THE UNIVERSITY OF MELBOURNE

FOOD20006 Food Microbiology & Safety

Helen Billman-Jacobe

Control of microorganisms in food

Ray and Bhunia Ch 33 Control by heat (thermal processing

Intended learning outcomes

Define bacteriostatic, bacteriocidal and bacteriolytic

Know the purpose of pasteurization and decimal reduction time

Describe the difference effects of low and high thermal processing methods

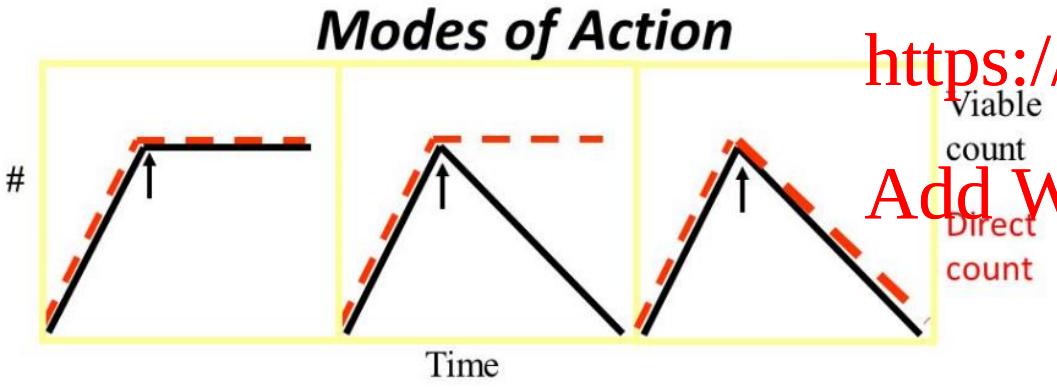
Express D, TDT and z values

Kill or inhibit growth

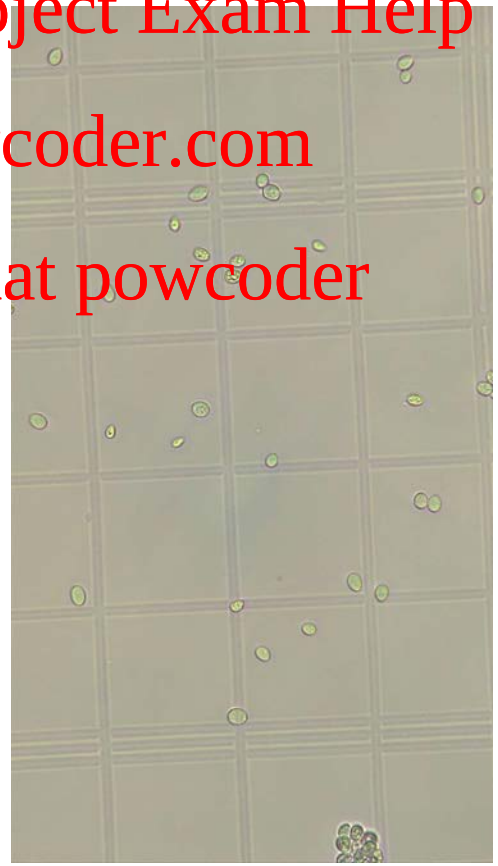
Bacteriostatic = can inhibit growth of bacteria without killing them

Bactericidal = can kill bacteria

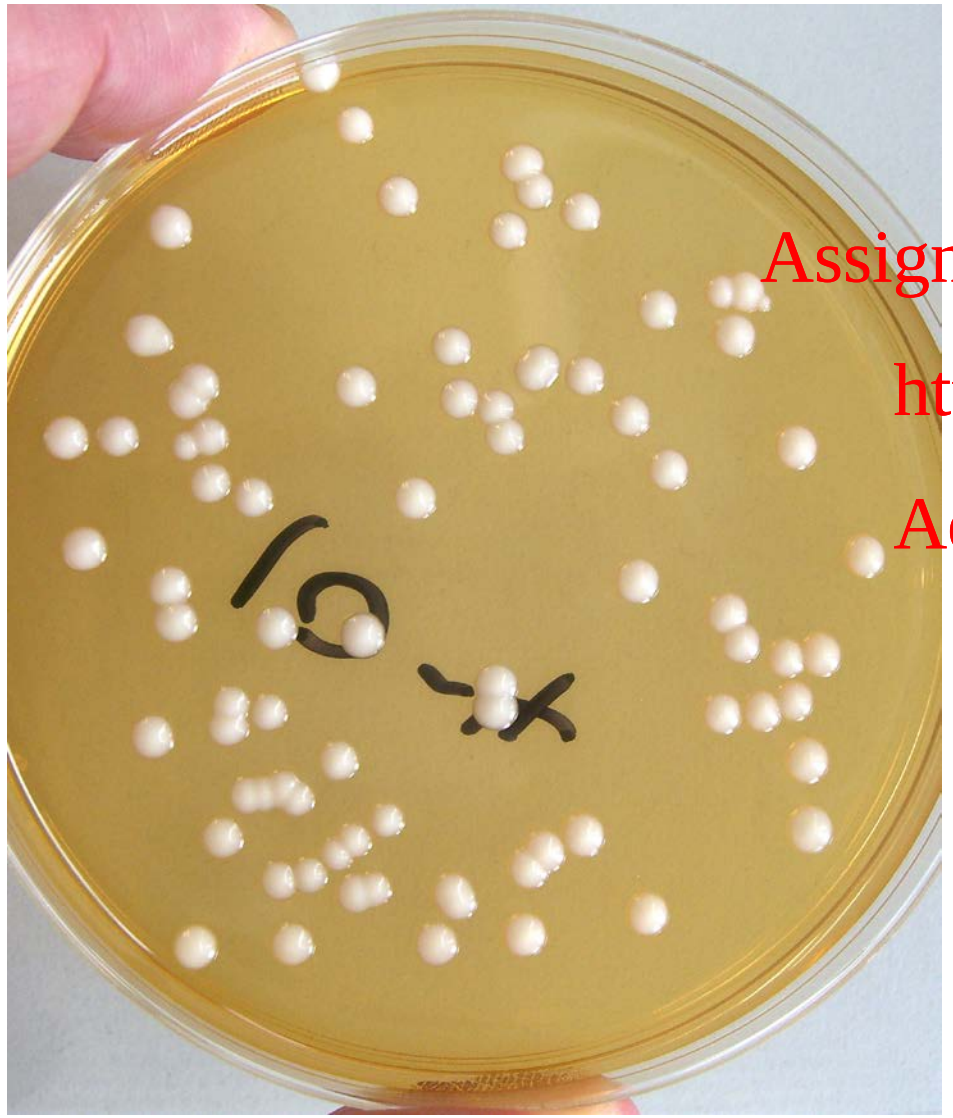
Bacteriolytic = lyses (breaks open) bacterial cells



- Bacteriostatic:
  - Inhibits growth
  - Non-lethal
  - Reversible
- Bacteriocidal
  - Kills
  - Irreversible
- Bacteriolytic
  - Kills
  - Cell lysis
  - Irreversible



Viable counts



Serial decimal dilution

Spread plate method

Known volume of diluted sample spread on plates

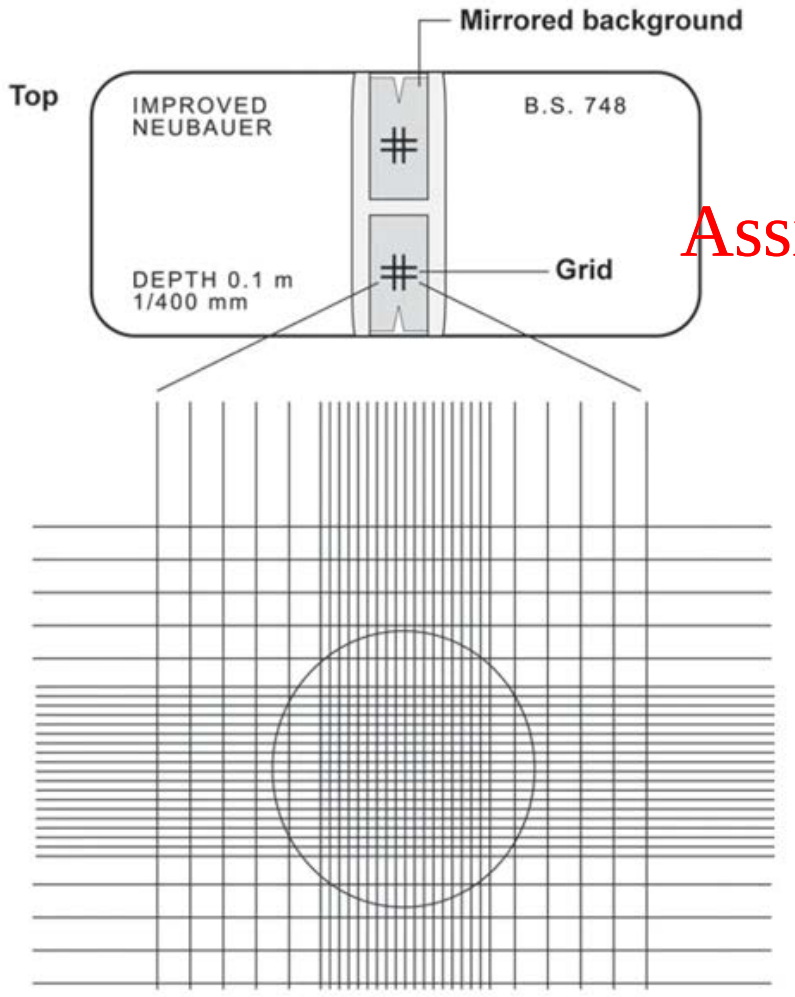
Count the colonies

Calculate cfu/ml

Only viable, culturable cells will form colonies

Colonies/(volume plated ml)X dilution factor) = cfu/ml

Direct counts



If cells can be viewed under a microscope then they can be counted using a special microscope slide, counting chamber = hemocytometer

1. Clean the chamber and cover slip with alcohol. Dry and fix the coverslip in position.
2. Harvest the cells. Add 10 µL of the cells to the hemocytometer. Place the chamber in microscope
3. Count the cells in the gridded square
4. Multiply by the conversion factor to estimate the number of cells per mL.
5. Prepare duplicate samples and average the count.

Neubauer chamber's counting grid

is 3 mm x 3 mm in size.

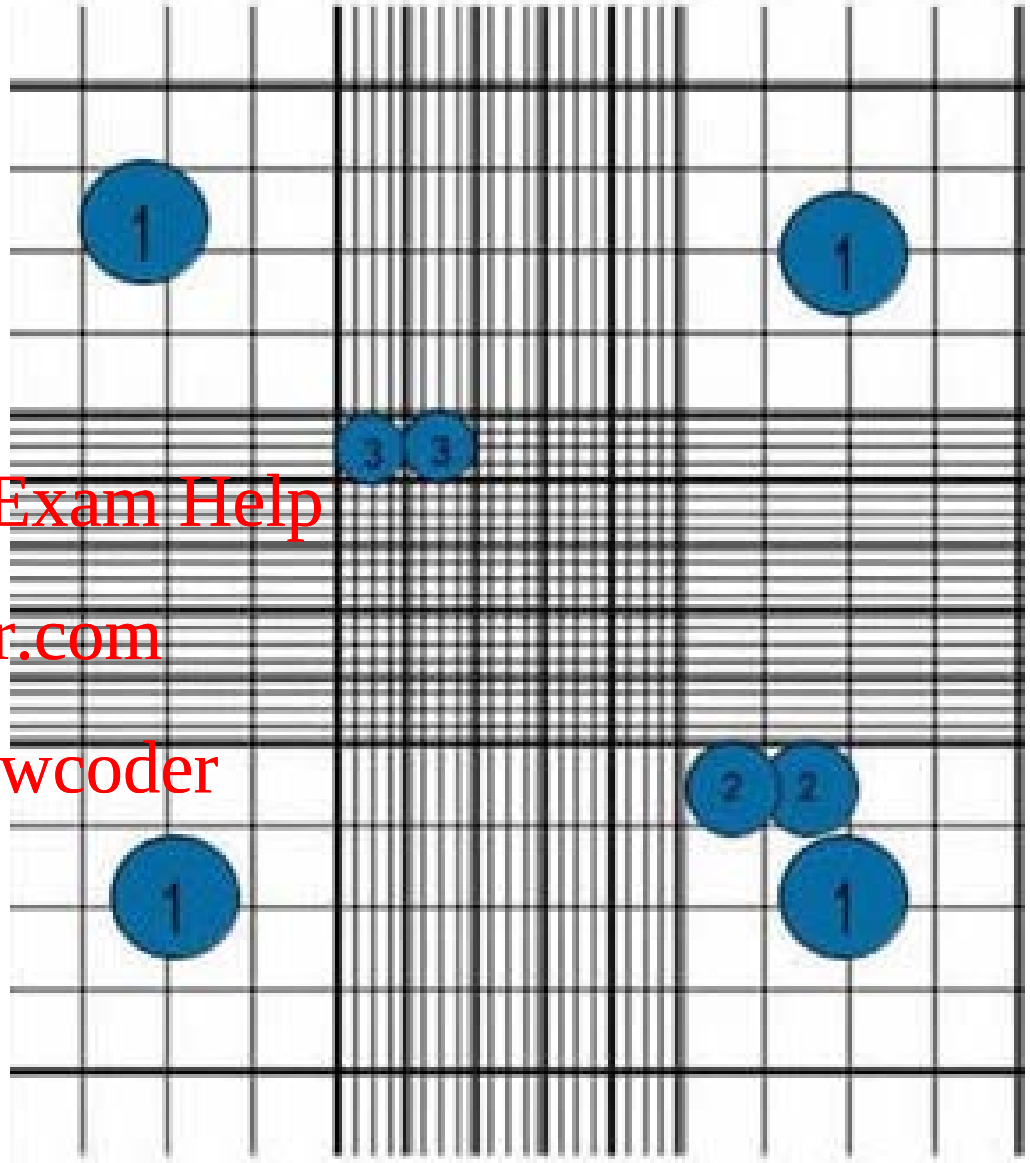
The grid has 9 square subdivisions of width 1mm 1

In case of blood cell counting, the squares placed at the corners are used for white cell counting. Since their concentration is lower than red blood cells a larger area is required to perform the cell count.

The central square is used for platelets or red cells . This square is split in 25 squares of width 0.2 mm (200 µm) 2

.

Each one of the 25 central squares is subdivided in 16 small squares. Therefore, the central square is made of 400 small squares and can be used to count yeast of bacteria 3



Counting cells

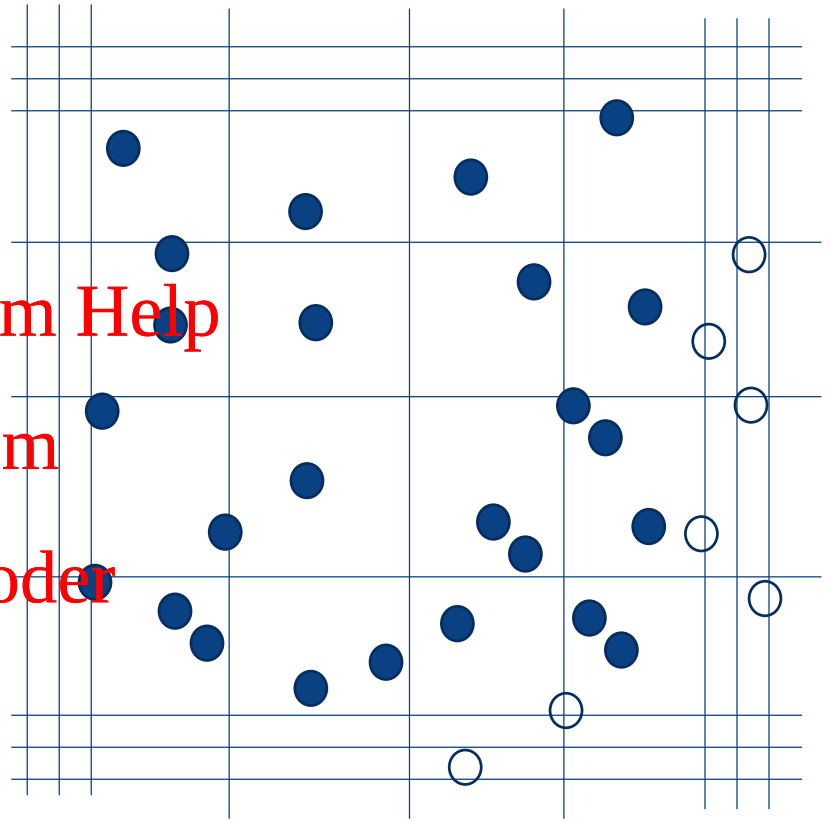
Look for the first counting grid square where the cell count will start.

This example is from a Neubauer improved chamber

Start counting the cells in the first square then move to the next

Different laboratories have different counting protocols, however, there is a popular unwritten rule that states:

"Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits, which should not be taken into account."



Calculation

Concentration = Number of cells x 10,000 / Number of squares

We apply the formula for the calculation of the concentration:

Concentration (cell / ml) = Number of cells / Volume (in ml)

The number of cells will be the sum of all the counted cells in all squares counted.

Since the volume of 1 big square is:

0.1 cm x 0.1 cm = 0.01 cm2 of area counted.

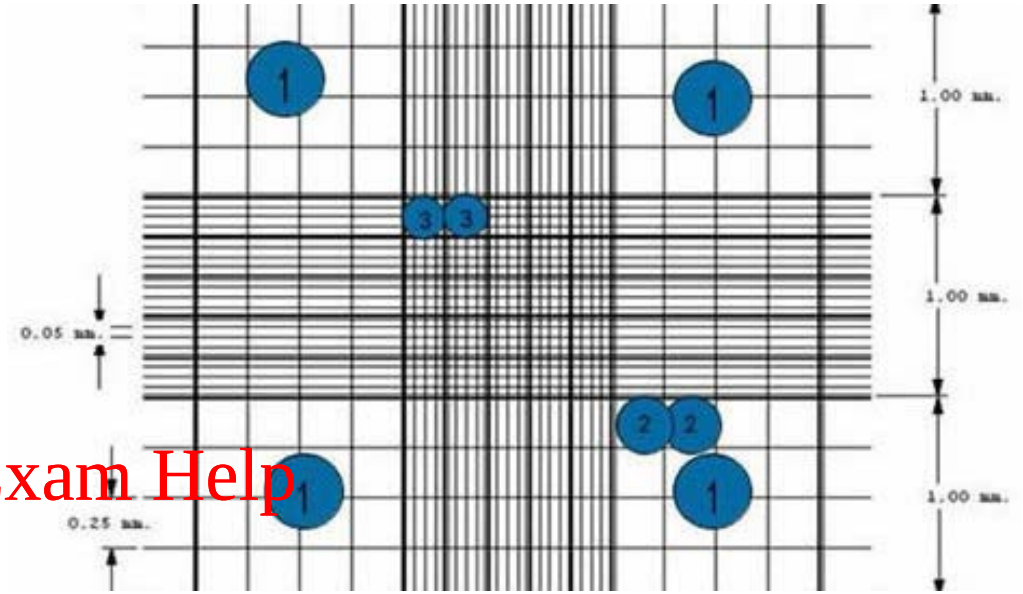
Since the depth of the chamber is 0.1mm:

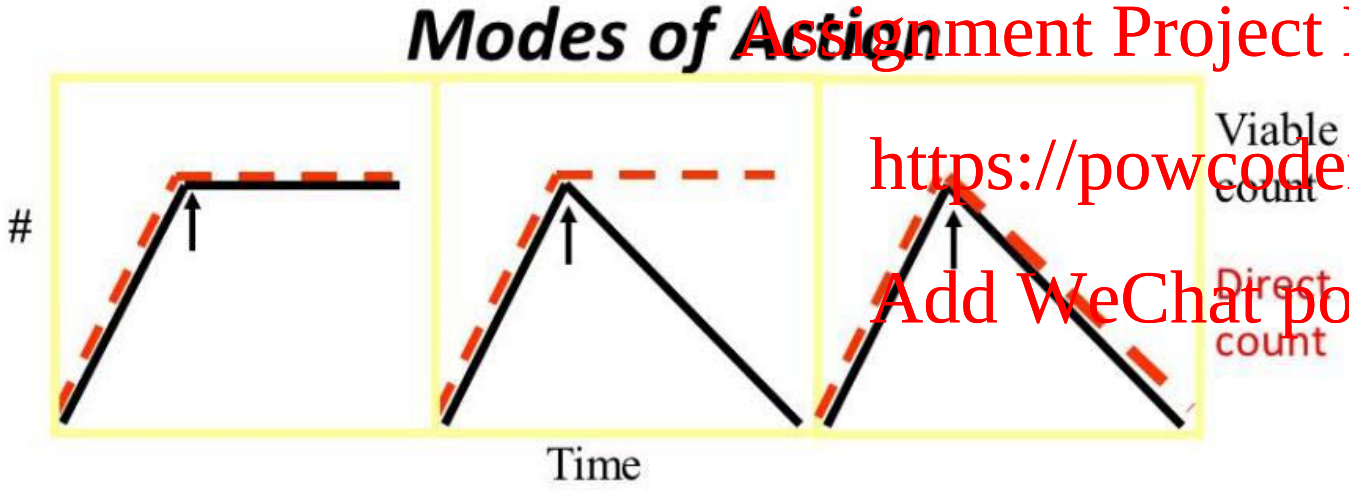
0.1 mm = 0.01 cm

0.01 cm2 x 0,01 cm = 0.0001 cm2 = 0.0001ml = 0.1 µl

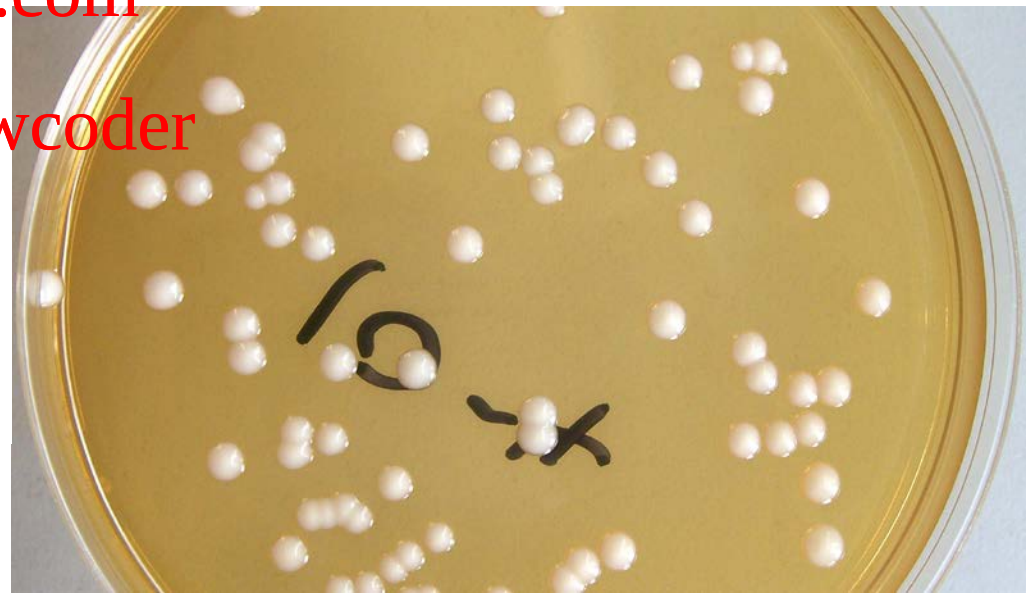
So, for the Neubauer chamber, the formula used when counting in the big squares is:

Limitations and advantages of counting methods





- Bacteriostatic:
- Bacteriocidal
- Bacteriolytic



Thermal treatment of food

The basic purpose for the thermal processing of foods is

- to reduce or destroy microbial activity
- reduce or destroy enzyme activity and
- to produce physical or chemical changes to make the food meet a certain quality standard.

Summary of thermal processing methods

Low-heat processing or pasteurization:

Foods are heated at temperatures below 100°C for a fixed time with the objectives to kill all pathogens, except spores, and ~ 90% of spoilage microbes, except thermoduric bacteria, spores and toxins.

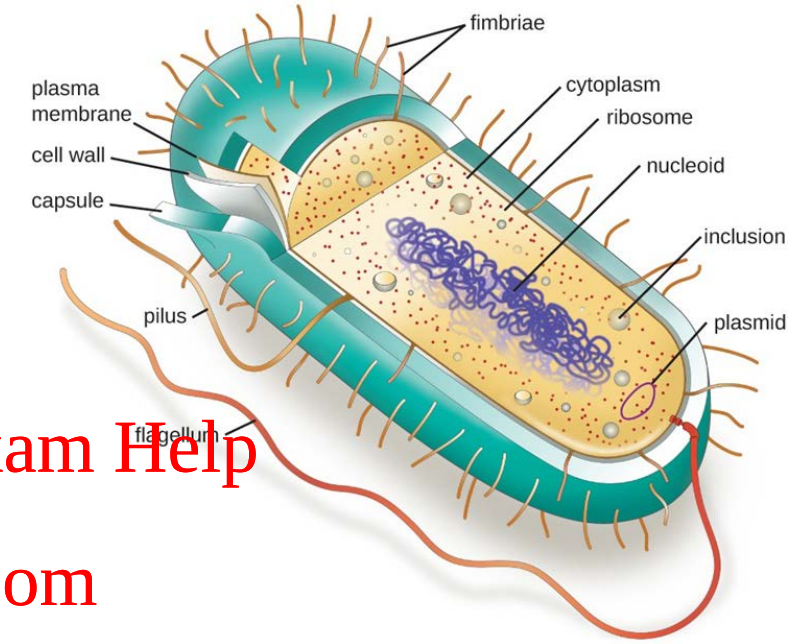
High-heat processed foods:

Foods are heated uniformly at 100°C or above for the time, which depends on a product and microbes to be killed. For low acid foods a 12 D treatment is given to destroy *C. botulinum* spores, and get commercial sterility.

Microwave heating:

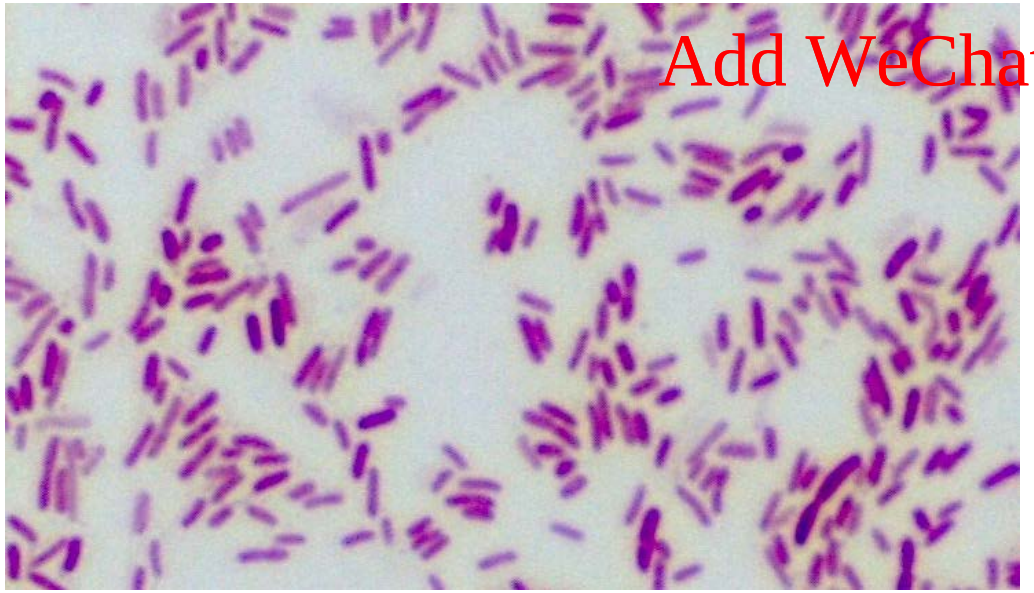
When frozen foods are treated with microwaves, the waves change polarity rapidly, making water molecules to move fast. The frictions of the water molecules generate heat, which kills bacteria. However, the food may not be heated uniformly and can have cold spots. (cannot assure complete destruction of pathogens)

Mechanism of thermal inactivation of microbes

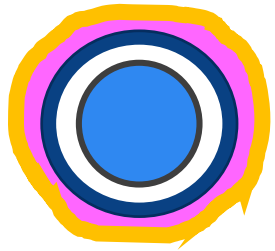


Vegetative cells

- injury to cell membrane (more permeable)
- DNA (strand breaks), ribosomal RNA breakdown
- Cell wall damage

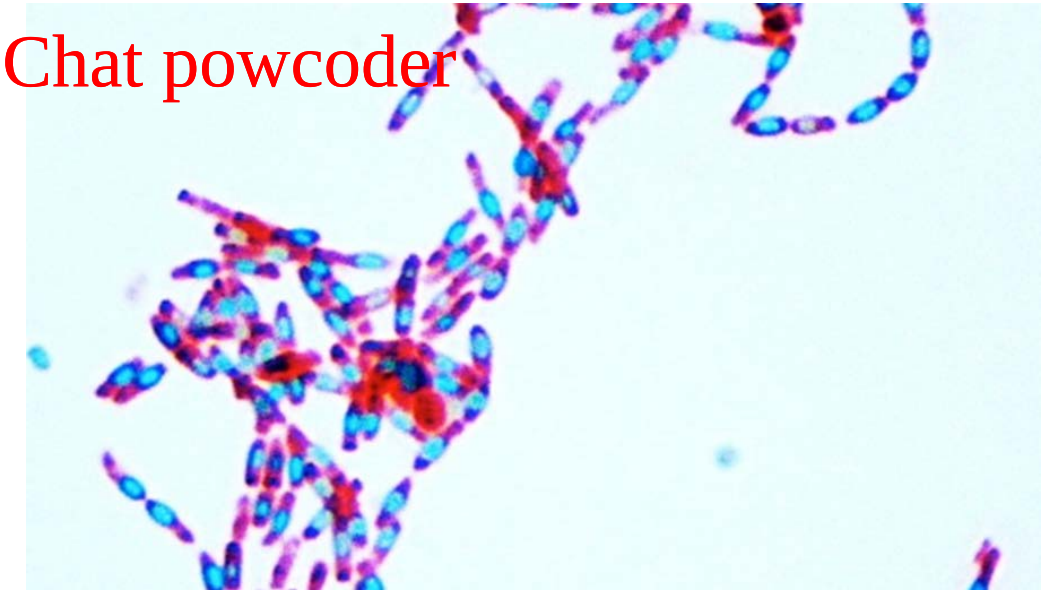


Mechanism of thermal inactivation of microbes



Bacterial Spores

- lose components of spore coat
- damage to layers that become membrane and wall
- cannot take up water for germination



Thermal treatment: time

Three common and important questions regarding thermal inactivation of bacteria in foods:

1. How much time (at x°C) is needed to kill bacteria present?
2. How do different bacteria vary in their sensitivity to heat?
3. What happens to kill rate as I increase the temperature?

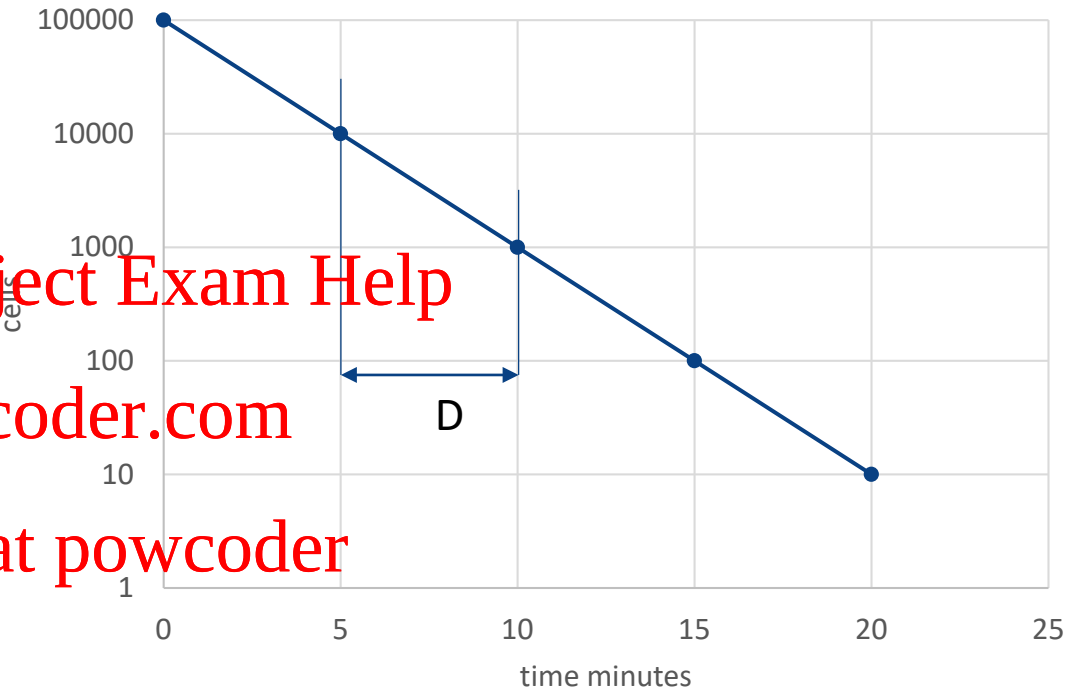
Three well known values:

D-value, Thermal Death Time, z-value

Decimal reduction time (D-value):

At a given temperature (C or F), the time takes (D min) to kill 90% (1 log) of a microbial population (cells, spores).

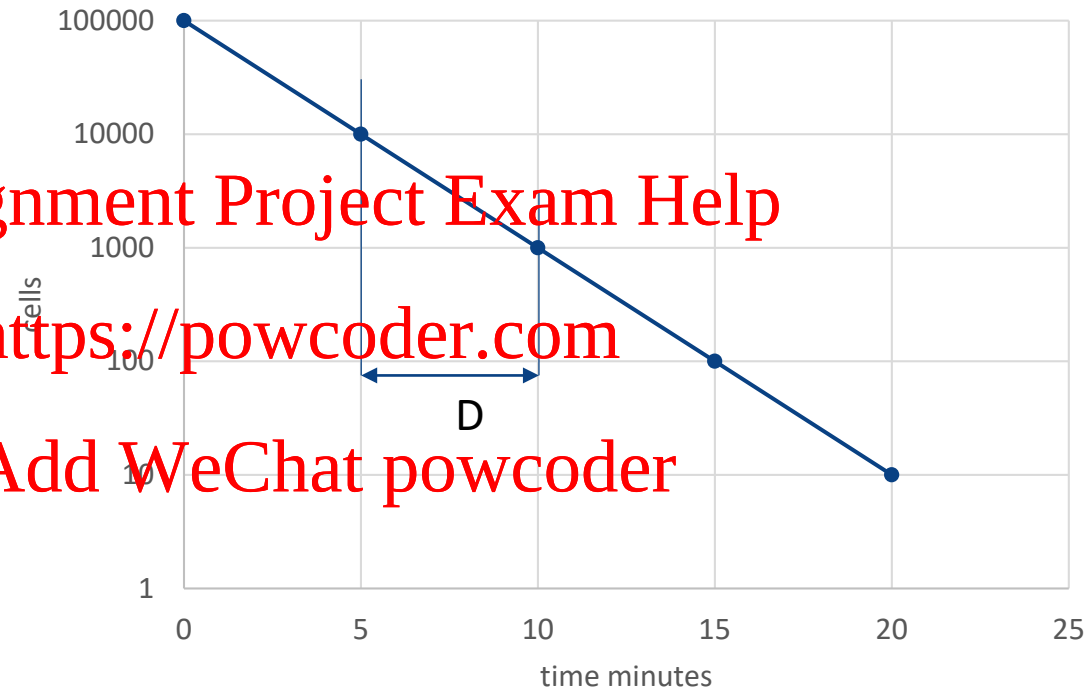
D-values can be used to determine relative heat sensitivity among species and strains, as well as, what heating time is required (D values), under a given time- temperature combination, to reduce a microbial population to certain level.



D value formula

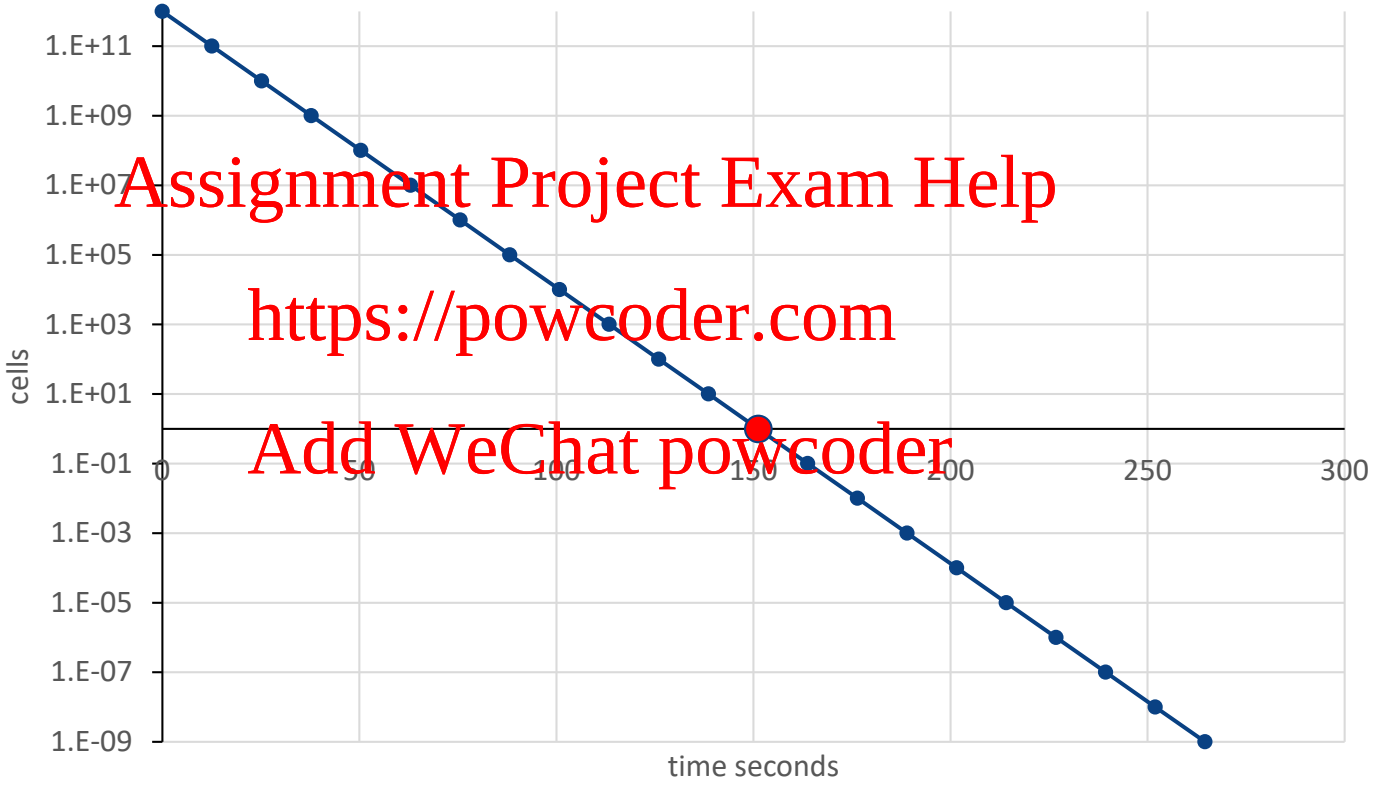
D_T value = $t_2 - t_1 /(\log N_0 - \log N_1)$

T = temperature
t_1 initial time
t_2 = final time
N_0 = initial population
N_1= final population



Thermal treatment

Microbial destruction in food by heat is expressed in terms of its exposure to a specific temperature for a period of time



Killing curve of C. botulinum: This curve presents the DR value (12.6 seconds) and the 12-D reduction (151 seconds) for *C. botulinum*. The killing agent is heat at 121°C.

12D process

Experience has shown that a process equivalent to twelve decimal reductions in the population of *C. botulinum* spores is sufficient for safety; this is referred to as a 12D process

Assuming an initial spore load of 1 spore/g of product, it can be shown that, for such a process, the corresponding probability of *C. botulinum* spore survival is 10^{-12}, or one in a million million.

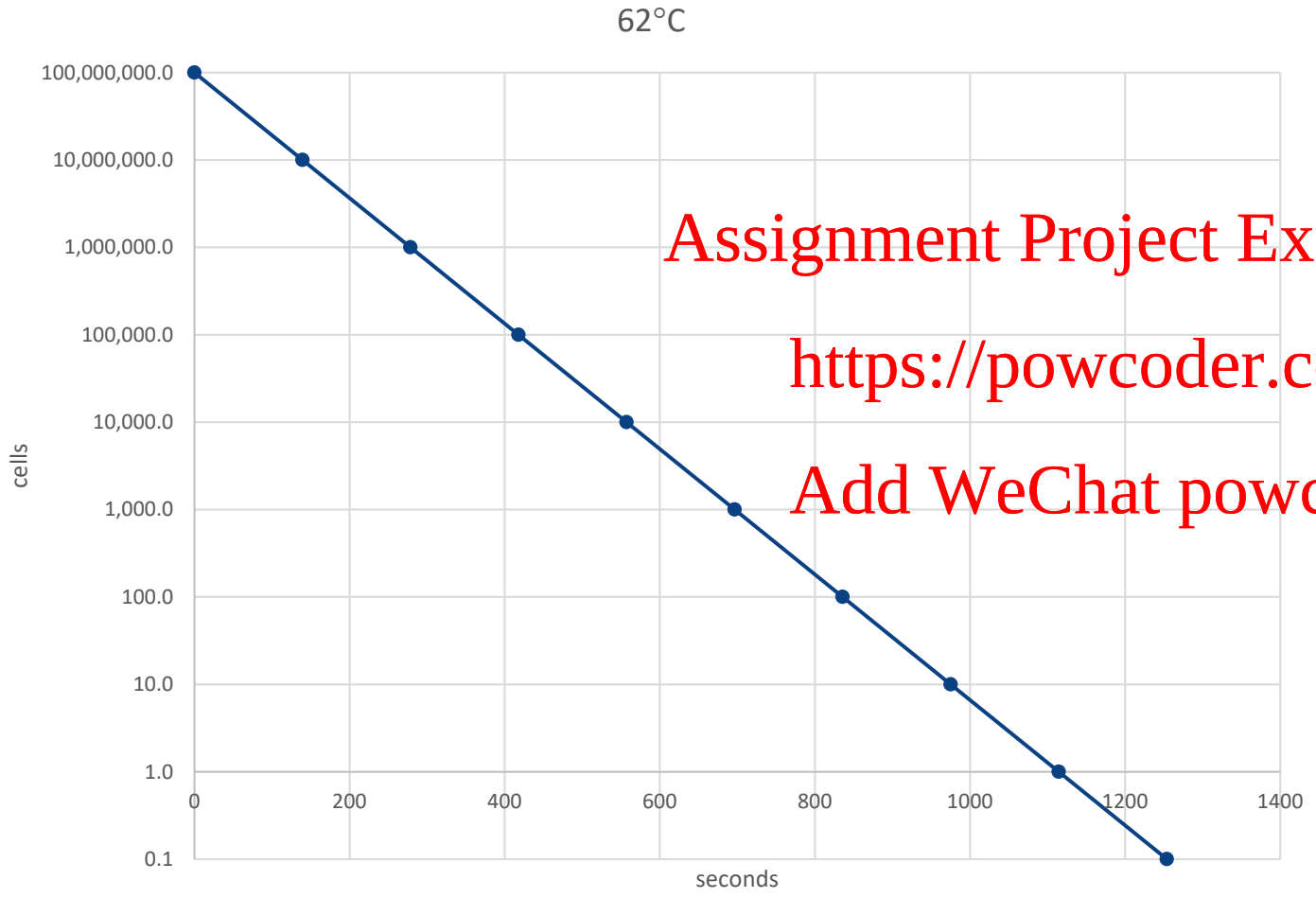
This implies that for every million million cans given a 12D process, and in which the initial load of *C. botulinum* spores was l/g, there will be only one can containing a surviving spore.

This is commercially acceptable

Decimal reduction times (D values) for bacterial spores

Organism	Approximate optimum growth temp. (°C)	D value (min) a/
B. stearothermophilus	55	D121.1 4.0 - 5.0
C thermosaccharolyticum	55	D121.1 3.0 - 4.0
D. nigrificans	55	D121.1 2.0 - 3.0
C. botulinum (types A & B)	37	D121.1 0.1 - 0.23
C.sporogenes (PA 3679)	37	D121.1 0.1 - 1.5
B. coagulans	37	D121.1 0.01 - 0.07
C. botulinum type E	30 - 35	D82.2 0.3 - 3.0

Thermal death time (TDT)

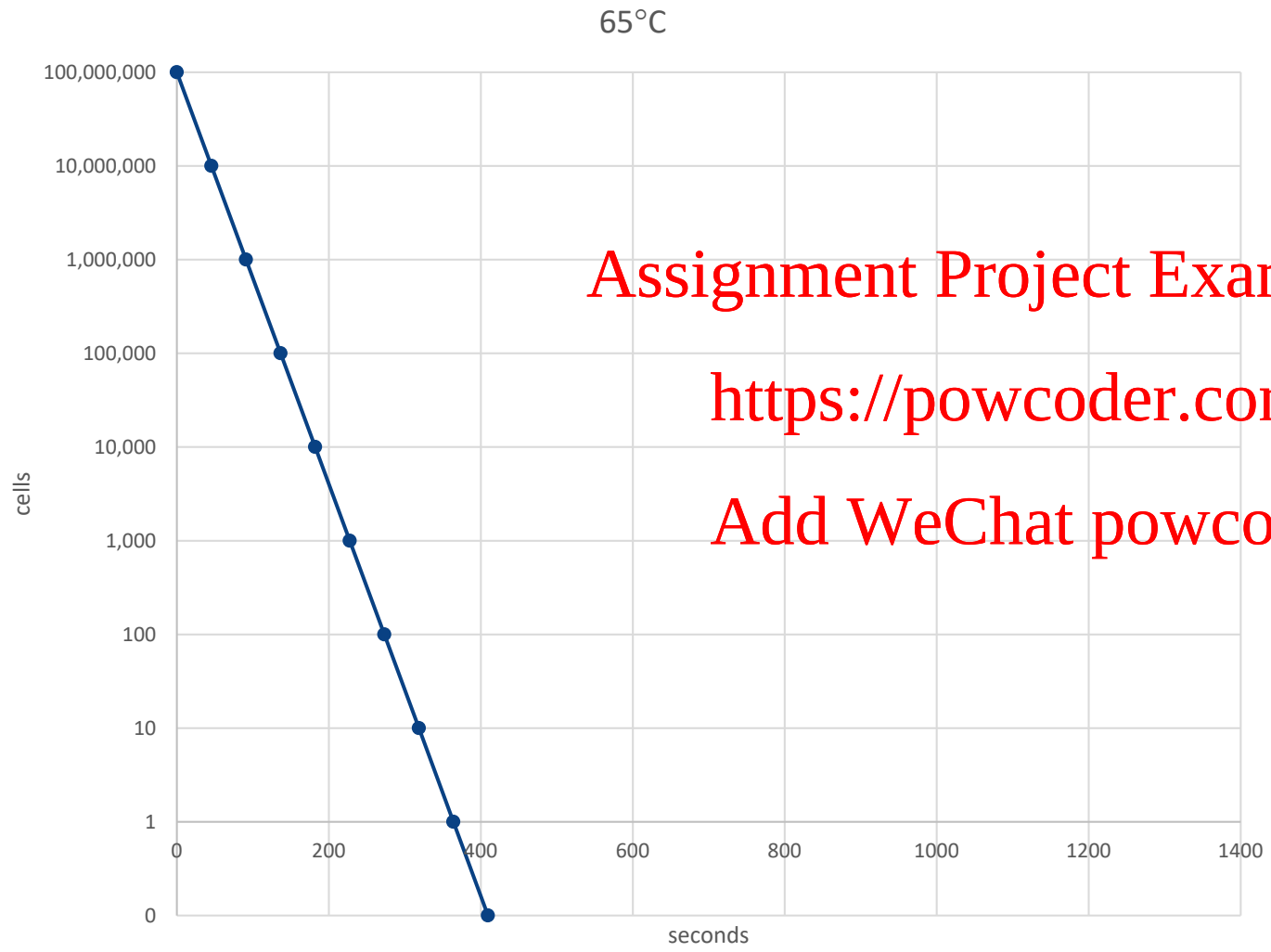


1235.7

TDT

-time required for complete destruction of specific number of microbial cells or spores at a specific temperature.

Thermal death time (TDT)



409.4

TDT

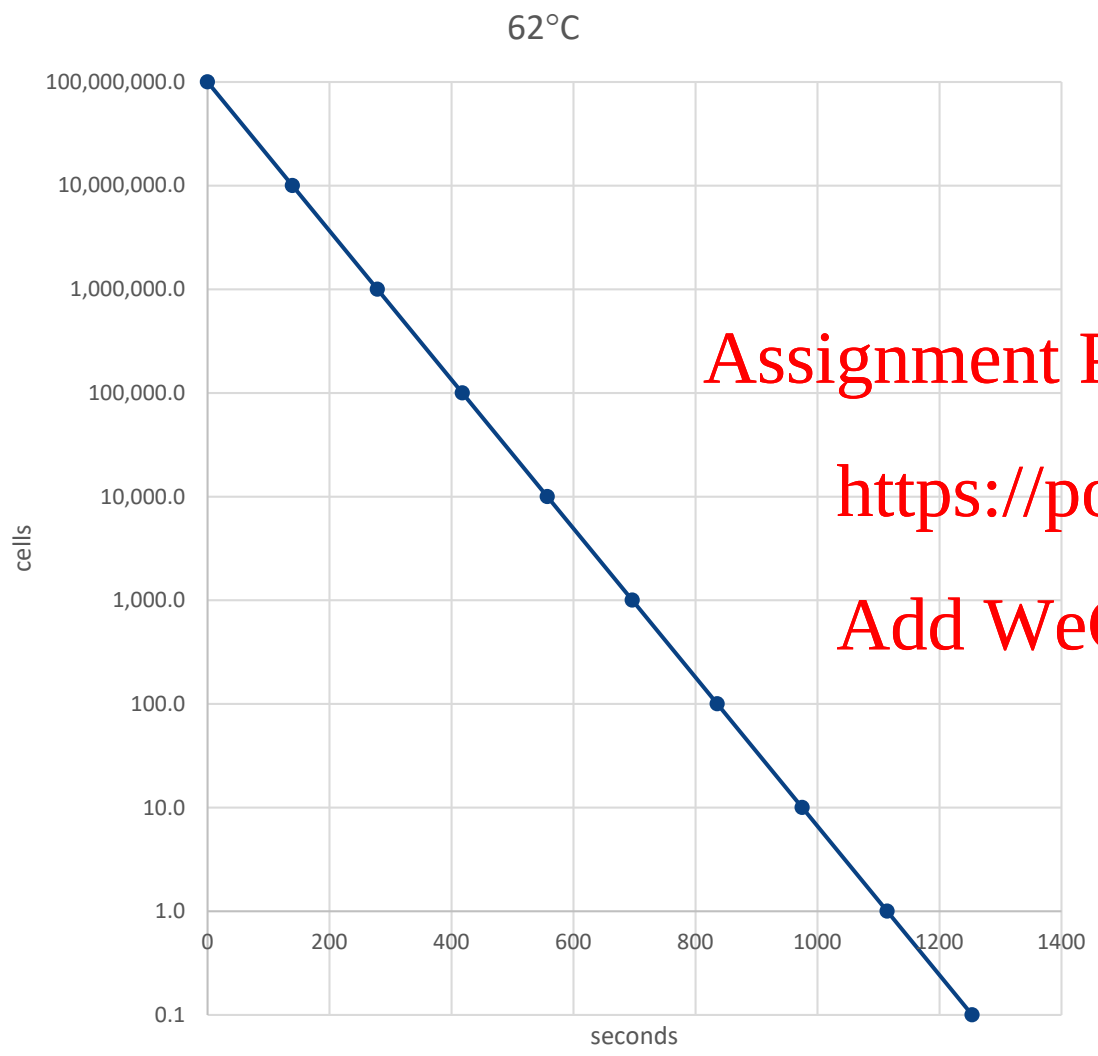
-time required for complete destruction of specific number of microbial cells or spores at a specific temperature.

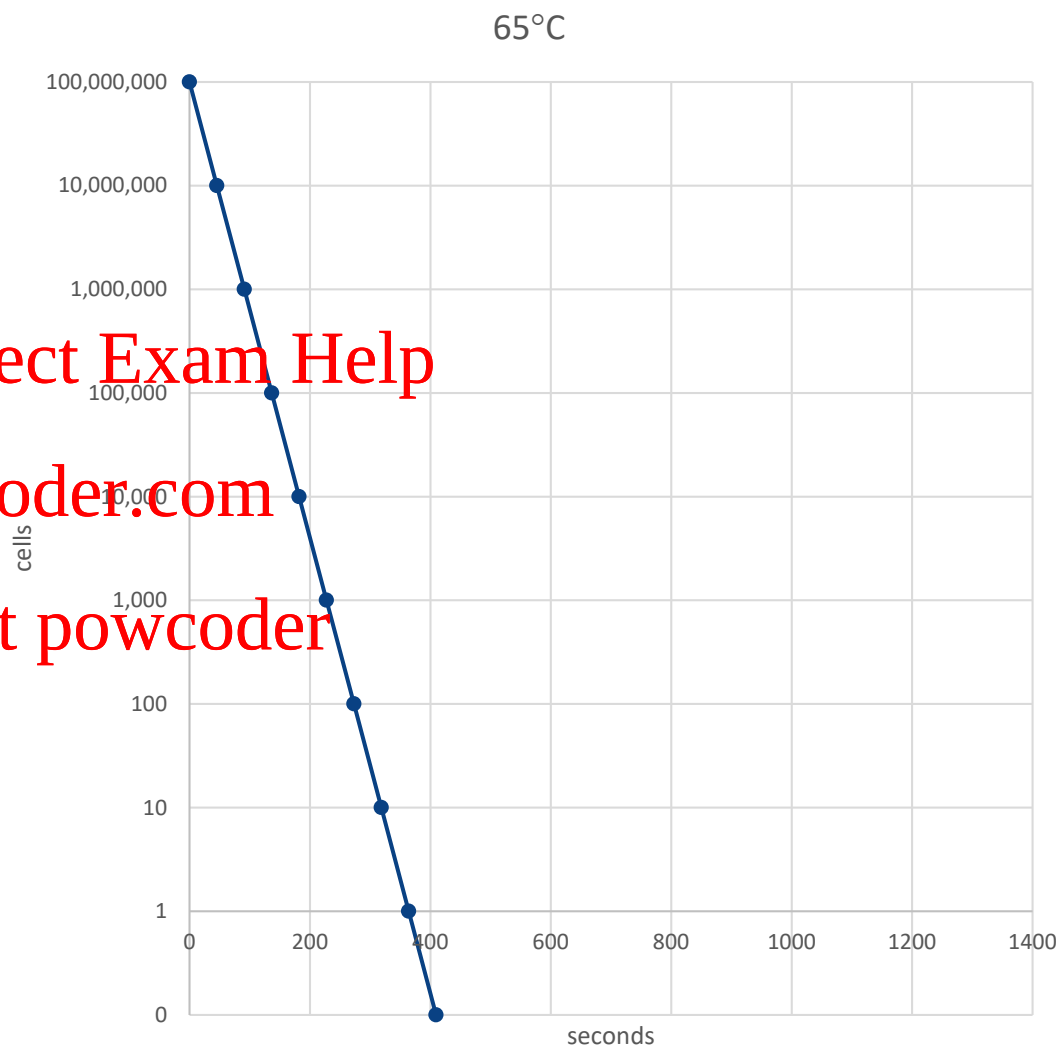
Higher temperature

Shorter D

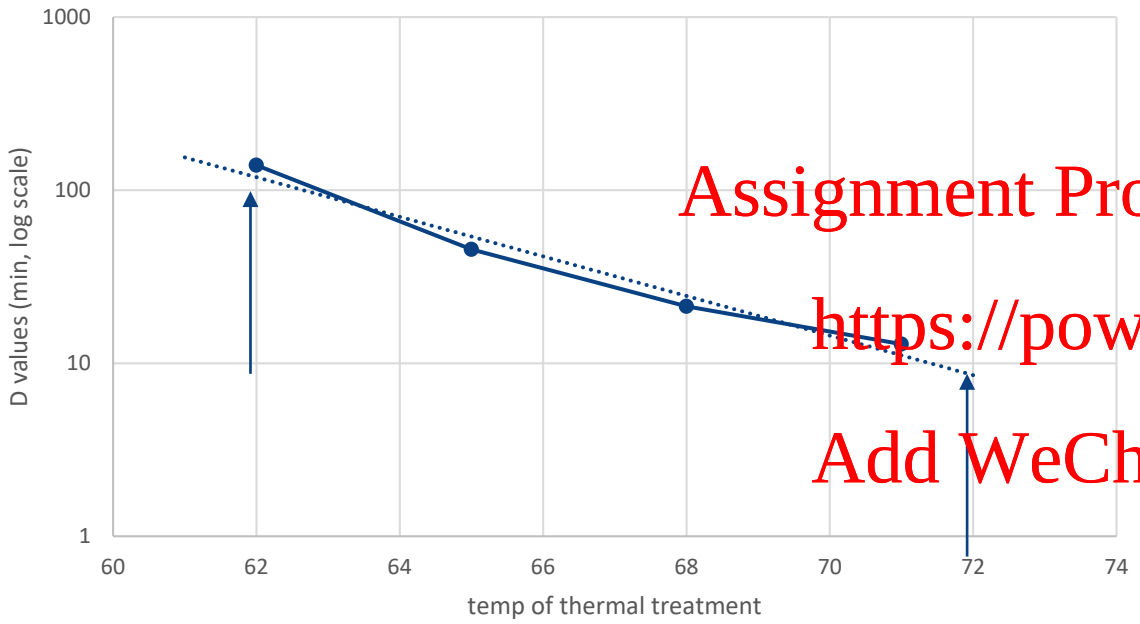
Shorter TDT

Slope of line steeper than at lower temp





Thermal death time (TDT) and Z value



The z-value is a measure of the change of the D-value with varying temperature

the slope of the curve gives Z value (time to reduce D-values by 1 log

The D value at 62°C is 139.9 sec and at 71°C it is 12.9

Ray and Bhunia Ch 33 Control by heat (thermal processing

Control by heat Pasteurisation

- uses temperatures below 100°C
- aim is to destroy all vegetative cells of pathogens, and a large proportion (90%) of spoilage microbes
- Time/Temp. combination used is designed to be minimum to achieve microbiological standards, and minimize thermal damage to food (quality)
- Thermoduric bacteria survive (e.g. spore-formers)
- Refrigeration normally used to delay spoilage
- Bacterial heat-stable enzymes can be a problem
- Milk is most obvious food item that is pasteurized

Thermal processing of dairy food from FSANZ Standard 4.2.4

(1) Milk must be pasteurised by –

(a) heating to a temperature of no less than 72°C and retaining at such temperature for no less than 15 seconds; or

(b) heating, using any other time and temperature combination of equivalent or greater lethal effect on any pathogenic micro-organisms in the milk

In the case of Ultra Heat Treatment (UHT) of milk, for example, temperatures of at least 132°C must be used to achieve commercial sterility.

Thermal processing of dairy food from FSANZ Standard 4.2.4

	All dairy produce (excluding ice cream) with						
	Milks with <10% fat and no added sweeteners and particles			Dairy produce with ≥10% fat and/or added sweeteners and concentrated dairy produce with > 15% total solids and particles			Ice Cream mixes with particles <1000 µm
Particle Diameter	<200 µm Ø	200 to <500 µm Ø	500 to <1000 µm Ø	<200 µm Ø	200 to <500 µm Ø	500 to <1000 µm Ø	
Minimum holding time (seconds)	Minimum Temperature (°C)						
1.0	81.6	-	-	84.4	-	-	-
2.0	79.0	81.6	-	81.8	84.4	-	-
3.0	77.6	79.0	-	80.4	81.8	-	-
4.0	76.5	77.6	81.6	79.3	80.4	84.4	-
5.0	75.7	76.5	79.0	78.5	79.3	81.8	-
6.0	75.1	75.7	77.6	77.9	78.5	80.4	-
7.0	74.6	75.1	76.5	77.4	77.9	79.3	-
8.0	74.1	74.6	75.7	76.9	77.4	78.5	-
9.0	73.7	74.1	75.1	76.5	76.9	77.9	-
10.0	73.3	73.7	74.6	76.1	76.5	77.4	85.5
11.0	73.0	73.3	74.1	75.8	76.1	76.9	-
12.0	72.7	73.0	73.7	75.5	75.8	76.5	-
13.0	72.4	72.7	73.3	75.2	75.5	76.1	-
14.0	72.1	72.4	73.0	74.9	75.2	75.8	-
15.0	72.0	72.1	72.7	74.8	74.9	75.5	79.5
30.0	70.7	70.8	70.9	73.5	73.6	73.7	-
60.0	69.4	69.4	69.5	72.2	72.2	72.3	-