STERILIZATION

notions: **STERILITÁS -- STERILITY**

ASZEPTIKUSSÁG -- ASEPTICITY

ELSZIGETELÉS, IZOLÁLÁS -- CONTAINMENT

Killing microbes

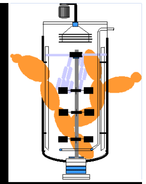
Protecting the environment from microbes

Protect the system from the microbes
aseptic operation=maintaining sterility

Patogenes
Víruses
vakcinatermelés
GMO-s
rDNS production problems

OECD 1986 – Recombinant DNA Safety Considerations
EC 1990 Council Directive on the Contained use od GMOs

GMO
I.: small risk
II.: others



STERILIZATION

CONTAMINATION

DECREASING YIELD
ALTERATION IN PROCESS BEHAVIOUR (KINETICS)

Extra work, money

- **PLUS STERILIZATION NEED**
- **WHOLE CHARGE GOES WRONG (SCALE DEPENDENT DEMAGE)**
- **PROBLEM AT DOWN-STREAM**

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METHODS for removal and killing of microbial cells

mechanical methods: filtration,

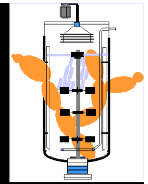
centrifugation,

flotation,

electromagnetic irradiation: UV, X ,

chemical methods: dezinfection,

heat.



Thermal death of microorganisms

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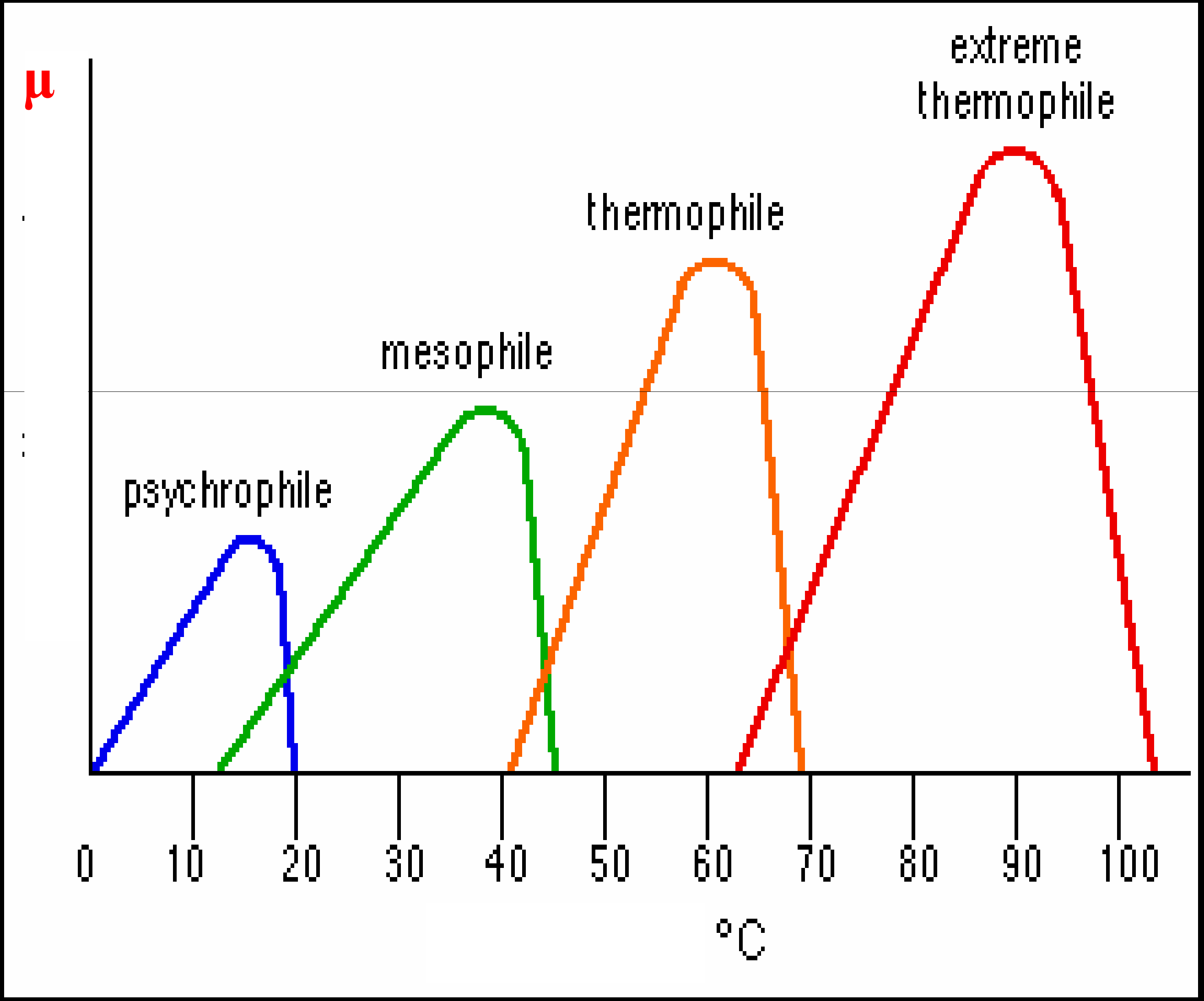
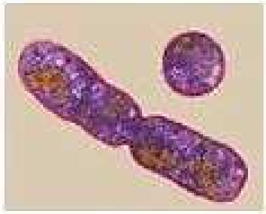
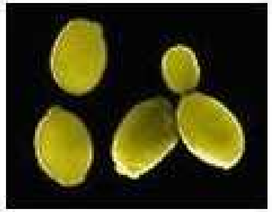
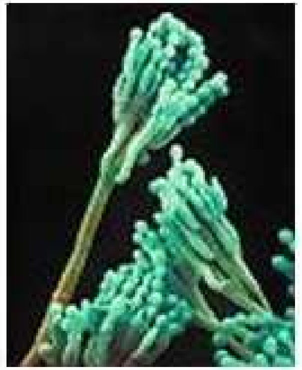
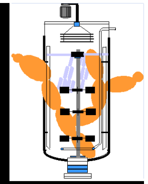
Temperature ranges of the growth of microbes



Relative heat resistency

Vegetative bacterium- and yeast cells	1	 	*E. coli* *S. cerevisiae* Vegetative cells
Conidiospores of phylamentous fungi	2-10		*P. chrysogenum* conidiospores
Viruses and bacteriophages	1-5		H1N1 influenzavírus
Bacterium spores	~10^6		*Bacillus anthracis* endospores



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- heat sensitivity depends upon (given species): life history of the cell, age of the cell
  (e.g.: cells from the exponencial growth phase are more sensitive than cells from the stacioner phase)

- cells are more sensitive against **moist** heat than against **dry** heat

- heat sensitivity (thermal death) increases with incr. temperature

- heat sensitivity depends on media
  pH, viscosity, osmotic pressure,
  presence of defending colloids,

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KINETICS OF THERMAL DEATH AT CONSTANT TEMP.

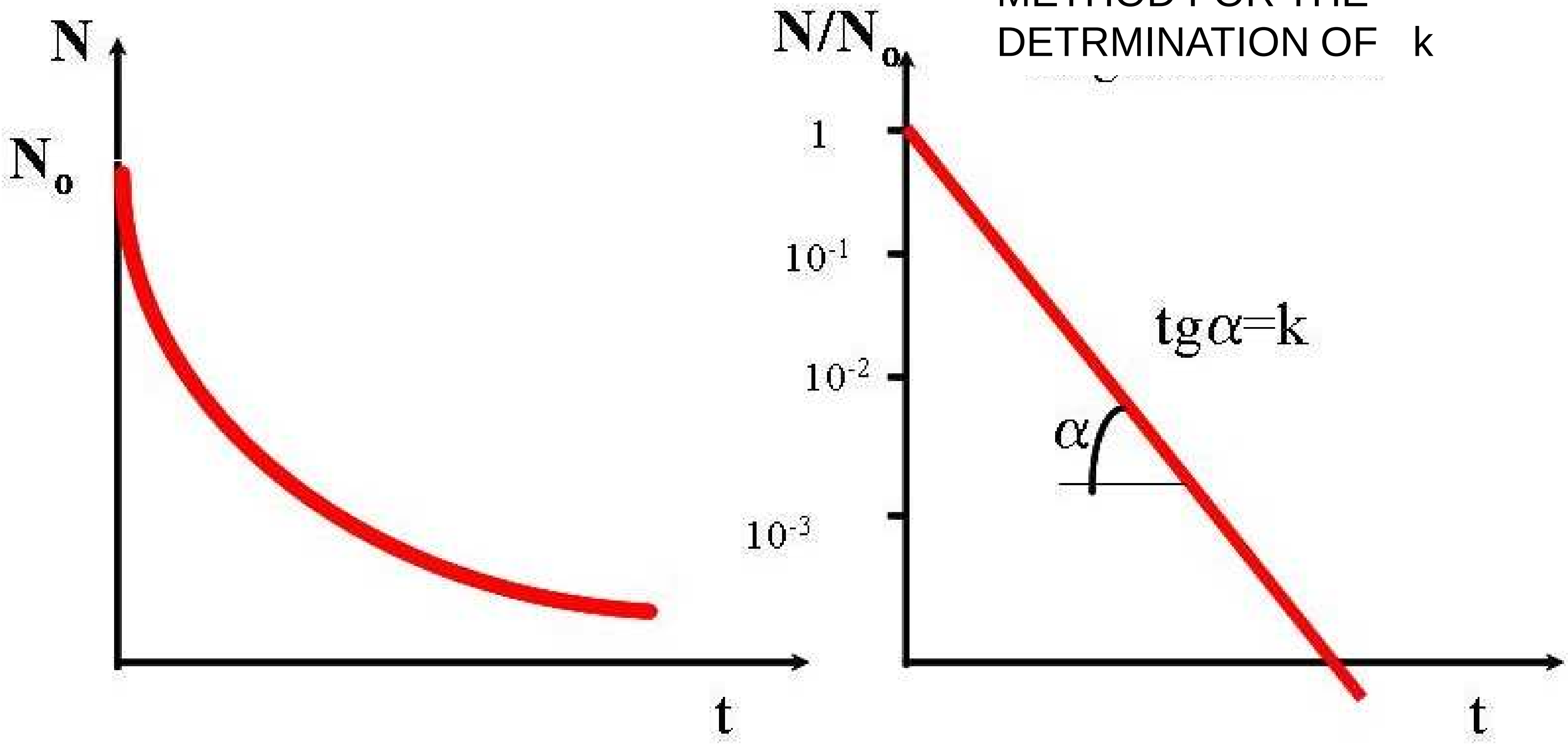
$$\boxed{\frac{dN}{dt} = -kN}$$

N number of living cells [pc/cm^3]

k thermal death rate (decay) constant [min^{-1}].

$$\int_{N_0}^{N} \frac{dN}{N} = \int_{N_0}^{N} d\ln N = -\int_0^t k\,dt \rightarrow \begin{cases} \ln \dfrac{N}{N_0} = -kt \\ N = N_0 e^{-kt} \end{cases}$$

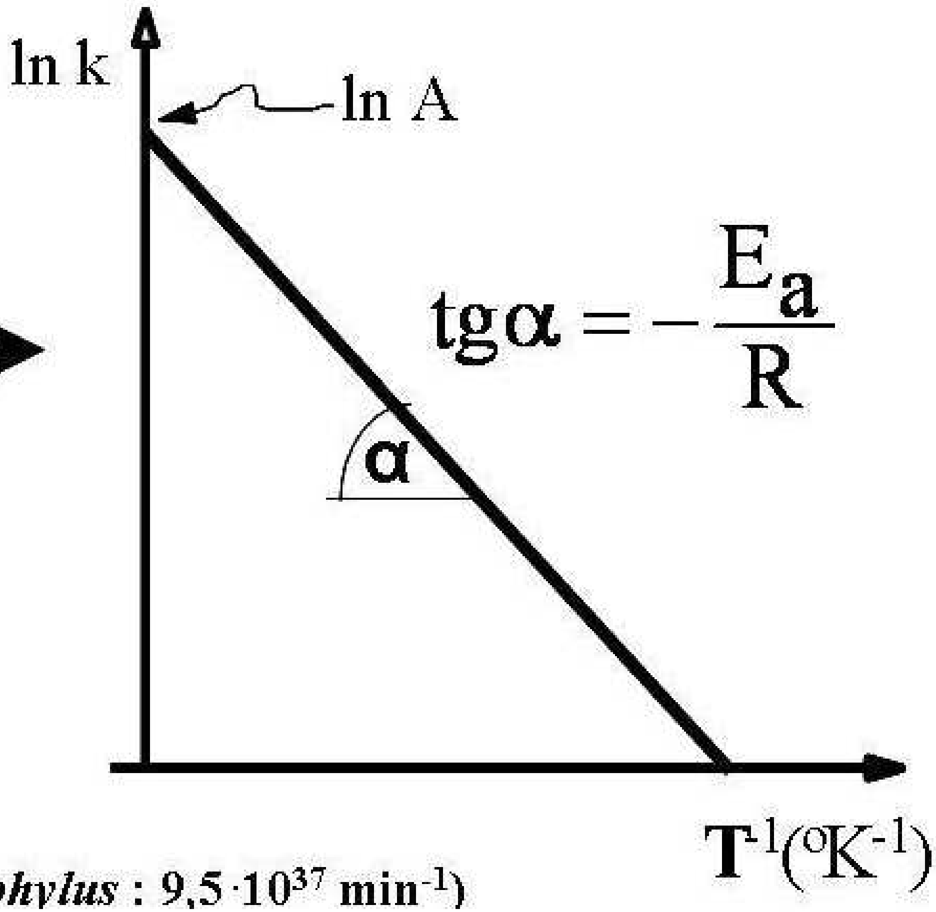
METHOD FOR THE DETRMINATION OF k



what k depends on?

microbe ....sort, „form”
medium
temperature

$$\ln k = \ln A - \frac{E_a}{R}\frac{1}{T}$$

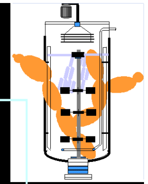


A: Empirical constant

(*Geobacillus stearothermophylus* : $9{,}5 \cdot 10^{37}$ min^{-1})

E_a: Apparent activation energy of thermal death (KJ/mol)

(*Geobacillus stearothermophylus* : 70 kcal/mol) **Determination method**



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Microbe	T[°C]	k[min^{-1}]	E_a [KJ/mol]
Bacillus subtilis			
(vegetative)	110	27	310
Bacillus subtilis			
(spores)	121,1	3	-
Bacillus			
stearothermophilus	104	0,051	283
(spores)	125	6,06	283
	130	17,52	283
Clostridium botulinum			
(spores)	104	0,42	344
Hemoglobin			
(heatdenaturation)	68	$6{,}3 \cdot 10^{-3}$	312

Medium components heat decay apparent activation energies [kJ/mol]	
Reaction between carbohydrates and proteins	**130,6**
B_1 vitamin decay	**87,9**
B_2 vitamin decay	**98,8**

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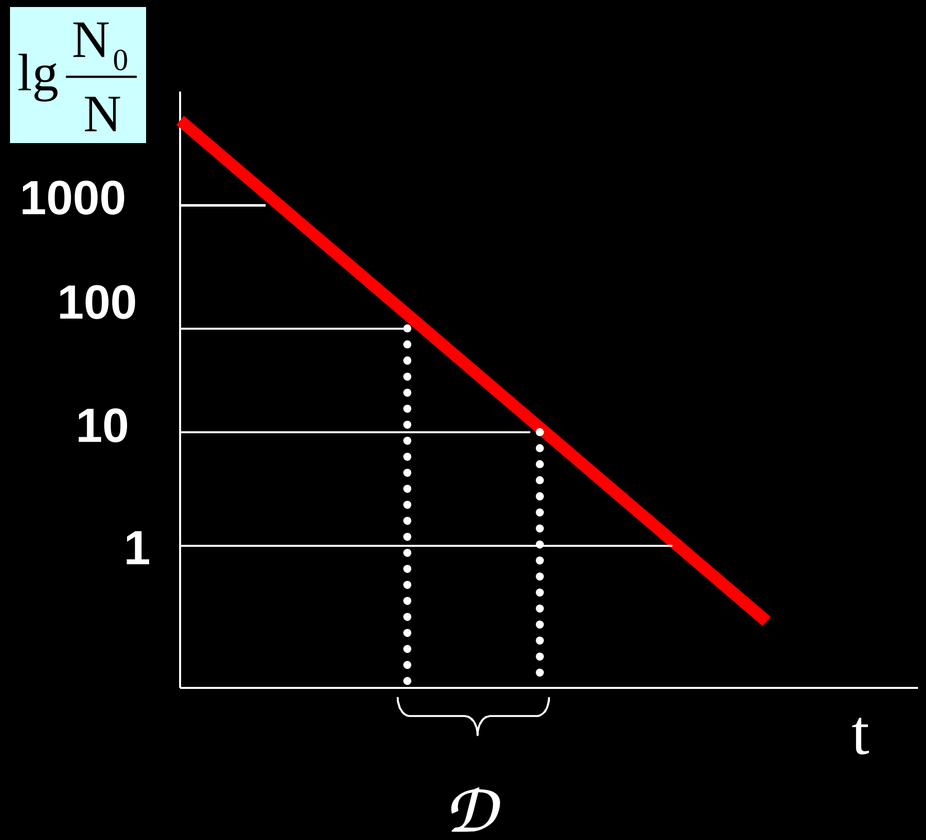
$$\frac{1}{k} = \bar{t}$$

Mean life span

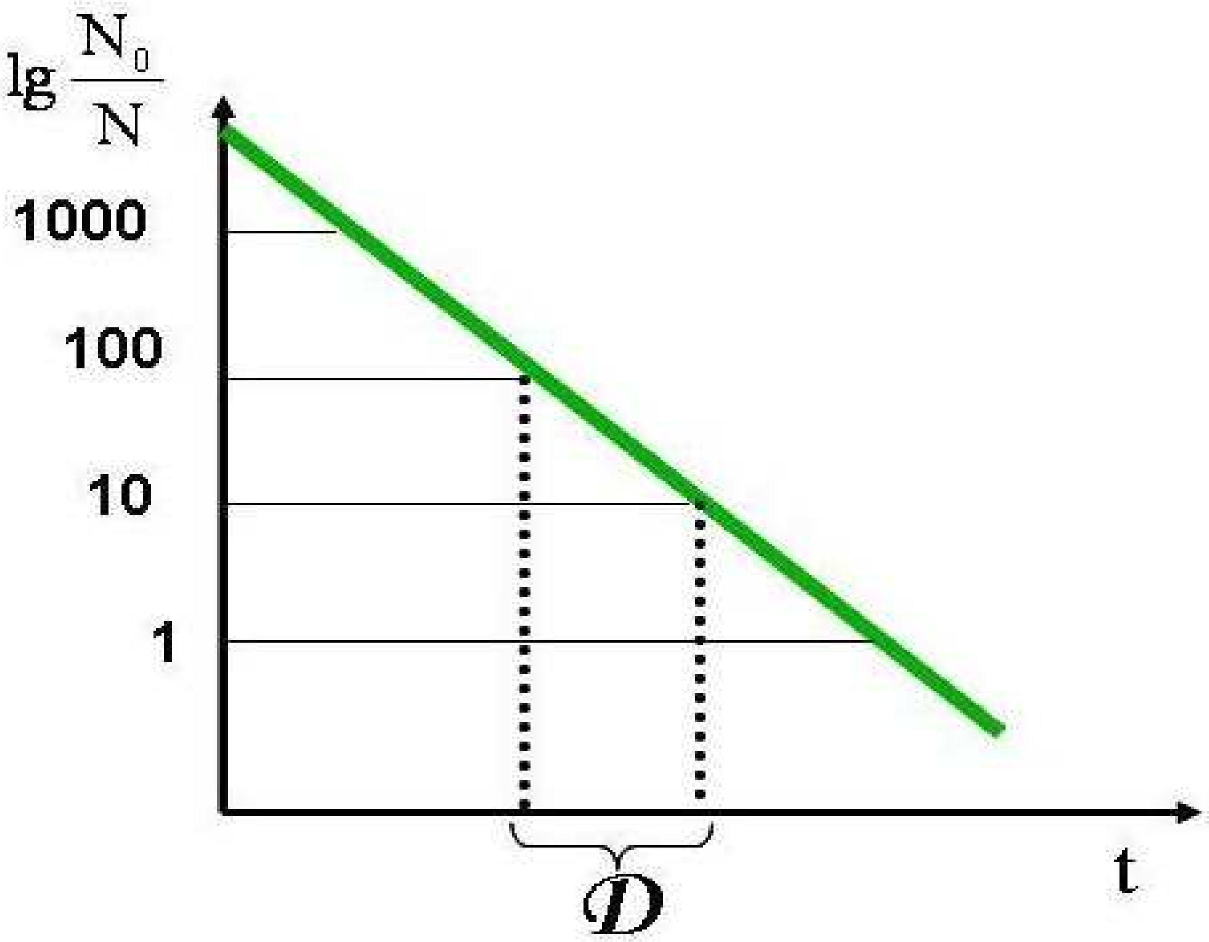
$$\frac{2{,}3}{k} = \mathcal{D}$$

decimal reduction time



$$\frac{1}{k} = \bar{t}$$ Mean life span

$$\frac{2{,}3}{k} = \mathcal{D}$$ decimal reduction time



Probabilistic approach of thermal death

Kinetic description is good if N_o>>1 !
Thermal death is also a stochastic process

Definition: **the life span of one cell (spore)is the length time during which the cell (spore) will just remain viable.**

mean life span of the population $$\bar{t} = \frac{1}{N_0}\sum_{i=1}^{\infty} N_i t_i$$

Life span

!

N_0 no of all the spores
N_i no of the spores with life span of t_i

Mean thermal decay constant $$\frac{1}{\bar{t}} = \bar{k}$$

STERILIZATION

CONDITIONS
If temp is the same everywhere in the vessel,
No growth, (!!!!)
Behaviour of the individual spores is independent of the others.

Probability of the event that at time t the no of the survivors is exactly N (N= 0,1,2,...N_o), follows a binomal distribution:

$$P_N(t) = \binom{N_0}{N} [p(t)]^N [1 - p(t)]^{(N_0 - N)}$$

$$p(t) = e^{-\bar{k}t}$$

probab. that one spore is survivor at time

$$P_N(t) = \frac{N_0!}{(N_0 - N)!N!} \left(e^{-\bar{k}t}\right)^N \left(1 - e^{-\bar{k}t}\right)^{(N_0 - N)}$$

STERILIZATION

$$P_N(t) = \frac{N_0!}{(N_0 - N)!N!}\left(e^{-\bar{k}t}\right)^N \left(1 - e^{-\bar{k}t}\right)^{(N_0 - N)}$$

What is the prob. that all the spores had already died by the time t ?

$$P_0(t) = \left(1 - e^{-\bar{k}t}\right)^{N_0} < 1$$

Always higher than 0:

$$1 - P_0(t) = 1 - \left(1 - e^{-\bar{k}t}\right)^{N_0} > 0$$

At a common sterilization process $N_0 >> 1$

$$1 - P_0(t) \cong 1 - e^{-N}$$

in which $N = N_0 e^{-\bar{k}t}$.

$$= 1 - e^{-N_0 e^{-\bar{k}t}} \approx N_0 e^{-\bar{k}t}$$

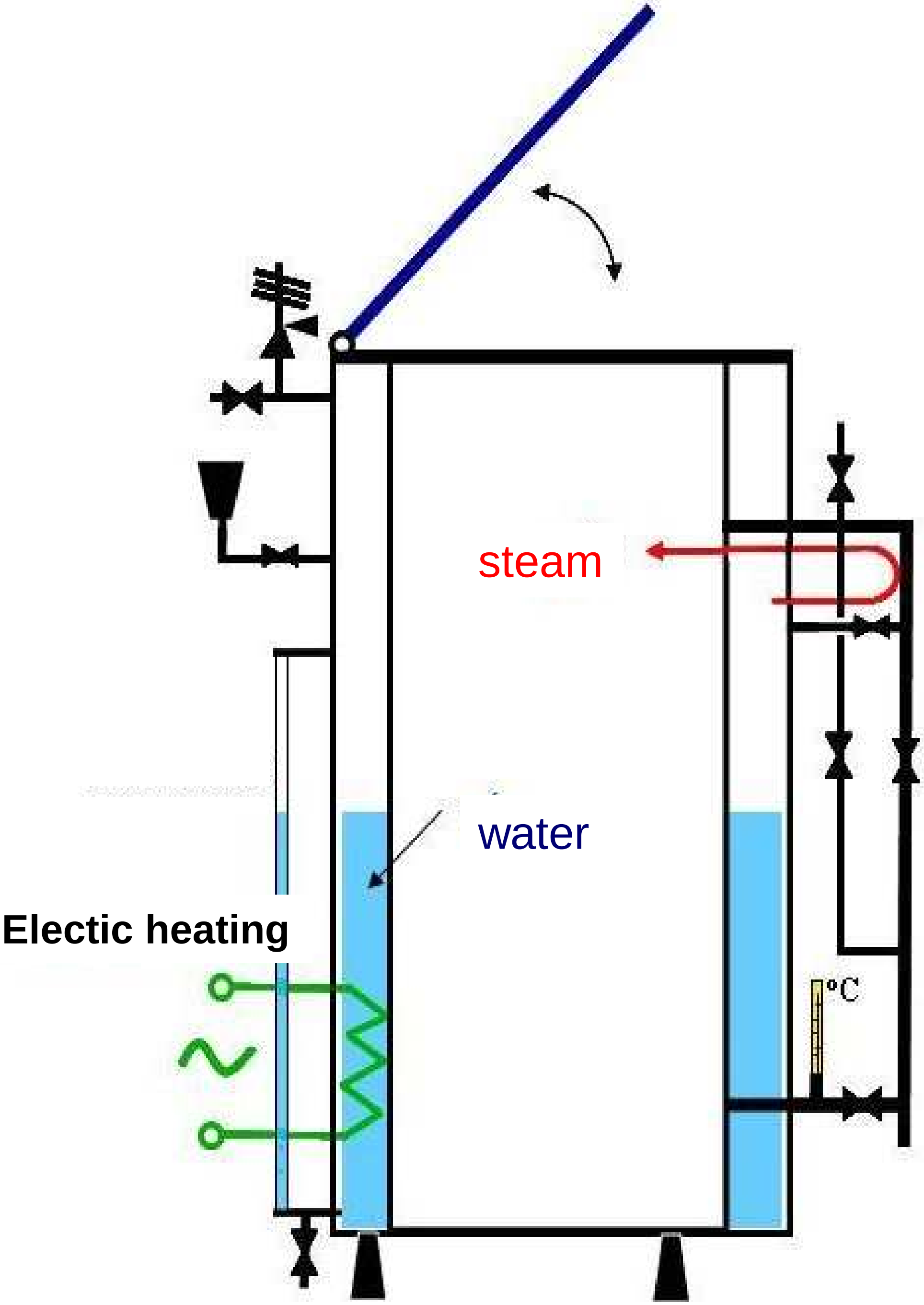
$e^{-x} \sim 1 - x + \ldots$ according to a Taylor serie

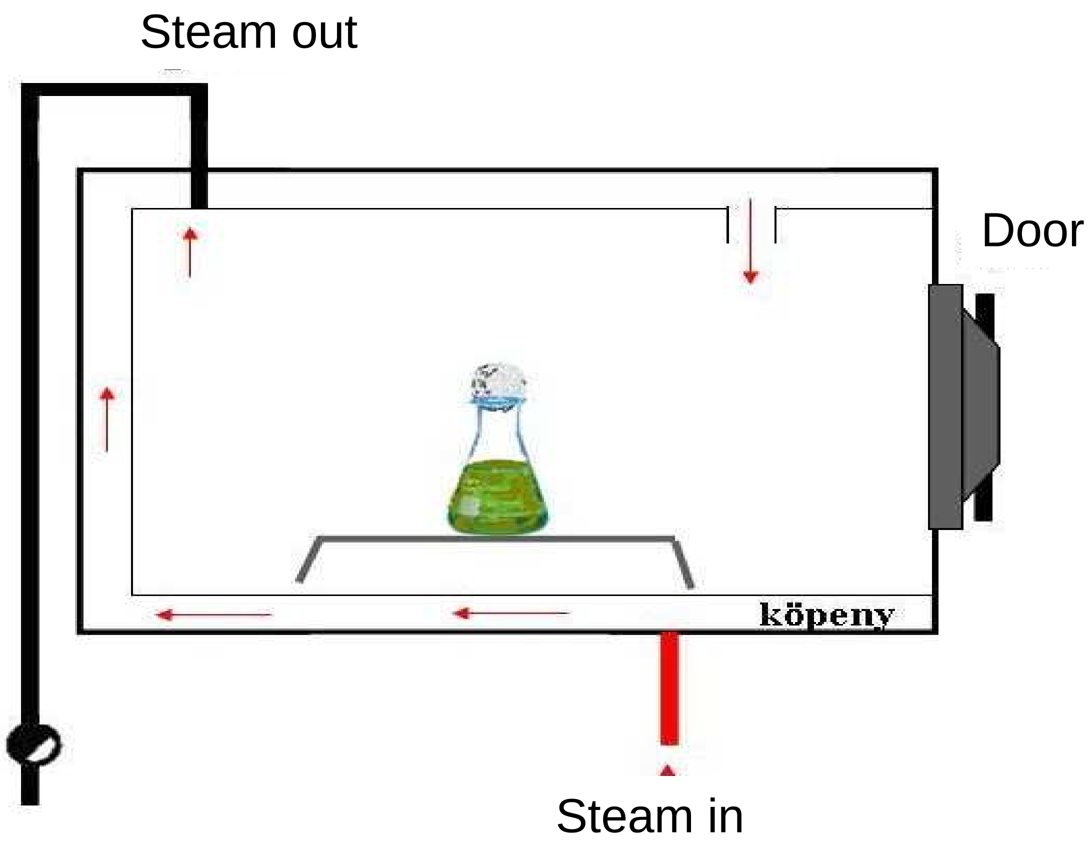
STERILIZATION

Sterility criterion? Pl .:

99,9% → $P_0(t)=0,999$ → $1-P0(t)=0,001=10^{-3}$

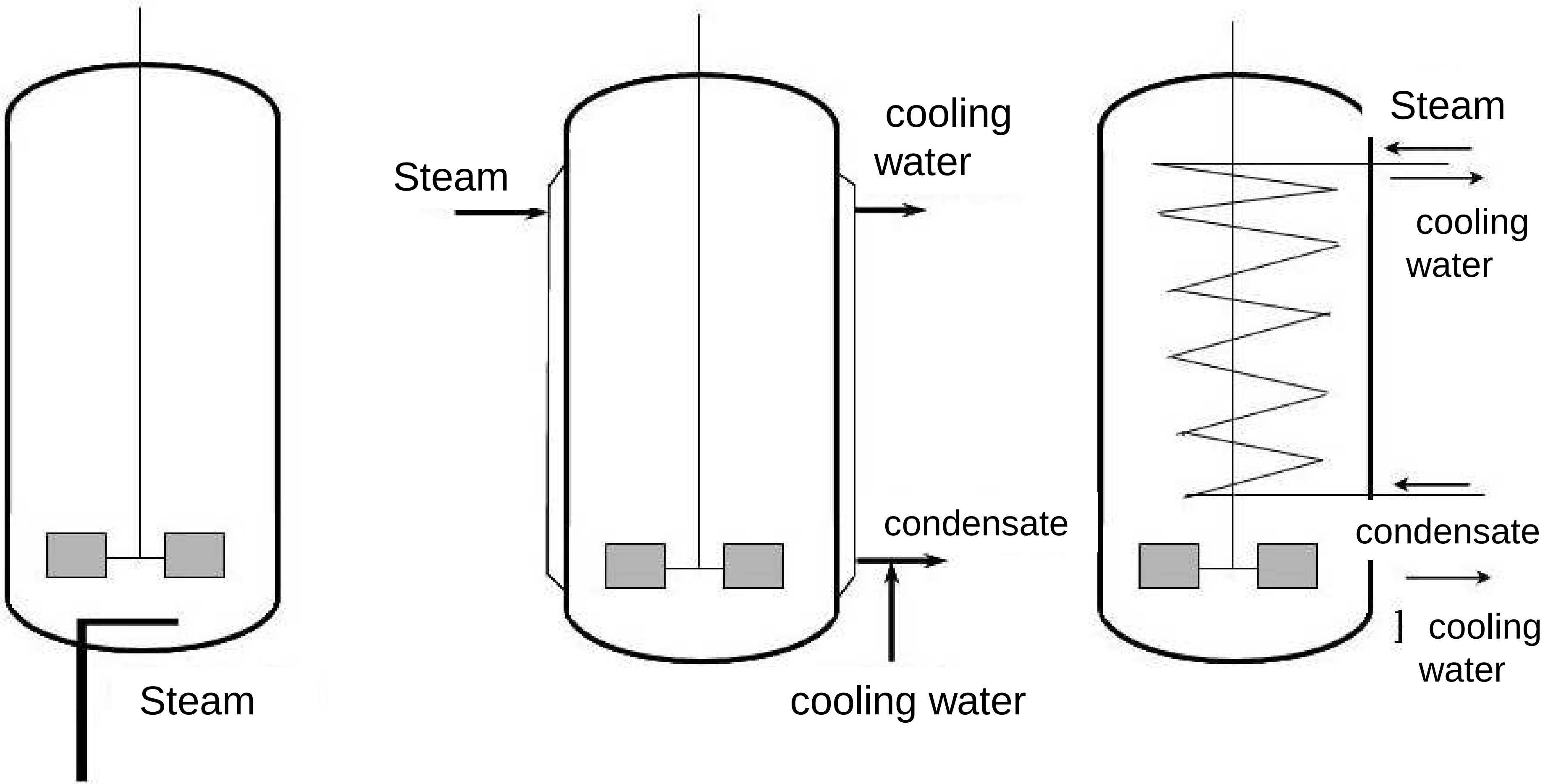
- **The probability that the sterilzitation had failed, i.e. there remained at least one survivor: 10^{-3}**
- **The probability that the sterilization succeeded, No remaining living cells : 0,999**
- **At every 1000th of sterilizations is allowed to occur one unsuccesful one. It is probable that one in thousand went wrong.**
- **After the sterilization process the number of survivors (Cell in the whole system)**


steam
water
Electic heating
°C


Steam out
Door
köpeny
Steam in

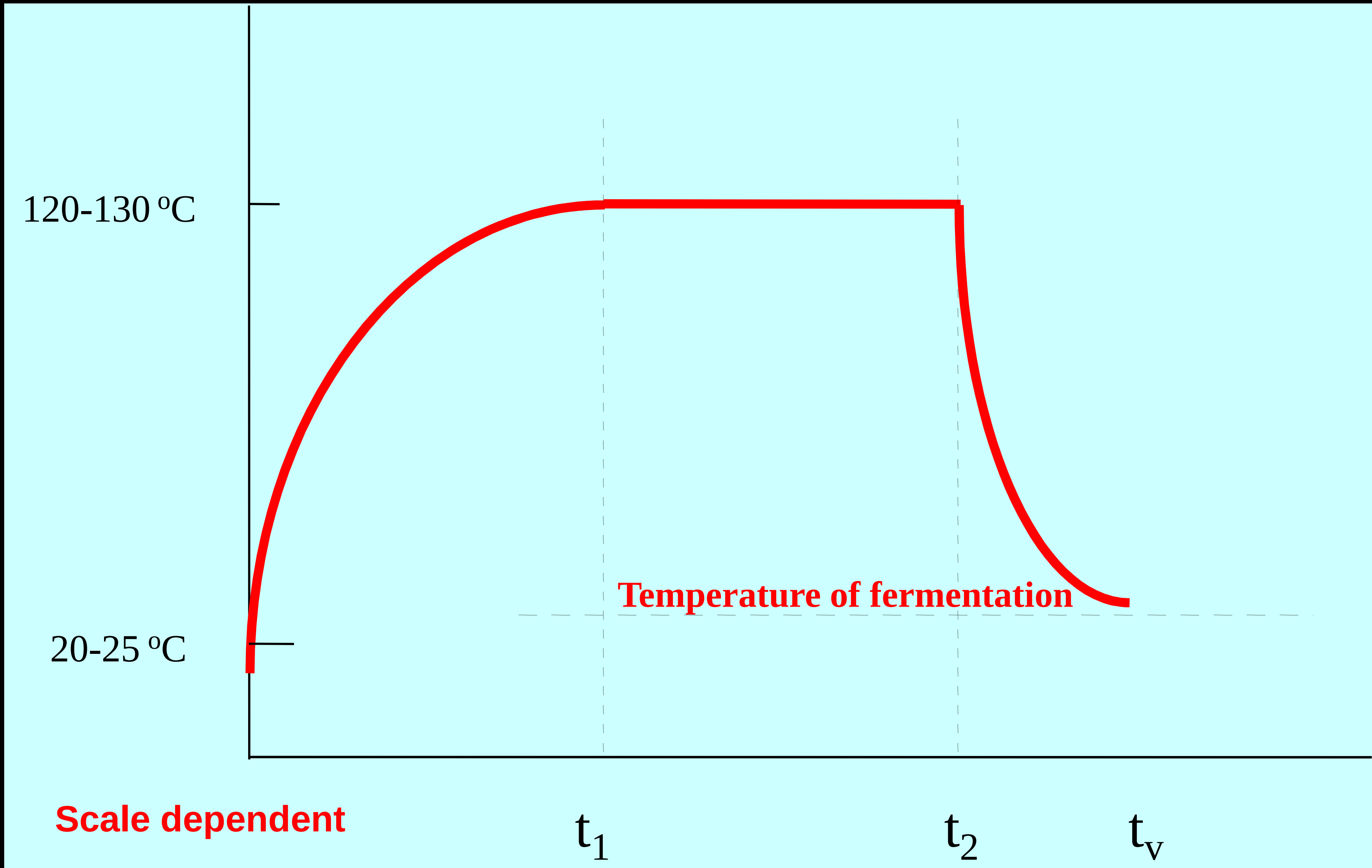

Consolidated

Batch sterilization of culture media

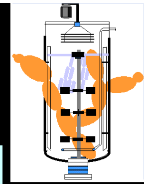


STERILIZATION

Heat penetration curve **Commeasurable sections!**



STERILIZATION



Thermal death during heating period:

$$\ln\frac{N_0}{N} = \int_0^{t_1} k\,dt = \nabla_{heating}$$

Thermal death during holding period:

$$ln\frac{N_1}{N_2} = k_{holding}\cdot(t_2 - t_1) = \nabla_{holding}$$

Thermal death during cooling period:

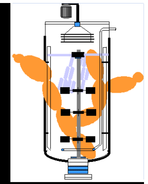
$$ln\frac{N_2}{N_v} = \int_{t_2}^{t_v} k\,dt = \nabla_{cooling}$$

$$\nabla = \nabla_{heat} + \nabla_{hold} + \nabla_{cool}$$

$$\ln\frac{N_0}{N_v} = \ln\left(\frac{N_0}{N_1}\frac{N_1}{N_2}\frac{N_2}{N_v}\right) = \ln\frac{N_0}{N_1} + \ln\frac{N_1}{N_2} + \ln\frac{N_2}{N_v}$$

e.g.: **0,20** **0,75** **0,05**

STERILIZATION



$$\nabla_{factor} = \ln \frac{N_0}{N}$$

Scale sensitive!

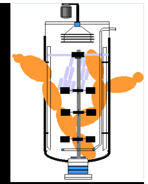
10^{-3} $\qquad$ $N_0 = 10^5$ / ml

$$100\ \text{liter} \qquad \frac{10^5.10^5}{10^{-3}} = 10^{13} \qquad \nabla = 32{,}2$$

$$10\ m^3 \qquad \frac{10^5.10^4.10^3}{10^{-3}} = 10^{15} \qquad \nabla = 36{,}8$$

$$100\ m^3 \qquad \frac{10^5.10^5.10^3}{10^{-3}} = 10^{16} \qquad \nabla = 39{,}2$$

10x: increases with 2,3



STERILIZATION

Continuous sterilization of culture media

Fermentor size limit productivity: (kg product/fermentor.m^3.year).

Advantages of the cont. Sterilization process:

-at higher temp.(130-140 ^{o}C) with shorter process time

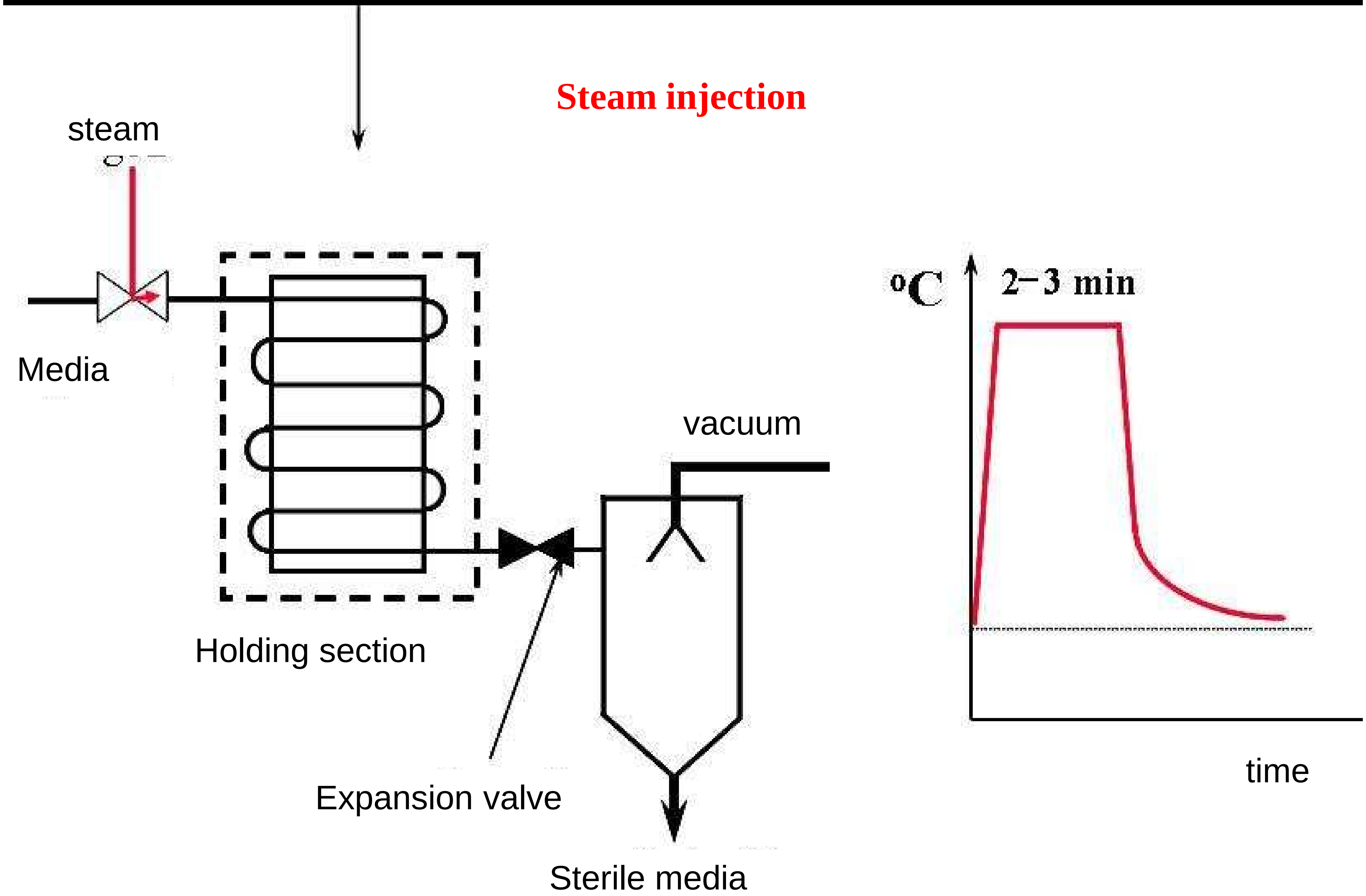
increased safety
less thermal decay of culture medium components

-the continuous process more reproducible,

-stable quality of the sterile media
this may increase the fermentation yield

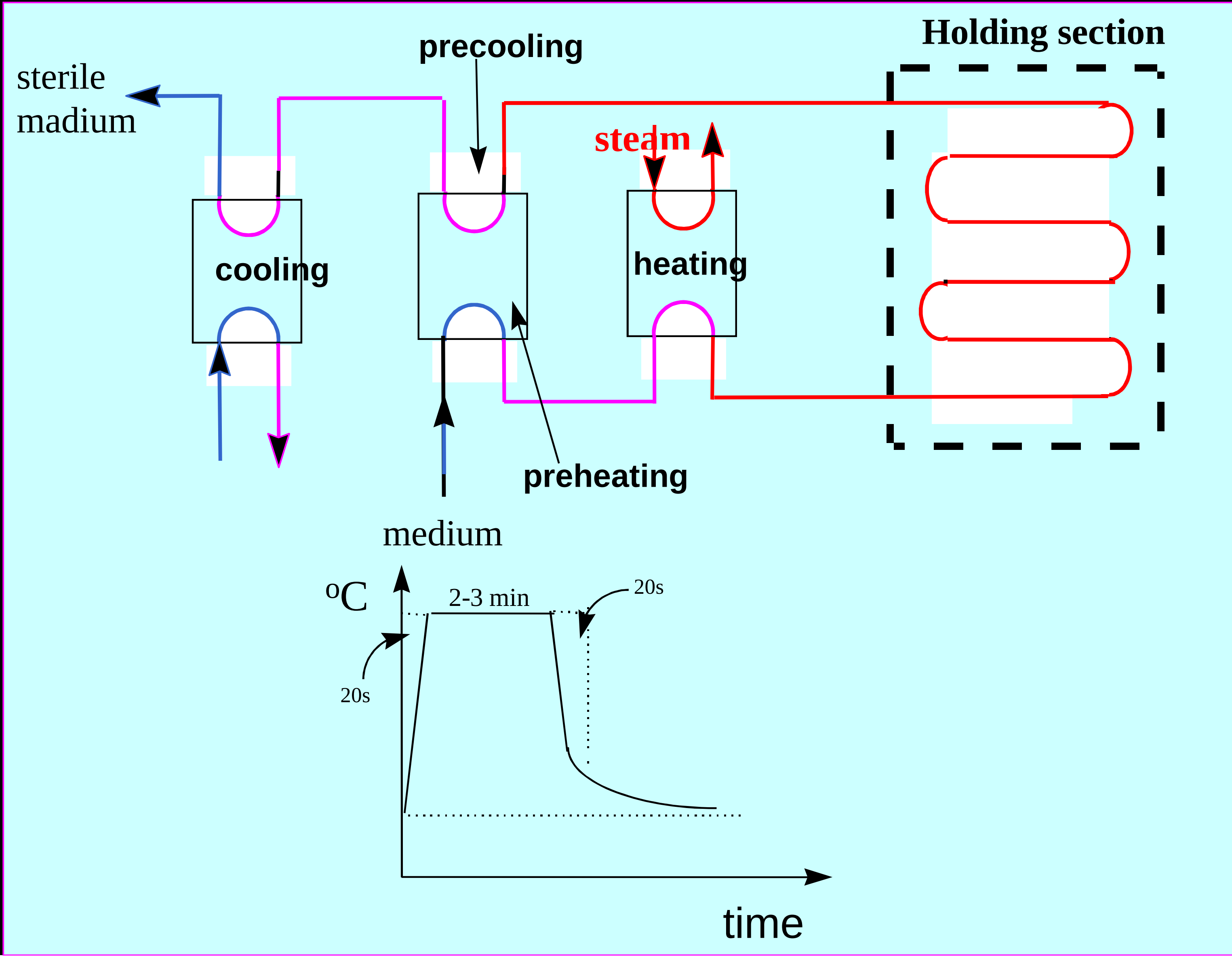
-cont. Ster. Equipment and the process easily controllable,
automatization possible.

Steam injection



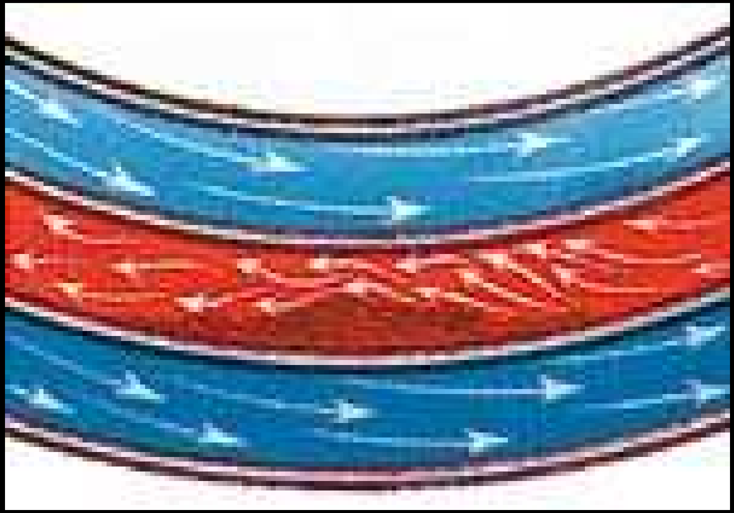
STERILEZÉS

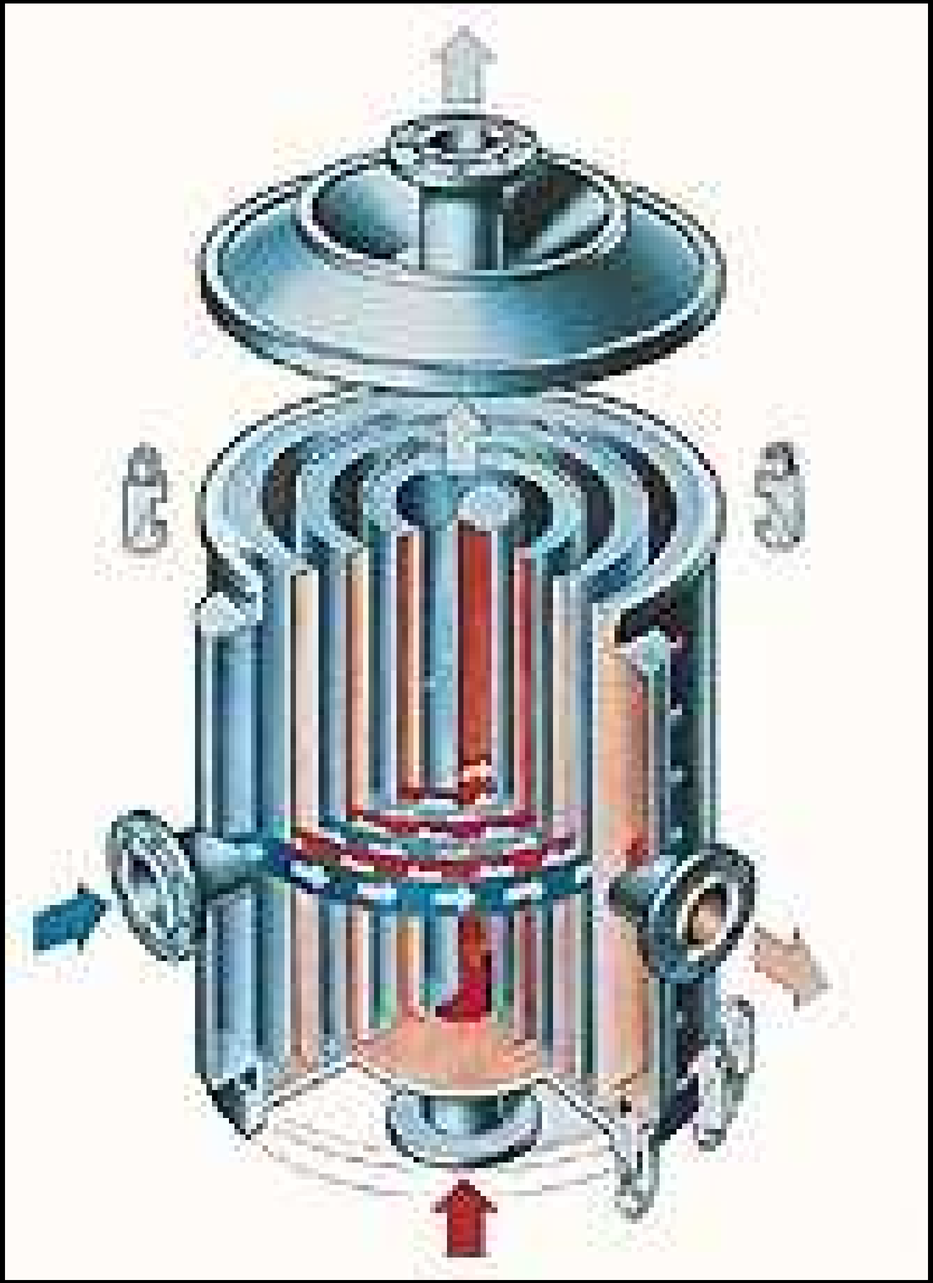
Plate and frame heat exchanger



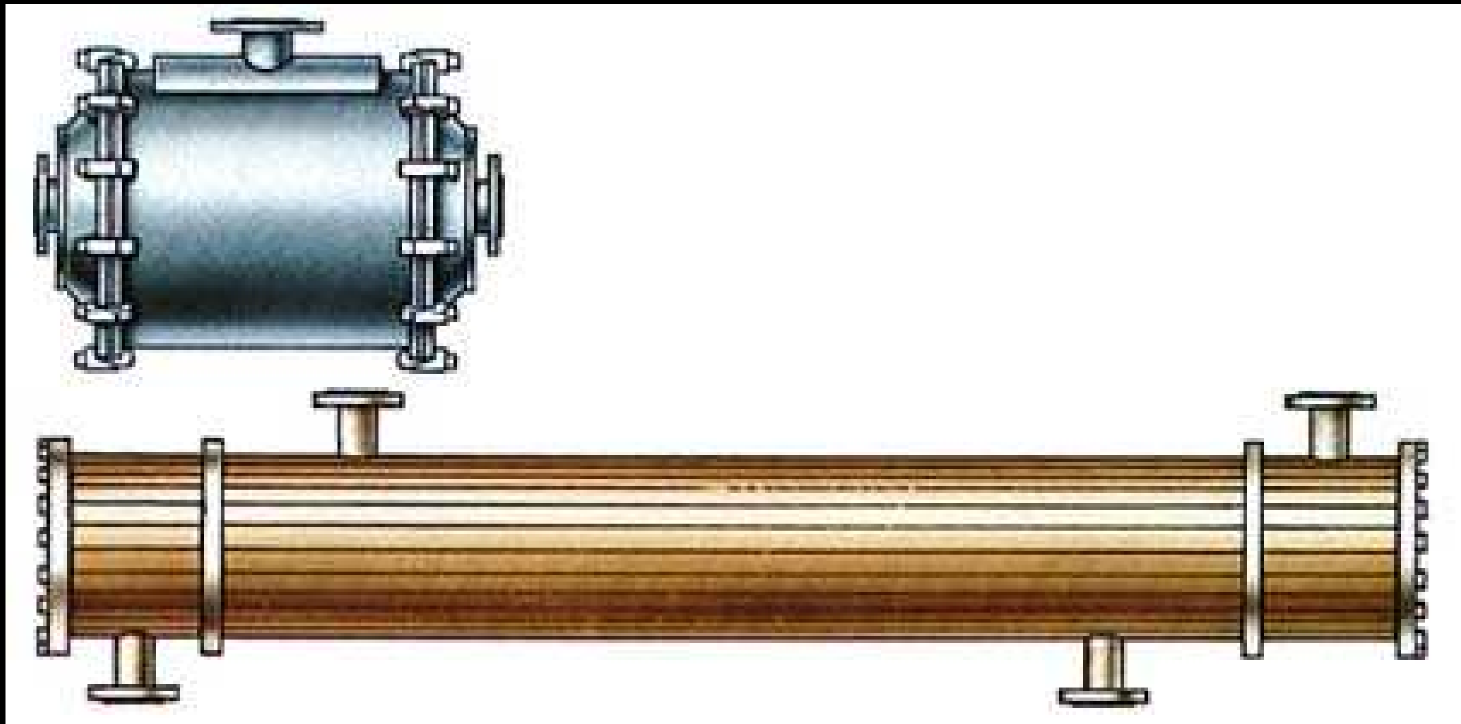
STERILIZATION

SPIRAL HEAT EXCH.





STERILIZATION

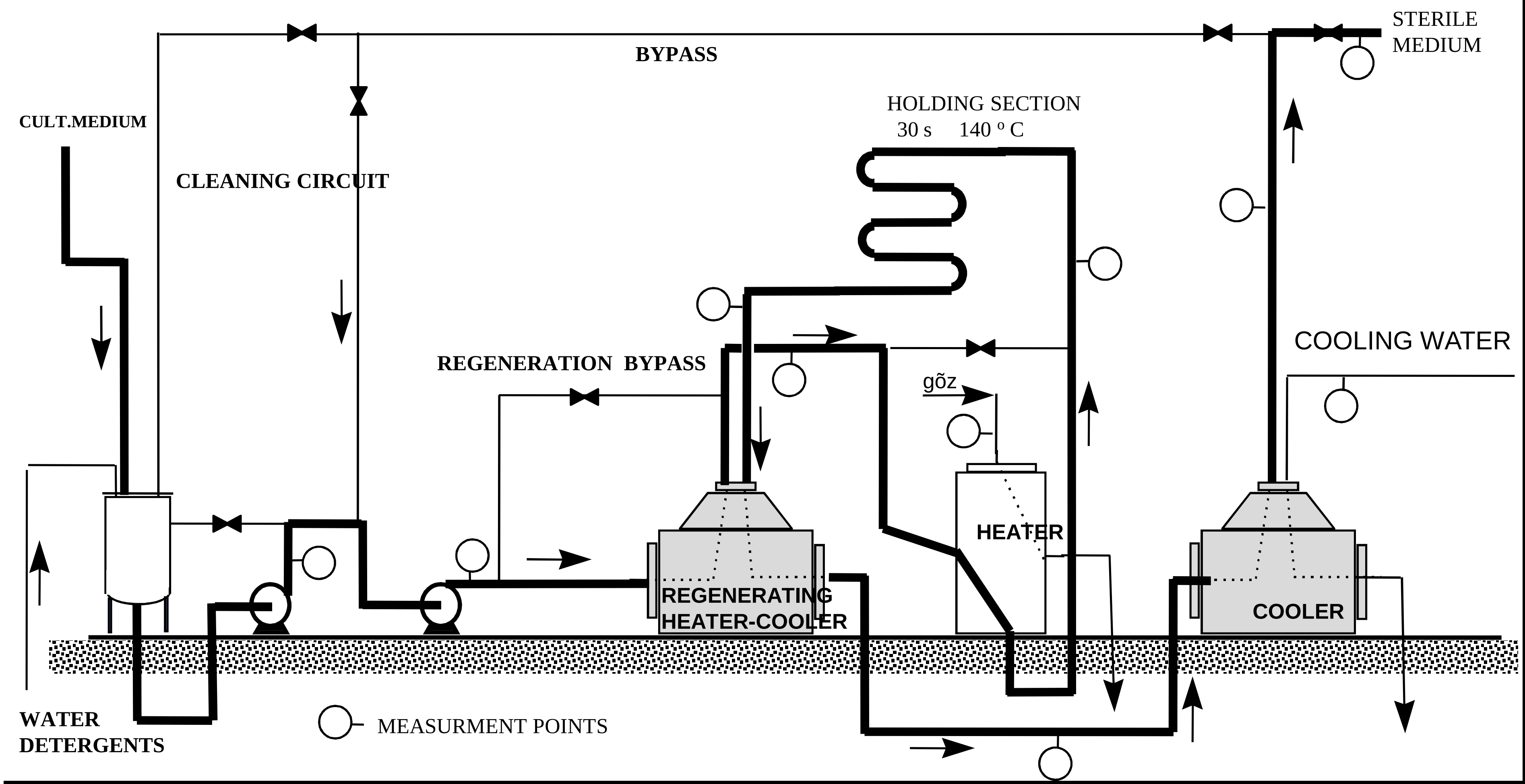


STERILIZATION

STERILIZATION

STERILIZATION

CONTINUOUS MEDIUM STERILIZATION PROCESS OUTLINE



STERILIZATION

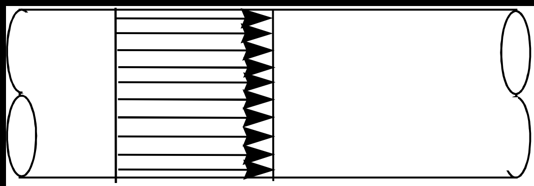
Continuous sterilizer (design) calculation

For holding section:

$$\ln\frac{N_0}{N_v} = k\Delta t = k\frac{L}{\frac{w}{q}}$$

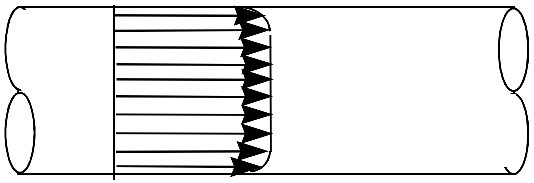
L – length of holding section tube (m)
w –volumetric rate (m^3/min)
q – tube cross sectional area (m^2)

BUT!!!



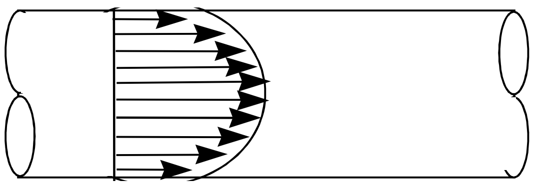
plug flow

$$\bar{u} = u_{max}$$



Turbulent stream

$$\bar{u} = 0{,}82 * u_{max}$$



Laminar stream

$$\bar{u} = 0{,}5 * u_{max}$$

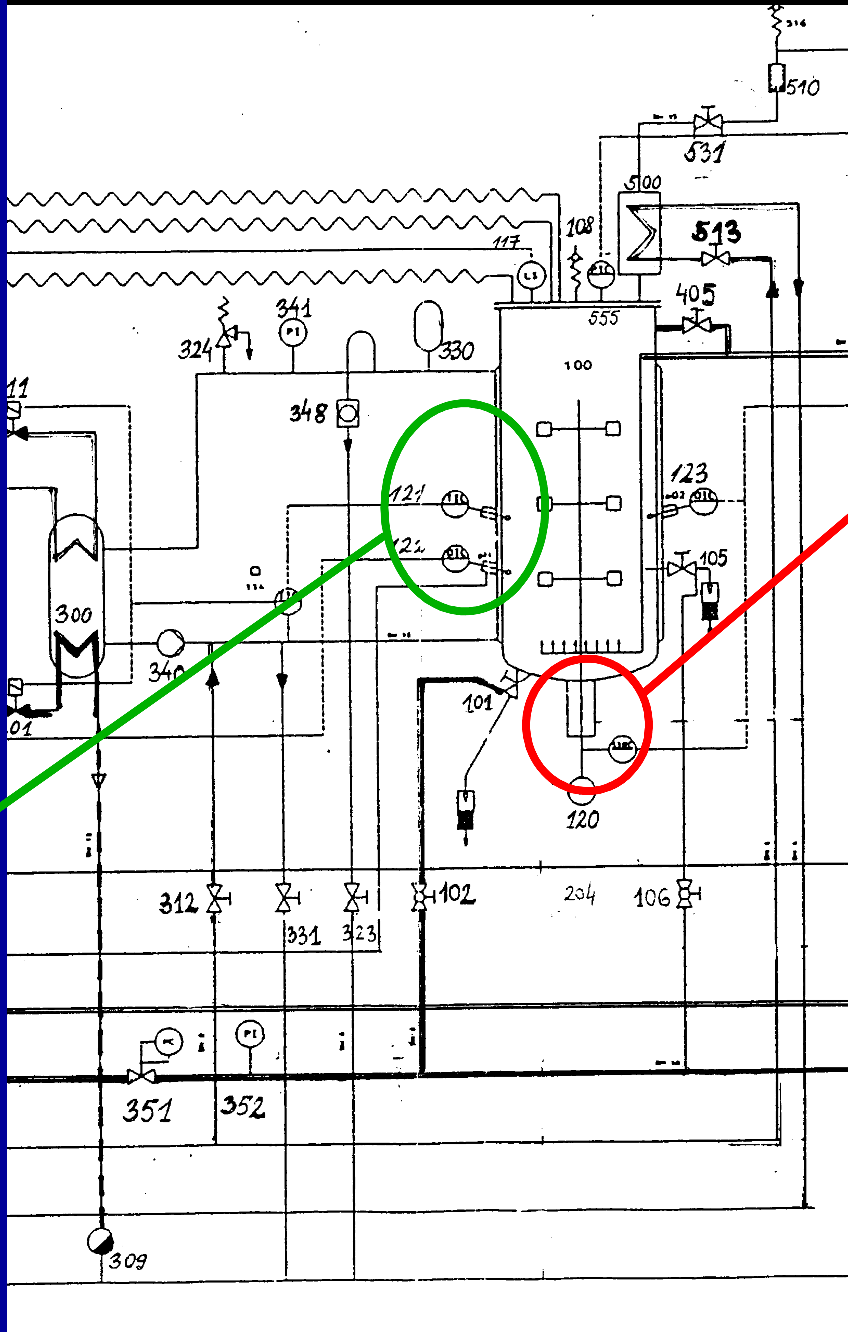
STERILIZATION

HOT POINTS

BOTTOM DRIVE

ELEKTRODES

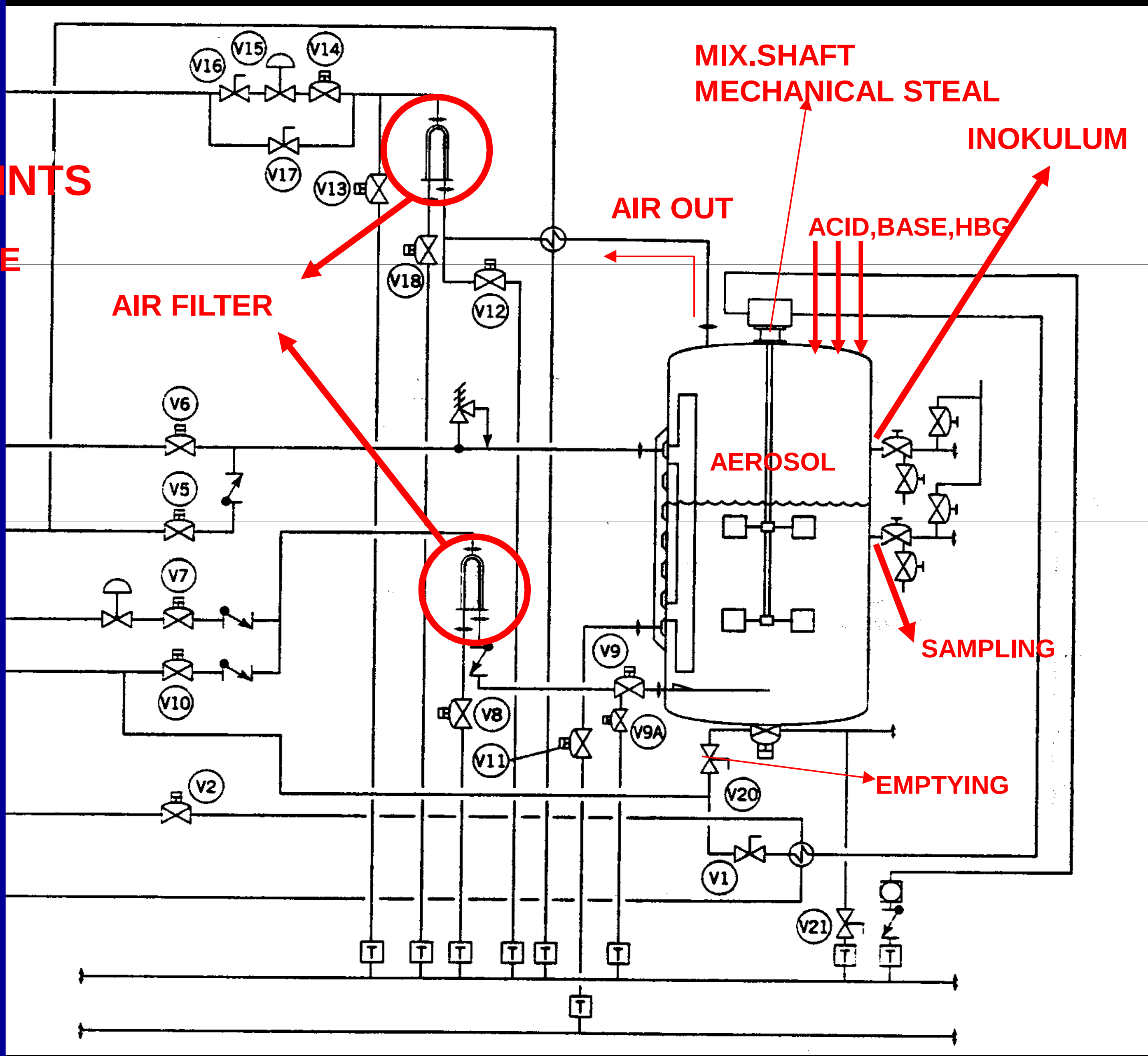
MECHANICAL SEAL



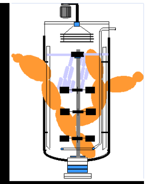
STERILEZÉS

HOT POINTS

TOP DRIVE



STERILIZATION



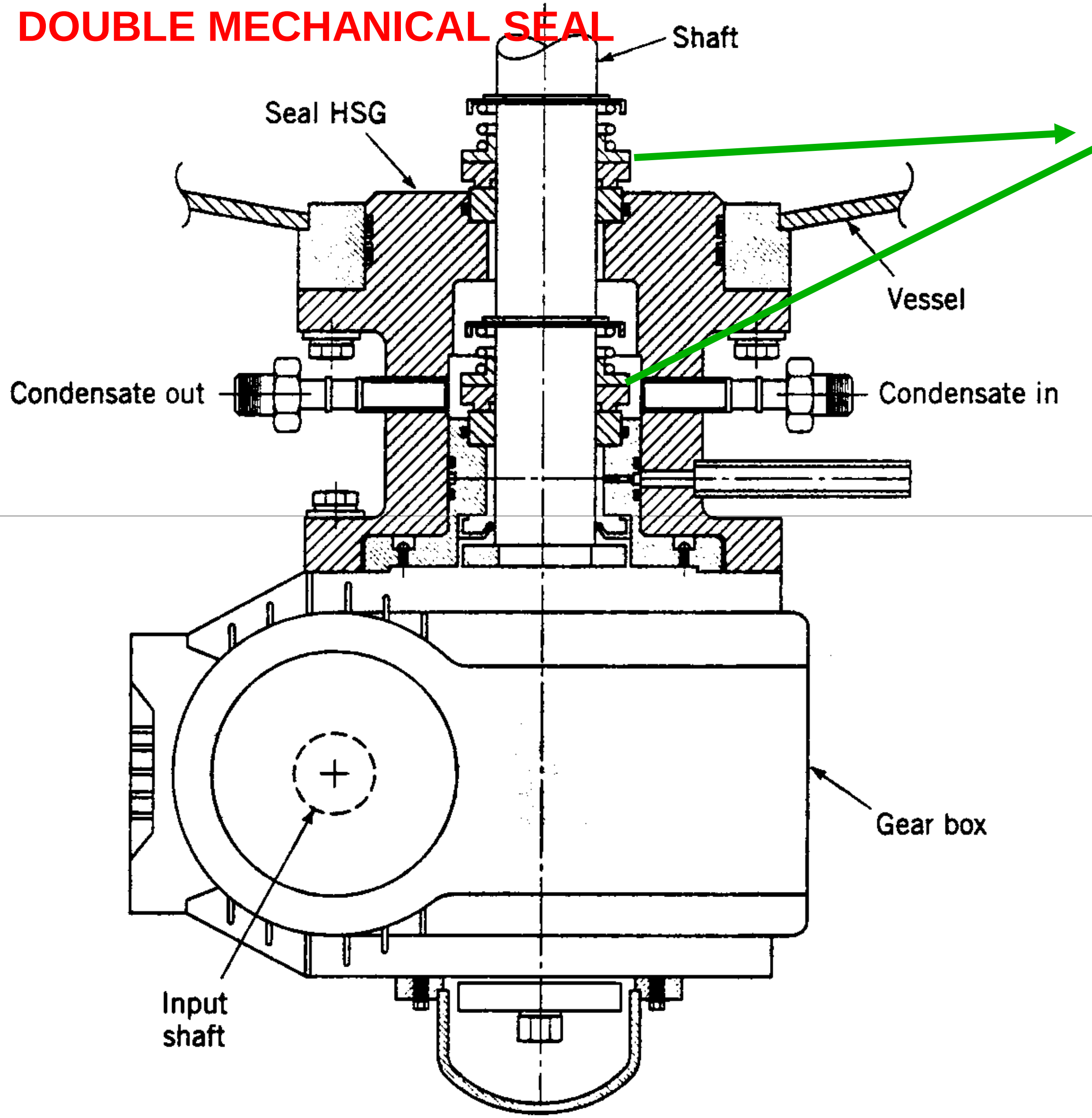
MIXING SHAFT
AIR IN, OUT
INGREDIENTS:
- PIPES
- VALVES

INOKULUM LINE
SENSORS
PUMPS

DOWN-STREAM: STERILE
NONSTERILE

STERILIZATION

DOUBLE MECHANICAL SEAL

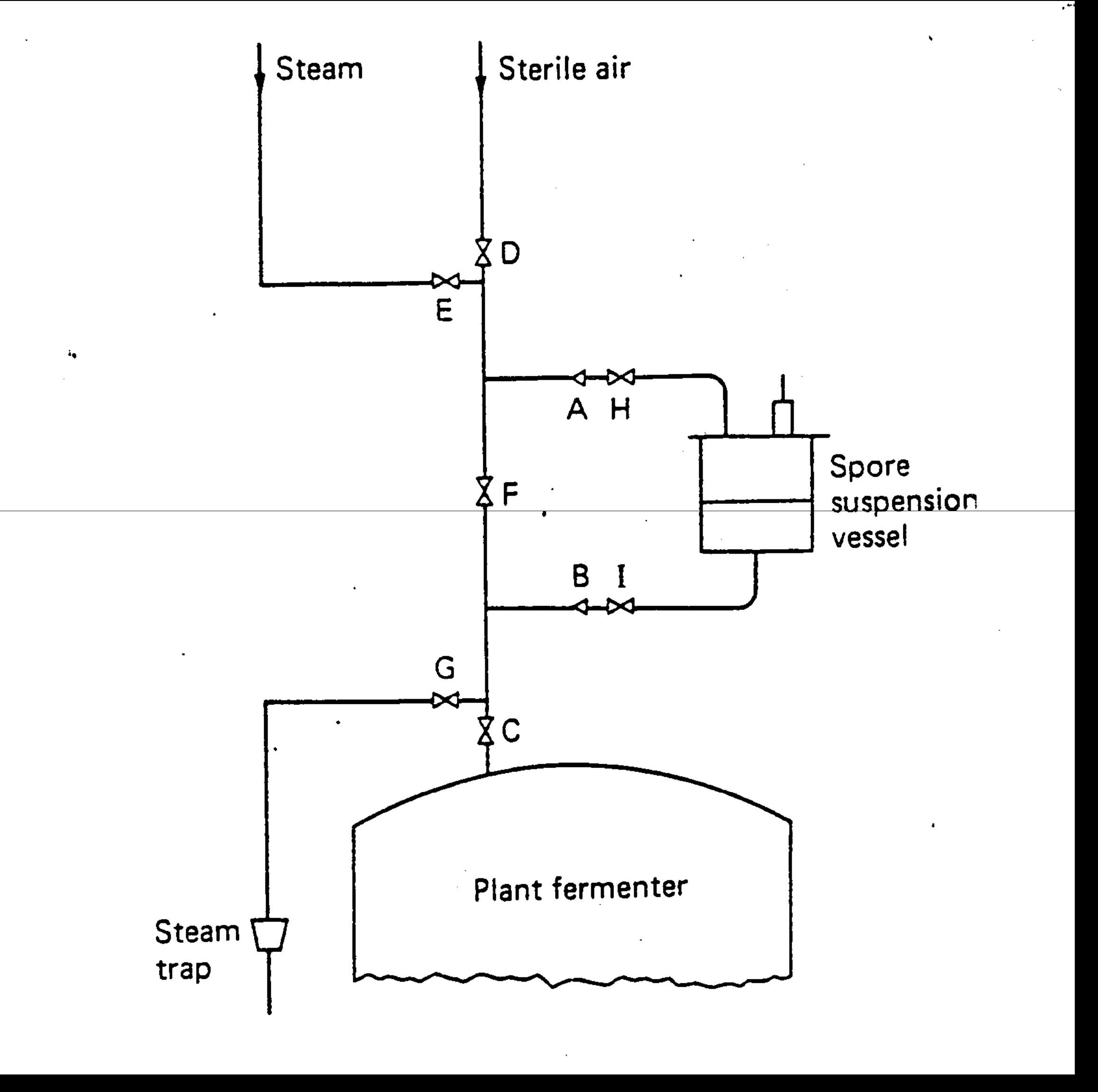


SLIPPING SURFACE

STERILE WATER – LUBRICATION

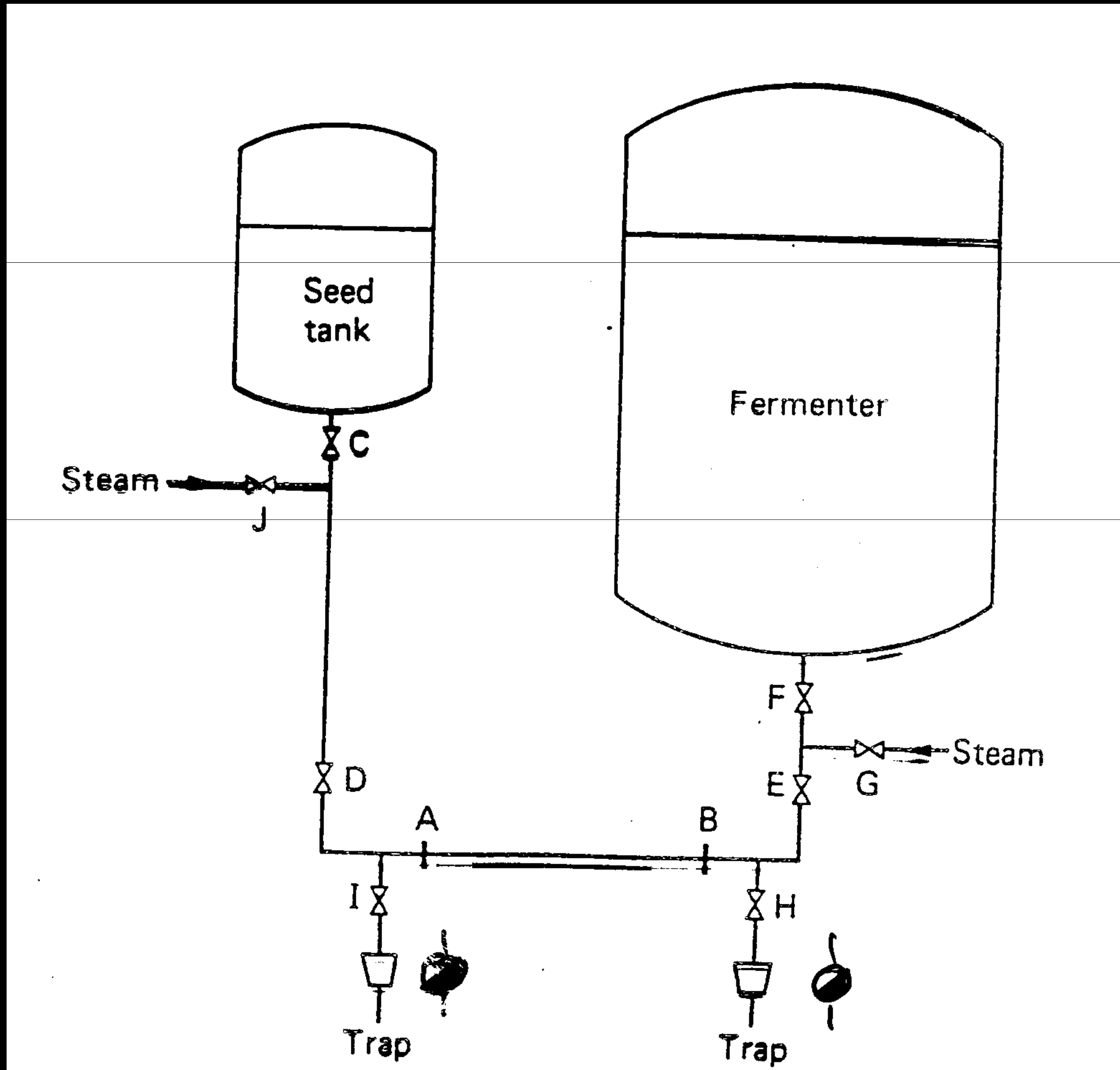
STERILIZATION

STERILE INOCULATIC



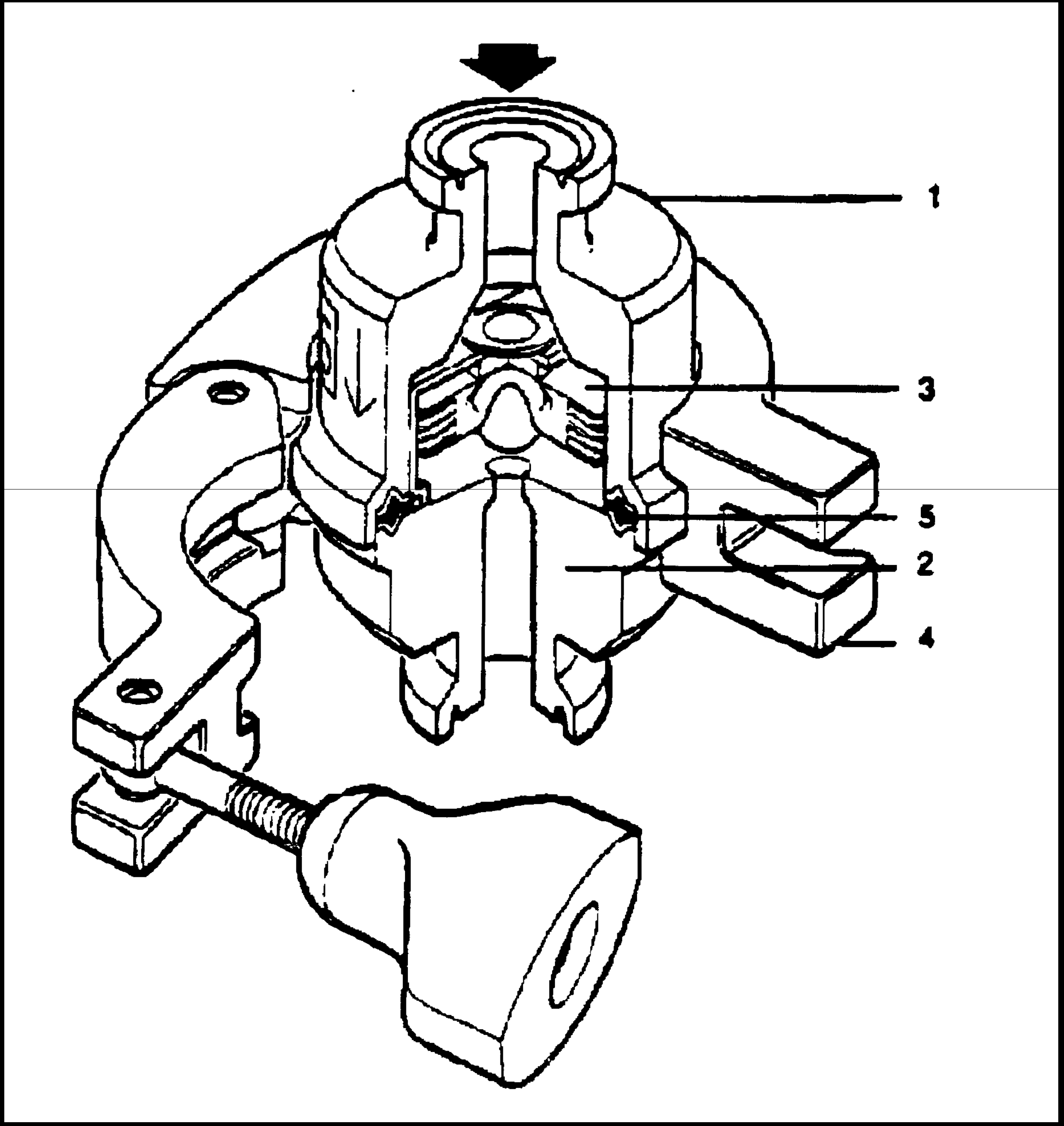
STERILIZATION

STERILE INOCULATION 2

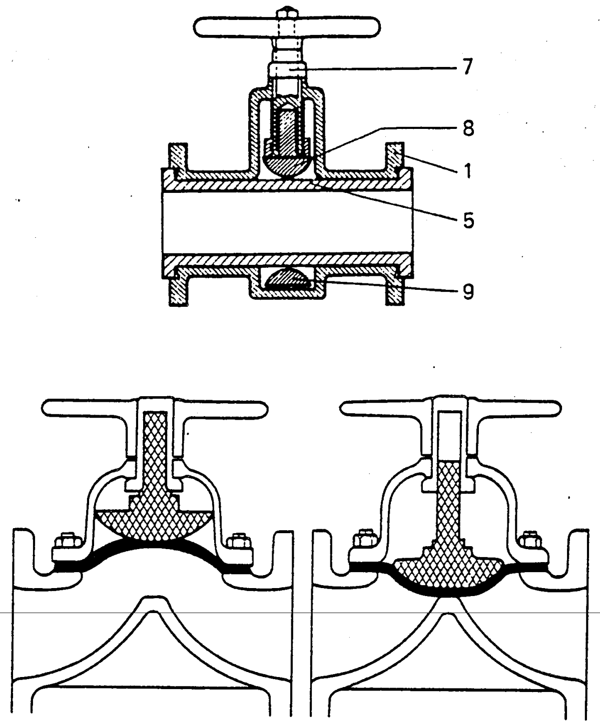


STERILIZATION

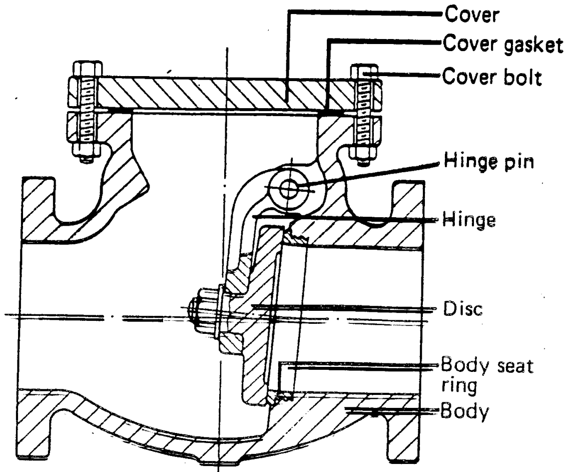
THERMODYNAMIC STEAM TRAP



STERILIZATION



MEMBRANE VALVES



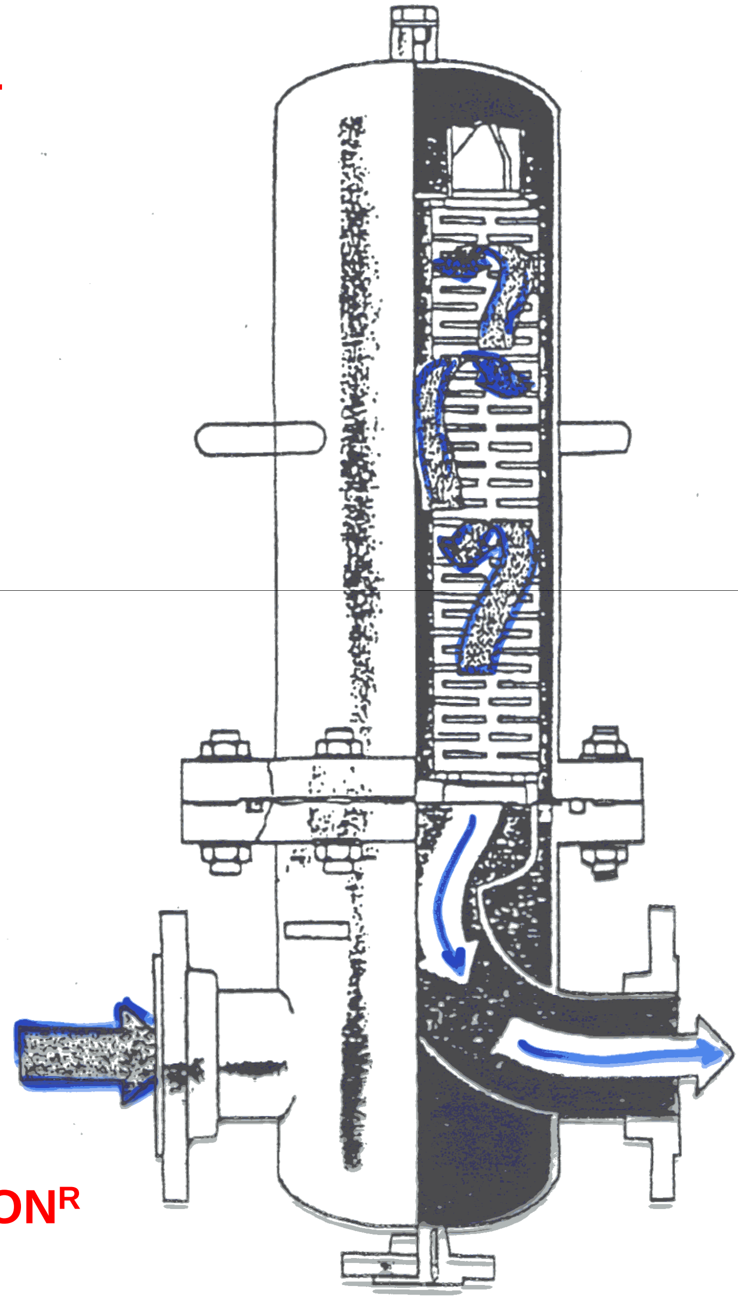
VISSZACSPÓSZELEP

STERILIZATION

AIR FILTRATION

Absolute filter

PALL



EMFLON^R

AIR FILTRATION

DEPTH FILTER

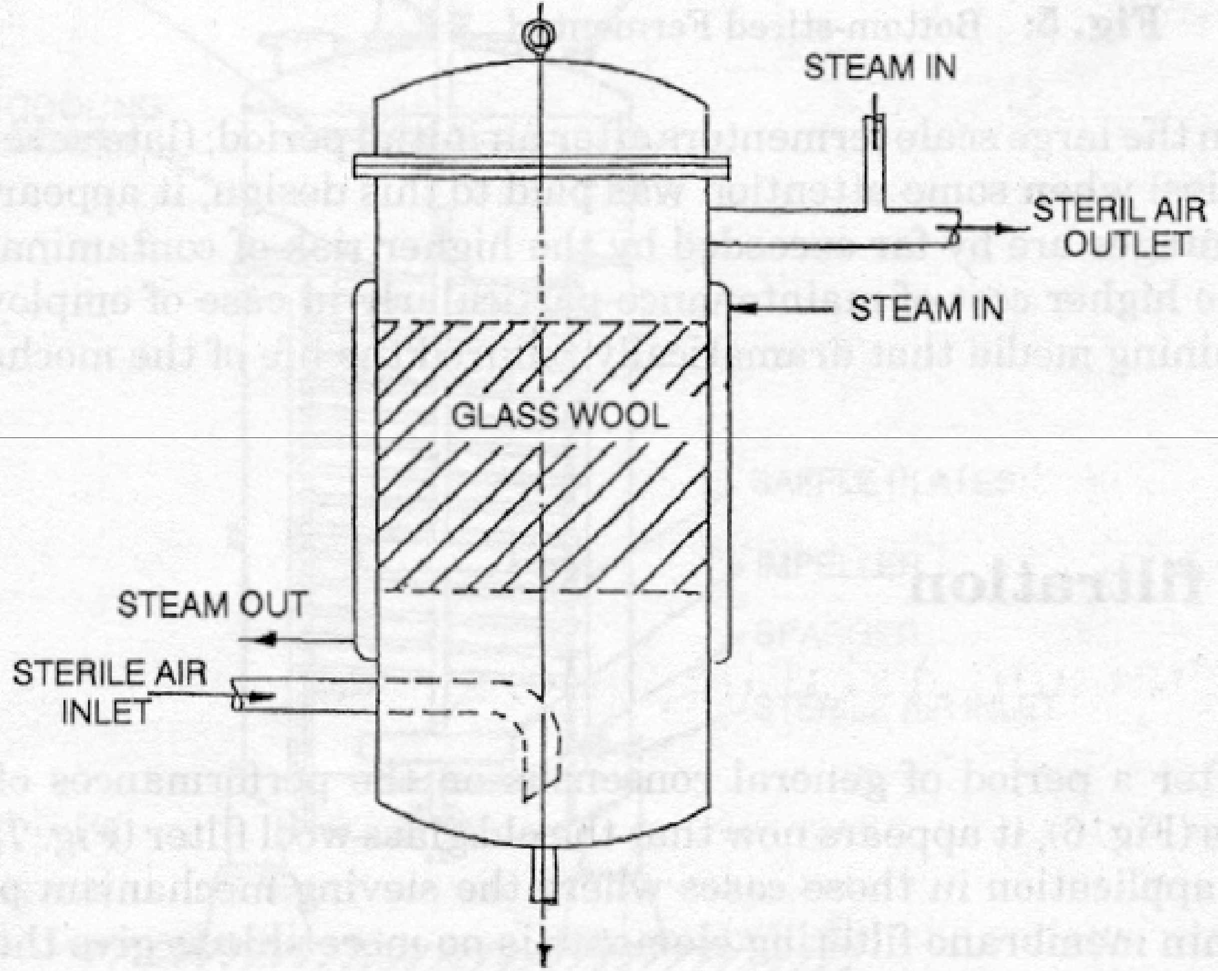


Fig. 7: Depth filter.

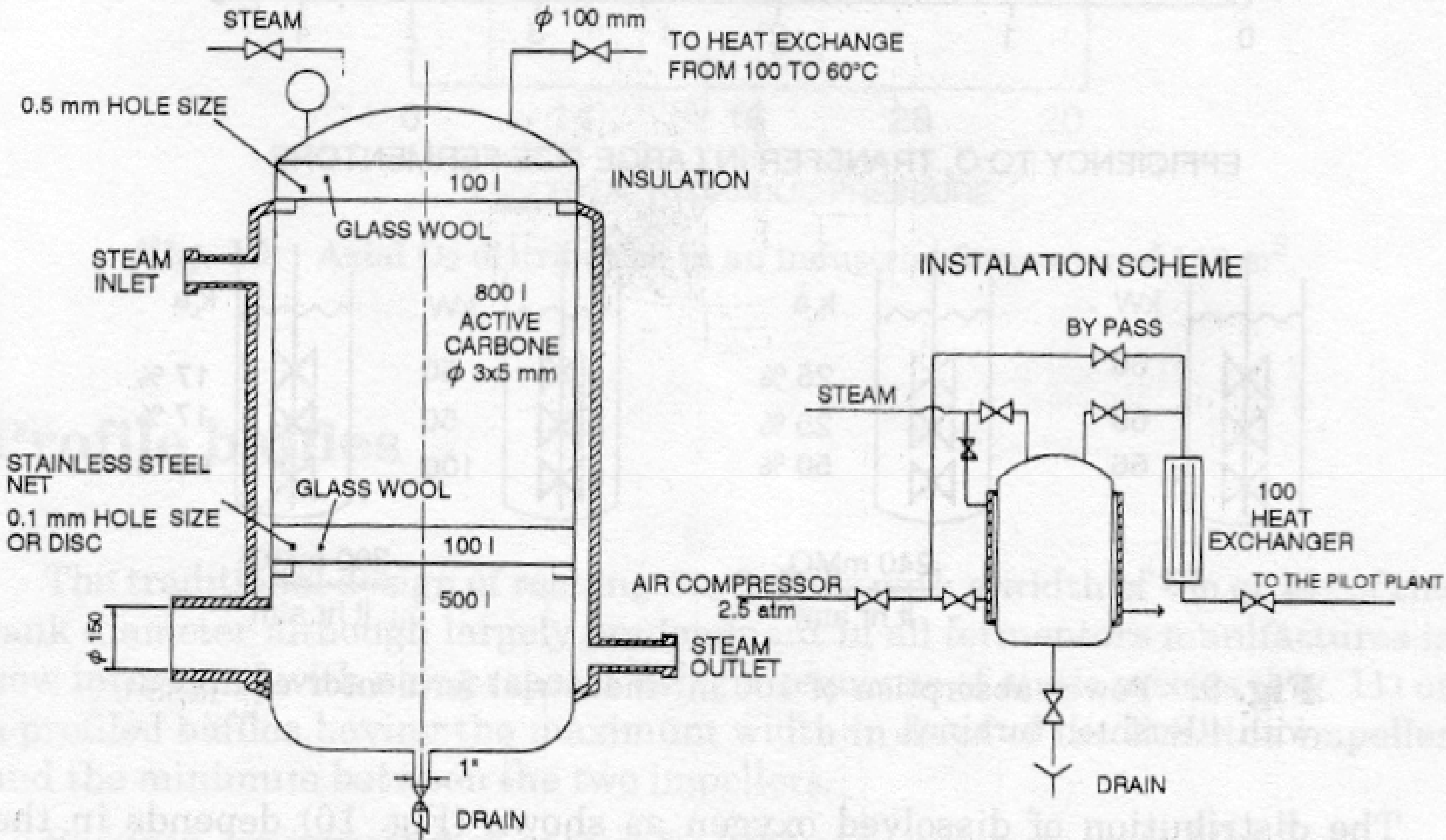
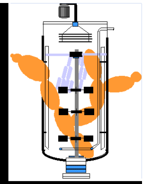


Fig. 8: Antiphage system.

STERILIZATION



CIP

(CLEANING IN PLACE)

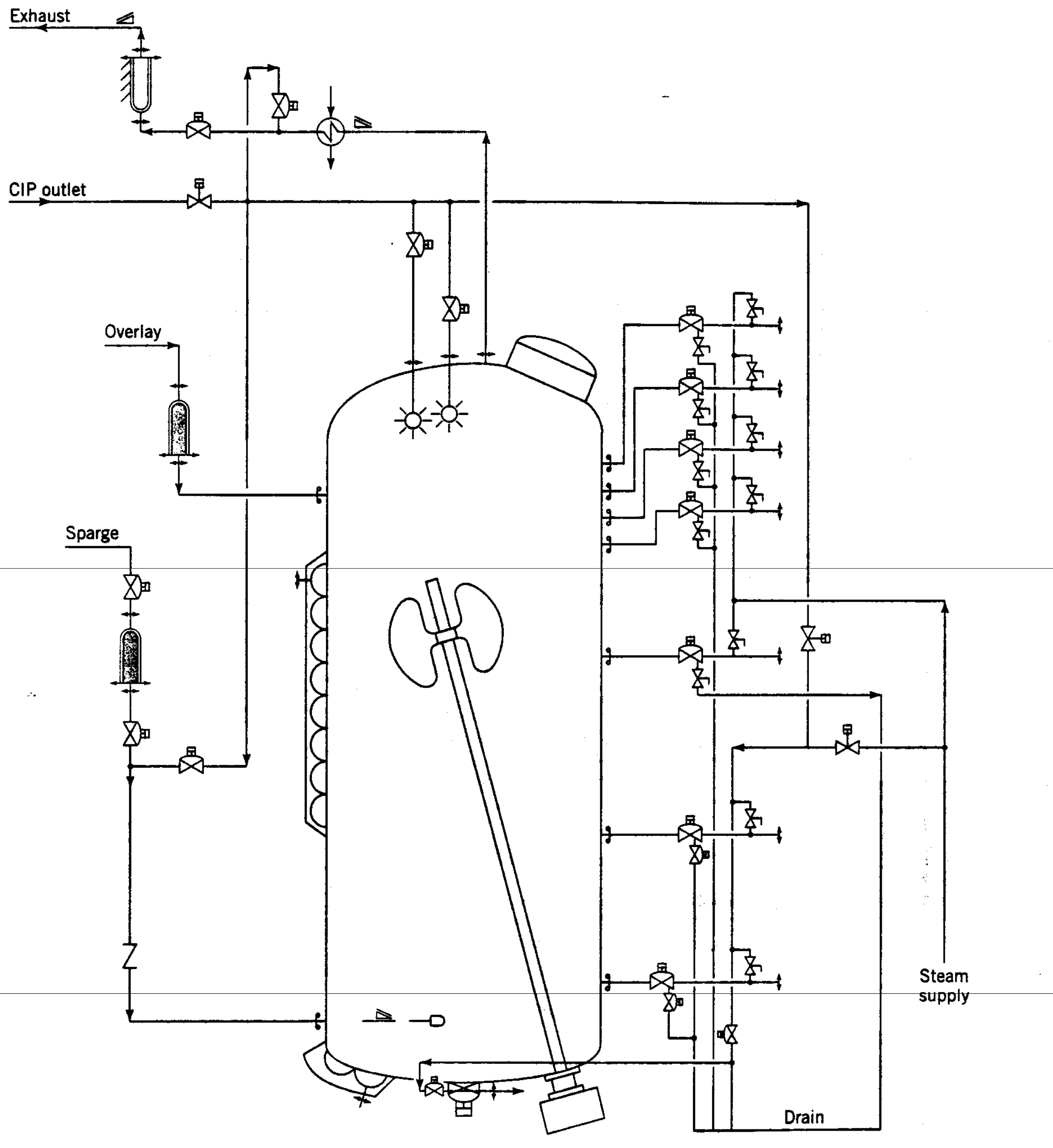


Figure 18. Simplified CIP piping diagram.