PRACTICALS TO THE INDUSTRIAL MICROBIOLOGY COURSE

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A Debreceni Egyetem fejlesztése a felsőfokú oktatás minőségének és hozzáférhetőségének együttes javítása érdekében
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DEBRECENI EGYETEM

Content

1. *Microbial stoichiometry*

Theoretical background:
Similarly to the chemical reactions, the stoichiometry of a microbial cultivation process enables the creation of proper mass balances. The left side of the equation contains the respective amounts of the medium components, the right side contains the amount of the newly formed biomass and those of the (biochemical) end-products. For a proper stochiometric equation, one needs to know the elemental composition of the micro-organism studied. This can vary greatly depending on the cultivation conditions and/or the actual physiological state of the cells; however, a range for most elements can be provided. As a percentage of the dry cell weight (DCW), the range is 40-60 % for carbon, 7-8 % for hydrogen, 7-15 % for nitrogen, 2-4 % for phosphorus, 0.1-1 % for sulphur and 0-38 % for oxygen. It is practically impossible to get a complete elemental composition (values never add up to 100 %), because all cells contain several minor components that will remain in the ash during elemental analysis, which is based on burning the biomass.

In the stoichiometric equation the formula of micro-organisms is given as „molar microbial mass", which is the elemental composition divided by the atomic mass of the respective elements (e.g., $C_{3.75}H_{6.45}O_{2.06}N_{0.63}$…+ ash). For simplicity, a so-called „C-mol formula for biomass" has been introduced, where the coefficient for carbon equals one, i.e., the formula is specified to carbon. Many micro-organisms have a C-mol formula of $CH_{1.8}O_{0.5}N_{0.2}$…+ ash (also known as standard biomass composition).

If all (growth) substrates and products are well-defined chemical compounds, one can set up a stoichiometric equation typical for the fermentation process under study. For the cultivation of chemoorgano-heterotrophic micro-organisms such as fungi, the most important substrate is carbon. Part of the carbon source incorporates into the newly formed biomass (ΔS_C), another part (ΔS_E) is used to generate energy.

$$\Delta S = \Delta S_C + \Delta S_E$$

To calculate ΔS_C, one needs to know the elemental carbon content of the carbon source and the biomass (α_2 and α_1, respectively), and the amount of the newly formed biomass (Δx), according to the equation

$$\alpha_2 \Delta x = \alpha_1 \Delta S_C$$

1.1. Exercise

For a baker's yeast fermentation we get the following stoichiometric equation: 0.585 $C_{12}H_{22}O_{11}$ + 3.15 O_2 + 0.61 NH_3 + other components → 100 g yeast DCW. Elemental composition of 100 g yeast DCW is the following: 44.70 g carbon; 6.16 g hydrogen; 31.20 g oxygen; 8.54 g nitrogen; 0.54 g sulphur; 1.09 g phosphorus.
What is the molar microbial mass and what is the C-mol formula of the yeast?

Solution

Calculation of molar microbial mass:

$C_aH_bO_cN_dS_eP_f\ldots$ + ash

a=C%/12=44.7/12=3.725

b=H%/1=6.16/1=6.16

c=O%/16=31.2/16=1.95

d=N%/14=8.54/14=0.61

e=S%/32=0.54/32=0.016

f=P%/30=1.09/30=0.036

$C_{3.725}H_{6.16}O_{1.95}N_{0.61}S_{0.016}P_{0.036}$…+ ash

Calculation of the C-mol formula:

$CH_{6.16/3.725}O_{1.95/3.725}N_{0.61/3.725}S_{0.016/3.725}P_{0.036/3.725}$ → **$CH_{1.65}O_{0.52}N_{0.16}S_{0.004}P_{0.009}$**

1.2.Exercise

Aspergillus niger – a filamentous fungus – is grown in defined liquid medium, with glucose (100 g/L) and ammonium ions being the sole carbon and nitrogen sources, respectively. By the end of the cultivation glucose is consumed completely, and the biomass grows to 32 g/L from 1.8 g/L. The fungal biomass contains 45% carbon, 33% oxygen, 6.45% hydrogen and 8.92% nitrogen. What is the elemental composition of the biomass and the stoichiometric equation of the fermentation?

Solution

First we calculate the conversion of carbon source into biomass.

Carbon content of biomass: $\alpha_2 = 0.45$

Carbon content of carbon source: α_1=72/180 = 0.4

$\alpha_2 * \Delta x = \alpha_1 * \Delta S_c$

Δx = 32-1.8 = 30.2 g/L

ΔS_c = 0.45*30.2/0.4 = **33.9 g/L**

Hence, **33.9%** of the carbon was built into the biomass, and **66.1%** was released as CO_2.

Secondly, the biomass elemental composition is calculated as follows:

C: 45/12=3.75

H: 6.45/1=6.45

O: 33/16=2.06

N: 8.92/14=0.63

$C_{3.75}H_{6.45}O_{2.06}N_{0.63}$

Thirdly, coefficients of the stoichiometric equation will be:

$C_6H_{12}O_6 + a\ O_2 + b\ NH_3 \rightarrow c\ (C_{3.75}H_{6.45}O_{2.06}N_{0.63}) + d\ CO_2 + e\ H_2O$

c: $0.339*6 = 3.75*c \rightarrow c = 0.542$

d: $0.661*6 = 1*d \rightarrow d = 3.96$

b: $b = c*0.63 \rightarrow b = 0.341$

e: $12+b*3 = c*6.45+2*e \rightarrow e = 4.76$

a: $6+a*2 = c*2.06+d*2+e \rightarrow a = 3.89$

The coefficients above will result in the following stoichiometric equation:

$C_6H_{12}O_6 + 3.89\ O_2 + 0.341\ NH_3 \rightarrow 0.542\ C_{3.75}H_{6.45}O_{2.06}N_{0.63} + 3.96\ CO_2 + 4.76\ H_2O$

1.3.Exercise

A bacterial strain grows aerobically on glucose and ammonium ions as sole carbon and nitrogen sources, respectively. The (standard) elemental composition is $CH_{1.8}O_{0.28}N_{0.25}$; 6 C-mol glucose will yield 1.5 C-mol biomass. What is the stoichiometric equation of this fermentation?

Solution:

$6\ CH_2O + a\ O_2 + b\ NH_3 \rightarrow 1.5\ CH_{1.8}O_{0.28}N_{0.25} + c\ H_2O + d\ CO_2$

d: $6 = 1.5 + d \rightarrow d = 4.5$

b: $b = 0.25$

c: $6*2 + 0.25*3 = 1.5*1.8 + c*2 \rightarrow c = 5.02$

a: $6 + a*2 = 1.5*0.28 + 5.02 + 4.5*2 \rightarrow a = 4.22$

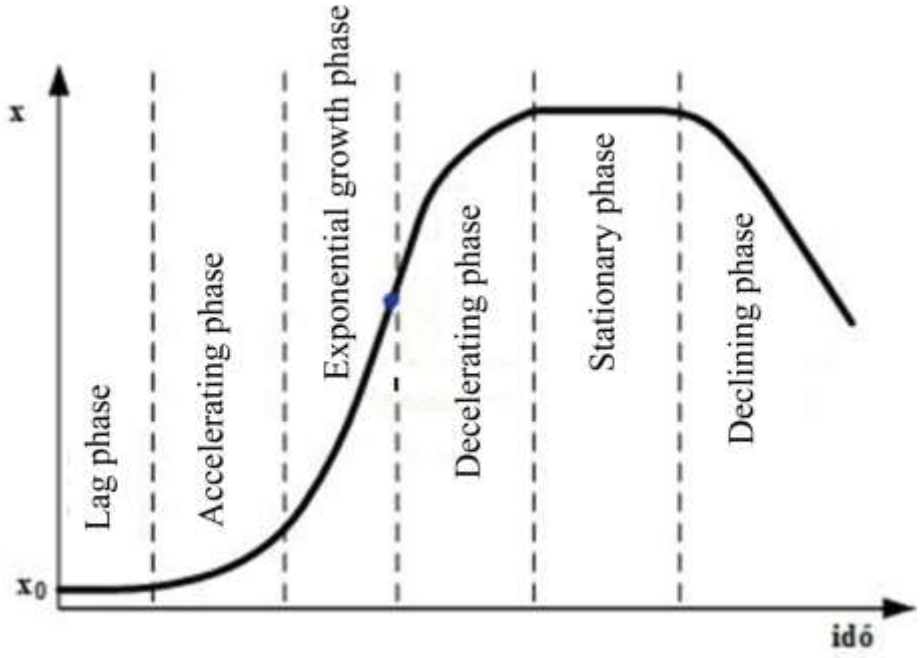
$6\ CH_2O + 4.22\ O_2 + 0.25\ NH_3 \rightarrow 1.5\ CH_{1.8}O_{0.28}N_{0.25} + 5.02\ H_2O + 4.5\ CO_2$

$CH_2O + 0.703\ O_2 + 0.041\ NH_3 \rightarrow 0.25\ CH_{1.8}O_{0.28}N_{0.25} + 0.836\ H_2O + 0.75\ CO_2$

2. *Growth and nutrient utilization of microorganisums*

Required knowledge:

Growth (= net biomass formation) of a batch culture can be divided into well-defined stages named as lag-, accelerating-, exponential-, deccelerating-, stationary-, and declining stage. Growth stops when an essential medium component gets depleted (= limiting component).



Growth rate at the exponential stage reflects the maximal specific growth rate of the culture attainable under the given experimental conditions. Depicting the natural logarithm of the biomass as a function of time gives a linear plot. Specific growth rate equals to the slope of the linear plot. The biomass-related yield coefficient ($Y_{x/Si}$, where i = substrate No. i.) is the measure of the rate of substrate conversion, i.e., the ratio of the increase of biomass (dx) and the consumption of substrate within a certain period of time (dS_i).

$$Y_{x/Si} = \frac{dx}{dS_i}$$

Effectively, yields provide quantitative info on the utilization of the growth medium components.

Another term used to characterize the kinetics of a fermentation is the productivity (J) that describes product formation rate. A fermentation product (P, given in g/L) could be the biomass formed, a primary or secondary metabolite of an enzyme.

$$J = \frac{dP\ (\frac{g}{L})}{dt\ (h)}$$

Substrate uptake rate is the rate by which a given medium component gets inside the cell, described as its concentration decrease within a certain period of time (volumetric rate). When the volumetric rate is specified to biomass, it is called specific substrate uptake rate.

The term inoculation in fermentation technology refers to the process of introducing new, exponential phase culture to a fresh, axenic growth medium. The size of the inoculum is given in percentage (%), i.e., the ratio of the volumes of the inoculum and that of the fermentation medium. Under batch conditions the amount of inoculum is critical for the kinetics of fermentation. Formation of the so-called secondary metabolites (a term used for metabolites that are not absolutely necessary for the survival of the organism) typically occurs in the idiophase of growth, hence a long lag- or exponential phase is economically disadvantageous. It is better to use an inoculum size (and subsequent scaling-up volumes) that gets the culture into production phase within the shortest possible period of time. The factor most critical in determining the size of inoculum during scaling up is the generation time (t_g) of the micro-organism used. Generation time is the period required for the doubling of cell number or cell biomass, and is defined as:

$$t_g = \frac{ln2}{\mu_x}$$

where μ_x is the specific growth rate.

The inoculum size is typically 1-2 % for a relatively fast growing bacterium (generation time: 0.3-0.7 h) and 5-10 % for slower growing filamentous fungi (generation time: 1-3 h). Yet another, crucial requirement for a successful scaling up process is the the growth stage of the inoculum culture: it has to be in the exponential phase at the time of inoculation. Otherwise, a long adaptation period will elongate fermentation time and thus increase production costs.

2.1.Exercise

The generation time of a yeast culture at the exponential stage is 0.5 hour. How much time is needed for the culture to arrive to a cell concentration of 10^6 per ml in a 10 m^3 (useful volume) vessel if

(1) the entire vessel (10 m^3) is inoculated with a 1-L culture containing 10^6 cells per ml?
(2) the 1-L inoculum culture containing 10^6 cells per ml is introduced first into a fresh medium of 100-L, and this culture – upon reaching a cell concentration of 10^6 per ml – will serve as inoculum for the 10 m^3 vessel?

Exponential phase to be reached in four hours after inoculation (the lag- and accelerating phase together last for 4 hours).

Solution:

In the exponential phase, the following equation can be used:

$$lnN = lnN0 + \frac{ln2t}{g}$$

where *N*: cell number, *N0*: initial cell number, *g*: generation time, *t*: time

(1)

V_1= 1 L
C_1= 10^6 per ml = 10^9 per L
N_0= C_1*V_1= 10^9
V_2= 10 m^3
C_2= 10^6 per ml
N= C_2*V_2= 10^{13}

substituting: $\ln(10^{13}) = \ln(10^9) + \frac{\ln 2t}{0.5}$

t= 49.7 h (+ 4 hours adaptation)

t= 53.7 h

(2)

V_1=1 L
C_1= 10^6 per ml = 10^9 per L
N_0= C_1*V_1= 10^9
V_2= 100 L
C_2= 10^6 per ml
N= C_2*V_2= 10^{11}

$\ln(10^{11}) = \ln(10^9) + \frac{\ln 2t}{0.5}$

t_1= 4.98 h (+ 4 h adaptation)

t_1= 8.98 h

V_2= 100 L
C_2= 10^6 per ml = 10^9 per L
N_0= C_1*V_1= 10^{11}
V_3= 10 m^3
C_3= 10^6 per ml
N= C_3*V_3= 10^{13}

$\ln(10^{13}) = \ln(10^{11}) + \frac{\ln 2t}{0.5}$

t_2= 4.98 h (+ 4 h adaptation)

t_2= 8.98 h

Altogether, 8.98h + 8.98 h = **17.96 hours** are required

2.2.Exercise

An *Aspergillus nidulans* culture is grown batchwise in defined, liquid medium, with glucose (10 g/L) being the sole carbon and ammonium-nitrat being the sole nitrogen source. Glucose depletion

and biomass formation are being monitored all through the fermentation. Elemental composition of the biomass is C: 45%, O: 33%, H: 6.45%, N: 8.92%.

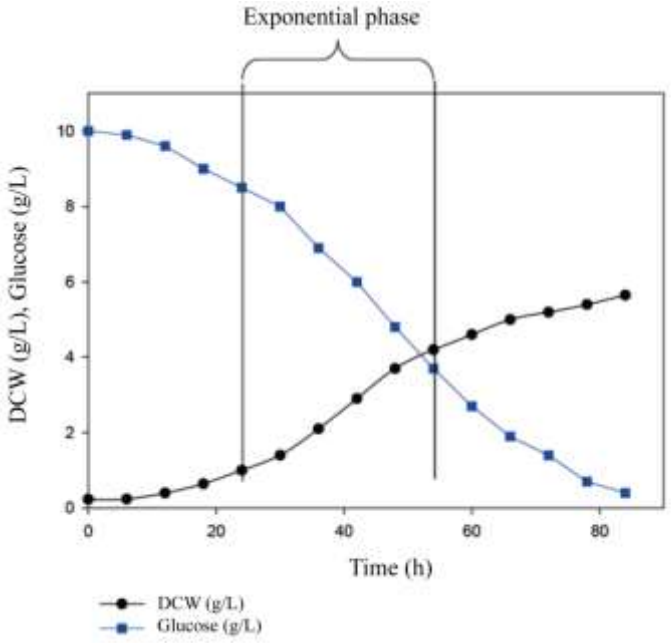
(1) Plot glucose consumption as well as biomass formation against fermentation time by using the data in the table below:

time (h)	DCW (g/L)	Glucose (g/L)
0	0.23	10
6	0.24	9.9
12	0.4	9.6
18	0.64	9
24	1	8.5
30	1.4	8
36	2.1	6.9
42	2.9	6
48	3.7	4.8
54	4.2	3.7
60	4.6	2.7
66	5	1.9
72	5.2	1.4
78	5.4	0.7
84	5.65	0.4

(2) Determine specific growth rate in the exponential phase!
(3) Calculate biomass yield!
(4) Calculate how much carbon (g/L) will incorporate into the biomass from the available carbon source (10 g/L)!
(5) Describe the elemental composition of the biomass as well as the stoichiometry of the fermentation!
(6) Determine substrate yield, oxygen yield, the productivity of the fermentation and the substrate consumption rate!
(7) What would be the maximal cell concentration with 15 g/L initial glucose concentration?

Solution

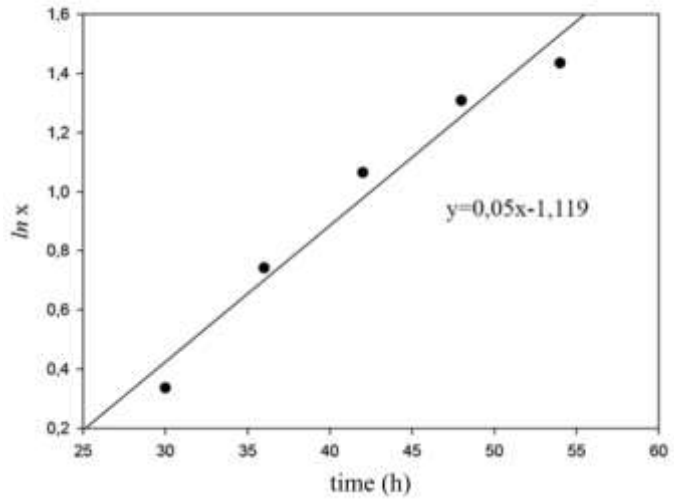
(1) Graphical representations of glucose consumption and biomass formation:



(2) Determination of the specific growth rate:

We use datapoints from the exponential phase, i.e., ln x values vs. time. Slope of the (linear) plot is identical to the specific growth rate, which in this case is **0.05 h^{-1}**.

time (h)	**DCW (g/L)**	***ln* x**
30	1.4	0.336
36	2.1	0.741
42	2.9	1.064
48	3.7	1.308
54	4.2	1.435



(3) Biomass yield:

Y = DCW formed (g/L) / carbon source (g/L)

$$Y = \frac{5.65\ ^{g}/_{L} - 0.23\ ^{g}/_{L}}{10\ ^{g}/_{L}} = \mathbf{0.542}$$

(4) Carbon-biomass conversion:

Carbon content of the biomass is: α_2=0.45

Carbon content of the carbon source is: α_1=72/180=0.4

$\alpha_2 * \Delta x = \alpha_1 * \Delta S_c \rightarrow \Delta S_c = 0.45*5.42/0.4 = 6.09$ g/L

60.9% of the carbon source becomes biomass, **39%** is released as CO_2.

(5) Elemental composition of the biomass and stoichiometry of the fermentation:

C: 45/12=3.75

H: 6.45/1=6.45

O: 33/16=2.06

N: 8.92/14=0.63

Elemental composition of the biomass is: **$C_{3.75}H_{6.45}O_{2.06}N_{0.63}$**

$C_6H_{12}O_6 + a\ O_2 + b\ NH_4NO_3 \rightarrow c\ (C_{3.75}H_{6.45}O_{2.06}N_{0.63}) + d\ CO_2 + e\ H_2O$

c: 0.609*6 = 3.75**c* →*c* = 0.974

d: 0.39*6 = 1**d* → *d* = 2.34

b: 2**b* = *c**0.63 → *b* = 0.306

e: 12+*b**4 = *c**6.45+2**e* →*e* = 3.47

a: 6+*a**2+*b**3 = *c**2.06+*d**2+*e* → *a* = 1.61

Knowing the stoichiometric coefficients, the equation is:

$C_6H_{12}O_6 + 1.61\ O_2 + 0.306\ NH_4NO_3 \rightarrow 0.974\ C_{3.75}H_{6.45}O_{2.06}N_{0.63} + 2.34\ CO_2 + 3.47\ H_2O$

(6) Substrate yield, oxygen yield, productivity of the fermentation, substrate consumption rate:

$Y_{x/Sc}$ = (0.974*(3.75*12+6.45+2.06*16+0.63*14))/180 = **0.504** (g biomass/g glucose)

$Y_{x/O}$ = (0.974*(3.75*12+6.45+2.06*16+0.63*14)/(1.61*2*16) = **1.76** (g biomass/g oxygen)

J = 5.42/84 = **0.064** g biomass/h

dS/dt =10/84=**0.119** g glucose/h

(7) Maximal cell concentration at 15 g/l initial glucose concentration:

Y = ΔX/ΔS = 0.542

$X_{max} = X_0 + Y*S_0$ = 0.23 + 0.542*15 = **8.43 g/L** biomass

2.3.Exercise

The methanol-biomass conversion of an aerobic yeast culture is 60%. The fermentor used has an oxygen transfer rate (OTR) of 100 mol/m^3*h. The C-mol formula of the yeast is $CH_{1.8}O_{0.5}N_{0.2}$. The sole nitrogen source of the culture is ammonia. Determine substrate yield, oxygen yield, productivity and substrate consumption rate!

Solution

$CH_4O + a\ NH_3 + b\ O_2 \rightarrow 0.6\ CH_{1.8}O_{0.5}N_{0.2} + c\ CO_2 + d\ H_2O$

c: $0.4 = c*1 \rightarrow c = 0.4$
a: $a*1 = 0.6*0.2 \rightarrow a = 0.12$
d: $4+a*3 = 0.6*1.8 + d*2 \rightarrow d = 1.64$
b: $1+b*2 = 0.6*0.5 + c*2 + d*1 \rightarrow b = 0.44$

Substrate yield:

Yeast C-mol mass is: 12+1.8+8+2.8 = 24.6
Metanol C-mol mass is: 12+4+16 = 32
$Y_{x/S}$ = (0.6*24.6)/(1*32) = **0.461 g biomass/g methanol**

Oxygen yield:

$Y_{x/O}$ = (0.6*24.6)/(0.87*32) = **0.53 g biomass/g oxygen**

Productivity:

$J=Y_{x/O} * OTR$
OTR: 100 mol/m^3*h = (100*32/1000) = 3.2 kg/m^3*h
J = 0.53*3.2=**1.696 kg sejt/m^3*h**

Substrate consumption rate:

dS/d t = J/Y=1.696/0.461 = **3.67 kg methanol/m^3*h**

2.4.Exercise

Glucose feed is required in a 10 m^3 fermentor, but for productivity reasons, glucose levels in the medium should not exceed 0.1 %. What feed rate and what glucose concentration should be applied for this level to keep if glucose uptake rate of the culture is 3 g/L*h?

Solution

Calculate the mass of 0.1% glucose in the fermentor:

10000 kg*0.1/100 = 10 kg →10 kg glucose has to be present continuously in the fermentor.

Substrate uptake rate:

3 g/L*h →for 10 m^3 →30 kg/h glucose must be fed.

Using a glucose solution of 50% (w/v) means 60 L water is needed for 30 kg glucose → feed rate is 60 L/h = **1 L/min**

2.5.Exercise

A bacterial culture is grown aerobically on glucose and ammonium as sole carbon and nitrogen sources, respectively. From 6 C-mol glucose, 2 C-mol biomass is formed, and there are no other products. The C-mol formula of the bacterium is $CH_{1.8}O_{0.28}N_{0.25}$. The molar heating value (calorific value, Qo) of organic compounds (including that of the biomass) is 112.6 kJ/g $_{ekvivalent}$. The calorific value of glucose is 2805 kJ/mol. Calculate the amount of heat released upon formation of one unit of biomass!

(1) Describe stoichiometric equation!
(2) Calculate the mass of 1 C-mol microbe!
(3) Calculate how many electrons are passed over oxygen upon burning of 1 C-mol microbe!
(4) Calculate the calorific value of 1 g biomass (ΔH_x)!
(5) Calculate substrate yield ($Y_{x/S}$)!
(6) Calculate oxygen yield! ($Y_{x/O}$)
(7) Calculate the amount of heat released!

Solution:

(1)

$6\ CH_2O + a\ O_2 + b\ NH_3 \rightarrow 2\ CH_{1.8}O_{0.28}N_{0.25} + c\ H_2O + d\ CO_2$

d: $6 = 2 + d \rightarrow d = 4$

b: $b = 0.25$

c: $6*2 + 0.25*3 = 2*1.8 + c*2 \rightarrow c = 4.575$

a: $6 + a*2 = 2*0.28 + 4.575 + 4*2 \rightarrow a = 3.56$

$6\ CH_2O + 3.56\ O_2 + 0.25\ NH_3 \rightarrow 2\ CH_{1.8}O_{0.28}N_{0.25} + 4.575\ H_2O + 4\ CO_2$

$CH_2O + 0.59\ O_2 + 0.041\ NH_3 \rightarrow 0.33\ CH_{1.8}O_{0.28}N_{0.25} + 0.76\ H_2O + 0.66\ CO_2$

(2)

the mass of 1 C-mol micro-organism is:

$m_{C\text{-}mol} = (1*12) + (1.8*1) + (0.28*16) + (0.25*14) = 21.78$ g/C-mol

(3)

The number of electrons passed over on oxygen upon burning of 1 C-mol microbe is:

oxidation number of carbon (C): +4
oxidation number of oxygen (O): -2
oxidation number og hydrogen (H): +1
oxidation number of nitrogen (N): 0

$\gamma = (1*4) + (1.8*1) + (0.28*(-2)) + (0.25*0) = 5.24$

(4)

The calorific value of 1 g biomass is:

$\Delta H_x = Q_O*\gamma = 112.6*5.24 = 590.02$ kJ/C-mol

ΔH_x = (590.02 kJ/C-mol) / (21.78g/C-mol) = 27.09 kJ/g biomass.

The calorific value of 1 g glucose:

ΔH_S = (2805 kJ/mol)/(180 g/mol) = 15.58 kJ/g glucose

(5)

The substrate yield is:

$Y_{x/S}$= (0.33*(12+1.8*1+0.28*16+0.25*14))/(12+2+16) = 0.24 g biomass/g glucose

(6)

The oxygen yield is:

$Y_{x/O}$ = (0.33*(12+1.8*1+0.28*16+0.25*14))/(0.59*2*16)=0.38 g biomass/ g oxygen

(7)

The heat released upon formation of 1 unit of biomass is:

$$Y_{x/Q} = \frac{Y_{x/S}}{(\Delta H_S - \Delta H_x * Y_{x/S})}$$

$Y_{x/Q}$ = 0.24/ (15.58- (27.09*0.24) = **0.026 g biomass/kJ**

$1/Y_{x/Q}$ = **38.46 kJ/g**

3. Sterilization

Required knowledge:
Sterilization is the process during which all (living) germs are killed and/or removed from the given system (e.g., growth medium, other liquids, gas, solid surfaces, etc.). It is among the most important upstream operations in the fermentation industry, as almost all processes are mono-cultural (= there can be only one organism – the producer strain – in the system). An improperly sterilized growth medium may result in a massive loss of time, resources and materials.

Of all the techniques available, sterilization by heat and mechanical filtering are the most widespread. The latter is used to sterilize incoming air/gas supply, while heat is used for liquids, the vessel and all the auxiliary equipments.

Over a certain temperature, all microorganism including the thermo-tolerant ones will perish at a measurable rate. Kinetics of heat-elicitated decay depends on the microorganism, its vegetative forms and the method of sterilization employed. Heat-resistant spores are usually more sensitive to steam than dry heat.

Time-course of cell death is an exponential function, and follows first-order kinetics:

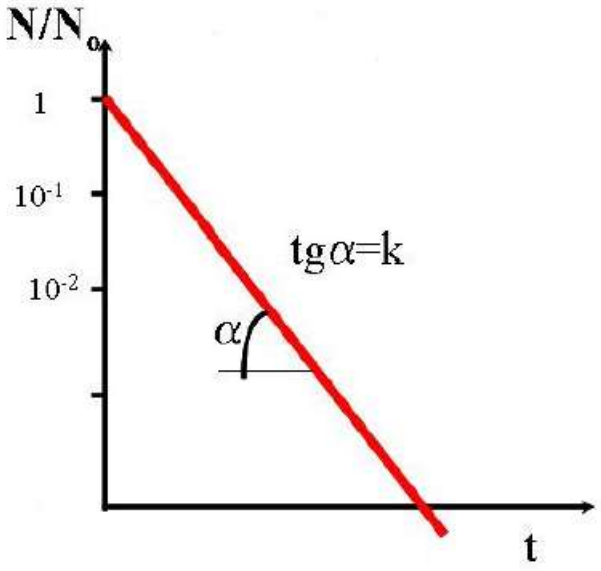
$$\frac{dN}{dt} = -k * N$$

where N = number of living germs (bits per cm^3), k = rate coefficient of heat decay (min^{-1}), *t* = time (min).
Integration of the above equation gives:

$$ln\frac{N_0}{N} = -kt$$

where N_0 = initial cell number, N = cell number after the time-period *t*.

At any given temperature, the value of *k* is strictly species-dependent. However, the value of *k* also varies with temperature. The *k* value for any given micro-organism can be calculated by knowing the initial cell number as well as the cell number after time *t* at a defined temperature. One must plot N and N_0 against time (t). The values of N/N_0 plotted against time yields a straight line, whose slope is identical to *k* at the given temperature.



Forrás: Sevella Béla: Biomérnöki műveletek és folyamatok (2012)

Rate coefficient of heat decay for a few microorganisms:

Microorganism	**T (°C)**	**k (min^{-1})**
Bacillus subtilis (vegetative)	110	27
Bacillus subtilis (spores)	121.1	3
Bacillus stearothermophilus (spores)	104	0.051
	125	6.06
	130	17.52
Clostridium botulinum (spores)	104	0.42

Forrás: Sevella Béla: Biomérnöki műveletek és folyamatok

Determination of the heat decay rate coefficient for a given microorganism is time-consuming. Therefore, in everydays' practice, *k* values for the vegetative spores of the thermophilic bacterium *Bacillus sterothermophilus* are employed to validate the sterilization process. In case of contamination from an unknown source, heat decay data for this thermophilic bacterium are used to determine the length and intensity (temperature) of sterilization.

k values for the spores of the thermophilic bacterium *Bacillus sterothermophilus*:

T (°C)	**k (min^{-1})**	**T (°C)**	**k (min^{-1})**	**T (°C)**	**k (min^{-1})**
100	0.019	111	0.267	121	2.538
101	0.025	112	0.336	122	3.16
102	0.032	113	0.423	123	3.929
104	0.051	114	0.531	124	4.881
105	0.065	115	0.666	125	6.056

106	0.083	117	1.045	127	9.293
107	0.105	118	1.307	128	11.494
108	0.133	119	1.633	129	14.2
110	0.212	120	2.037	130	17.524

Forrás: Sevella Béla: Biomérnöki műveletek példatár (2001)

The likelihood of a viable germ remaining in the system never falls to zero after sterilization, but by choosing a sufficiently long and intense protocol, this likelihood can be minimized. The fermentation industry works with a generally accepted likelihood of 0.01%. Hence, criterium for a sufficient sterilization is $1\text{-}P_0(t) = 10^{-2} – 10^{-4}$, where $P_0(t)$ is the likelihood of all germs being dead after sterilization period *t*.

Sterilization of the growth medium is an *in situ* process, i.e., it usually happens inside the fermentor. The heating and cooling periods may also contribute to heat decay, and hence must be taken into consideration during designing the protocol, which is to contain the minimally safe holding period (i.e., the time effectively spent at the ultimate sterilization temperature).

The heat decay index (∇) is the natural logarithm of the ratio of the initial and final germ numbers. Overall heat decay index is the sum of the heat decay indexes of the three periods (heating, holding and cooling periods).

$$\nabla_{Total} = \nabla_{Heating} + \nabla_{hold} + \nabla_{cooling}$$

$$\nabla_{total} = ln\frac{N_{initial}}{N_{final}} = ln\frac{N_{initial}}{N_{Heating}} + ln\frac{N_{Heating}}{N_{hold}} + ln\frac{N_{hold}}{N_{final}}$$

During batch sterilization, the heating, holding and cooling periods contribute to the success of sterilization, typically in the following proportion:

$$\frac{\nabla_{\text{heating}}}{\nabla_{total}} = 0.2\ ;\ \frac{\nabla_{hold}}{\nabla_{total}} = 0.75\ ;\ \frac{\nabla_{cooling}}{\nabla_{total}} = 0.05$$

Typical parameters during *in situ*, batch sterilization of growth media are:

temperature: 121 °C

overpressure inside the vessel: 1.1 -1.2 bar

holding period: 20-60 perc

3.1.Exercise

100-L growth medium contains 10^7 unknown spores per ml. Heat decay rate coefficient of the spores at 121°C is 3.2 min^{-1}. How long should the minimal holding period be, if the sterilization criterium is 99.99%? (heat decay during heating and cooling shall not be considered).

Solution

$P_0(t) = 0.9999 \rightarrow 1-P_0(t)=10^{-4} = N$

$k_{121}=3.2\ min^{-1}$

$N_0 = 10^7 * 10^3 * 10^2 = 10^{12}$ spores

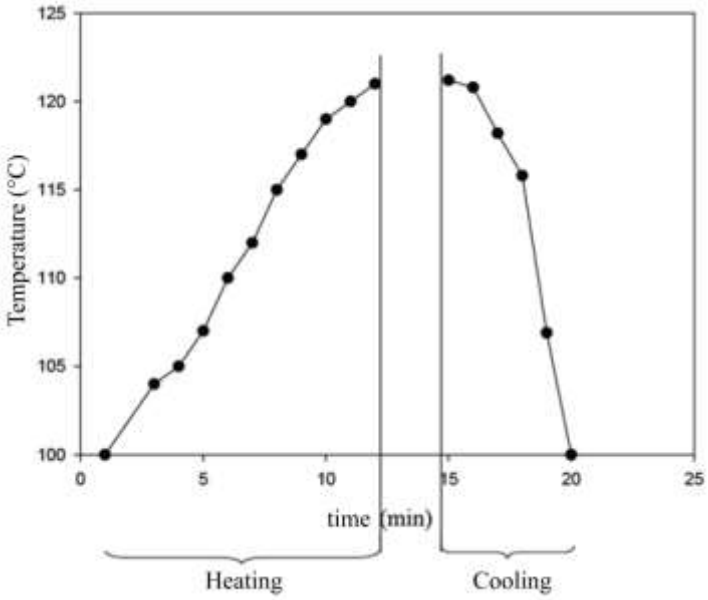
$$ln\frac{N_0}{N} = -kt$$

$\ln(10^{12}/10^{-4})=-3.2t$

t = 11.51 min

3.2.Exercise

100-L growth medium is contaminated with unknown spores; their concentration is 10^8 per mL. Heat penetration curve of the fermentor during the warming and cooling periods is shown below. How long should the minimal holding period be, if the sterilization criterium is 99.99%?



Solution

From the sterilization criterium, value of N_{final} is:

$P_0(t)=0.9999 \rightarrow 1-P_0(t)=\mathbf{10^{-4}} = N_{final}$

Overall heat decay index is:

$$\nabla_{total} = ln\frac{N_{initial}}{N_{final}}$$

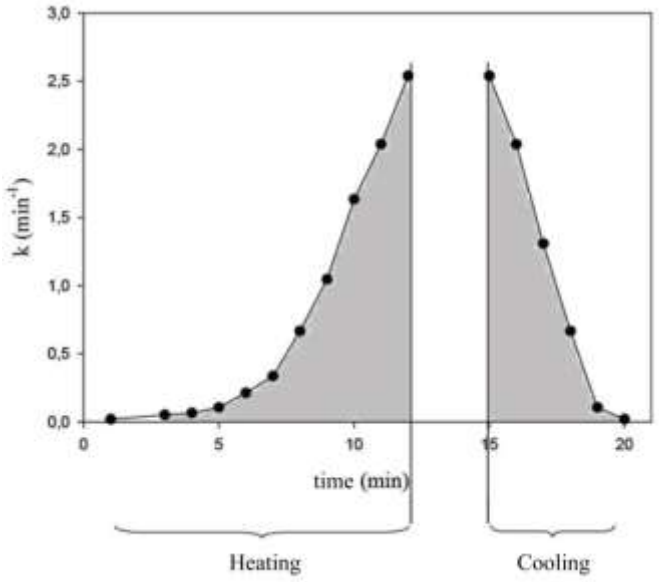
$N_{initial} = 10^8 * 10^3 * 10^2 = 10^{15}$ spores

$\nabla_{total} = \ln(10^{15}/10^{-4}) = \mathbf{43.74}$

Heat decay indexes for the heating and cooling periods are:

A plot of *k* against *t* (time) can be drawn by using the heat decay rate coefficient data of the spores of *Bacillus sterothermophilus* (see table below). Graphical integration of such curve will provide us with the warming and cooling indexes.

Heating			Cooling		
t (min)	**T (°C)**	**k (min^{-1})**	**t (min)**	**T (°C)**	**k (min^{-1})**
1	100	0.019	15	121.2	2.538
3	104	0.051	16	120.8	2.037
4	105	0.065	17	118.2	1.307
5	107	0.105	18	115.8	0.666
6	110	0.212	19	106.9	0.105
7	112	0.336	20	97.7	0.019
8	115	0.666			
9	117	1.045			
10	119	1.633			
11	120	2.037			
12	121	2.538			



Calculating the areas under curve yields the $\nabla_{heating}$ és $\nabla_{cooling}$ indexes, respectively.

$\nabla_{heating}$: 7.46

$\nabla_{cooling}$: 5.39

$\nabla_{hold}=\nabla_{total}-(\nabla_{heating}+\nabla_{cooling})$ = 30.89

k_{121}=2.538

$\nabla_{hold} = k_{121}*t$ →**t = 12.17 min**

4. Culture of aerobic microorganisms in a bioreactor

Required knowledge:

On technical scale, the microorganisms that produce marketable metabolites are grown in large cylindrical vessels called fermenters. Metabolism of these "industrial" microorganisms (bacteria, fungi) is usually oxygen-dependent (aerobic), hence a sufficiently high level of oxygen in the growth medium is a critical prerequisite.

Solubility of oxygen in the growth medium is influenced by three factors: (1) temperature, (2) partial gas pressure of oxygen, and (3) the presence of other, soluble compounds. Saturation levels of oxygen increase with increasing partial pressure and with decreasing temperature. To a varying extent, the presence of other solutes in the medium also decreases oxygen solubility, which means that dissolved oxygen levels are necessarily lower in a growth medium of any composition than in pure water. Maximal available oxygen levels in a growth medium primarily depend on the type and concentration of the electrolites as well as of the organic compounds. Solubility of a gas in water to be calculated by Henry's law.

$$\text{solubility [mol/dm}^3\text{]} = \text{partial pressure [bar]} / k_H \text{ [mol/dm}^3\text{*bar]}$$

where k_H is the Henry-constant of the studied gas at a given temperature.

Henry's law helps to determine the maximal solubility of a certain gas in liquids, but it does not provide any information on the kinetics of dissolution, e.g., that how long it will take to achieve maximal solubility under the given conditions. Rate of oxygen absorption in water is described by the following equation:

$$\frac{dC}{dt} = K_L a(C^* * C)$$

K_L: mass transfer coefficient [cm*s^{-1}], i.e., the reciprocal of the resistances to the transfer of oxygen from gas to liquid.
a: gas/liquid interface area per liquid volue [cm^2 cm^{-3} = cm^{-1}],
C*: saturation concentration of oxygen [mg/dm^3],
C: actual dissolved oxygen concentration [mg/dm^3].

It is practically impossible to individually determine K_L and a, as their values depend on a variety of liquid and/or vessel characteristics. However, their product can be determined rather easily, hence the two terms are combined in the term K_La, the volumetric mass transfer coefficient [s^{-1}]. K_La is a measure of the aeration capacity of a fermenter. The larger the K_La the higher the aeration capacity of the system.

There are several experimental methods to determine the K_La of a fermentor. The sulphite oxidation method does not require the measurement of dissolved oxygen concentrations. Instead, it relies on the converson rate of a solution of sodium sulphite to sodium sulphate in the presence of copper or cobalt ions as catalysts:

$$Na_2SO_3 + 1/2\ O_2 \rightarrow Na_2SO_4$$

The rate of reaction is such that as oxygen enters the solution it is immediately consumed during the oxidation of sulphite. Therefore, the sulphite oxidation rate is equivalent to the oxygen transfer rate, and the dissolved oxygen concentration is zero. K_La is calculated from the equation:

$$\text{Oxygen transfer rate} = K_La * C^*$$

Oxygen transfer rate is determined as follows. The vessel is filled up with a 0.5 M solution of sodium sulphite containing 10^{-3} M Cu(II) ions, and aerated and agitated at fixed rates. Samples are taken regularly and added to excess iodine solution which reacts with the unconsumed sulphite. The level of sulphite is determined by a back titration with sodium thiosulphate solution. The volumes of the thiosulphate titrations are plotted against sample time and the oxygen transfer rate is calculated from the slope of the graph.

The sulphite oxidation method is simple, but will give accurate results only under perfectly clean conditions, in pure water as a solvent (it is of course not working in growth media). It is time-consuming (up to 3 hours), and is inaccurate in the presence of even tiny amounts of surface-active contaminants such as amino acids, fatty acids, proteins, lipids. In addition, rheology of a sodium sulphite solution is completely different from that of a fermentation growth medium, especially when mycelial organisms such as fungi is cultivated. However, it is useful upon testing the relative impact of aeration-related elements (baffles, impellers) on the oxygen transfer rate.

Estimation of the K_La of a fermentor by gassing-out techniques is based on monitoring the increase of dissolved oxygen concentration by a probe (sensor) in a solution (could be a fermentation growth medium as well) during agitation and aeration, after dissolved oxygen concentration had been decreased to a low level. A plot of dissolved oxygen concentration against time will yield a saturation curve. At any time-point, the oxygen transfer rate will be equal to the slope of the tangent drawn at the respective dissolved oxygen concentration-point.

Lowering the dissolved oxygen concentration can be achieved by two techniques – the static method and the dynamic method. In the first, the oxygen concentration is lowered by gassing the liquid with nitrogen gas. Integration of the equation that describes the increase in dissolved oxygen concentration yields:

$$\ln (C^* - C) = - K_Lat$$

Thus, a plot of ln (C* - C) against time will yield a straigt line with a slope being $-K_La$. This technique is rapid (takes up to 15 minutes) and may utilize the cell-free fermentation medium.

The dynamic method utilizes the respiratory activity of a growing microbial culture in the vessel to lower the dissolved oxygen level before aeration and agitation kicks off. This method has the advantage of being carried out during a fermentation process, hence it provides a more realistic assessment of the efficiency of the fermentor. Supply of air is halted, which results in a linear decline of the dissolved oxygen concentration due to the ongoing respirator activity of the cells. Once the dissolved oxygen level is low enough, aeration is resumed. The observed increase in the dissolved oxygen concentration will be the difference between the transfer of oxygen into the solution and the uptake of oxygen by the cells, as shown by equation X.

$$\frac{dC}{dt} = K_L a(C^* * C) - x\ QO2$$

where x = biomass concentration, QO_2 = specific respiration rate.

Linearization of this equation will yield a straight line, with its slope being equal to $-1/K_L a$.

The dynamic method has only one major disadvantage: the range over which the increase in dissolved oxygen concentration is measured. Because of the presence of living (microbial) cells during measurements, one cannot allow the dissolved oxygen concentration drop below a critical level. Otherwise, respiration rate would be limited by the lack of available oxygen and the term QO_2 would not remain constant during the measurement.

In order to achieve high $K_L a$ values, the fermentor must be intensively aerated. Liquid must be agitated continuously to mix up and homogenize the components of the medium, to dispergate air bubbles and to separate gas and liquid phases. Speed of the impeller depends on a variety of factors such as mechanical sheer force, mixing time, specific energy provision, power consumption of the electric motor behind the mechanical agitator.

In any agitated vessel, strong mechanical sheer force may cause stress that is harmful to the cells. Hence, mechanical sheer force – which is frequently the most critical element during scale-up – is to be reduced as much as possible. Sheer force is proportional to the peripheral speed of the impeller and the impeller type used. Using the same impeller geometry at larger scales will enhance sheer force; hence, to keep the sheer force constant during scale-up, rotational speed of the impeller in the bigger vessels must be lowered relative to the smaller ones.

The phrase “mixing time” (t_k) refers to the period needed for an ingoing substrate to achieve identical concentrations everywhere inside the vessel, so biological and mass transfer reactions will proceed at the same rate. Mixing time can be calculated by determining the dimensionless mixing number (N_k), which depends on the type of the impeller used.

$$N_k = t_k * N$$

Mixing number, in turn, is dependent on the dimensionless Reynolds number (*Re*). Reynolds number is a fundamental characteristic in fluid mechanics, and is mostly used to qualify flow types.

If the flow of the fluid is laminar or turbulent, *Re*<10 and *Re*>1000, respectively. In a transition range, its value is 10<*Re*<1000. If the flow fells into the turbulent range, the mixing number (N_k) is constant.

$$Re = \frac{N * D_i^2 * \rho}{\mu}$$

where N = rotational speed, D_i = impeller diameter, ρ = fluid density, μ = fluid viscosity.

Power absorption of the impeller depends on the geometry of the vessel and of the stirrer, and the density and viscosity of the fluid. The typical power output range of an impeller is characterized by the dimensionless power number (N_p).

$$N_p = \frac{P}{\rho N^3 D_i^5}$$

where P = external power from the agitator, N = impeller rotational speed, D_i = impeller diameter, ρ = liquid density

Thus, the power number is the ratio of the external force exerted (P) to the inertial force imparted ($pN^3D_i^5$) to the liquid.

Values for P at various values of N, D, μ and p can be determined experimentally, and that will in turn allow to calculate the Reynolds and the power numbers. A plot of the logarithm of the power number against the logarithm of the Reynolds number yields a graph which is termed the power curve (Figure X). A power curve for a baffled vessel agitated by a flat-blade turbine can be divided into three defined zones, each containing a different type of fluid flow. (1) the laminar or viscous flow zone, where the logarithm of the power number linearly decreases with an increase in the logarithm of the Reynolds number. The power absorbed within this range is the sole function of liquid viscosity. The Reynolds number < 10; (2) the transient zone, where the relationship between the two variables is not consistent. Reynold number is between 10 and 1000; (3) the turbulent flow zone, where the power number is constant and independent of the Reynolds number, which is > 1000. Viscous (laminar) flow is rare in the fermentation industry; flow characteristics are either turbulent or transient.

Aeration of a liquid decreases the power consumption of the agitator because aerated liquid (= growth medium) is less dense than the unaerated one due to the air bubbles. The ratio of gassed to ungassed power consumption is referred to as aeration number, a dimensionless term (N_a).

$$N_a = \frac{linear\ air\ velocity}{rotational\ speed\ of\ the\ impeller} = \frac{U_G}{v_k} = \frac{Q \Big/ \frac{D_i^2 \pi}{4}}{\pi N D_i}$$

where Q = volumetric air flow rate, D_i = impeller diameter, N = rotational speed.

The aeration number is a useful tool when agitation variables (impeller tip speed, impeller diameter) are varied over a wide range. However, it is important to remember that the greatest power demands often occur during *in situ* sterilization of the medium, e.g., when the system is not gassed. The agitation motor should therefore be sufficiently powerful to be capable of agitating the ungassed system, too. On the other hand, if the impeller is unable to disperse the incoming air then the impeller is becoming flooded. Flooding is the phenomenon where the air-flow dominates the flow pattern and is due to an inappropriate combination of air flow rate and agitation speed.

Part of the energy introduced by the impeller into the vessel is converted into heat, that increases the demand for cooling water. The amount of heat introduced depends on the geometry of the impeller, the fermentor in general, also the rheology of the growth medium, and can be precisely determined experimentally (provided that temperature is well measurable). During measurement, the growth medium is not aerated, while the cooling water is drained from the duplicator and filled up with air instead. Under such conditions, the agitated fluid will be warming up, the extent of which is proportional to the mechanical heat coming off the agitator.

Fermentations require cooling. The extent of cooling depends on the heat introduced by the agitator and the metabolic heat produced by the microorganisms. This latter factor primarily depends on the specific growth rate of cells, which in turn is proportional to the oxygen uptake rate (OUR). Higher oxygen demand involves more heat produced during growth. A linear correlation is generally found between metabolin heat production and OUR, with a value of 518 ± 12 KJ / mol oxygen. This value is independent of the organism tested.

By increasing the volume (= scale) of the fermentor, the surface of the liquid grows by the second-, while its volume grows by the third power, i.e., the larger the fermentors are, the more challenging their cooling is. Pilot-scale vessels can be effectively cooled via water introduced into the duplicator (= the double walled space around the vessel). On technical scale, this is insufficient, and internal heat exchangers are needed. These built-in heat exchangers are running parallel with the wall of fermentor, and hence also function as baffles that make liquid flow turbulent.

4.1.Exercise

How many grams of oxygen can be solved water at 25 °C and and

(1) atmospheric pressure?
(2) at 0.4 bar overpressure?
(3) at the bottom of a 10 meters high column of water, with an additional 0.4 bar overpressure?

Solution

C_{O2} (mol/dm^3) = K_H (mol/dm^3*bar) *$p_{(O2)}$(bar)

K_{HO2} (25 °C-on)= 1.3*10^{-3} mol/dm^3*bar

Oxygen makes up 21% (v/v) of air.

(1) At atmospheric pressure:

C_{O2} = 1.3*10^{-3} mol/dm^{3}bar *0.21*1 bar = 2.73*10^{-4} mol/dm^3 → **8.7 mg/dm^3**

(2) at 0.4 bar overpressure:

C_{O2}=1.3*10^{-3} mol/dm^{3}bar *0.21*1.4 bar = 3.8*10^{-4} mol/dm^3 → **12.2 mg/dm^3**

(3) at the bottom of a 10 meters high water column, with an additional 0.4 bar overpressure:
$p_{hidrostatic}$= ρ*g*h = 1000 kg/m^3*9.81m/s^2*10 m = 98100 Pa = 0.98 bar
C_{O2}= 1.3*10^{-3} mol/dm^3bar *0.21*(1 bar + 0.4 bar + 0.98 bar)= 6.49*10^{-4} mol/dm^3 **→20 mg/dm^3**

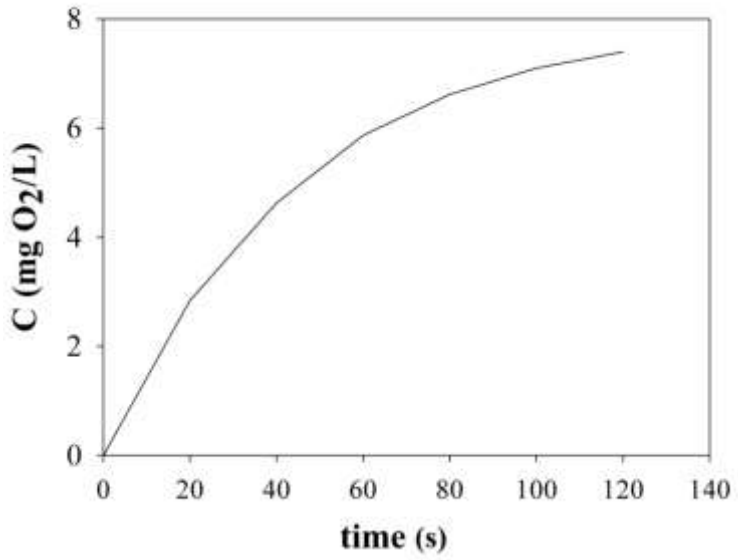
4.2.Exercise

The K_La of a fermentor can be estimated from the saturation rate of oxygen at a given agitation and aeration. The following experiment must be undertaken: the vessel is filled up with water, and the oxygen is flushed out with nitrogen gas. The oxygen sensor falls to zero % (indicating a dissolved oxygen (DOT) level of 0 mg/L). Aeration and agitation are resumed, and DO levels are periodically read.
The following table contains DO data to calculate K_La.

time (s)	C (mgO_2/L)
0	0
20	2.84
40	4.63
60	5.87
80	6.62
100	7.1
120	7.4

Solution

Plot oxygen saturation kinetics! (DO as a function of time)!



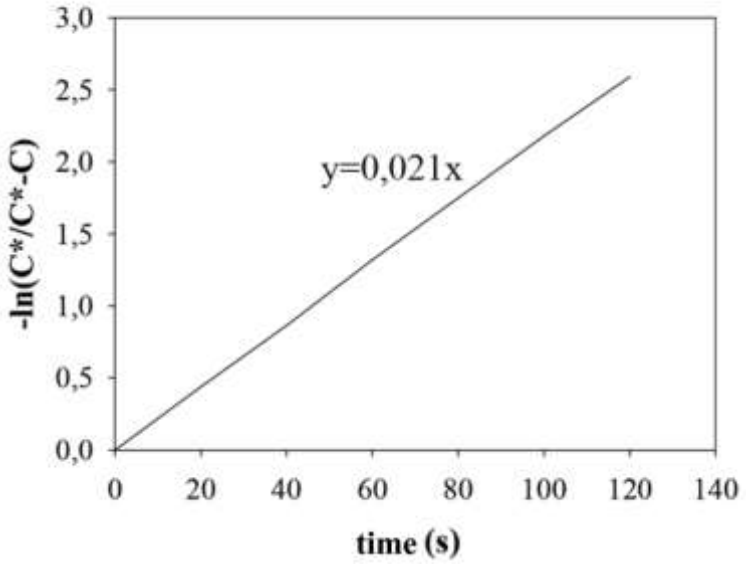
Linearize the values:

-ln(C*/C*-C)

where C* is the saturation concentration of oxygen (= 8 mg/L)

time (s)	-ln(C*/C*-C)
0	0
20	0.438
40	0.864
60	1.32
80	1.75
100	2.18
120	2.59

Plot the linearized values as a function of time!



Slope of the straight line equals K_La (in s^{-1} dimension).
$tg\alpha = K_La = 0.021\ s^{-1} =$ **75.6 h^{-1}**

4.3.Exercise

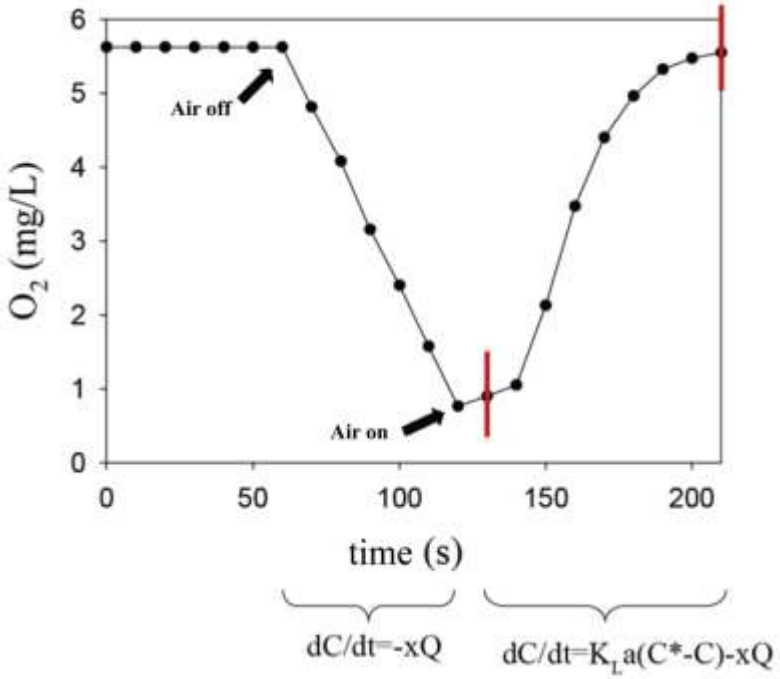
We wish to determine the K_La value of a fermentor by the dynamic method, during cultivation of a *Pseudomonas putida* strain. Aeration paused for a minute, then resumes. DOT data are taken every 10 minutes (see table below). Calculate K_La and oxygen transfer rate (OTR)!

time (s)	mg O_2/dm^3	time (s)	mg O_2/dm^3	time (s)	mg O_2/dm^3
0	5.625	80	4.08	160	3.4725
10	5.625	90	3.1575	170	4.4025
20	5.625	100	2.4	180	4.965
30	5.625	110	1.575	190	5.325
40	5.625	120	0.765	200	5.475

50	5.625	130	0.9	210	5.55
60	5.625	140	1.05		
70	4.815	150	2.13		

Solution

Plot oxygen saturation kinetics! (DO as a function of time)!



Calculate respiratory activity from the oxygen uptake data taken between air off and air on!

$$dC/dt = -xQ$$

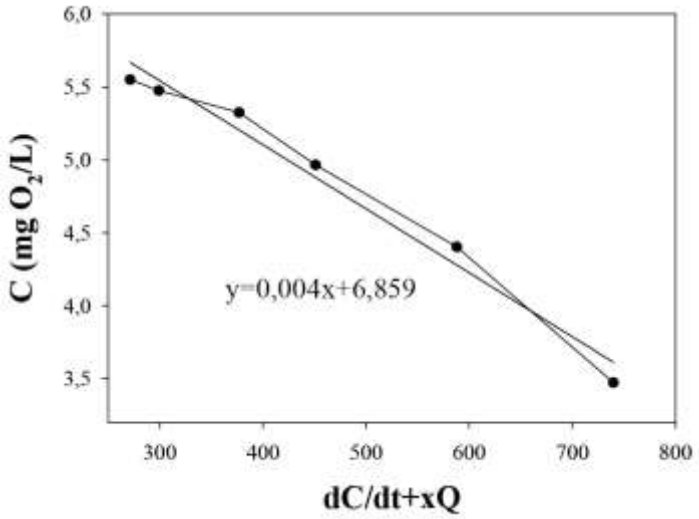
where x = dry cell weight of the culture, Q = specific respiratory rate.

dC/dt = (5.625 mgO_2/L – 0.9 mgO_2/L) / (130s -60s) = (4.725 mgO_2/L)/0.0194h = 243.5 $mgO_2/L*h$

xQ= 243.5 $mgO_2/L*h$ **→7.6 mmol O_2/L*h**

K_La can be calculated from the oxygen saturation data taken after the resumption of aeration and agitation. In order to this, one must first linearize the curve between the two time-points indicated by red colour. Axis "X" refers to the values of dC/dt + xQ, while axis "Y" refers to the respective dissolved oxygen values. The slope of the straight line that arises after linearization is:

$$tg\alpha = -1/K_La$$



tg α= -0.004 →K_La = **<u>250 h^{-1}</u>**

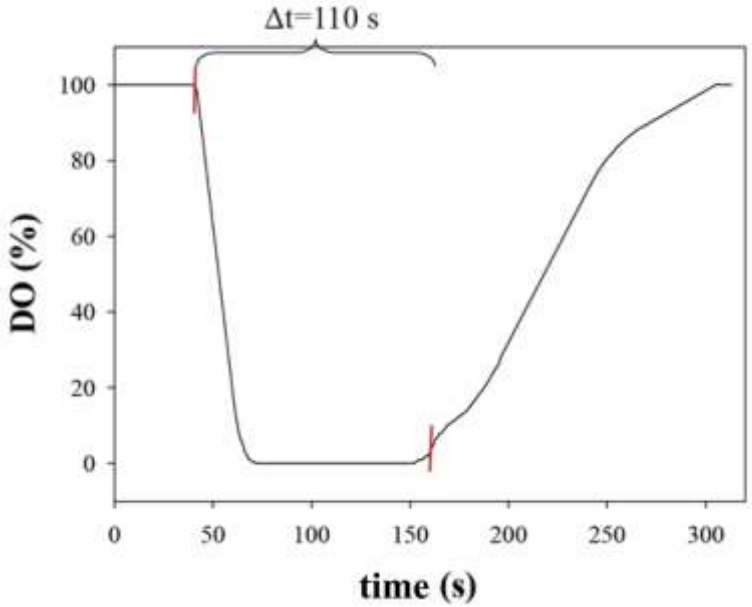
Oxygen transfer rate (OTR) is:

OTR = $K_La(C^*-C)$ = 250 h^{-1}*(5.6 mgO_2/L - 0.765 mgO_2/L) =

1208 $mgO_2/L*h$ → **37.7 mmol O_2/L*h**

4.4.Exercise

The K_La of a fermentor must be estimated by the sulphite oxidation method at a given agitation and aeration. The fermentor is filled up with water (100 L, 25 °C), the actual overpressure is 0.4 bar. Under such conditions, the saturation concentration of oxygen is 12.2 mg/L. 65g Na_2SO_3 is now introduced to the vessel, and DOT levels are periodically read. The length of the period between the time-points of the start of decrease and that of the renewed increase in the DOT levels is 110 s.



Solution

Calculate how many moles of oxygen reacted with 65 g Na_2SO_3:

$$Na_2SO_3 + 1/2O_2 \rightarrow Na_2SO_4$$

M_{Na2SO3}= 126 g/mol

65g/126g = 0.515 mol Na_2SO_3 was used →**0.258 mol O_2 was used**

Calculate the oxygen transfer rate of the system:

$$OTR = \frac{used\ O_2[mmol]}{V_{water}[kg]\Delta t[h]}$$

OTR = 258 mmol/100 kg*0.03 h = **86 mmol/kg*h**

Calculate K_La at a given aeration and agitation rate:

$$OTR = K_L a C^*$$

OTR = 86 mmol/kg*h = 2752 mgO_2/kg*h

K_La = OTR/C*= (2752 mgO_2/kgh)/(12.2 mgO_2/kg) = **225.5 h^{-1}**

4.5.Exercise

A bactarial fermentation runs at 30 °C. The oxygen uptake rate (OUR) is 40 mmol/L*h. Compare the brutto air demands at (1) 5 L-, (2) at 100 L- and (3) at 10 m^3- scale, if air utilization efficiency is 15% in all cases. At 100 L and at 10 m^3, an overpressure of 0.45 bar is applied. Calculate the amount of the incoming air in both L/min and VVM.

Solution

Calculate how many kilograms of O_2 is needed hourly for a 1 m^3 culture:

OUR = 40 mmol/L*h= 1.28 kg/m^3*h

(1) In case of a 5 L-fermentation:
Density of air at 30 °C and atmospheric pressure is 1.149 kg/m^3
O_2 utilization at atmospheric pressure is 1.149 kg/m^3*0.21*0.15= 0.036 kg/m^3

The amount of oxygen needed for the culture is:
1.28 kg/m^3*h *0.005 m^3= 6.4*10^{-3} kg/h

The required amount of air is (6.4*10^{-3}kg/h)/(0.036kg/m^3) = 0.178m^3/h= **2.96 L/min**
Air introduced into one unit of culture is: (2.96 L/min) / 5L = **0.59 VVM**

(2) In case of a 100 L-fermentation:
Density of air at 30 °C and 0.45 bar overpressure is 1.149 kg/m^3*1.45 = 1.666 kg/m^3
O_2 utilization at 1.45 bar esetén: 1.666 kg/m^3*0.21*0.15 = 0.052 kg/m^3
The amount of oxygen needed for the culture is: 1.28 kg/m^3h *0.1 m^3 = 0.128 kg/h
The required amount of air is: (0.128 kg/h)/ (0.052 kg/m^3) = 2.46 m^3/h = **41.02 L/min**
Air introduced into one unit of culture is: (41.02 L/min)/100 L = **0.41 VVM**

(3) In case of a 10 m^3-fermentation:
Density of air at 30 °C and 0.45 bar overpressure is 1.149 kg/m^3*1.45 = 1.666 kg/m^3
O_2 utilization at 1.45 bar esetén: 0.052 kg/m^3
The amount of oxygen needed for the culture is: 1.28 kg/m^3h*10 m^3= 12.8 kg/h
The required amount of air is: (12.8 kg/h)/ (0.052 kg/m^3) = 246 m^3/h = **4102 L/min**
Air introduced into one unit of culture is: (4102 L/min)/10^4L = **0.41 VVM**

4.6.Exercise

Egy 150 L-es fermentorban a keverő átmérője 196 cm, termelői fermentorban pedig 1.1 m. A keverőelemek típusa megegyezik.

(1) Mennyi a keverők kerületi sebessége, ha mindkét fermentorban 200 rpm a kevertetés?
(2) Mennyi a termelői fermentorban a keverő fordulatszáma, ha a keverő kerületi sebessége 2.04 m/s?

Solution

(1) N_{150}= 200 rpm= 3.33 s^{-1}
D_i= 0.196 m

$$v_k = D_i \pi N$$

v_k= 0.196 m*3.14*3.33 s^{-1}= **2.04 m/s**
$N_{termelői}$= 3.33 s^{-1}
D_i= 1.1 m

v_k= 1.1 m*3.14*3.33 s^{-1}= **11.5 m/s**

(2)
$v_k = D_i \pi N$
2.04 m/s = 1.1 m*3.14*N

N= 2.04/(1.1*3.14)= 0.59 s-1= **35.4 rpm**

4.7.Exercise

Egy kisüzemi fermentorban a folyadék térfogata 0.1 m^3, viszkozitása 10^{-2} Pas, sűrűsége 1000 kg/m^3. 3 db Rusthon keverő található benne, melynek átmérői 196 cm. Számolja ki a keveredési időt, ha a fordulatszám:

(1) 100 rpm
(2) 200 rpm
(3) 300 rpm
(4) 400 rpm

Solution

(1)

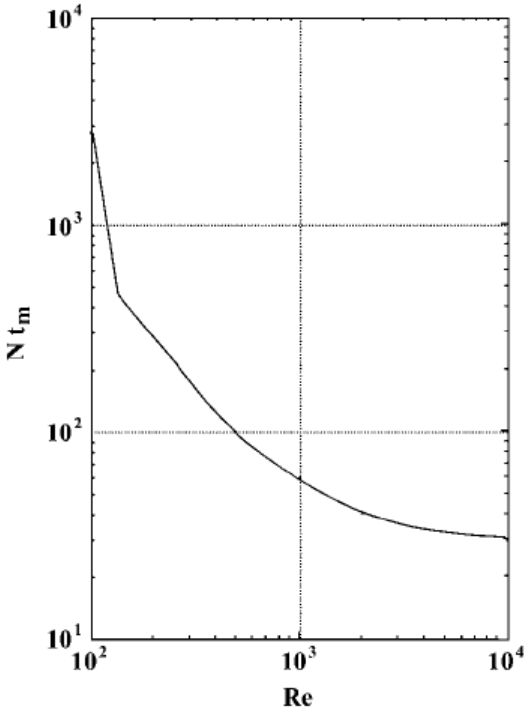
Számolja ki a keverési Reynolds számot:

$$Re = \frac{N * D_i^2 * \rho}{\mu}$$

Ahol, N: a fordulatszám (s^{-1})
D_i: a keverőelem átmérője (m)
ρ: a folyadék sűrűsége (kg/m^3)
μ: a folyadék viszkozitása (Pas)

Re = (1.667 s^{-1} *0.196^2 m *1000 kg/m^3)/0.01 Pas = **6403.9** → Az áramlás turbulens tartományba esik.

Turbulens áramlás esetén a keverési szám (N_k) egy konstans értéket vesz fel, amit az alábbi ábráról lehet leolvasni. A diagram a Rushton keverő típusra vonatkozik.



Keverési szám függése a Re számtól.

(Forrás: Shiego Katho, Fumitake Yoshida: Biochemical Engineering)

Turbulens áramlás esetén a ke: **30**

A keverési szám: $N_k = t_k * N$

Ahol, t_k: keverési idő (s)

N: fordulatszám (s^{-1})

$30 = t_k * 1.667\ s^{-1}$ → **t_k= 18 s**

(2) 200 rpm esetén: **t_k=9 s**

(3) 300 rpm estén: **t_k= 6 s**

(4) 400 rpm esetén: **t_k= 4.5 s**

4.8.Exercise

A fermentor with an inner vessel diameter of 490 cm and with 50 mm wide buffles contains 100 L medium, whose density is 1000 kg/m^3, its viscosity is 10^{-3} Pas. Agitation is done with 3 six-blade Rushton impellers, each having a diameter of 196 cm. Agitation rate is 150 rpm, aeration rate is 90 L/min. How much energy is introduced into one unit of volume in case of (1) agitation only, (2) combined agitation and aeration?

Solution

Fluid volume: V= 100 L = 0.1 m^3

Agitator rotational speed: N= 150 rpm =2.5 s^{-1}

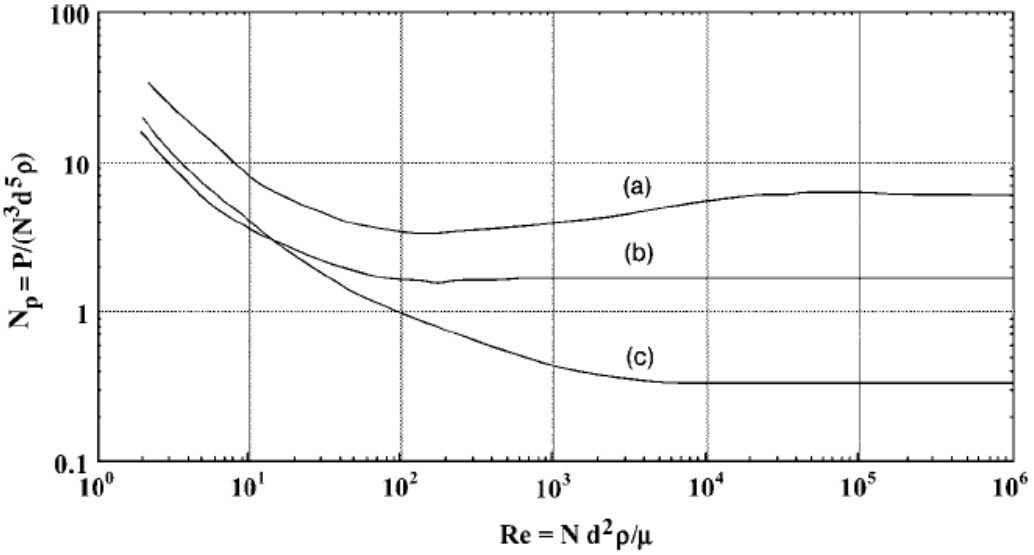
Fluid density: ρ= 1000 kg/m^3

Fluid viscosity: $\mu = 10^{-3}$ Pas
Fermentor diameter: D_T= 490 cm = 0.49 m
Rushton turbine diameter: D_i= 196 cm = 0.196 m
Number of Rushton impellers: 3
Buffles width: w_t= 50 mm
Incoming air: Q= 90 L/min = 0.09 m^3/min

(1) Calculate Reynolds number!

$$Re = \frac{N * D_i^2 * \rho}{\mu}$$

Re = (2.5 s^{-1}*0.196^2 m*1000 kg/m^3)/0.001 Pas = **9.6*10^4** → the flow is turbulent.
Value of the dimensionless Power number (N_p) can be read from the diagram below.



Correlation between Reynolds number and Power number (a) 6-blade Rushton turbine impeller with baffles, (b) 2-blade impeller with baffles, (c) 3-blade impeller with baffles.
(Source: Shiego Katho, Fumitake Yoshida: Biochemical Engineering)

In case of turbulent flow, Power number of the Rushton turbine impeller is N_p=6

$$N_p = \frac{P}{\rho N^3 D_i^5}$$

$$P = N_p N^3 D_i^5 \rho$$

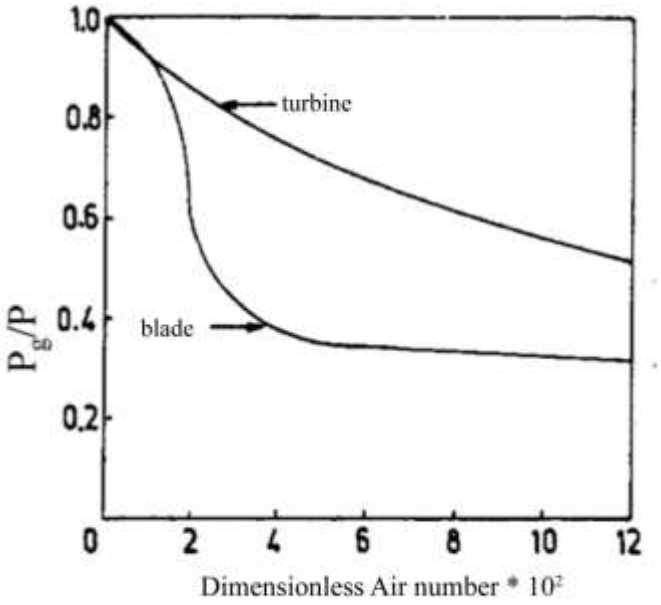
P= 6*2.5^3 s^{-1}*0.196^{5}m*1000 kg/m^3= 27.11 W →for one impeller
For three impellers combined: 3*27.11= 81.35 W
Energy provision (agitation only): P/V= **813.5 W/m^3**

(2) With aeration:
Q = 0.09 m^3/min = 1.5*10^{-3} m^3/s
Dimensionless air number is:

$$N_a = \frac{linear\ air\ flow}{impeller\ rotational\ speed} = \frac{U_G}{v_k} = \frac{Q \Big/ \frac{D_i^2 \pi}{4}}{\pi N D_i}$$

N_a= [1.5*10^{-3} m^3/s /(0.196^{2}m*3.14/4)]/(3.14*2.5 s^{-1}*0.196 m) = 0.032

P_G/P ratio can be determined from the diagram below.



P_G/P vs. Air number

(Source: Sevella Béla: Biomérnöki műveletek és folyamatok)

P_G/P= 0.8 →P_G= 0.8*P= 0.8*81.35W= 65.08W

Energy provision with aeration:

P_G/V= 650.8 W/m^3

4.9.Exercise

Egy 10 m^3 hasznos térfogatú fermentorban a 3 db keverőelem átmérője 1.1 m. Folyadék sűrűsége 1000 kg/m^3. Mennyi legyen a maximális fordulatszám és levegőztetés, ha az egységnyi térfogatba bevitt energia nem lehet több 813.5 W/m^3. Levegőztetés során a levegőztetési szám 0.032 legyen.

Solution

P/V= 813.5 W/m^3

D_i= 1.1 m

Keverőelemek száma: 3

Folyadék térfogata: V= 10 m^3

Folyadék sűrűsége: ρ= 1000 kg/m^3

Turbulens áramlás esetén a Rushton turbina teljesítményszáma: N_p=6

N_a=0.032

Számolja ki a maximális fordulatszámot:

Teljesítményfelvétel levegőztetés nélkül: P= 10*813.5= 8135 W

$$P = N_p N^3 D_i^5 \rho$$

N^3= 8135 W/(1.1^{5}m*6*1000 kg/m^3) = 0.841

N= 0.943 s^{-1}= **56.63 rpm**

Számolja ki a bemenő levegő mennyiségét Na= 0.032 eseté:

$$N_a = \frac{lineáris\ légsebesség}{leverő\ kerületi\ sebessége} = \frac{U_G}{v_k} = \frac{Q \Big/ \frac{D_i^2 \pi}{4}}{\pi N D_i}$$

Q= 0.032*0.943 s^{-1}*1.1m*3.14*((1.1^{2}m*3.14)/4)=0.099 m^3/s= 5.94 m^3/min= **5940 L/min**

4.10. Exercise

Egy fermentort pontosan 100 kg vízzel feltöltjük majd a kezdeti hőmérsékletét 25 °C-ra állítjuk. Ezt követően kiürítjük a duplikátort és elindítjuk a kevertetést 300 rpm-el. Azt figyeltük meg, hogy a víz hőmérséklete 2 óra alatt 5 °C-al emelkedett meg.

(1) Becsülje meg a keverő által leadott energiát 120 órás fermentáció esetén!
(2) Becsülje meg a keverő által felvett teljesítményt, ha a keverő átmérője 196 cm és teljesítményszáma 6.
(3) Az egységnyi térfogatba bevitt energiának hány százaléka alakul hőenergiává?

Solution

(1) Becsülje meg a keverő által leadott hőmennyiséget:

Δt= 2 h

ΔT= 5 °C

A víz fajhője: 4.18 kJ/kg*°C

$$Q_k = (C*m*\Delta T)/\Delta t$$

Q_k = (4.18 kJ/kg°C*100 kg* 5 °C)/2 h= **1045 kJ/h**

120 órás fermentáció alatt 300 rpm kevertetéssel:

Q_k= 120 h*1045 kJ/h= 1.25*10^5 kJ = **125.4 MJ**

(2) A keverő által felvett teljesítmény:

D_i= 196 cm = 0.196 m

N_p= 6

Kevertetés: 300 rpm = 5 s$^-$1

Nem levegőztetett rendszer esetén:

$$P = N_p N^3 D_i^5 \rho$$

P= 6*5^3 s$^-$1*0.196^{5}m*1000 kg/m^3= 216.94 W

3 keverőre esetén: **650.82 W**

Egységnyi térfogatba bevitt energia: 650 W/0.1 m^3= **6.5 kW/m^3**

(3)

Ha az összes energia hővé alakul:

Q_{mech}= P*V*t

Q_{mech}= 6.5 kW/m^3*0.1 m^3*120 h= 78 kWh

1kWh= 3600 KJ

78 kWh= 2.8*10^5 kJ= **280 MJ**

Hány százaléka alakul hővé a keverő által bevitt energiának?

125.4 MJ/280 MJ= **44.7%**

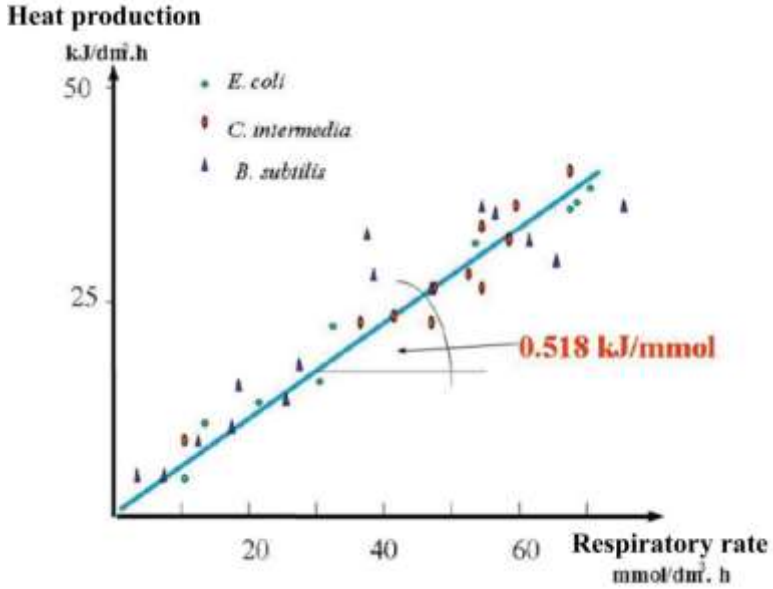
4.11. Exercise

Respiratory activity of an *E. coli* culture is 40 mmol/L*h. Volume is 100 L, temperature is 30 °C, fermentation time is 120 h. Oxygen Uptake Rate (OUR) is considered constant. How much heat is generated by the culture?

Solution

Metabolic heat production: Q_{met} =V* OUR*tgα

tgα can be read from the diagram below:



Oxygen uptake rate vs. metabolic heat production

(Source: Sevella Béla: Biomérnöki műveletek és folyamatok)

Q_{met}= 100 L*40 mmol/Lh *0.518 kJ/mmol= 2072 kJ/h

In 120 h →248640 KJ = **248.64 MJ**

4.12. Exercise

Egy 120 órás fermentáció során a tenyészet metabolikus hőtermelése 248.6 MJ, míg a keverő által leadott energia 125 MJ. Számolja ki mennyi a hűtővíz igényét a 30 °C-os fermentációnak, ha a

hűtővíz hőmérséklete 20 °C. A duplikátorból kilépő víz hőmérséklete minimum 5 °C-al mindig alacsonyabb, mint a fermentáció hőmérséklete. A számolás leegyszerűsítése érdekében tekintse a hűtővíz és a fermentlé hőkapacitását egyenlőnek.

Solution

Az elvonandó hőmennyiség: $Q_{elv}= Q_{met}+Q_k= 248.6$ MJ+125 MJ= 373.6 MJ

Számolja ki mennyi lesz az átlagos hőmérsékletváltozás a duplikátorban:

$\Delta T_{átl}= ((T_{flé}-T_{be})+(T_{flé}-T_{ki}))/2= ((30-20)+(30-25))/2= 7.5$ °C

A víz fajhője: C= 4.18 kJ/kg*°C

$Q_{elv}= C*m*\Delta T$

373600 kJ= 4.18 kJ/kg°C*m*7.5 °C

m= 11917 Kg →11.9 m^3 →**<u>99.1 L/h</u>**

4.13. Exercise

Számolja ki mennyi egy 10 m^3-es 60 óráig tartó fermentációnak a hűtővíz igénye, ha a mikroba légzésének intenzitása 30 mmol/L*h és a keverő egységnyi térfogatba bevitt teljesítménye 1.5 kW/m^3. A keverő által bevitt energiának 44%-a hőenergiává alakul. A fermentációs hőmérséklet 30 °C, míg a hűtővízé 15 °C. A duplikátorból kilépő víz hőmérséklete minimum 5 °C-al mindig alacsonyabb, mint a fermentáció hőmérséklete.

Solution

$Q_{met}= V*$ OUR*tgα

$Q_{met}= 10^4$ L*30 mmol/Lh *0.518 kJ/mmol = $1.55*10^5$ kJ/h

60 h alatt →$9.3*10^6$ kJ = **$9.3*10^3$ MJ**

$Q_{mech}= P*V*t$

$Q_{mech}= 1.5$ kW/m^3*10 m^3*60 h= 900 kWh

900 kWh= $3.24*10^6$ kJ= $3.24*10^3$ MJ →

A keverő által bevitt energia 44%-a alakul hővé: **$1.42*10^3$ MJ**

$\Delta T_{átl}= ((T_{flé}-T_{be})+(T_{flé}-T_{ki}))/2= ((30-15)+(30-25))/2= 10$ °C

A víz fajhője: C= 4.18 kJ/kg*°C

$Q_{elv}= C*m*\Delta T$

$Q_{elv}= 9.3*10^3$ MJ + $1.42*10^3$ MJ = $1.07*10^4$ MJ= $1.07*10^7$ kJ

$m= Q_{elv}/(C*\Delta T)$

m= $1.07*10^7$ kJ /(4.18 kJ/kg°C *10°C)= $2.55*10^5$ kg

1 óra alatt: 4266 kg/h = **4.2 m^3/h**

Megjegyzés: a példa levezetése során figyelmen kívül lett hagyva, hogy a levegőztetett rendszerben kisebb a keverő által bevitt teljesítmény és a bemenő levegő a hő egy részét elvonja.