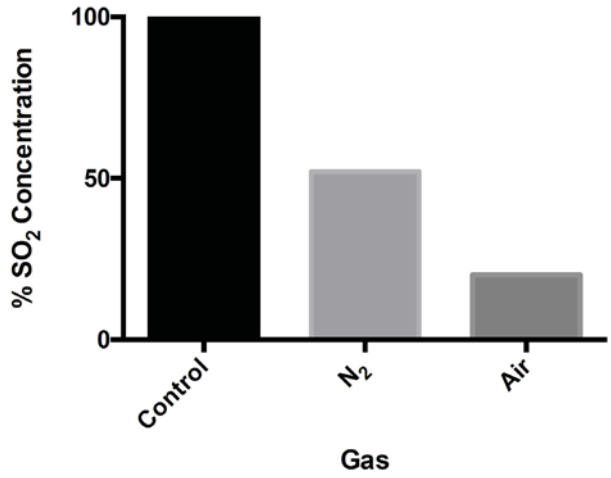

FIG. 13: Percent of SO_2 remaining in steep water treated by RVGA

ference in disinfection between treated and untreated samples: Approximately 50% of bacteria are inactivated in air plasma. The overall observation is that air is more effective at sterilization and neutralization than N_2 (Fig. 14).

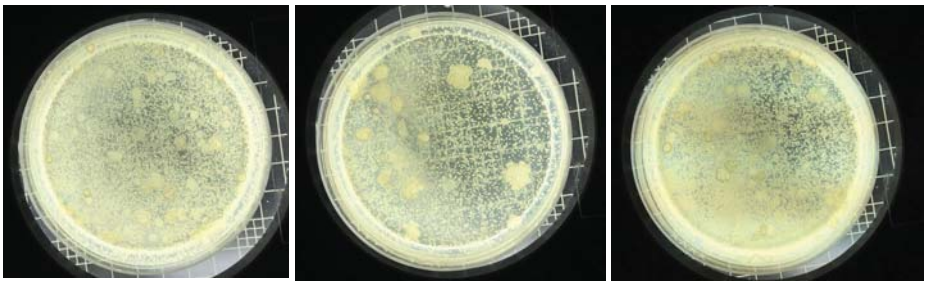

FIG. 14: Disinfection results of steep water treated by RVGA. (Left) Control; (center) plasma treatment with air; (right) plasma treatment with nitrogen

D. FVGA

The SO_2 remaining in steep water treated by FVGA is close to the desired result: approximately 40% reduction is observed (Fig. 15). The rest of the experiments are focused on disinfection because thus far there has been no clear effect with other plasma systems. To assist in the sterilization effect, NAC, ethanol, and H_2O_2 are used as additives before treatment. Because NAC also reacts with iodine solution, the titration results are significantly higher than without NAC, but we do see a large reduction in the amount of titrant used for treated samples. For H_2O_2, we see a decrease in SO_2 concentration, but apparently H_2O_2 neutralizes some of SO_2 before treatment (Fig. 15).

Titration is not performed for samples with added ethanol, because subsequent experiments focus on disinfection.

Similar to other plasma systems, there is very little disinfection effect with FVGA (without additives). Multiple passes of treatment seem to yield better results: Although unclear in the photographs (Fig. 16), there are single colonies on the dish with multiple-pass-treated steep water. However, even nine passes do not result in much difference in results with three passes (Fig. 16). The addition of ethanol and H_2O_2 produces a >6 log reduction. Samples with ethanol before treatment are found to be the same as original steep water samples, but results after treatment show complete inactivation of bacteria. With the H_2O_2 concentration ranging from 0.3% to 10%, a significant disinfection effect is observed in up to 1% of H_2O_2 (Fig. 17).

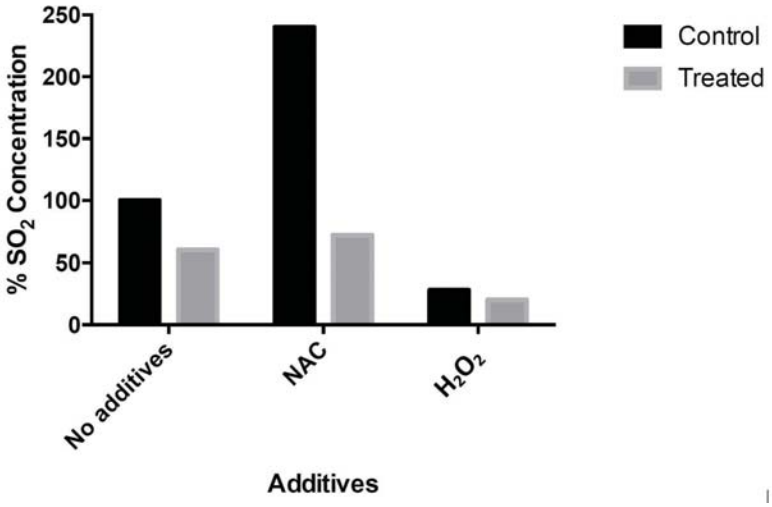

FIG. 15: Percent of SO_2 remaining in treated steep water by FVGA with and without additives

FIG. 16: Disinfection result of steep water after multiple passes treatment using FVGA. (Left) Control; (center) three passes; (right) nine passes

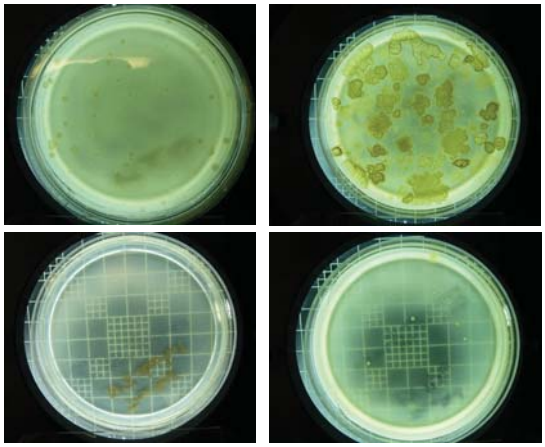

FIG. 17: Sterilization results with additives using FVGA. First column: (top) 10% ethanol steep water before treatment; (bottom) 10% ethanol steep water after treatment. Second column: (top) 1% H_2O_2 steep water before treatment; (bottom) 1% H_2O_2 steep water after treatment

IV. DISCUSSION AND CONCLUSION

A number of reports on inactivation of bacteria in liquid claim that a combination of ultraviolet (UV) radiation and reactive oxygen species comprise the primary killing mechanism.[4,7–11] CSL contains a high concentration of organic materials and minerals that readily react with reactive oxygen and reactive nitrogen species generated in plasma, blocking their antimicrobial effects. In addition, the effect of UV is completely absorbed by this water (Fig. 18).[12,13]

In this article, we have presented the effects of cold plasma and warm plasma on disinfection of CSL. Warm plasma clearly has a stronger effect, especially with the addition of a 1% volume of 30% H_2O_2 solution (or a 0.3% volume of pure H_2O_2). Hydrogen peroxide alone, even at a 10% addition, has little effect on disinfection; however, when CSL is activated by plasma, we observe complete inactivation of pathogens present. We hypothesize that this effect is due to reaching a threshold at which the hydrogen peroxide produced in plasma, in combination with the added H_2O_2, overcomes the pathogen load.

SO_2 reduction may be attributed to high temperature in the plasma channels, leading to the formation of SO_4^- or SO_3 gas. Both depend on temperature.[14]

$$3H_2SO_3 \leftrightarrows 2H_2SO_4 + H_2O + S$$

$$SO_2 + O_3 \leftrightarrows SO_3 + O_2$$

Finally, analyzing the power input into plasma as compared with the volume of CSL that was treated, we see that all the discharges deliver ~0.1 kWh/L of CSL treatment efficiency or ~$20 to ~$100 per 1000 gallons of CSL treatment efficiency (Table 1). The

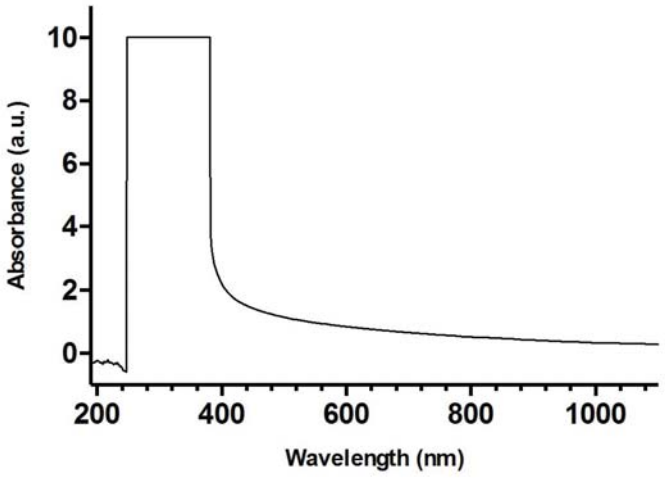

FIG. 18: UV-VIS absorbance spectra of typical CSL sample. VIS, Visible; a.u: abitrary unit

TABLE 1: Calculation of productivity of the four plasma systems

System	Power (W)	Liquid volume (mL)	Treatment time (s)	CSL Treatment productivity (kWh/L)
DC spark in liquid	12	25	600	0.08
DBD with ultrafine misting	6	25	600	0.04
Reverse vortex gliding arc	200	35	60	0.10
Forward vortex gliding arc	125	12	60	0.17

attractiveness of these treatments throughout the industry remains an open question, based primarily on the complexity of the treatment, coupled with the cost associated with it.

In summary, we have presented four plasma systems with potential for SO_2 reduction/removal and disinfection of CSL. With the addition of 0.3% H_2O_2 to CSL before plasma treatment, we observe the most significant effect of SO_2 reduction by ~50% and complete inactivation of pathogens (~5–6 log reduction). The systems we report here are not yet ready for commercial-scale operation, so this presents the biggest challenge for the future work in this field. We plan to scale up these systems in the future.

REFERENCES

1. Liggett RW, Koffler H. Corn steep liquor in microbiology. Bacteriol Rev. 1948;12(4):297.
2. Lawford HG, Rousseau JD. Corn steep liquor as a cost-effective nutrition adjunct in high-performance Zymomonas ethanol fermentations. Appl Biochem Biotechnol. 1997;63(1):287–304.

3. Gutsol A, Vaze ND, Arjunan KP, Gallagher Jr, MJ, Yang Y, Zhu J, Vasilets VN, Fridman A. Plasma for air and water sterilization. Plasma Assist Decontam Biol Cheml Agents. 2008:21–39.
4. Wilson A, Staack D, Farouk T, Gutsol A, Fridman A, Farouk B. Self-rotating DC atmospheric-pressure discharge over a water-surface electrode: regimes of operation. Plasma Sources Sci Technol. 2008;17:045001.
5. Robinson RD, Gutsol K, Rabinovich A, Fridman AA. Plasma acid production in a gliding arc plasmatron. 2012;2(4):249–58.
6. Fridman G, Peddinghaus M, Ayan H, Fridman A, Balasubramanian M, Gutsol A, Brooks A. Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air. Plasma Chemi Plasma Proc. 2006;26(4):425–42.
7. Yang Y, Kim H, Starikovskiy A, Fridman A, Cho YI. Pulsed multichannel discharge array in water with stacked circular disk electrodes. IEEE Trans Plasma Sci. 2011;39(11):2624–5.
8. Yamatake A, Fletcher J, Yasuoka K, Ishii S. Water treatment by fast oxygen radical flow with DC-driven microhollow cathode discharge. IEEE Trans Plasma Sci. 2006;34(4):1375–81.
9. Burlica R, Shih KY, Locke BR. Formation of H2 and H2O2 in a water-spray gliding arc nonthermal plasma reactor. Ind Eng Chem Res. 2010;49(14):6342–9.
10. Cho M, Chung H, Yoon J. Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. Appl Environ Microbiol. 2003;69(4):2284–1.
11. Traylor MJ, Pavlovich MJ, Karim S, Hait P, Sakiyama Y, Clark DS, Graves DB. Long-term antibacterial efficacy of air plasma-activated water. J Phys D: Appl Phys. 2011;44(47):472001.
12. Gouda MK, Swellam AE, Omar SH. Production of PHB by a Bacillus megaterium strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. Microbiol Res. 2001;156(3):201–7.
13. Santos DK, Rufino RD, Luna JM, Santos VA, Salgueiro AA, Sarubbo LA. Synthesis and evaluation of biosurfactant produced by Candida lipolytica using animal fat and corn steep liquor. J Petrol Sci Eng. 2013;105:43–50.
14. Zhou S, Wang S, Chen C, Xu X. Thermal degradation of ethanolamine in CO2 capture with SO2. In: Cleaner Combustion and Sustainable World. New York: Springer; 2013. p. 1267–73.