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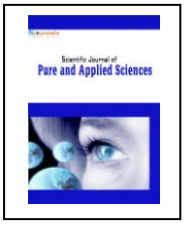

Original article

Modelling the effect of temperature on specific death rate of the micro-flora of raw cotton wool

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ABSTRACT

Cotton wool for use in medicine must be sterilized and heat treatment is the most common sterilization technique for bulk processing of agricultural materials. Modelling the effect of temperature variations on specific death rate of the micro-flora of raw cotton wool was carried out in this project. The raw cotton wool was collected from a farmer in Kano State, the most important cotton wool producing state of Nigeria. The micro-flora was determined by inoculating and incubating with 0.85% NaCl diluents and the number of bacteria determined by plate count method. As the sterilization temperatures were varied with increased time, the thermal death rates and thermal death time of the micro-flora were determined for each temperature and these was used in the modelling. The model lnK = 83.9 – 34190(1/T) shows that the sterilization temperature has a linear relationship with the death rate constant and it is in line with Arrhenius model.

1. Introduction

Cotton is a soft, fluffy staple fibre that grows in a boll, or protective capsule, around the seeds of cotton plants of the genus *Gossypium*. The fibre is almost pure cellulose. It is a single cell fibre and develops from the epidermis of the seed (Rollins, 1965). An elongation period continues for 17-25 days after flowering. Cotton consists of cellulosic and non-cellulosic material (Karmakar, 1999).

The outer most layer of the cotton fibre is the cuticle, covered by waxes and pectins and this surrounds a primary wall built of cellulose, pectins, waxes and proteinic material (Tayme and balser, 1970). The inner part of the cotton fibre comprises the secondary wall, subdivided into several layers of parallel cellulose fibrils and the lumen (Karmakar, 1999).

Like other materials of vegetable origin, the susceptibility or resistance of cotton to bio deterioration depends on the content of cellulose, lignin and other organic constituents. The presence of non-cellulosic components such as lignin and waxes decreases susceptibility; in contrast, pentosans and pectins increase susceptibility.

From the time Cotton grows on the boll, on through the stages of manufacture and use, the fibres are subject continually to contamination with micro-organisms. Included in this micro-organic flora may be the pathogenic organisms responsible for disease. There may also be other groups less dangerous to health but capable of multiplying rapidly under favourable conditions and causing the cotton fibres' to be stained with mildew or to lose strength and deteriorate in other ways. The utilization of cotton therefore may be seriously affected by the action of micro-organisms. Often time's cotton must be sterilized to free it of pathogenic organisms, and also of the non pathogens which damage it as already indicated. Also in bacteriological studies of the effect of these microorganisms on the fibres, it is essential that the cotton be sterilized. Many of both these groups of organisms have a sporulating stage, during which they are very resistant to the usual means of sterilization (Humfeld et al., 1973). It is generally believed that these spores are not killed until their protoplasm is coagulated. Since wool is a protein and since the protoplasm of the spores is also largely protein in nature, it becomes evident that the destruction of the spores without damaging the wool is by no means an easy problem.

The main agents of deterioration are micro organisms (bacteria, *actinomycetes* and fungi) and insects, (Menier, 1988). An indispensable condition to start a bacterial attack is a high water content; this condition is favoured by the high hygroscopicity of cotton.

The micro organisms most frequently mentioned in degradation of wool and other protein fibres are bacteria of the genus Bacillus (*B. mesenthericus* and *B. subtilis*), *Proteus Streptomyces fradiae*), Micro-fungi are not very frequently involved in biodeterioration of protein fibres but species of *Aspergillus, Fusarium* and *Trichoderma* are sometimes reported(Menier,1988.,Kowalik,1980).

During sterilization, these bacteria and other micro flora of processed cotton wool are destroyed. The aim of this work is to study the effect of sterilization on the micro-flora of processed cotton wool and then develop a model equation. A survey of literature indicated that relatively little work has done to determine the effect of temperature on the micro flora of raw cotton wool.

Sterilization is defined as the process where all the living microorganisms, including bacterial spores are killed. Sterilization can be achieved by physical, chemical and physiochemical means (Sridhar, 2008). Great difficulty has been experienced in devising a method of Sterilisation of wool which does not change its original state. Autoclaving wool has been shown by Stahl et al (1950), to lessen resistance to microbial attack. Although sterilisation with organic solvents has been shown by Humfield, Elmquist, and Kettering (1937) and McQuade (1964) to be effective and apparently not to affect keratin, this method leads to the removal of the suint and wool wax (Truter1956). The use of ethylene oxide by the method of Noval and Nickerson (1959), according to these authors causes little change to wool.

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents (Yashmin, 2007). Moist heat is thought to kill microorganisms by causing denaturation of essential proteins. Death rate is directly proportional to the concentration of microorganisms at any given time. The time required to kill a known population of microorganisms in a specific suspension at a particular temperature is referred to as thermal death time (TDT). Increasing the temperature decreases TDT, and lowering the temperature increases TDT. Processes conducted at high temperatures for short periods of time are preferred over lower temperatures for longer times (Kenneth 2009).

2. Materials and methods

2.1. Sample collection

The samples used in this study were obtained from a farmer in Kano State, out of which 24-gram of the sample was soaked in water and left for 3 days, so that the microbes can be submerged in the water.

2.2. Experimental procedure

The number of bacteria on the wool sample was estimated by the plate count method (Ibe et al, 2007) method that was also used by Mulcock and Philippa (1965). After the 3 days 9mil of the solution (microbial) was poured into 4 test tubes. 1mil of diluents (0.85% Nacl) was carefully poured into tube A and then used to dilute other test tubes by dilution count method. A volume of 0.1mil of the content of the tubes was each poured into a petric dish and put into an incubator at 37^{o}C for 22 hour to determine the microbial density.

Also 1gram weight of the contaminated bacterial solution was poured into 6 petric-dishes each and incubated. After 22 hours, the dishes were brought out and put into the autoclave one at a time. The numbers of colonies left in the petric- dishes was recovered after a given time intervals, which was gradually increased from 2 to 12 minutes. The overall reduction rate of the microbes (bacterial) was noted for every 2 minutes increase at a constant temperature of 121^{o}C, this was repeated for temperature of 123^{o}C, 125^{o}C, and 127^{o}C respectively.

The number of bacteria on the samples was estimated by plate count method and the colony – forming unit of the bacteria was calculated using;

$CFU = \frac{1}{DF} * A * \frac{1}{V}$ (Ibe et al, 2007. Willey, 2008)

Where;

CFU=Colony Forming unit of Bacterial.

DF = Dilution Factor

V= Volume

A = Number of Surviving Organisms.

2.3. Determination of the thermal death rate constant

The destruction of organisms by heat at a specific temperature follows a rate equation of the form (Opara, 2002; Casolari, 1994).

$$\frac{dN}{dt} = -KN \quad \ldots\ldots (1)$$

Where K= Rate Constant (min-1) which is a function of temperature

N = number of viable organisms

t = time

Integrating (1) with boundary condition that N=No at t=0

$$N = No\ e - kt \quad \ldots\ldots (2)$$

Linearising equation (2).

lnN = lnNo – kt.....(3)

A plot of lnN (surviving colonies as heating progressed), against sterilization time (t) gives the death rate constant (k) . It also indicates the rate of depletion of bacteria as time increases for a constant temperature.

2.4. Effect of temperature on specific death rate

The effect of temperature on the values of the reaction rate constant K follows an Arrhenius type relationship (Opara, 2002; Casolari, 1994).

$$K = Ae^{-\frac{E}{RT}} \quad \ldots\ldots (4)$$

linearising

$$lnK = \ln\left(Ae^{-\frac{E}{RT}}\right)$$

$$lnK = lnA + lne^{-\frac{E}{RT}}$$

$$lnK = lnA - \frac{E}{RT} \quad \ldots\ldots (5)$$

Where k = Rate Constant

A = Frequency Factor

E = Activation Energy

R = Universal gas Constant

T = Temperature

A plot of lnk against the reciprocal of temperature can be used to deduce the relationship between the death rate constant and sterilization temperature.

3. Results and discussion

The results are summarised in Table 1. It can be observed that as sterilization temperature was increased, the rate of elimination of the bacteria increases, also as the time for each temperature were increased, more of the bacteria are eliminated.

At temperature of 121^{o}C; from the table, the rate of elimination of the colonies formed by the bacteria increases from time 2- 6min but between time 6 – 8 min the rate is lowered and then from time 8 – 12 min, the rate is constant.

At temperature of 123^{o}C; the rate of elimination of the bacteria shows a fluctuating trend with the peak at time 6 – 8 min.

At temperature of 125^{o}C, the rate of elimination of the bacteria was constant at time 2 – 4 min, at time 6 – 8 min the rate of elimination of the bacteria was highest after which rate of elimination of the bacteria lowered.

At temperature of 127^{o}C; the rate of elimination of the bacteria shows a fluctuating trend with the highest at time 2 -4 min.

The fluctuating rate of elimination of bacteria of the raw cotton wool shows that the raw cotton wool is composed of different species of bacteria that requires different temperatures and time for it elimination.

Table 1

The effect of temperature on the colony forming unit of bacteria at different time.

Time	For 121^{o}C	Rate of reduction of colonies	For 123^{o}C	Rate of reduction of colonies	For 125^{o}C	Rate of reduction of colonies	For 127^{o}C	Rate of reduction of colonies
T(min)	Y(CFU/ml)	Y_n-Y_{n+2} (121^{o}C)	Y(CFU/ml)	Y_n-Y_{n+2} (123^{o}C)	Y(CFU/ml)	Y_n-Y_{n+2} (125^{o}C)	Y(CFU/ml)	Y_n-Y_{n+2} (127^{o}C)
0	197	3	200	5	197	7	200	10
2	194	14	195	35	190	30	190	40
4	180	22	160	20	160	30	150	30
6	158	8	140	46	130	35	120	36
8	150	16	94	6	95	30	84	38
10	134	16	88	28	65	21	46	34
12	118		60		44		12	

4.1. Determination of the Thermal Death Rate Constant and Thermal Death time

From Fig. 1; At 121^{o}C, Substituting in equation (3) $\ln N_1 = 5.328 – 0.043t$. It can be observed that as the time is increased, the bacteria are eliminated with a death rate constant of 0.043 Also the correlation coefficient (R^2 = 0.971) shows that the data is acceptable. Calculating the period of time when only one of the bacteria would be viable (Thermal death time) ln1 = 5.328 – 0.043t, therefore t = 123.91min. At 123^{o}C; Substituting in equation (3) $\ln N_2 = 5.430 – 0.102t$, it can be observed that as the time is increased, more of the bacteria are eliminated with a death rate constant of 0.102 Also the correlation coefficient (R^2 = 0.951) shows that the data is acceptable. Calculating the period of time when only one of the bacteria would be viable. ln1 = 5.430 – 0.102t, therefore t = 54.3min. At 125^{o}C; Substituting in equation (3) $\ln N_3 = 5.478 – 0.128t$, it can be observed that as the time is increased, more of the bacteria are eliminated with a death rate constant of 0.128. Also the correlation coefficient (R^2 = 0.941) shows that the data is acceptable. Calculating the period of time when only one of the bacteria would be viable. ln1 = 5.478 – 0.128t, therefore t = 42.80min. At 127^{o}C; Substituting in equation (3) $\ln N_4 = 5.714 – 0.212t$, it can be observed that as the time is increased, the bacteria are eliminated faster with a death rate constant of

0.212. Also the correlation coefficient (R^2 = 0.832) shows that the data is acceptable. Calculating the period of time when only one of the bacteria would be viable. ln1 = 5.714 – 0.212t, therefore t = 27min.

Comparing the period of time required for only one of the bacteria to be viable, it can be obsevered that at higher temperature shorter time is required to kill the microorganisms and therefore lower is the heat induced damage to the cotton wool than at lower temperature. Also increasing sterilization temperature increases the thermal death rate constant.

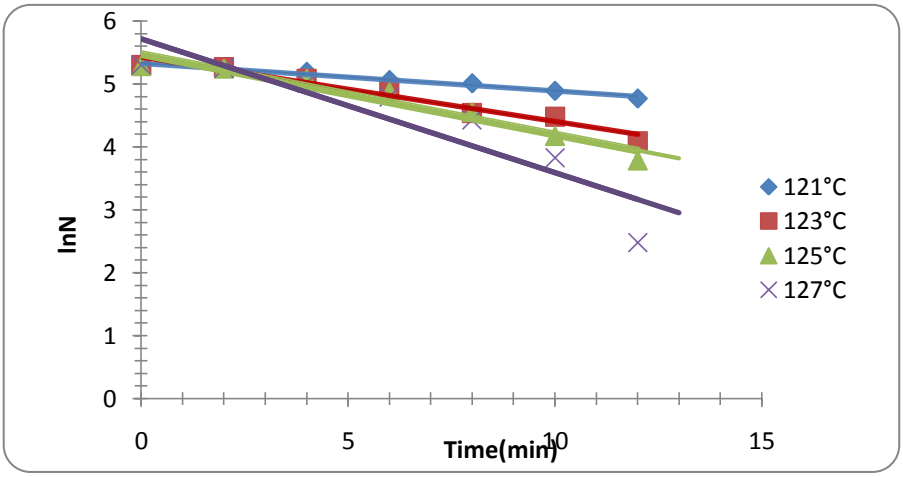

Fig. 1. Determination of the various death rate constant.

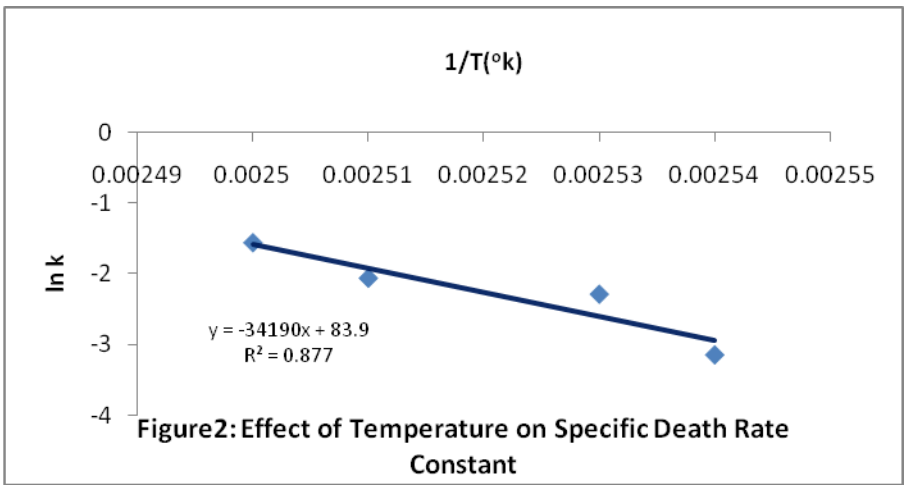

Fig. 2. Effect of temperature on specific death rate constant.

4.2. Arrhenius relationship

Determination of Activation Energy
From Arrhenius equation

$$lnK = lnA - \frac{E}{RT}$$

From Fig. 2; The slope = -E/ R and from the plot of lnk against 1/T, the activation energy can be calculated as:
Slope = -34190= -E/R
Therefore; E= R*34190 and R = gas constant=8.314J/gmol-k
E= 8.314J/gmol-k * 34190= 284255.66 J/gmol

The Frequency Factor is calculated from the intercept as:

lnA = 83.9

Also from Fig. 2: it shows a very nearly linear relationship (R^2= 0.877) between the natural log of the reaction rate and the inverse of the temperature in K. This confirms that the data agrees with Arrhenius equation. Therefore the Arrhenius relationship (model equation) between the death rate constant of the bacteria and temperature is:

lnk = 83.9 – 34190(1/T).

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