DETERMINATION OF TDP AND TDT VALUE FOR SPOILAGE BACTERIA

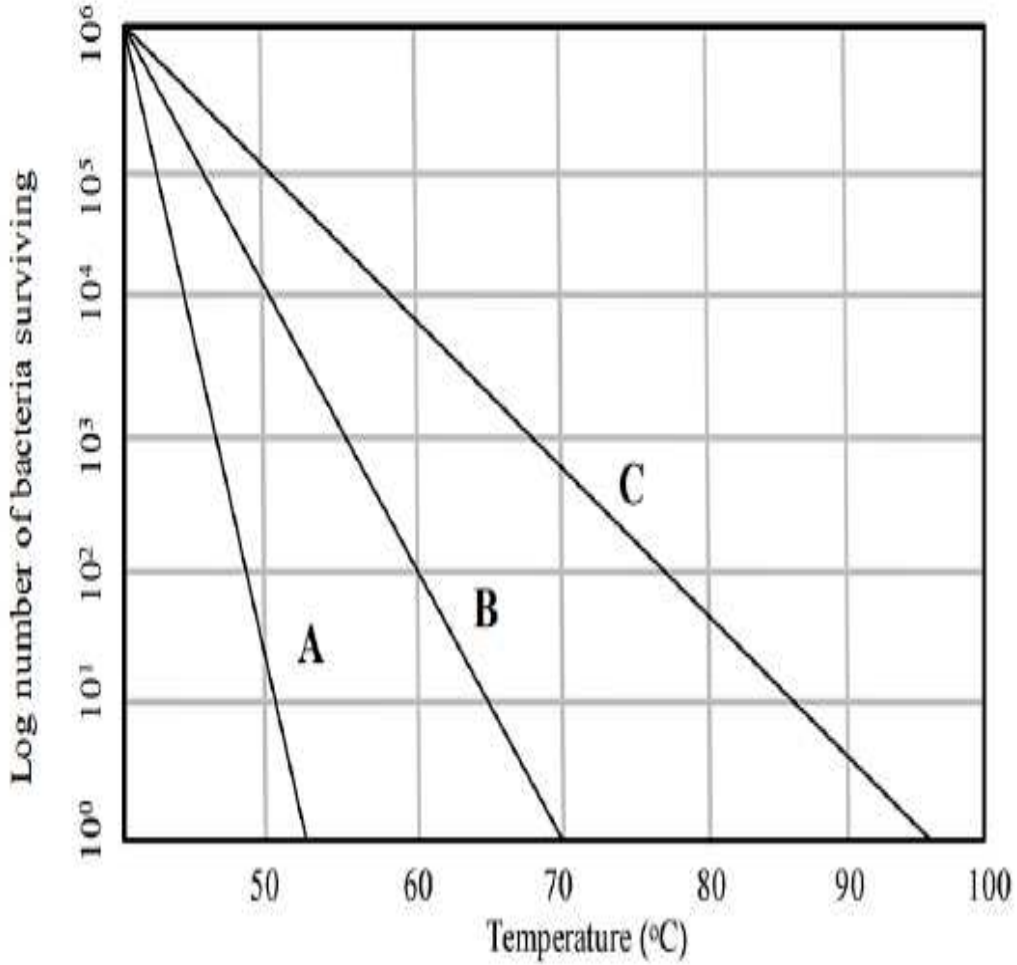
Introduction

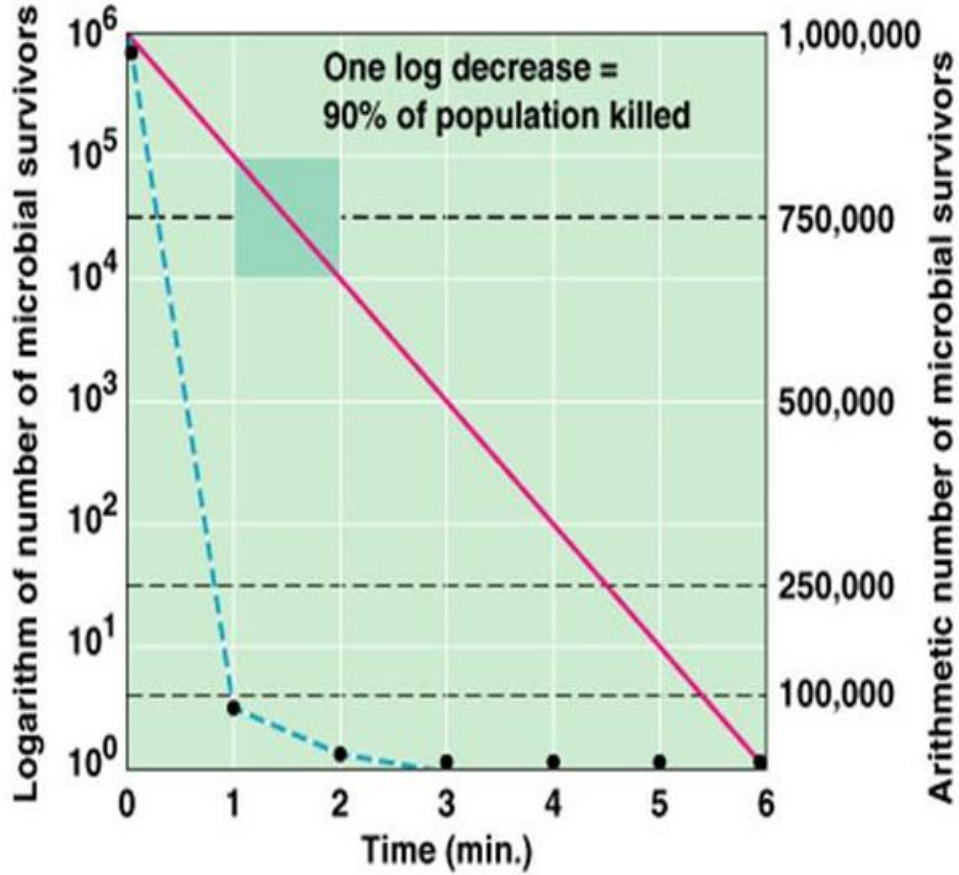
- Heat is one of the oldest methods of food processing and preservation.
- The use of high temperature to preserve food is based on its destructive effects on microorganisms and their spores.
- The killing of microorganisms by heat is supposed to be caused by the denaturation of the proteins and especially by the inactivation of enzymes required for metabolism.
- The heat treatment necessary to kill organism or their spores varies with the kind of organism.

Determining the Effectiveness of Heating Methods

- TDP :thermal death point (TDP) is the lowest temperature at which all the microbes in a liquid will be killed in 10 minutes under definite conditions .
- TDT: the thermal death time (TDT) that is the shortest time necessary to kill all micro-organisms in a suspension at a specific temperature and under defined conditions.
- D-value: This is the decimal reduction time, or the time required to destroy 90% of the organisms.

Organisms	Time (min)	Temperature
S aureus	0.20 – 2.20	150°F
Coxiella burnetti	0.50 – 0.60	150°F
Mycobacterium hominis	0.20 – 0.30	150°F
E. coli	20 – 30	57.3°C
B. coagulans	13.7	95°C
Bacillus licheniformis	2.4	95°C


Log number of bacteria surviving
10^6
10^5
10^4
10^3
10^2
10^1
10^0
A
B
C
50
60
70
80
90
100
Temperature (°C)


Logarithm of number of microbial survivors
10^6
10^5
10^4
10^3
10^2
10^1
10^0
One log decrease =
90% of population killed
1,000,000
750,000
500,000
250,000
100,000
Arithmetic number of microbial survivors
0
1
2
3
4
5
6
Time (min.)

Applications

- Determining TDT in canned food and dairy products can be used in preservation of them using minimum heat as a treatment .
- It has also found applications in cosmetics, producing salmonella-free feeds for animals (e.g. poultry) and pharmaceuticals.
- In the food industry, it is important to reduce the number of microbes in products to ensure proper food safety. This is usually done by thermal processing and finding ways to reduce the number of bacteria in the product. Time-temperature measurements of bacterial reduction is determined by TDT and TDP.

DETERMINATION OF TDP

Procedure

Prepare a saline suspension of the spoilage causing agent and adjust cell density to 0.1 A660 nm

Add 0.5 ml culture to St. Medium tubes

Adjust the temperature of a water bath (for example 80 °C) to the desired temperature using a thermometer dipped in a suspension tube with water .

Immerse one tube with the suspension of the isolate in water bath. Remove the tube after **10 minutes** and immediately cool under running tap water.

Adjust the temperature of water bath next to next desired temperature and repeat the above step

Continue till all the tubes are exposed to the desired temperature for **10 minutes** each.

Add 4.5 ml of St. Nutrient broth to all the tubes

Also inoculate 0.5 ml of unheated culture to 4.5 ml broth as positive control, 0.5 ml of heat killed culture to 4.5 ml of broth as negative control and 5 ml of broth to a tube as medium control

Incubate all the tubes at desired temperature for 24 hours (or 48 hours / more depending on the microorganism)

Determine the TDP of the culture on the basis of turbidity obtained in the tubes

Tube no	Volume of culture in (ml)	Temp at which culture is exposed °C	Condition	Volume of nutrient broth added (ml)
1	0.5	50	Keep the tubes at respective temperature for **10 minutes**. Cool	4.5 ml
2	0.5	60		4.5 ml
3	0.5	70		4.5 ml
4	0.5	80		4.5 ml
5	0.5	90		4.5 ml
6	0.5	100		4.5 ml

1	Positive control	0.5 ml (unheated culture)	4.5 ml broth
2	Negative control	0.5 ml (heat killed culture)	4.5 ml broth
3	Medium control	-	5 ml broth

Observation table

SR.No	Temperature in °C	Result
1	50	+
2	60	+
3	70	+
4	80	_
5	90	_
6	100	_
7	Positive control	+
8	Negative control	_
9	Medium control	_

Keys :
+ = growth
- = No growth

DETERMINATION OF TDT

Procedure

Prepare a saline suspension of the spoilage causing agent and adjust cell density to 0.1 A660 nm

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Add 0.5 ml culture to St. Medium tubes

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Adjust the temperature of a water bath to the desired temperature using a thermometer dipped in a suspension tube with water .

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Immerse all the tubes with the suspension of the isolate in the water bath

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Remove tubes at regular intervals and immediately cool it under running tap water

Add 4.5 ml of St. Nutrient broth to all the tubes

Also inoculate 0.5 ml of unheated culture to 4.5 ml broth as positive control, 0.5 ml of heat killed culture to 4.5 ml of broth as negative control and 5 ml of broth to a tube as medium control

Incubate all the tubes at desired temperature for 24 hours (or 48 hours / more depending on the microorganism)

Determine the TDT of the culture on the basis of turbidity obtained in all the tubes .

Tube no	Volume of culture in (ml)	Time of exposure in minutes (temperature :80° C)	Condition	Volume of nutrient broth added
1	0.5	2	Cool	4.5 ml
2	0.5	5		4.5 ml
3	0.5	10		4.5 ml
4	0.5	15		4.5 ml
5	0.5	20		4.5 ml
6	0.5	25		4.5 ml

1	**Positive control**	**0.5 ml (unheated culture)**	**4.5 ml broth**
2	Negative control	0.5 ml (heat killed culture)	4.5 ml broth
3	Medium control	-	5 ml broth

Observation table

SR.No	Time interval (min)	Result
1	2	+
2	5	+
3	10	-
4	15	-
5	20	-
6	25	-
7	Positive control	+
8	Negative control	_
9	Medium control	_

Keys :

+ = growth

- = No growth

Result & conclusion

- The Thermal death time of spoilage bacteria is 10 minutes at 80 °C
- The thermal death point of spoilage bacteria is 80 ° C at 10 minutes.

THANKYOU...