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Finding effective plasma process factors on yeast deactivation by numerical simulation and RSM

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Abstract

In recent years, there has been an increasing interest in the application of plasma technology in food preservation technologies. Plasma is nonthermal physical processing that has a high potential in the field of food processing. In this study, a mathematical model was investigated for yeast deactivation during plasma treatment. The definitive screen design was used to investigate the factors that affect yeast deactivation by plasma. Four factors of voltage (A: 20- 30 kV), Vessel diameter (B: 40- 60 mm), process temperature (C: 20-40ºC), and type of plasma media (air or water) were selected. Then the treatment was simulated by COMSOL software. The responses of reaction kinetics coefficient, the ozone concentration, and final deactivation time were analyzed by definitive screen design expert to find the effective model parameters and process optimization. The results show that plasma treatment in water can have the strongest effect than air plasma. The changes in the number of microorganisms have a linear relationship with process time at different voltage-temperature conditions, but the ozone concentration dramatically changes at different combinations of voltage and temperature. The analyzed data show the k_{reac} is affected significantly by the diameter of the vessel and the 221 types of process media (water or air). The ozone concentration only depends on the type of plasma media and the final 223 process time significantly depends on vessel diameter and type of media. Also, in plasma treatment, media type had a significant effect on all 3 responses, while the effect of temperature was only on final process time. For example, at temperature 20ºC the ozone concentration decreased at the first time of treatment and then stay constant, but at 30ºC, the ozone production increased with treatment time. This study showed when an RSM design was applied for designing the experiment which considers different process factors, the results can significantly differ from the study on only one-factor. In plasma treatment, media type had a significant effect on all 3 responses, while the temperature shows its effect only on final process time. Thus it can be concluded that with proper selecting of plasma media, this technology can be used for deactivation of food microorganisms.

Keywords: Plasma treatment, Microorganism deactivation, CFD simulation, RSM design

Introduction

Increasing the shelf- life of raw food materials is an important aspect of food processing. Traditional technologies such as thermal processing can reduce the deterioration of food materials. However, they also have some disadvantages on nutritional, color, taste, and texture characteristics of food products. In

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recent years, there has been an increasing interest in the application of plasma technology in food preservation technologies. Plasma is nonthermal physical processing that has a high potential in the field of food processing (Xiang et al., 2019). Plasma is the fourth state of material and consists of gas molecules and charged particles. This technology is categorized into equilibrium and non-equilibrium plasma (Tabibian et al., 2020). In the recent decade, the atmospheric pressure of nonthermal plasma has gained significant attention for use in food processing (Perinban et al., 2019). The plasma technology has been used for inactivating the bacteria (Pankaj et al., 2018; Perinban et al., 2019; Xiang et al., 2018), viruses (Bourke et al., 2018; Guo et al., 2018), enzymes (Misra et al., 2016; Surowsky et al., 2013), removing the pesticide and antibiotic residues (Bourke et al., 2018; Chizoba Ekezie et al., 2017; Perinban et al., 2019), and altering the functional properties of food (Chizoba Ekezie et al., 2017; Muhammad et al., 2018) and packaging materials (Pankaj et al., 2014; Perinban et al., 2019). One of the most significant current research on plasma application in food preservation is the non-thermal effect on microorganisms' deactivation. So far, there has been very little research to simulate the effect of the parameters on the plasma deactivation process. Wang et al. (2020) simulate the gas-phase surface discharge plasma on the sterility of the water containing *Z. rouxii* LB (B- WHX- 12- 54). They found that yeast concentration slowing down its inactivation and the reactor diameter does not affect the inactivation process (Wang et al., 2020).

The advantage of using COMSOL simulation is that it is helpful to study more detailed process conditions (Chilka & Ranade, 2019). However, when we consider multiple process factors during simulations, the application of computational fluid dynamics (CFD) modeling leads to having a high number of runs that make them hard to analyze. Thus, using RSM (response surface methodology) techniques for reducing the CFD simulation can be helpful to minimize the CFD runs and data analysis time. When a certain response is dependent on several factors, RSM can be used as a collection of statistical and mathematical techniques that are used to improve, optimize and develop such processes (Sumic et al., 2016). The response surface methodology was used to determine factors affecting bacterial deactivation during plasma treatment through setting up a mathematical model. A key advantage of using the response surface methodology (RSM) is that it has the statistical ability to reduce the total treatments in a multi-independent factors study (Misra et al., 2013), it can be a suitable choice before numerical calculations by CFD methods to reduce the final runs behind considering all possible factors including a unit food process. The objective of this study was to investigate a mathematical model consisting of temperature, voltage, vessel diameter, and the type of plasma medium for yeast deactivation during plasma treatment.

Materials and methods

Microbial species

The strain *Z. rouxii* LB (B- WHX- 12- 54), was selected to investigate the deactivation effect of plasma treatment conditions. Data were obtained from Wang et al. (2020) to simulate the deactivation process.

The experimental design

At first, we studied the effect of temperature (20 and 30°C) and voltage (15 and 20kV) to validate the data obtained from Wang et al. (2020) for simulation. After validation and simulation set-up, the design was created by Design- Expert software v.11 to further simulation in COMSOL. The definitive screen design was used to investigate the factors that affect plasma deactivation. Four factors of voltage (A: 20- 30 kV), Vessel diameter (B: 40- 60 mm), the process temperature (C: 20- 40°C), and the type of plasma media (air or water) were selected. Table 1 shows 14 combinations of four factors that were simulated in COMSOL for investigating the factors affecting the bacterial inactivation.

Table 1- Definitive screen design in response surface methodology

Run	Voltage (kV)	Diameter (mm)	Temperature (°C)	Media
1	30	50	20	air
2	20	40	40	air
3	25	50	30	water
4	30	40	40	water
5	30	60	20	water
6	20	50	40	water
7	30	40	30	air
8	30	60	40	air
9	20	60	20	air
10	20	40	20	water
11	20	60	30	water
12	25	60	40	air
13	25	40	20	water
14	25	50	30	air

Experimental setup

A cylinder reactor with 40 mm diameter and 300 mm length was selected. A quartz tube was used at the center of the reactor (d= 10 mm, h= 270). A magnetic field was established through two inner stainless electrodes (15 kV, 50 Hz) inside the quartz tube. The plasma media between the quartz tube and the reactor was selected as the ground electrode. The gas flow inside the tube had a rate of 0.003 m/s. since the gas phase can produce chemical components such as ozone (Wang et al., 2020), in this study the ozone was selected as an indicator for simulation. The high-energy electrons can conduct the reaction between oxygen radicals and oxygen. This leads to ozone generating which presents in most plasmas and can have a deactivating effect on microbial population of the food surfaces. Wang et al. proposed that voltage and reactor dimensions can affect ozone concentration (Wang et al., 2020). Thus these factors were selected for this study. Also, we find in our pretreatment test that the temperature and the media inside the reactor can have an effective role in ozone production. Thus these four factors were selected for investigating their effect on ozone production and microbial deactivation rate.

Model definition in COMSOL

The simulation in COMSOL multiphysics 5.3a software was run for a 2D geometry (Fig. 1) in four modules of bubbly flow k-ε, transport of diluted species (for water/ air as the media), transport of diluted species (for microbial removal from the studied media), and the magnetic field.

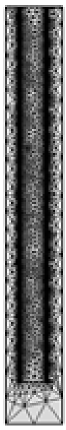


Fig. 1. The 2D geometry of reactor simulated in COMSOL

Bubbly flow k-ε

This module was used to simulate the ozone bubble rise in the reactor. The gas density is assumed negligible in comparison with the liquid density. The RANS k-ε equations were solved for liquid and ozone bubbles. The density and diameter of ozone bubbles were set as 2.14 kg/m^3 and 3.21 mm, respectively. The ozone diffusion coefficient was 1.74× 10^{-9} m^2/s.

The l and g are subscribed related to the liquid and gas, respectively.

$$\emptyset_l\rho_l\frac{\partial u_l}{\partial t}+\emptyset_l\rho_l(u_l.\nabla)u_l=\nabla.[-pI+\emptyset_l(\mu_l+\mu_T)(\nabla u_l+(\nabla u_l)^T)]+\emptyset_l\rho_l g+F \tag{1}$$

$$\rho_1\nabla.(u_l)=0, \qquad u_l=u \tag{2}$$

$$\frac{\partial\emptyset_g\rho_g}{\partial t}+\nabla.N_{\rho_g\emptyset_g}=-m_{gl}, \qquad \emptyset_g\rho_g=rhogeff \tag{3}$$

$$N_{\rho_g\emptyset_g}=\emptyset_g\rho_g u_g, \qquad u_g=u_l+u_{slip}-\mu_T\frac{\nabla\emptyset_g}{\rho_l\emptyset_g} \tag{4}$$

The transport of diluted species

The deactivation of microbial cells had a dependency on ozone concentration. The reaction rate was calculated based on Fick's law as:

$$\frac{\partial c_i}{\partial t}+\nabla.(-D_i\nabla c_i)+u.\nabla c_i=R_i \tag{5}$$

$$N_i=-D_i\nabla c_i+uc_i \tag{6}$$

During simulation, the R_i was the reaction rate of ozone and microbial cells which defied as:

$$R_i=-k_{reac}c_{o_3} \tag{7}$$

Which the k_{reac} is the death rate constant. The death rate of a microorganism in a determined condition follows the first- order kinetics. Thus, we can show the death rate by Ibarz & Barbosa-Cánovas, (2002); Valentas, Rotstein, & Singh, 1997):

$$N=N_0\exp(-k_{reac}t) \tag{8}$$

When this equation is plotted in semilogarithmic coordinates, a straight line with $-k$ as the slope is obtained which is called the thermal death curve (Ibarz & Barbosa-Cánovas, 2002).

Initial and boundary conditions

The outlet boundary was selected at the top of the reactor as a free surface and the motions on the surface were ignored. The ozone inlet boundary was at the bottom of the reactor. the ozone flux rate was as:

$$n.N_1=n.\left(uc_{0.j}\right) \tag{9}$$

The pressure point constraint was added on the outlet boundary (p= 0). The microorganism concentration in the reactor was set at 1.5× 10^4 CFU/ml. the initial temperature of plasma was set as the T based on the RSM design.

Problem-solving

The COMSOL multiphysics 5.3a was used to solve four modules based on turbulent RANS k-ε. The processor was a surface desktop Intel® Core™ i5-4300U, 2.50 GHz, RAM 4 GB, and Windows 10 64-bit operating system. The relative tolerance was 0.01. the data were recorded every 1 min. The inactivation was simulated for 20 min.

Simulation validation and statistics procedures

The simulated data for k_{reac} were used for validation with experimental data. After validation, the RSM design factors were set for each run in COMSOL and the deactivation time, the ozone concentration at the end of deactivation time, and the final k_{reac} at the deactivation time were recorded as the results. The response variables were fitted to a second-order polynomial model (Equation (10)) which is generally able to describe the relationship between the responses and the independent variables.

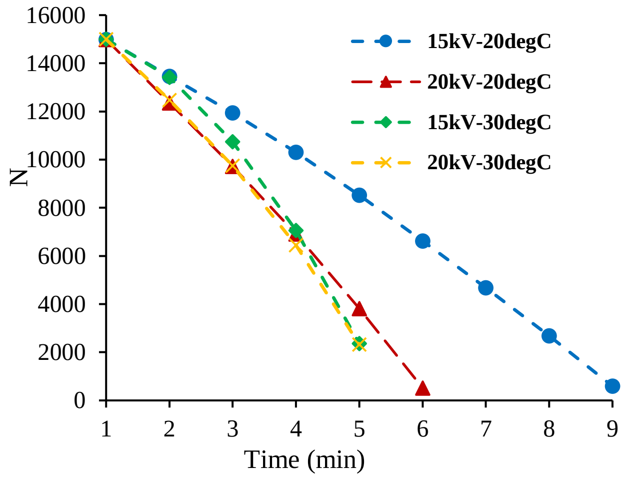
$$Y=\beta_0+\sum_{i=1}^{2}\beta_i X_i+\sum_{i=1}^{2}\beta_{ii}X_i^2+\sum_{i<i=1}^{2}\beta_{ij}X_{ij} \tag{10}$$

where Y is the response, X_i and X_j are the independent variables affecting the response, and β_0, β_i, β_{ii}, and β_{ij}, are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively. To evaluate model adequacy and determine regression coefficients and statistical significance, the analysis of variance (ANOVA) was used. The Design- Expert v.11 was used for RSM statistical analysis. The results were statistically tested at the significance level of p= 0.05. The adequacy of the model was evaluated by the coefficient of determination (R^2), model p-value, and lack of fit testing (Aliakbarian et al., 2018; Lisboa et al., 2018; Majeed et al., 2016) and the coefficient of variation (CV). The CV is a measure of deviation from the mean values, which shows the reliability of the experiment. In general, CV< 10% indicates better reliability (Islam Shishir et al., 2016). The final optimum parameters proposed by RSM were selected to simulate the optimum conditions with the CFD method. The proposed and the simulated data were compared to study the accuracy of final optimum conditions proposed by RSM.

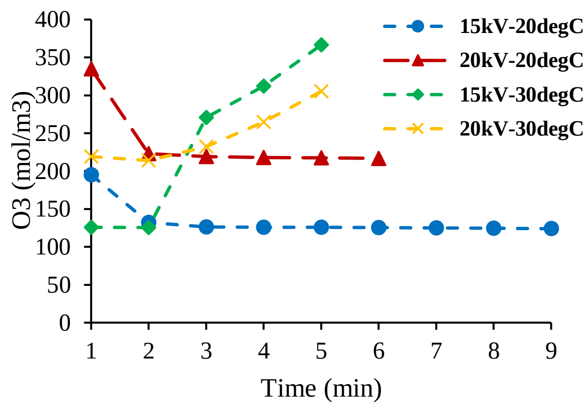
Results and discussion

Deactivation kinetics and Simulation validation

In microbial deactivation, if equation 8 is plotted as a semi-logarithmic coordinate graph named "The thermal death curve", we can consider the slope as k_{reac} (Ibarz & Barbosa-Cánovas, 2002). The temperature of deactivation can change the slope of this curve. However, the effect of the ozone concentration during the process should be considered. Especially when it is obvious that the temperature changes can affect the ozone concentration produced by the plasma.



(a)



(b)

Fig. 2. The changes of number of microorganisms (a) and ozone concentration at different combination of voltage and temperature.

Figure 2 shows the results of pretreatment to find the role of temperature and voltage on deactivation time. According to Figure 2, any changes in the number of microorganisms have a linear relationship with the process time at different voltage- temperature conditions. However, the ozone concentration dramatically changes at different combinations of voltage and temperature. Our finding is in agreement with Tabibian et al. (2020), Wang et al. (2020) and Yuan et al. (2019). Figure 2a shows that at 15 kV and 20°C the time required for microorganism reduction is significantly longer than the 15 and 20kV at 30°C. This shows that temperature can have a significant effect on deactivation by plasma. Figure 2b, also shows that changes in ozone concentration during plasma treatment have a direct relation with temperature. At temperature 20°C, the ozone concentration decreased at the beginning of treatment and then stayed constant, but at 30°C, the ozone production increased with treatment time. With respect to Figure 2a, the deactivation kinetics can be calculated.

We know the units of rate constants are the cycles per time units (t^{-1}) (Heldman & Lund, 2007; Valentas et al., 1997) and k_{reac} depends on the process temperature, food, and microorganism type and growth state (vegetative, or spore form) (Ibarz & Barbosa-Cánovas, 2002). The k_{reac} (m^3/ mol.s) as a function of time at 20°C for initial concentration of microorganism (N_0) was 1.5×10^4 CFU/ml which is shown in Figure 3.

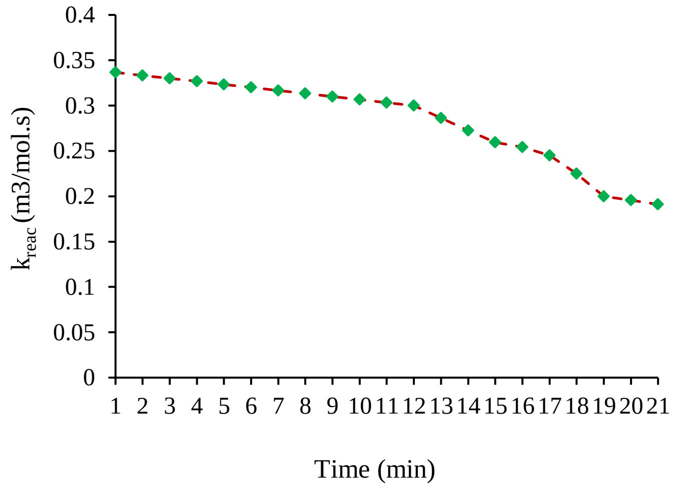


Fig. 3. The k_{reac} (m^3/mol.s) as a function of time at 20°C for initial concentration of *Z. rouxii* (N_0) was 1.5× 10^4 CFU/ml

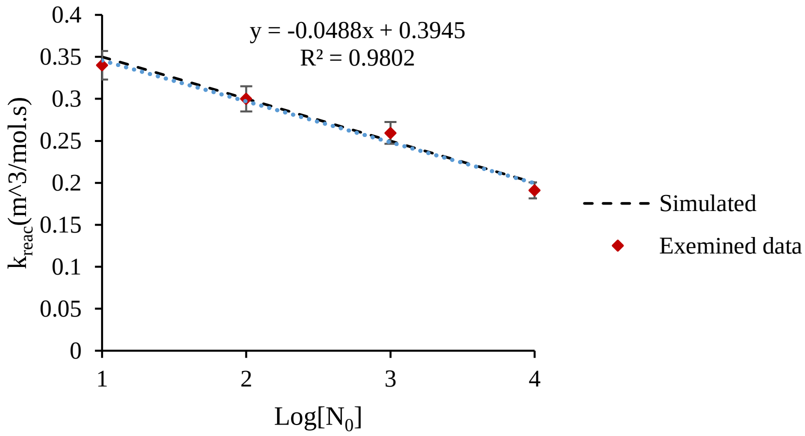
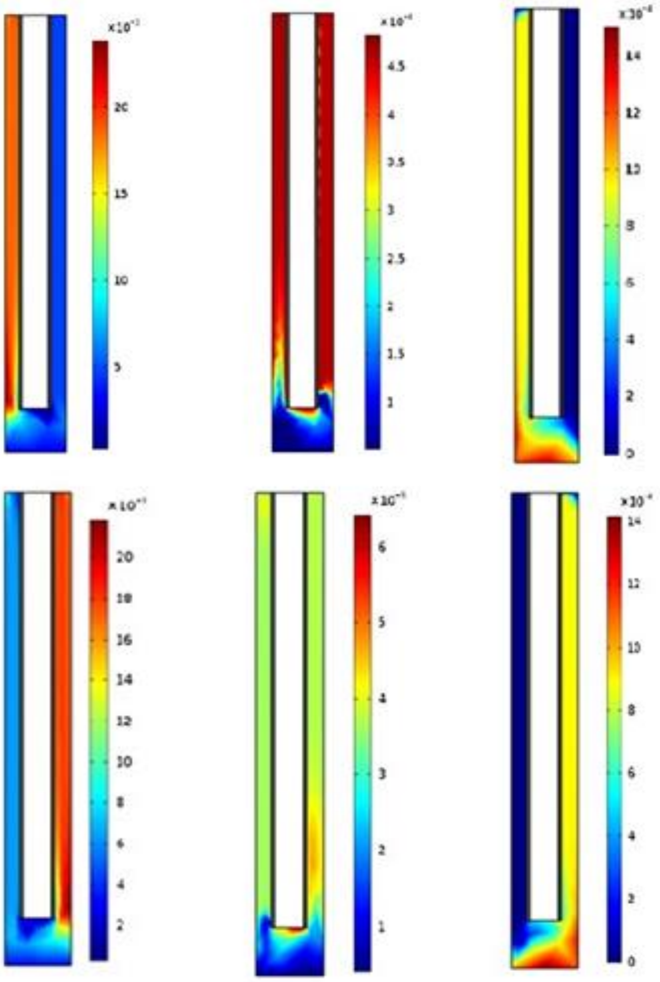


Fig. 4. The simulated and experimental data of k_{reac} (m^3/mol.s) as a function of time at 20°C for initial concentration of *Z. rouxii* (N_0) was 1.5× 10^4 CFU/ml

Finally, Figure 4 shows the simulated data compared with experimental data for k_{reac} obtained from Wang et al. (2020). The regression coefficient R^2= 0.9802 shows a good agreement between experimental and simulated data. When the initial concentration is increased, the k_{reac} (or death rate constant) decreased.

The simulation results in Figure 5, also validate that the temperature can have a significant effect on the velocity magnitude of liquid phase (m/s) and ozone phase dispersion coefficient (m^2/s) in a plasma treatment in water. The minimum velocity of liquid phase displacement due to ozone bubbles (Wang et al., 2020), was 5 m/s at 20°C, but with 10°C increase in treatment temperature leads to a 1.5

fold decrease in minimum velocity of the liquid phase.



(a) (b) (c)

Fig. 5. Velocity magnitude of liquid phase (m/s) (a), gas phase dispersion coefficient (m^2/s) (b), volume fraction of gas phase (c) during plasma treatment

We know that plasma can generate reactive oxygen species (ROS) (Zhang et al., 2013) such as atomic oxygen and hydroxyl radicals in water (Surowsky et al., 2014). The ROS produced in plasma- activated water (PAW), has an important role in bacterial inactivation. Especially atomic oxygen which reacts with hydrogen compounds and leads to protein oxidation and etching processes (Sakudo et al., 2019; Surowsky et al., 2014). The etching effects such as bacterial spore shrinkage contributes especially in oxygen gas plasma but not in nitrogen plasma (Sakudo et al., 2019). The ROS can initiate the breaking of single-stranded DNA, oxidation in amino acid and unsaturated fatty acids, protein cross- links, and cleavage in peptide bond (Surowsky et al., 2014). Ozone is formed as a consequence of combining atomic oxygen in presence of water (Surowsky et al., 2014). Thus it can be explained that when we use the gas as the plasma medium, the mechanism of plasma deactivation depends on the type of gas (Sakudo et al., 2019). The reactive species produced by gas, the UV radiated from the magnetic field, the electric field are involved in plasma deactivation in plasma with the medium of gas (Sakudo et al., 2019). Reactive nitrogen species (RNS) are produced when the gas is nitrogen or ambient air. One of them is nitric oxide which then contributes to oxidation reactions with ozone and makes some products such as nitric oxide, nitrite, and oxygen (Surowsky et al., 2014). Nitric oxide and nitrite can play as a strong anti- microbial agent, especially for gram-positive microorganisms. Since conductivity, temperature, and pH have the same role as electrode material, electric field characteristics, and gas composition or medium type, the result of this study about the effect of temperature on ozone concentration and plasma deactivation is in agree with Bruggeman & Leys (2009), Locke et al. (2006) and Thagard et al. (2009). This complexity of the role of different factors on microorganism deactivation by plasma leads to the investigation of other factors that affect the ozone concentration during plasma deactivation of microorganisms.

Data analyzing and model definition

After validating the results, we simulate the 14 treatments of RSM to find models for responses. The CFD simulation of plasma treatment showed a better efficiency for studying the plasma treatment conditions. When the RSM was used for investigating the most effective factors in plasma treatment, it was possible to study more than one factor in the process. the simulation was based on data obtained from Wang et al. (2020). The results for yeast deactivation under different levels of factors shown in Table 2, analyzed for finding the significant factors. Responses were k_{reac}, ozone concentration, at the end of deactivation, and final treatment time (min) for reaching the minimum amount of microorganism concentration.

Table 2- The Responses of Definitive screen design in RSM

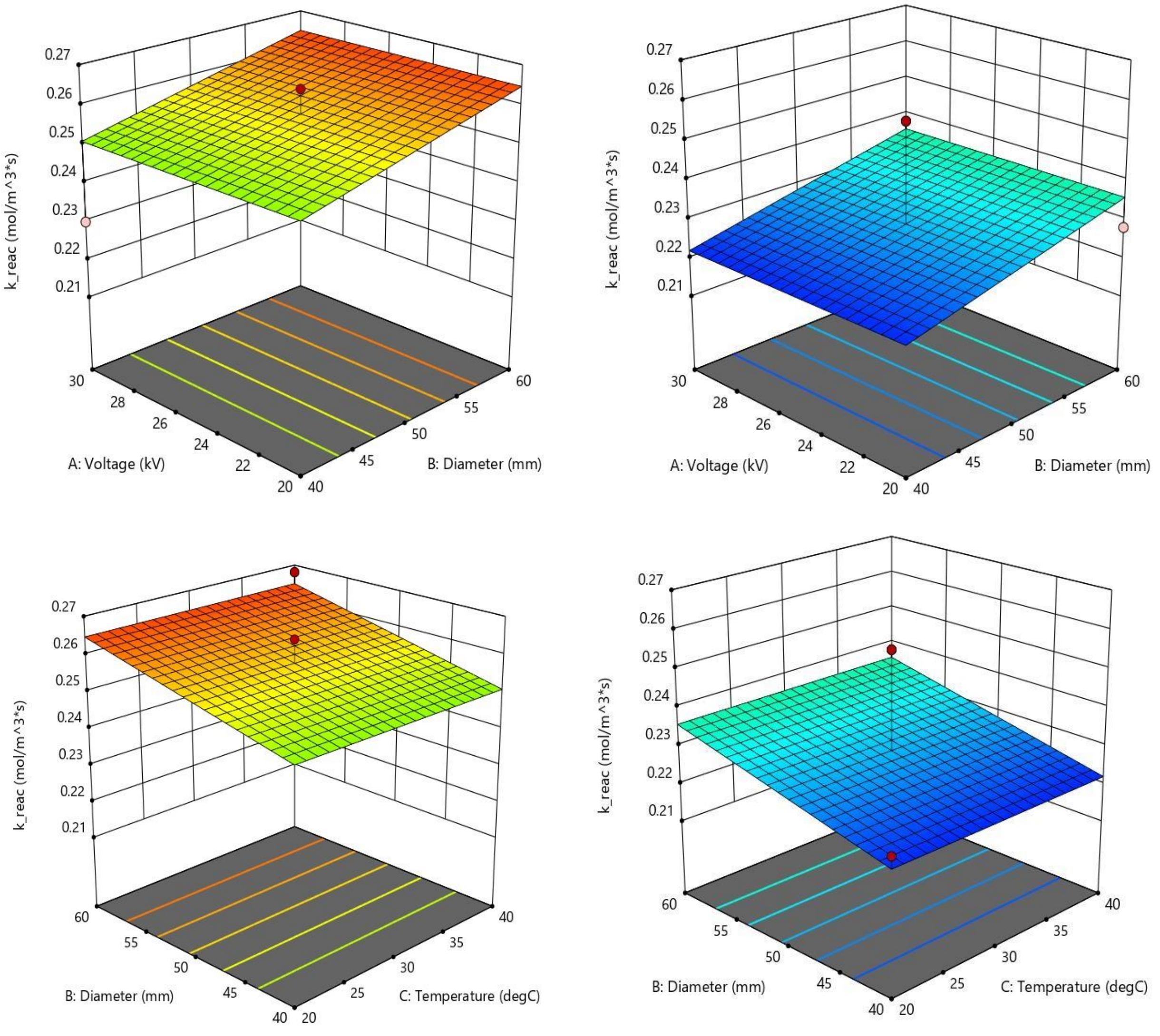
Run	Voltage (kV)	Diameter (mm)	Temperature (°C)	Media	O_3 (mol/m^3)	Deactivation time (min)
1	30	50	20	air	50	6
2	20	40	40	air	47.5	5.1
3	25	50	30	water	125	3
4	30	40	40	water	125	2.9
5	30	60	20	water	125	3.05
6	20	50	40	water	125	3
7	30	40	30	air	75	3.25
8	30	60	40	air	55	6.55
9	20	60	20	air	55	6.95
10	20	40	20	water	125	2.95
11	20	60	30	water	125	3.1
12	25	60	40	air	52.5	6.65
13	25	40	20	water	125	3.05
14	25	50	30	Air	50	6.2

The analyzed data show that the k_{reac} is affected significantly by the diameter of the vessel and the types of process media (water or air). The effect of media in plasma treatment on all 3 responses was highly significant. The ozone concentration only depends on the type of plasma media and the final process time significantly depends on vessel diameter and type of media. The models obtained for each response are as bellow:

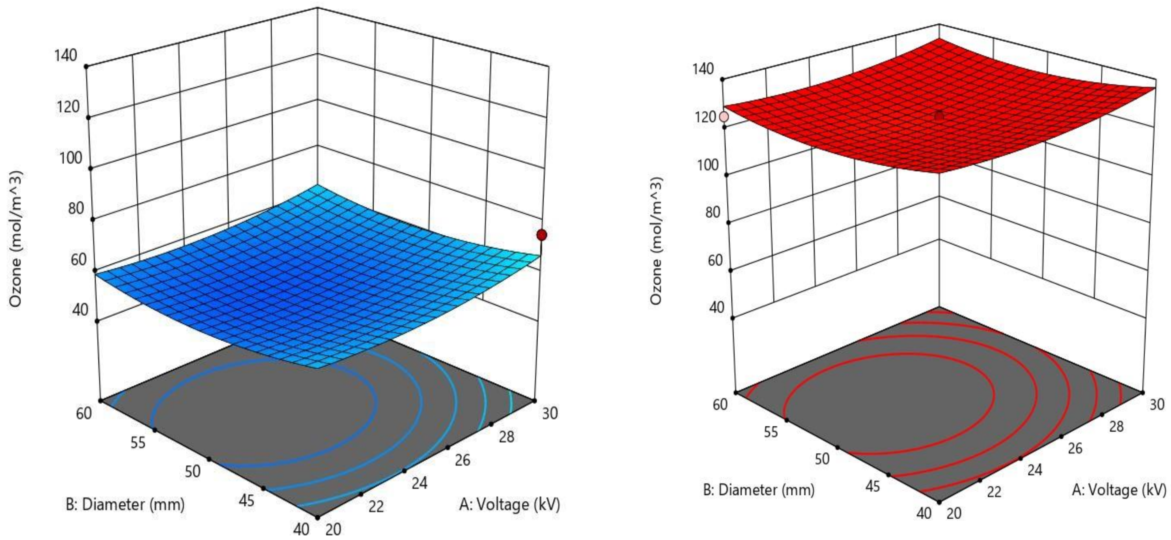
$$k_{reac} = 0.2433 + 0.0069B + 0.0144D \qquad R^2 = 0.9396\ P = 0.0011 \quad (11)$$

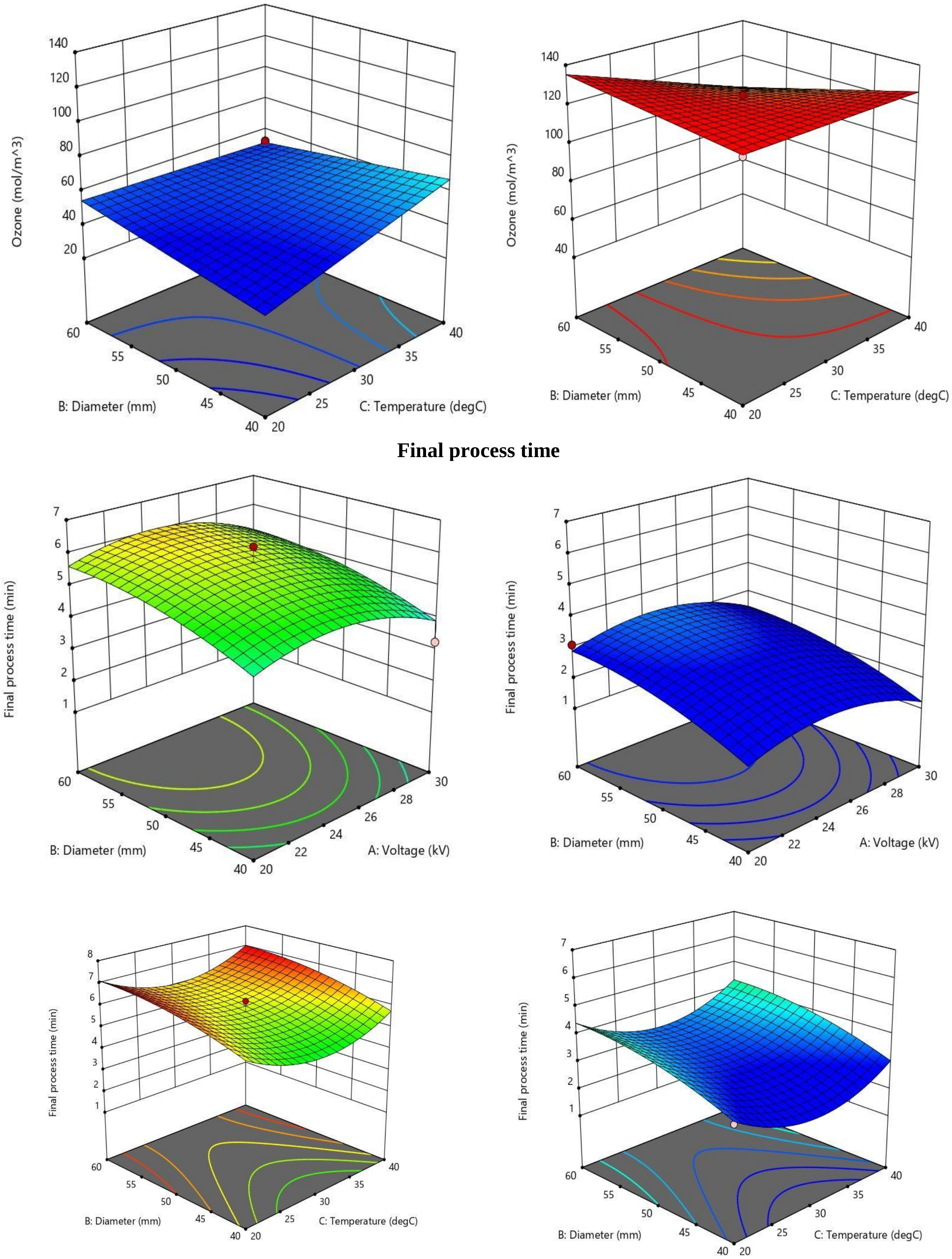
$$Ozone = 87.5 - 35.04D \qquad R^2 = 0.9898\ P = 0.0001 \quad (12)$$

$$Final\ process\ time\ (water/air) = 4.37 + 0.6351B + 1.34D + 1.02C^2 \qquad R^2 = 0.9396\ P = 0.0028 \quad (13)$$


k_reac (mol/m^3*s)
0.27
0.26
0.25
0.24
0.23
0.22
0.21
A: Voltage (kV)
20
22
24
26
28
30
B: Diameter (mm)
40
45
50
55
60
C: Temperature (degC)
20
25
30
35
40

Ozone


Ozone (mol/m^3)
40
60
80
100
120
140
B: Diameter (mm)
40
45
50
55
60
A: Voltage (kV)
20
22
24
26
28
30



Air **Water**

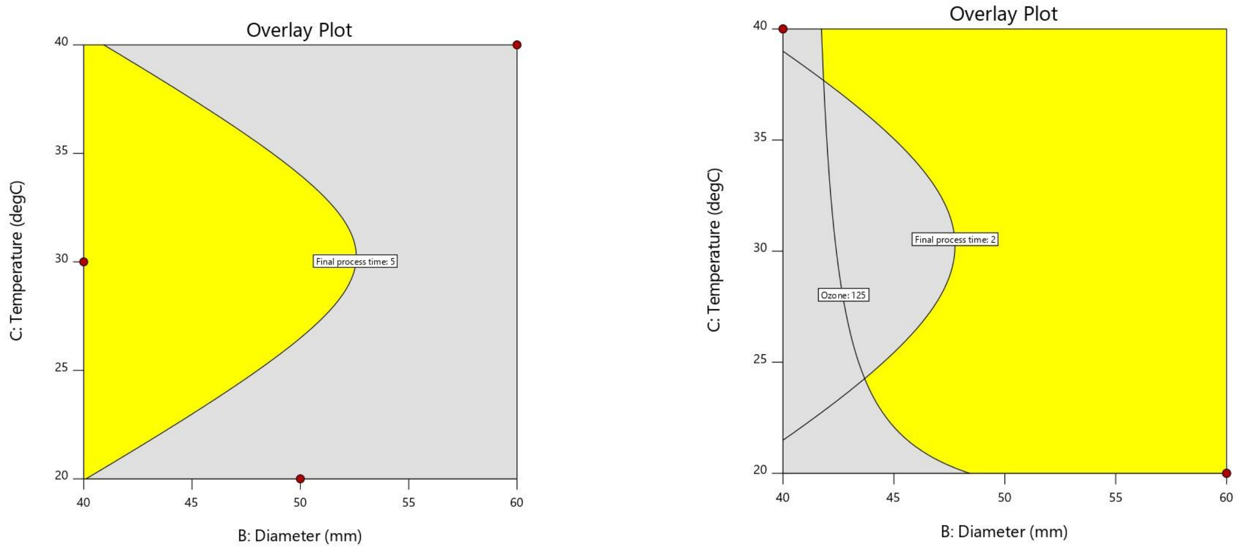
Fig. 6. Responses for k_{reac}, ozone concentration, at the end of deactivation, and final treatment time (min) at 30℃

During data analysis, it was demonstrated that the medium had a highly significant effect (p< 230 0.0001) on k_{reac} and ozone concentration responses. The final process time was affected by the diameter of the reactor vessel and the square of temperature. The variation of response by studied factors is shown in Figure 6. It can be seen that the responses are significantly different at two different reaction mediums (air and water). As can be seen in Figure 6, the ozone production in air plasma is lower than the water plasma. Meanwhile, the processing time is higher at air plasma which is related to lower ozone production during air plasma treatment. This result is according to the basis of production of second plasma species from ozone in water (such as hydrogen peroxide) (Julák et al., 2018; Perinban et al., 2019).

Liao et al. reviewed the influence of non-thermal plasma on microbial inactivation. They explained when researchers added the oxygen to the dielectric barrier discharge (DBD) plasma for deactivation of *E.coli* and Staphylococcus in cheese slices, more oxygen-based radicals were produced and lead to a strong antibacterial effect (Basaran et al., 2008; Liao et al., 2017). Thus the results from RSM are in agreement with other results from literature (Liao et al., 2018; Xiang et al., 2019).

Process optimization

Optimization aimed to reduce the processing time. The k_{reac} was set at the lowest and the ozone was set at the highest amount of 0.22 and 125, respectively. The final process time was set for 1 to 5 minutes. Figure 7 shows the graphical results of optimization. In this study, the optimium conditions were 29.79V of voltage, 46.34 mm of vessel diameter, and 4.86 min of process temperature with water as the media for plasma discharge.



Air **Water**

Fig. 7. The graphical optimization of final deactivation time for plasma treatment

Conclusion

The results of this study showed that for the deactivation of microorganisms, the plasma treatment in water can have the strongest effect than air plasma. Also, during studying the effect on plasma, a combination of factors should be considered. It has demonstrated that study one factor such as temperature on process responses such as ozone concentration, the results will be restricted by other factors that are ignored. This study showed when an RSM design was applied for designing the experiment which considers different process factors, the results can significantly differ from the only one-factor study. In plasma treatment, media type had a significant effect on all 3 responses, while the temperature shows its effect only on final process time. Thus it is recommended to use RSM or other statistical methods for studying various factors during plasma treatments.

Conflict of Interest

We have no conflicts of interest.

Nomenclature

φ	Phase volume fraction [m/m^3]
ρ	Density [kg/m^3]
u	Velocity vector [m/s]
μ	Viscosity [Pa.s]
g	Gravity vector [m/s^2]
$N_{\rho_g \emptyset_g}$	Flux vector [$mol/m^3.s$]
c	Concentration of the species [mol/m^3]
D	Diffusion coefficient [m^2/s]
R	Reaction rate expression for the species [$mol/m^3.s$]
N	Flux vector [$mol/m^3.s$]
k_{reac}	Reaction rate coefficient [$m^3/mol.s$]

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یافتن فاکتورهای موثر فرآیند پلاسما بر فعالیت مخمراز طریق شبیه‌سازی و روش سطح پاسخ

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چکیده

در این مطالعه، یک مدل ریاضیاتی برای غیرفعال کردن مخمر از طریق تیمار پلاسما مورد بررسی قرار گرفت. از طرح غربالگری تشخیصی برای جستجوی فاکتورهایی که در غیرفعال کردن با پلاسما موثر هستند، استفاده شد. چهار فاکتور ولتاژ (۲۰- ۳۰ کیلوولت) (A)، قطر ظرف (۴۰- ۶۰ میلی‌متر) (B)، درجه حرارت فرآیند (۲۰- ۴۰ درجه سانتی‌گراد) (C) و نوع محیط پلاسما (هوا یا آب) در این مطالعه بررسی شدند. سپس تیمارها با نرم‌افزار COMSOL شبیه‌سازی شدند. پاسخ‌های ضریب سنتیک واکنش، غلظت ازون و زمان غیرفعال شدن نهایی توسط طرح غربالگری فاکتورها در نرم‌افزار دیزاین اکسپرت تحلیل شدند تا پارامترهای موثر مدل ریاضیاتی و شرایط بهینه تعیین شوند. نتایج نشان دادند که تیمار با پلاسما در محیط آبی می‌تواند اثر قوی‌تری نسبت به هوا داشته باشد. همچنین در تیمار با پلاسما، نوع محیط اثر بارزی بر هر سه پاسخ داشت، در حالیکه درجه حرارت تنها بر زمان فرآیند موثر بود. بنابراین می‌توان نتیجه گرفت که با بررسی و انتخاب مناسب محیط، می‌توان از تکنولوژی پلاسما برای غیرفعال‌سازی میکروارگانیسم‌ها در مواد غذایی استفاده کرد.

واژه‌های کلیدی: تیمار پلاسما، غیرفعال‌سازی میکروارگانیسم، شبیه‌سازی عددی، روش سطح پاسخ.

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