International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 10(4): 423-428, August 2017
DOI: 10.5958/2230-732X.2017.00052.3

FOOD TECHNOLOGY

Optimization of Operating Conditions for Sterilization of Aseptic Food Packaging Material

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Paper No. 597 Received: 9-5-2017 Accepted: 6-7-2017

ABSTRACT

Sterilization of the packing material is essential in order to prevent spoilage caused by microorganisms found on the packing material. Hydrogen peroxide was used for sterilization of packaging material. Bacteriological effectiveness of sterilization of packaging material was tested with *Bacillus subtilis* ATCC 6633 as target organism as it is the most resistance to hydrogen peroxide. The initial count of spore taken for experimental purpose was 10^8 cfu/ml. In this study, the operating factors were hydrogen peroxide concentration (1.6-18.4 % v/v), temperature (26.4-93.6 °C) and treatment time (9.6-110.4 s) while the investigated response was log cycle reduction. The experimental data was fitted with second order regression equation. The operating conditions were optimized using response surface methodology. The optimum values of hydrogen peroxide concentration, temperature and treatment time were found to be 8.05 % v/v, 77.20 °C and 80.54 s respectively at desirability value of 0.39. The log cycle reduction was found to be 6.10 under optimized condition. The optimized condition may be employed for sterilization of food packaging material in aseptic packaging.

Highlights

- Sterilization of packaging material is essential for aseptic packaging.
- Hydrogen peroxide is used for sterilization of packaging material.
- The operating conditions of sterilization of packaging materials i.e. hydrogen peroxide concentration, temperature and treatment time is found using response surface methodology.

Keywords: Sterilization, packaging material, temperature, time, regression equation, packaging

Packaging plays a very vital role in extending the shelf life of food materials (Patel *et al.* 2016). Aseptic processing and packaging (APP) is currently of major importance in the food industry for delivery of safe food products with extended shelf-life (ESL) and reduced need for refrigeration. APP is a method in which presterilized liquid food is packed and sealed in presterilized container under aseptic environment to keep the products for prolonged period at ambient conditions. This technology is very useful for temperate countries where refrigeration is not prevalent as a means to preserve foods. The most important example of an aseptically processed and packed food is Ultra-High-Temperature (UHT) processed milk which undergoes ultrahigh-temperature-short-time sterilization with subsequent delivery and rapid sealing into presterilized containers. Spore forming *bacilli* present on equipment surface and packaging material cause spoilage of aseptically packed food products and hence decontamination of the packing material is essential in order to prevent spoilage. Sterilization of food packaging material is prerequisite for successful production of aseptically packaged products and it is carried out by using various chemical and physical methods used in aseptic filling of sterile products. The most common sterilants used for aseptic packing applications worldwide are hydrogen peroxide, gamma irradiation, hot air/steam, UV light,

halogens or combinations of these methods as well as a combination of peroxy acetic acid and hydrogen peroxide.

Among these sterilants, H_2O_2 is the most popular worldwide for sterilization of packaging material as it does not leave toxic residues in the food (Smith and Brown, 1980). It is reported that the traces of residual of H_2O_2 left in food is less than 0.25 ppm does not have any harmful effect when consumed. Concentrated solutions of H_2O_2 at high temperature (60-80 ºC) are generally used to increase sporicidal activity. In general, bacterial spores are more resistant to H_2O_2 than are vegetative cells, and spores are generally the major targets in peroxide sterilization. The first successful aseptic filling system for cartooning the aseptic Tetra Pak of 1961 used a combination of H_2O_2 and heat for the sterilization of the surface of container material (Burton, 1988). Hydrogen peroxide sterilization followed by hot air is commercially used as in–line sterilants for packaging materials. Dry heat sterilization is slow and not suitable for heat sensitive materials like many plastic items. The heavy shielding necessary to prevent radiation leakage from ionizing radiation source makes the system impractical to use for sterilization of film. At present, plastic films that are used for packaging are sterilized by soaking in hydrogen peroxide at high concentration. The packaging material (i.e. plastic laminates with cardboard, films of thermo formable plastics and laminates) are taken from a reel and dipped into a bath of aqueous 30-33% hydrogen peroxide (Reuter 1988) and 4-5 log cycles reduction has been claimed. Hydrogen peroxide kills a wide variety of organisms and *viruii*. Both linear and non linear relationships between log number of survivors and time of exposure to different concentrations of hydrogen peroxide have been reported.

Literatures are not available for sterilizing food packaging film by means of hydrogen peroxide at low concentration and its effect on log cycle reduction of spore of *B. Subtilis* ATCC 6633. Hence, sterilization of film at low concentration with suitable treatment time and temperature has been tried which could be beneficial for low capacity APP systems. The main objective of this study is to optimize the critical operating parameters of sterilization of food packaging material using response surface methodology (RSM) in order to confirm the safety of this sterilization method. The optimized operating condition is expected to be applied in aseptic processing and packaging (APP) system for sterilization of food packaging film.

MATERIALS AND METHODS

Preparation of test organism

Bacteriological effectiveness of the process was tested with *Bacillus subtilis* as target organism as it is the most resistant to hydrogen peroxide (Smith and Brown, 1980). First of all 50 ml nutrient broth media solution in four 100 ml flask was sterilized in autoclave for 15 min at 121 °C. Pure culture of *B. subtilis* (ATCC 6633) obtained from National Collection Centre, Dairy Bacteriology Division, NDRI, Karnal, India was inoculated in each flask and kept into incubator at 37 °C for seven days. After sufficient growth of strain, flasks were taken out from incubator and then nutrient agar surface was inoculated with those cultures and kept into incubator for growth at 37 °C for 24 hours. The spore crop was grown on the surface of nutrient agar for 4 days at 37°C. Sterile distilled water was added to each Petri dish simultaneously scraping gently the agar surface with sterile rod. The scraped strain was then added into sterile distilled water. The liquid containing cells from all Petri dishes was pooled, centrifuged and washed thrice with sterile distilled water. The cell obtained from centrifugation were suspended in sterile water and heated at 80 °C for 10 minutes to kill the vegetative cells and then spore suspensions were stored at 4°C approximately for 2 months. Standard plate count was done to enumerate the concentration of spores. Initial concentration was 10^{10} cfu/ml and it was diluted with sterile distilled water to make its concentration up to 10^8 cfu/ ml. This concentration was used for experimental study and finally the results were calculated for a reference initial count for the ease of interpretation.

Preparation of H_2O_2 solution of different concentrations

A commercially stabilized 30 % (v/v) concentrated solution of food grade hydrogen peroxide (Merck, Calcutta) was diluted with sterilized distilled water to get required concentrations of hydrogen peroxide

solution and stored at 4°C. Hydrogen peroxide concentrations in the range of 1.6-18.4% v/v were used to sterilize the packaging film in experiments.

Procedure of sterilization of packaging material

The packaging material was tested for sterility by using hydrogen peroxide solution at different levels of concentration, temperature and treatment time of packaging material. For estimation of the number of bacteria in inoculated packaging material before and after sterilizing, standard plate count method was used. First of all the nutrient agar media (Hi Media, Mumbai, India) was prepared by taking nutrient agar and distilled water in recommended proportions and sterilized the flask at 121 °C for 15 minutes. The other necessary glass and plastic wares like Petri dishes, test tubes, plastic tips etc along with the nutrient agar media were sterilized at 121°C for 15 minutes in the autoclave.

After that all sterilized materials except the bacterial cell solution were taken to the laminar flow chamber and UV light was kept ON for 20 to 30 minutes. Then 2 × 2 cm^2 of the plastic packaging film was taken. First the film was thoroughly dipped and shaken into spore suspension and then treated with different concentration of H_2O_2 maintained at different temperatures for different treatment times. The H_2O_2 solution temperature was maintained using water heater monitored by digital temperature controller.

Determination of survivors

After exposing the film for required combination of concentration of hydrogen peroxide solution, temperature and treatment time, it was immediately taken out and placed aseptically into 10 ml sterile dilution blank and properly shaken for few seconds to ensure separation of surviving spores from film and uniform distribution of surviving spores. This spore suspension was used to get the final bacterial count after the treatment.

The standard plate count method was used up to the required dilutions to determine the number of spore survivors. After plating, the Petri dishes were kept in the incubator for 24 hours at 37 °C. The initial bacterial load on the packaging film was also calculated using the film without any treatment following the same method. Then colony count was done using colony counter for all the dilutions and the average value was taken as a result of the treatment.

Design of Experiment

Sterilization of food packaging material is an important operation in aseptic processing and packaging. Testing of packaging material sterilization is essential before commercial production. For testing purpose, several operating parameters such as concentration of hydrogen peroxide, temperature of solution and treatment time of packaging material with hydrogen peroxide were selected and central composite rotatable design (CCRD) was used to optimize the operating condition using response surface methodology (RSM). A Central Composite Rotatable Design (CCRD) including 20 experiments formed by 6 central points and 6 (λ = 1.68) axial points to 2^3 full factorial design (Montgomery, 2001) was used (Table 2). The dependent variable taken as response is log cycle reduction (LCR) of microbial load which is the log of ratio of initial to final bacterial count.

Experimental data were fitted to a second order polynomial model and the generalized second order polynomial model used in the response surface analysis was as follows:

$$\eta = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j \quad \ldots(1)$$

Levels of independent variables were coded using following equation:

$$X_i = \frac{\varepsilon_i - \varepsilon_{i(maen)}}{\varepsilon_{i(maen)} - \varepsilon_{i(\min)}} \quad \ldots(2)$$

Where,

X_i = Coded value of the independent variable,

ε_i = Actual value of the factor, i in original units,

ε_i (mean) = Mean of high and low levels of the factor, i and

ε_i (min) = Minimum value of the factor, i

The values of hydrogen peroxide concentration, temperature and treatment time were coded within +1 and –1 level. Levels of variation and codes of variables are presented in Table 1.

Table 1: Levels and codes of independent variables in optimization of sterilization of packaging material

Variables	Code	Levels				
		-1.68	-1	0	+1	+1.68
H_2O_2 Concentration, %	X_1	1.6	5	10	15	18.4
H_2O_2 Temperature, °C	X_2	26.4	40	60	80	93.6
Treatment Time, s	X_3	9.6	30	60	90	110.4

After conducting each experiment, microbial load was found out by using standard plate count method. Dilution technique was followed to ease in counting of the colonies.

Analysis of Experimental Data

Data obtained from the operating variables combinations formulated with central composite rotatable design were used for developing response surface equation. Response surface methodology was used to evaluate the second order nonlinear response surface equation for getting the maximum log cycle reduction. The results of the central composite rotatable design were analyzed with Design Expert Ver. 7.0.0 (Stat-Ease 2005) software to get the optimum values of the variables i.e. concentration of hydrogen peroxide solution, temperature of solution and treatment time of the packaging material with hydrogen peroxide solution. Significant terms in the model for the response were found by analysis of variance (ANOVA). Model adequacies were checked by R^2, adj-R^2 and pre-R^2.

RESULTS AND DISCUSSION

Destruction of spores by hydrogen peroxide

The destruction of spores when treated with hydrogen peroxide under various combination of operating conditions in term of log cycle reduction (LCR) has been presented in Table 2. From the table it is clear that with rise in concentration, temperature and treatment time, LCR increases. Toledo (1975) has also reported that the antimicrobial power of hydrogen peroxide increases as the temperature rises. To visualize the combined effects of two factors on response, the response surface plots were generated as the function of two independent variables, while keeping the other variable at the central value (Fig. 1-3). These plots imply the positive effect of concentration of H_2O_2 solution, temperature and treatment time of packaging material on log cycle reduction. The increase in LCR is due to destruction of spore protein and degradation of outer spore layers including spore coats and cortex when exposed to hydrogen peroxide. Spore coats are disrupted by oxidizing sporicidal agents such as hydrogen peroxide which may cause extraction of spore coat material, facilitating the penetration of hydrogen peroxide into the cortex and protoplast. Setlow and Setlow (1993) believe that hydrogen peroxide, or possibly the free hydroxyl radicals resulting from its degradation, gained access to the core of spores of certain *B. subtilis* mutants and killed these spores at least in part by DNA damage.

Table 2: Log cycle reduction for various combination of hydrogen peroxide concentration, temperature and treatment time

Sl. No.	X_1 coded (real%)	X_2 coded (real °C)	X_3 coded (real, s)	LCR
1	+1(15)	+1(80)	+1(90)	8.0
2	+1(15)	+1(80)	-1(30)	3.46
3	+1(15)	-1(40)	+1(90)	1.4
4	+1(15)	-1(40)	-1(30)	0.51
5	-1(5)	+1(80)	+1(90)	4.92
6	-1(5)	+1(80)	-1(30)	1.77
7	-1(5)	-1(40)	+1(90)	0.67
8	-1(5)	-1(40)	-1(30)	0.23
9	+1.68 (18.4)	0(60)	0(60)	6
10	-1.68 (1.6)	0(60)	0(60)	1.2
11	0 (10)	+1.68 (93.6)	0(60)	7.36
12	0 (10)	-1.68(26.4)	0(60)	0.14
13	0 (10)	0(60)	+1.68(110.4)	6.88
14	0 (10)	0(60)	-1.68 (9.6)	0.67
15	0 (10)	0 (60)	0(60)	3.9
16	0 (10)	0(60)	0(60)	4.2
17	0(10)	0(60)	0(60)	3.8
18	0(10)	0(60)	0(60)	3.6
19	0(10)	0(60)	0(60)	3.9
20	0(10)	0(60)	0(60)	3.8

Optimization of Packaging Material Sterilization Operating Conditions

The second order regression analysis of the experimental data (Table 2) was performed to get second order polynomial model. The analysis of variance (ANOVA) for LCR is presented in Table 3. Regression equation coefficients of the proposed models and statistical significance of all main effects

calculated for response were obtained and effects being not significant ($p > 0.05$) were stepped down from models without damaging the model hierarchy (Table 3). The model terms hydrogen peroxide concentration, temperature and treatment time, and interaction between temperature and treatment time were found to be significant ($p<0.05$). Analysis of the regression coefficients showed that the effect of temperature on LCR was more than concentration of hydrogen peroxide.

Table 3: ANOVA evaluation of linear and interaction term for response variable

Source of variation	Sum of squares	df	Mean square	F value	p-value Prob>F
Model	102.15	4	25.54	30.21	<0.0001
X_1	14.05	1	14.05	16.62	0.0010
X_2	55.30	1	55.30	65.43	<0.0001
X_3	27.74	1	27.74	32.82	<0.0001
X_2X_3	5.06	1	5.06	5.98	0.0273
Lack of Fit	12.49	10	1.25	32.29	
Pure Error	0.19	5	0.039		
Total	**114.83**	**19**			

The response surface equation were obtained in terms of coded values and real values taking hydrogen peroxide concentration, temperature and treatment time as independent variables against the LCR as response after neglecting the non-significant terms at 5% significance level.

Regression equation in terms of coded values is given by following equation:

$$LCR = 3.32 + 1.01* X1 + 2.01* X_2 + 1.43* X_3 + 0.79*X_2*X_3 \quad \ldots(3)$$

Regression equation in terms of real values is given by following equation:

$$LCR = -2\ .83 + 0.203* X_1 + 0.0211* X_2 - 0.032* X_3 + 1.325\ E - 0.003* X_2 X_3 \quad \ldots(4)$$

A good fit was obtained with a coefficient of determination ($r^2=0.89$) which showed that the model developed was adequate for the experimental data. The predicted coefficient of determination of 0.75 was in reasonable agreement with the adjusted coefficient of determination of 0.86. Second order regression equation obtained in this study was

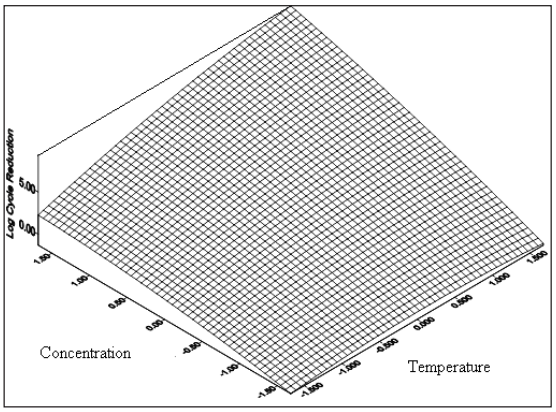

Fig. 1: Surface plot of effect of temperature and concentration on log cycle reduction at constant treatment time (60 s)

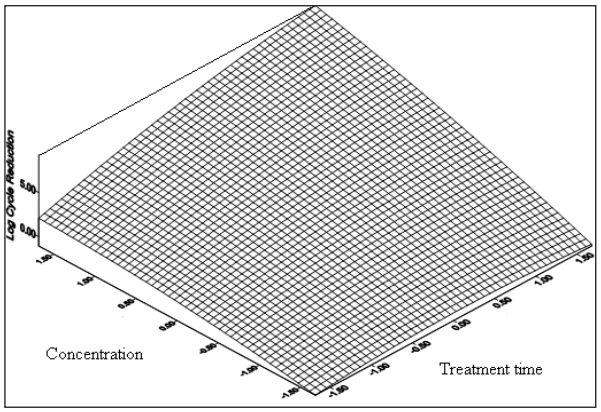

Fig. 2: Surface plot of effect of concentration and treatment time on log cycle reduction at constant temperature (60 °C)

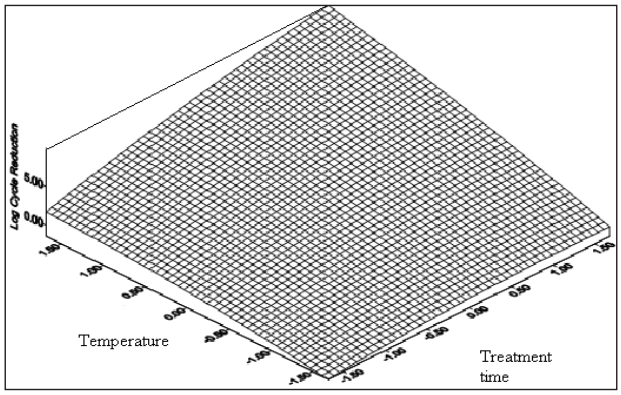

Fig. 3: Surface plot of effect of temperature and treatment time on log cycle reduction at constant concentration (10 % v/v)

utilized for optimization of operating conditions for the response. The numerical optimization technique (Design Expert software) was used to get the optimum values of the variables i.e. concentration of hydrogen peroxide solution, temperature and treatment time of the packaging material with hydrogen peroxide solution. The optimum condition was optimized by minimizing the operating variables within the level used for the study. The lower limit and upper limit of LCR fixed for optimization were 4 and 8. The optimized value of concentration, temperature and treatment time were found to be 8.05% v/v, 77.20 °C and 80.54s respectively at desirability value of 0.39. At this optimum operating condition, the maximum LCR was found to be 6.54 for *Bacillus subtilis* ATCC 6633, which is more than 4 LCR required for aseptic packaging. This optimized operating condition may be useful for application in sterilization of packaging materials**.**

CONCLUSION

Sterilization of packaging film is an essential step before filling sterilized food products to extend shelf-life. In this study, inoculated sample was exposed at hydrogen peroxide concentration of 1.6-18.4 % % v/v at temperature of 26.4-93.6 °C and at contact time of 9.6-110.4 second. A second order regression model was proposed which described the experimental results satisfactorily and based on the proposed model, the optimum operating condition for sterilization of packaging material was found be 8.05 % v/v, 77.20 °C and 80.54 s respectively at desirability value of 0.39. Under this condition, LCR was found to be 6.10 for *Bacillus subtilis* ATCC 6633.

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