Medium Design

Biochemical Engineering

ChE-311

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Agenda

- Medium in industry
- Law of the minimum
- Stoichiometric models
- Medium evaluation
- Elemental balances
- Energy yields
- Maximum yields

https://www.google.com/url?sa=i&url=https%3A%2F%2Fpixels.com%2Ffeatured%2F10-human-90-bacteria-microbiology-print-gift-tee-noirty-designs.html%3Fproduct%3Dthrowpillow&psig=AOvVaw2MCi6WaEilDT4xwC6eAoZb&ust=1741960506296000&source=images&cd=vfe&opi=89978449&ved=0CBQQjRxqFwoTCKDw-_Wah4wDFQAAAAAdAAAAABAE

Objectives for medium design in industry

Objectives

- Maximize productivity (by optimizing cell density, conditions for product synthesis)
- Minimize media costs

Requirements for a good medium

1. Business requirements

- Ensure product quality (limit aggregation, fragments etc.)
- Ensure media availability and quality (e.g., GMP)
- Respect health, safety and environment regulation (e.g., no antibiotics)

2. Nutritional requirements

- Provide carbon, nitrogen, phosphor and sulfur source
- Provide essential elements (K, Mg, Fe and trace elements)
- Provide specific nutrients (e.g., vitamins, amino acids, etc.)

3. Other physicochemical requirements

- Ensure buffering capacity (limit pH variability)
- Limit viscosity (ensure good mixing, flat temperature profile, flat dissolved oxygen profile)
- Limit/prevent catabolite repression
- Include induction system for genetic engineered strains
- Respect physiological constraints (e.g., ionic strength, substrate inhibition)

…thus, the five main functions of media are:

1) Supply all essential nutrients necessary for growth and allow production of biomass of defined composition.

2) Ensure “optimum” and “constant” growth conditions over a certain period of time.

3) Control the physiological performance of a microbial culture, e.g.
   - aerobic versus denitrifying growth
   - glucose versus acetate as carbon/energy source
   - nitrate versus ammonia as N-source...

4) Control the maximum specific growth rate (μ_{max}).

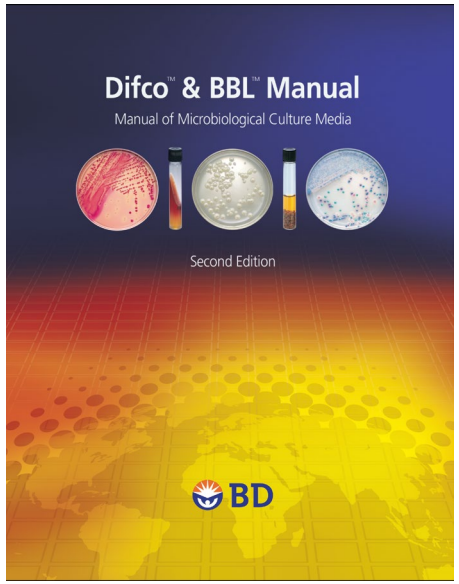
5) Control growth limitation by one specific nutrient according to the “law of the minimum”.

Note: In English language the plural of medium is **media** and not mediums.

Microbial growth media

Media	Purpose
Complex	Grow most heterotrophic organisms
Defined	Grow specific heterotrophs; often mandatory for chemoauto-trophs, photoautotrophs and for microbiological assays
Selective	Suppress unwanted microbes, or encourage desired microbes
Differential	Distinguish colonies of specific microbes from others
Enrichment	Similar to selective media but designed to increase the numbers of desired microorganisms to a detectable level without stimulating the rest of the bacterial population
Reducing	Growth of obligate anaerobes

https://sciencephotogallery.com/featured/bacterial-growth-on-culture-media-daniela-beckmann.html?product=art-print



https://microbiologyclass.net/fermentation-media/

pH optimum for growth

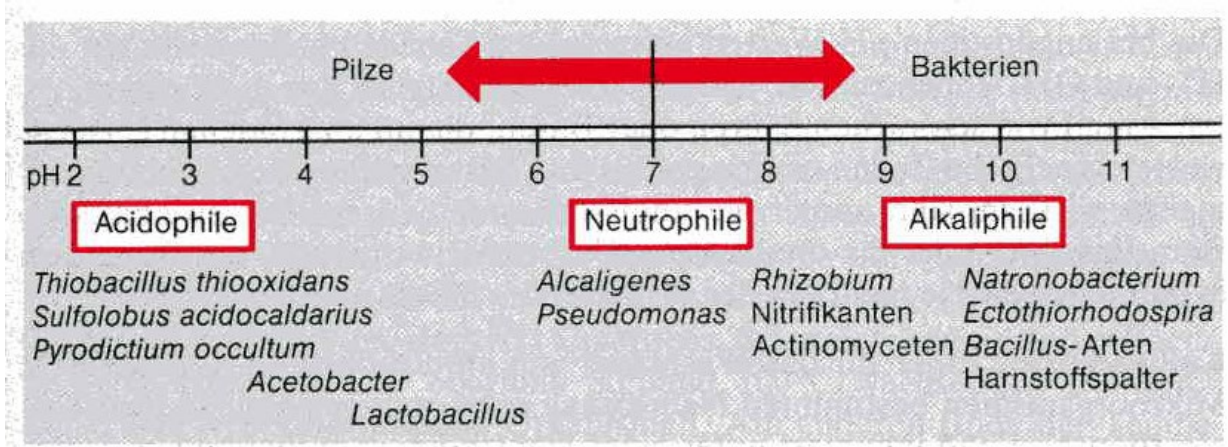


Abb. 6.1 Von Pilzen und verschiedenen Bakterien bevorzugte oder tolerierte pH-Bereiche

Organism	Minimum	Optimum	Maximum
Bacteria	2-5	6.5-7.5	8-11
Yeasts	2-3	4.5-5.5	7-8
Molds	1-2	4.5-5.5	7-8

Aspergillus niger: pH 7: oxalic acid, pH 2: citric acid

Typical medium composition

Factors	Range of concentration [% w/v]	Examples
Carbon source	0.5 - 20	Glucose, sucrose, starch, molasses, dextrins, alcohols, corn meal, glycerol, lipids
Nitrogen source	0.1 - 10	Ammonia gas, ammonium salts, casein hydrolysates, glutamic acid, nitrates, peptones, urea, yeast extract
Phosphorus	0.1 - 2	Corn steep liquor, phosphates
Sulfur	0.1 - 1	Methionine, proteins, sulfates
Other nutrients	<1	Iron salts, magnesium salts, oxygen, potassium, trace elements, vitamins

Defined medium

The defined medium consists of many substrates that are all - **without exception**- well-described and their chemical structure is known.

Table 6.1. *Example of a simple synthetic nutrient solution*

K_2HPO	0.5 g
NH_4Cl	1.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$FeSO_4 \cdot 7H_2O$	0.01 g
$CaCl_2 \cdot 2H_2O$	0.01 g
Glucose	10.0 g
Water	1000 ml
Trace element stock solution	1 ml

Table 6.2. *Trace element stock solution*

$ZnCl_2$	70 mg
$MnCl_2 \cdot 4H_2O$	100 mg
$CoCl_2 \cdot 6H_2O$	200 mg
$NiCl_2 \cdot 6H_2O$	100 mg
$CuCl_2 \cdot 2H_2O$	20 mg
$NaMoO_4 \cdot 2H_2O$	50 mg
$Na_2SeO_3 \cdot 5H_2O$	26 mg
[$NaVO_3 \cdot H_2O$	10 mg]
[$Na_2WO_4 \cdot 2H_2O$	30 mg]
HCl (25%) 1.0 ml	
Distilled water	to 1000 ml

[ ], required by only a few organisms.

Table 6.3. *Well-established solution of vitamins for soil and water bacteria*

Biotin	0.2 mg
Nicotinic acid	2.0 mg
Thiamine	1.0 mg
4-Aminobenzoate	1.0 mg
Pantothenate	0.5 mg
Pyridoxamine	5.0 mg
Cyanocobalamine	2.0 mg
Distilled water	100 ml

2–3 ml of the solution are added per 1000 ml nutrient solution

Note:
It is well-known that all trace metals easily form highly insoluble phosphate salts and precipitate in growth media. This can be avoided by the addition of metal-chelating agents such as EDTA, NTA, or sometimes also carboxylic acids such as citrate or tartrate.

Function of trace elements

Element	Cellular function
Chromium (Cr)	Required by mammals for glucose metabolism; no known microbial requirement
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Certain proteins, notably those involved in respiration, for example, cytochrome *c* oxidase; or in photosynthesis, for example, plastocyanin; some superoxide dismutases
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme of photosystem II in oxygenic phototrophs
Molybdenum (Mo)	Present in various flavin-containing enzymes; also in molybdenum nitrogenase, nitrate reductase, sulfite oxidase, DMSO-TMAO reductases, some formate dehydrogenases, oxotransferases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles (for example, aldehyde:ferredoxin oxidoreductase of *Pyrococcus furiosus*)
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Present in the enzymes carbonic anhydrase, alcohol dehydrogenase, RNA and DNA polymerases, and many DNA-binding proteins

Microbial nutrition: Growth factors

Organic compounds are required by some bacteria (particularly pathogens).

E.g.: Streptococcus, Lactobacillus, Leuconostoc (lactic acid bacterium): Complex vitamin requirements

Vitamin	Function
p-Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; β-decarboxylations; some CO_2 fixation reactions
Cobalamin (B_{12})	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD^+; electron transfer in oxidation–reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN, FAD in flavoproteins involved in electron transport
Thiamine (B_1)	α-Decarboxylations; transketolase
Vitamins B_6 (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell

Anaerobic growth media

HIMEDIA®

Technical Data

Anaerobic Agar

M228

Intended Use:

Anaerobic Agar is recommended for the cultivation of anaerobic bacteria, especially *Clostridium* species and other anaerobic organisms from clinical and non-clinical samples.

Composition**

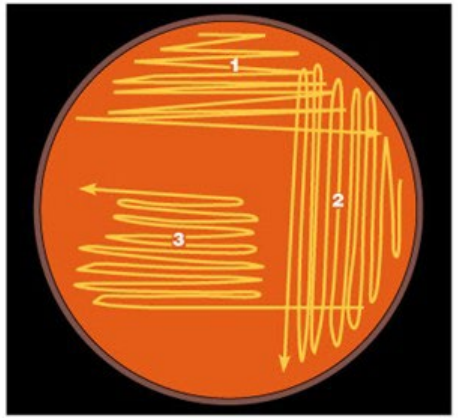
Ingredients	Gms / Litre
Tryptone	20.000
Dextrose (Glucose)	10.000
Sodium chloride	5.000
Sodium thioglycollate	2.000
Sodium formaldehyde Sulfoxylate	1.000
Methylene blue	0.002
Agar	20.000
Final pH (at 25°C)	7.2±0.2

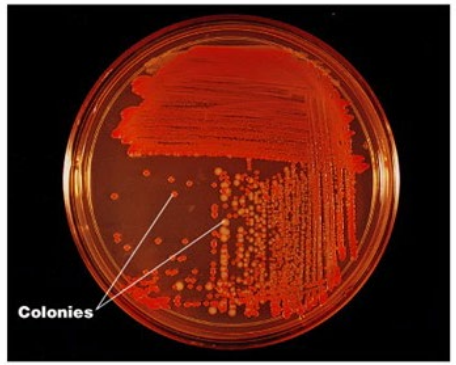
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

http://himedialabs.com/TD/M228.pdf





Agar plates are usually prepared with 15-20 g L^{-1} of agar

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Principle And Interpretation

Anaerobic Agar was originally designed for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates (1). This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible (2,3). Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements (3). Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor (4).

This medium contains sodium thioglycollate and sodium formaldehyde sulphoxylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen. Casein enzymic hydrolysate and dextrose provide essential nutrients while sodium chloride maintains osmotic equilibrium.

Dispense 50-60 ml medium per 95 x 20 mm plate. For best results, use porous tops for the plates during solidification to get the dry surface. Inoculation can be done by streaking or smearing. Cover the inoculated plate with sterile Brewer Anaerobic Petri dish cover. Incubate aerobically, as desired. When standard plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium. After solidification, incubate anaerobically as required by particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

Type of specimen

Clinical- stool, blood, abscess

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

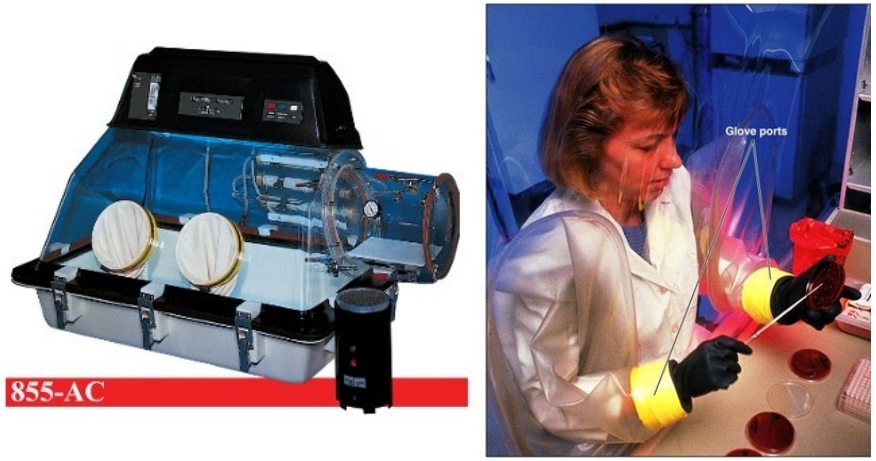
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Important information!

Please refer disclaimer Overleaf.

Anaerobic growth chambers

Glovebox for anaerobic microorganisms



https://www.biocompare.com/Lab-Equipment/9730-Anaerobic-Chambers/

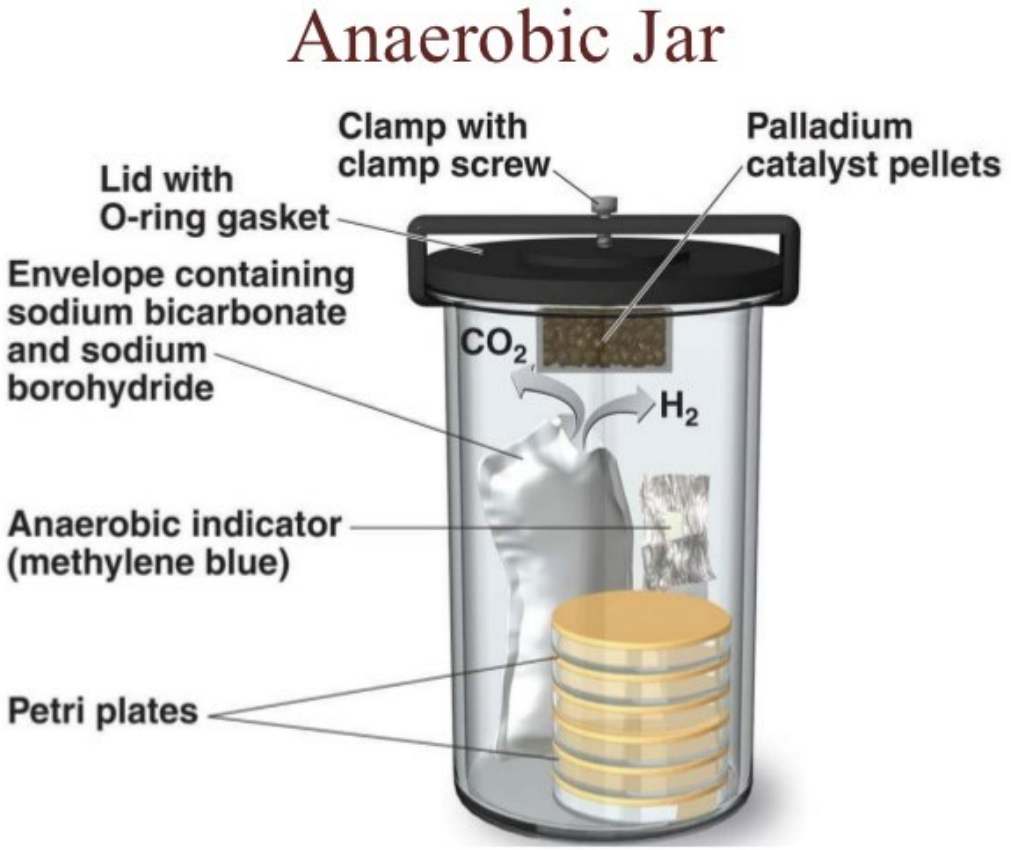


Figure 6.6

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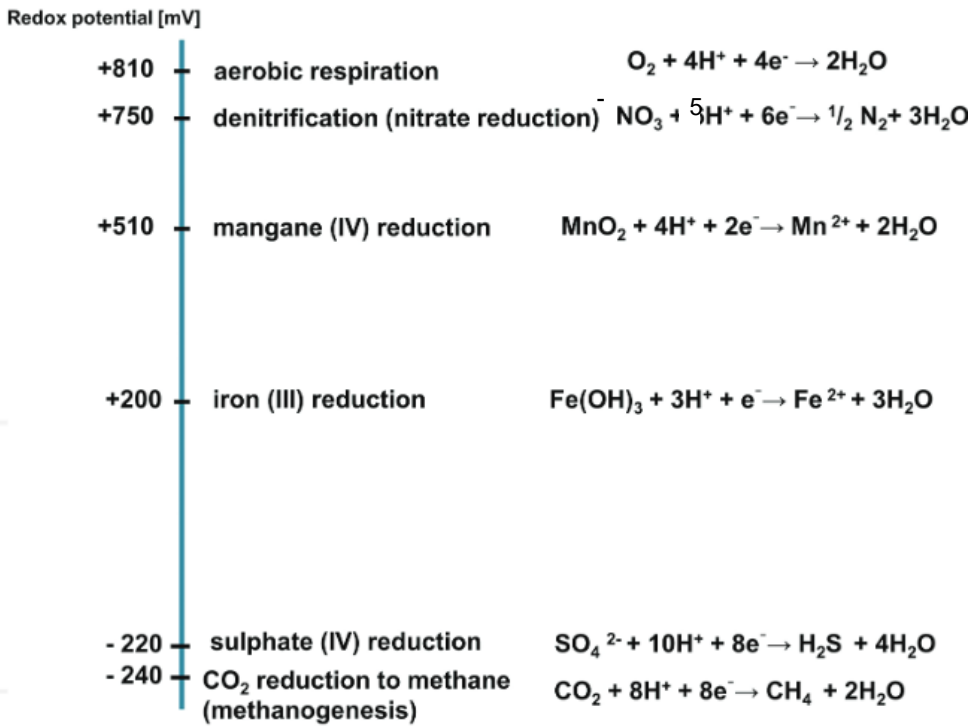
Energy and biomass yields including anaerobic growth



Growth under anaerobic conditions results in lower energy and growth yields and slower specific growth rates.

Electron donors

Molecular hydrogen:	Y_{x/H_2}	= 12 g/mol
Thiosulfate:	Y_{x/S_2O_3}	= 4 g/mol
Fe^{2+}:	$Y_{x/Fe^{2+}}$	= 0.35 g/mol
NH_4^+ to NO_2^-:	Y_{x/NH_4^+}	= 1.3 - 2.6 g/mol
NO_2^- to NO_3^-:	Y_{x/NO_2^-}	= 0.9 - 1.8 g/mol

Electron acceptors

Molecular oxygen:	Y_{X/O_2}	= 10_a – 42_b g/mol
NO_3^- to N_2	Y_{X/NO_3^-}	= 27 g/mol$_c$
NO_2^- to N_2	Y_{X/NO_2^-}	= 17 g/mol$_c$
N_2O to N_2	Y_{X/N_2O}	= 9 g/mol$_c$

a for growth with reduced substrates such as methane or n-alkanes

b for growth with more oxidized substrates such as glucose

c for growth of *Paracoccus denitrificans* with glutamate as carbon substrate

Stoichiometry as a tool

Cell growth and product formation are complex processes reflecting the overall kinetics and stoichiometry of the thousands of intracellular reactions that can be observed in a cell.

For many process calculations, we wish to compare potential substrates in terms of ***cell mass yield***, ***product yield***, *or* ***evolution of heat***. Also we need to know how close to its thermodynamic limit a system is operating.

If a system is close to its thermodynamic limit, it would be unwise to try to improve production through mutation or genetic engineering.

Law of the minimum

One nutrient limits the amount of biomass that can be produced in a system. All other nutrients are in excess.

Justus von Liebig, 1840

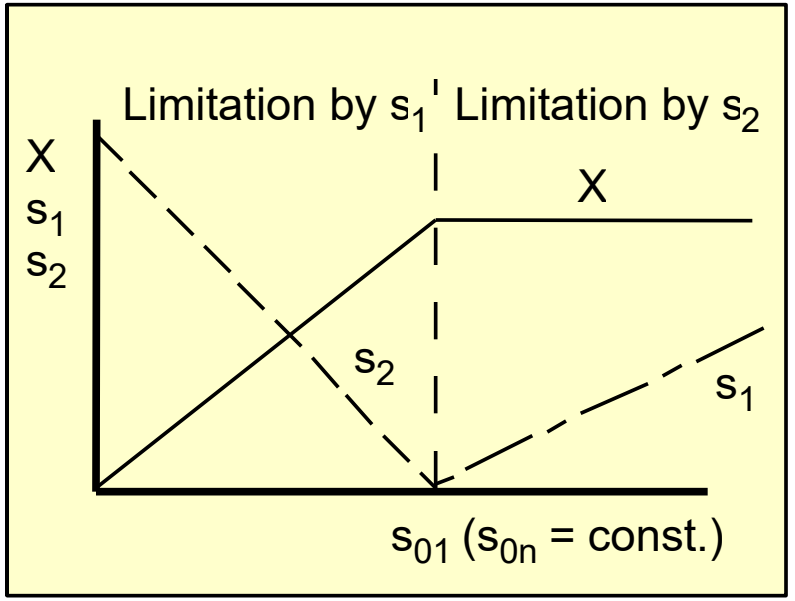
Freiherr Justus von Liebig

12. 5. 1803 – 18. 4. 1873

http://rs-maxdorf.bildung-rp.de

Kaliapparat

Growth limitations by nutrients



Stoichiometric limitation

Stoichiometry according to Monod model

There is almost a constant relationship between the growth rates of biomass and of the substrate uptake.

$$Y_{x/s} = -\frac{r_x}{r_s} = const.$$

$$r_x = \frac{dx}{dt}$$

$$r_s = \frac{ds}{dt}$$

} Valid only in exponentially growing cultures

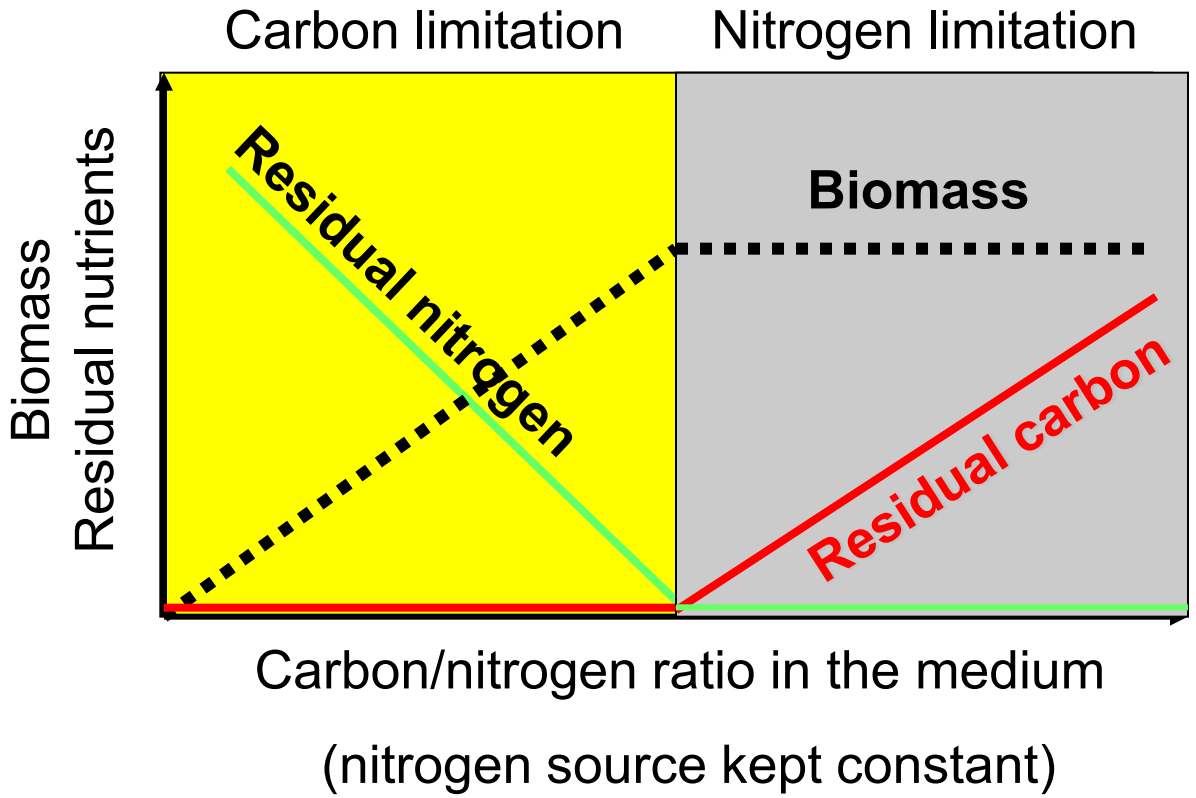
Generally, Yx/s is considered to be a constant value under exponential growth conditions.

$$Y_{x/s} = \frac{\Delta x}{\Delta s} = \frac{x - x_0}{s_0 - s}$$

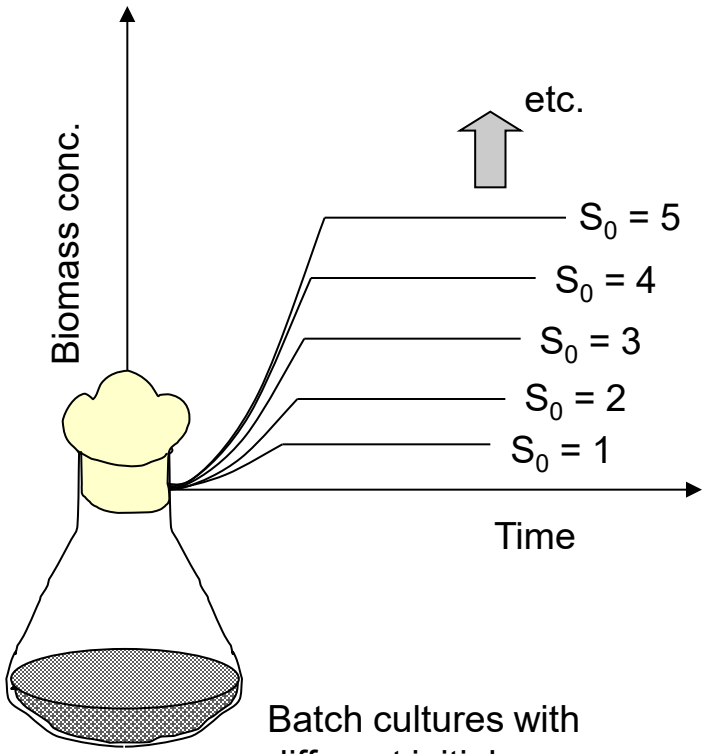
[g g^{-1}] or [g mol^{-1}]

The law of the minimum in the view of a microbiologist

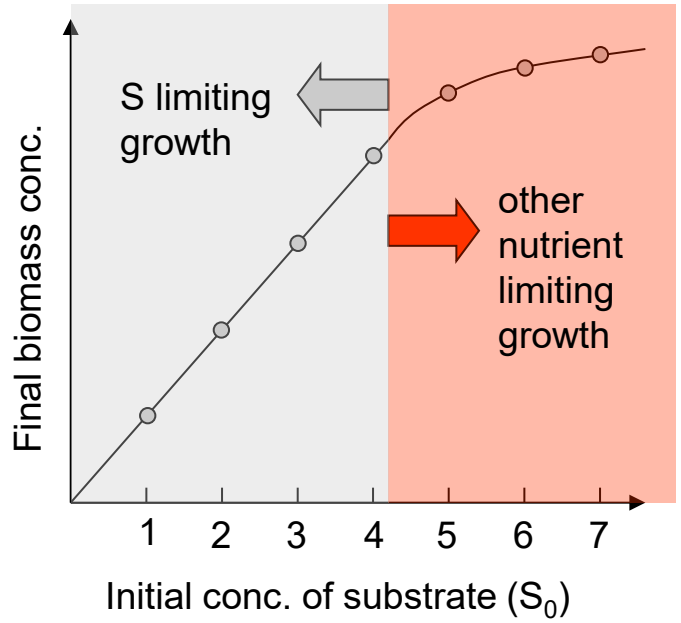
$X = Y_{X/C} * C$ $X = Y_{X/N} * N$



Testing a medium for the growth-limiting substrate



Batch cultures with different initial concentrations of S_0



Medium check

TABLE 4.4 Examples of cu

Defined culture medium for *Escherichia coli*

K_2HPO_4	7 g
KH_2PO_4	2 g
$(NH_4)_2SO_4$	1 g
$MgSO_4$	0.1 g
$CaCl_2$	0.02 g
Glucose	4–10 g
Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo)	2–10 μg each
Distilled water	1000 ml
pH 7	

(a)

Concentration of DW that can be produced from N = [conc. N in medium] x $Y_{X/N}$:

1 g $(NH_4)_2SO_4$ contains ~ 0.21 gN

0.21 gN x 8 gCDW/gN = 1.7 gCDW

Concentration of DW that can be produced from C = [conc. C in medium] x $Y_{X/C}$:

4-10 g Glucose contains ~ 1.6-4 gC

1.6-4 gC x 1 gCDW/gC = 1.6-4 gCDW

* **With 4 g glucose there is just enough nitrogen in the medium,**

-> **but if 10 g glucose is supplied, there is not enough nitrogen in this medium!**

Theoretical approach of designing a medium

Systematics of elemental balancing for microbial reactions

Several ways of setting up elemental balances are described in the literature. The first applies directly chemical formulas and the related stoichiometric coefficients.

$$CH_{sH}O_{sO} + Y_{O/S}\,O_2 + Y_{N/S}NH_3 \longrightarrow$$

$$Y_{X/S}CH_{xH}N_{xN}O_{xO} + Y_{C/S}CO_2 + Y_{W/S}H_2O$$

Another useful concept uses C-moles (one atom of C in each molecule) with correspondingly modified stoichiometric coefficients.

Elemental composition of *Escherichia coli*

(From R.Y. Stanier, E.A. Adelberg and J. Ingraham, 1976, The Microbial World, *4th edn, Prentice-Hall, New Jersey)*

Element	*% dry weight*
C	50
O	20
N	14
H	8
P	3
S	1
K	1
Na	1
Ca	0.5
Mg	0.5
Cl	0.5
Fe	0.2
All others	0.3

Biomass is represented by

$CH_{\alpha}O_{\beta}N_{\delta}$

The formula is a reflection of the biomass composition

90-95% of biomass can be accounted for by four major elements: C, H, N, and O.

Elemental balances

We need to know the stoichiometry of the growth process i.e. number of substrates and products.

Accurate estimation of biomass and **biomass composition** (elemental analysis).

Consider a biological reaction in which only biomass, CO_2 and H_2O are formed (i.e. no extracellular products) from a carbohydrate substrate:

$$CH_{sH}O_{sO} + Y_{O/S}\,O_2 + Y_{N/S}NH_3 \longrightarrow Y_{X/S}CH_{xH}N_{xN}O_{xO} + Y_{C/S}CO_2 + Y_{W/S}H_2O$$

Elemental balances

$CH_{sH}O_{sO}$ represents 1 C- mole of carbohydrate substrate

$CH_{xH}N_{xN}O_{xO}$ represents 1 C- mole of biomass

One C- mole represents the amount of a compound containing one carbon atom:

e.g.
1 mole glucose = $C_6H_{12}O_6$
(molecular weight = 180)

1 C-mole glucose = CH_2O
(C- mole formula weight (CMFW) = 30)

Exercise: determine C-mole formula and CMFW of:
ethanol (C_2H_5OH, acetate (CH_3COOH), glycerol ($C_3H_8O_3$) etc.

Elemental balances

$$CH_{sH}O_{sO} + Y_{O/S}\,O_2 + Y_{N/S}NH_3 \longrightarrow$$

$$Y_{X/S}CH_{xH}N_{xN}O_{xO} + Y_{C/S}CO_2 + Y_{W/S}H_2O$$

An elemental balance over the stoichiometric equation yields:

C: $1 = Y_{X/S} + Y_{C/S}$

H: $sH + 3Y_{N/S} = Y_{X/S}\,xH + 2Y_{W/S}$

N: $Y_{N/S} = Y_{X/S}\,xN$

O: $sO + 2Y_{O/S} = Y_{X/S}xO + 2Y_{C/S} + Y_{W/S}$

Therefore 5 yield coefficients (plus enthalpy yield) for 4 conservation balance constraints (atoms C, H, N, O plus energy) therefore degree of freedom 5-4 = 1

In principle measuring just one yield allows determination of all others.

Elemental balances

To make a carbon balance one requires:

C: $1 = Y_{X/S} + Y_{C/S}$

Knowledge of biomass yield $Y_{X/S}$ and carbon dioxide yield $Y_{C/S}$ (note: these are C- molar yields):

$Y_{X/S}$ = C- mole biomass formed per C- mole substrate consumed

$Y_{C/S}$ = mole CO_2 formed per C- mole substrate consumed

(note: 1 C-mole CO_2 = 1 mole CO_2)

This requires measurement of:

Biomass (cell dry weight and elemental analysis), substrate (e.g. glucose), carbon dioxide (infra-red CO_2 analyzer).

Elemental balances

To establish a nitrogen balance we need:

$$\mathbf{N:}\ \ Y_{N/S} = Y_{X/S}\, x_N$$

Simply require knowledge of biomass yield $Y_{X/S}$ and fraction of nitrogen in biomass.

This requires measurement of:

Biomass (cell dry weight and elemental analysis) and of the substrate (e.g., glucose).

Therefore:

We need to know the elemental composition of biomass: how much C, H, N, and O (i.e., knowledge of x_H, x_N and x_O).

Example

$$C_wH_xO_yN_z + a\ O_2 + b\ H_gO_hN_i \longrightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O$$

Balance for C, H, O, N and RQ:

C balance: $w = c + d$

H balance: $x + bg = c\alpha + 2\ e$

O balance: $y + 2a + bh = c\beta + 2\ d + e$

N balance: $z + bi = c\delta$

RQ = moles CO_2 produced / moles O_2 consumed = d/a

Elemental composition of different organisms

Microrganism	% weight C	H	N	O	Ash	C-mole formula	Formula wt
A. aerogenes	48.7	7.3	13.9	29.0	8.9	$CH_{1.78}N_{0.24}O_{0.33}$	22.5
K. aerogenes	50.6	7.3	13.0	29.0	8.0	$CH_{1.74}N_{0.22}O_{0.43}$	22.5
Bacteria	53.0	7.3	12.0	19.0	8.0	$CH_{1.67}N_{0.20}O_{0.27}$	20.7
Bacteria	47.1	7.8	13.7	31.3		$CH_{2.00}N_{0.25}O_{0.50}$	25.5
Yeast	47.0	6.5	7.5	31.0	8.0	$CH_{1.67}N_{0.13}O_{0.40}$	23.5
Yeast	50.3	7.4	8.8	33.5		$CH_{1.75}N_{0.15}O_{0.50}$	23.9
Yeast	44.7	6.2	8.5	31.2		$CH_{1.64}N_{0.16}O_{0.52}$	26.9
C.utilis	50.0	7.6	11.1	31.3		$CH_{1.82}N_{0.19}O_{0.47}$	24.0
C.utilis	50.3	7.7	11.0	30.8		$CH_{1.82}N_{0.19}O_{0.46}$	23.9
C.utilis	46.9	7.2	10.9	35.0		$CH_{1.84}N_{020}O_{0.56}$	25.6
General:						$\boldsymbol{CH_{1.8}O_{0.5}N_{0.2}}$	***24.6***

Elemental composition of cells ***varies between*** **species** and as function of growth conditions, e.g., carbon source, nitrogen limitation, oxygen limitation, growth rate, etc.

Elemental analysis required (C, H, N) on **dried** biomass (water- free, since any water present would give values for H and O).

Residual water and ash content must be determined.

Elemental balances

(When one metabolic product also formed)

$$CH_{sH}O_{sO} + Y_{O/S}\,O_2 + Y_{N/S}NH_3 \longrightarrow$$

$$Y_{X/S}CH_{xH}N_{xN}O_{xO} + Y_{P/S}CH_{pH}N_{pN}O_{pO} + Y_{C/S}CO_2 + Y_{W/S}H_2O$$

An elemental balance over the stoichiometric equation yields:

C: $1 = Y_{X/S} + Y_{P/S} + Y_{C/S}$

H: $s_H + 3Y_{N/S} = Y_{X/S}\,x_H + Y_{P/S}\,p_H + 2Y_{W/S}$

N: $Y_{N/S} = Y_{X/S}x_N + Y_{P/S}p_N$

O: $s_O + 2Y_{O/S} = Y_{X/S}x_O + Y_{P/S}p_O + 2Y_{C/S} + Y_{W/S}$

Therefore 6 yield coefficients (plus enthalpy yield) for 4 conservation balance constraints (atoms C, H, N, O plus energy) therefore degrees of freedom 6- 4 = 2.

In principle measuring just two yields allows determination of all others.

Elemental balances

(When one metabolic product is also formed)

To prepare a carbon balance, we require:

$$\mathbf{C:} \quad \mathbf{1 = Y_{X/S} + Y_{P/S} + Y_{C/S}}$$

Knowledge of biomass yield $Y_{X/S}$, product yield $Y_{P/S}$ and carbon dioxide yield $Y_{C/S}$ (note: these are C- molar yields):

$Y_{X/S}$ = C- mole biomass formed per C- mole substrate consumed
$Y_{P/S}$ = C- mole product formed per C- mole substrate consumed
$Y_{C/S}$ = mole CO_2 formed per C- mole substrate consumed (note: 1 C-mole CO_2 = 1 mole CO_2)

This requires measurement of:

- biomass (cell dry weight and elemental analysis)
- substrate (e.g., glucose)
- carbon dioxide (infra- red CO_2 analyzer)
- metabolic product, e.g., ethanol, acetate etc.

Elemental balances

To prepare a nitrogen balance we require:

$$\text{N:} \quad Y_{N/S} = Y_{X/S}x_N + Y_{P/S}p_N$$

Simply require knowledge of biomass yield $Y_{X/S}$ product yield $Y_{P/S}$ and and fraction of nitrogen in biomass (x_N) and product (p_N) .

This requires the measurement of:

- biomass (cell dry weight, elemental analysis)
- substrate (e.g. glucose)
- product

Degree of reduction

Elemental balances provide little information about the energetics of the bioprocess- although they are very useful in determining the precision of measurements and the presence of metabolic products due to shifts in metabolism.

The degree of reduction can be used in association with elemental balances to provide thermodynamic information such as heat production by the process.

The degree of reduction, γ, of a compound is defined as the number of equivalents of available electrons per C-mole of the compound.

The available electrons are those electrons in a compound which would be transferred to oxygen during combustion (oxidation) to CO_2, H_2O and NH_3.

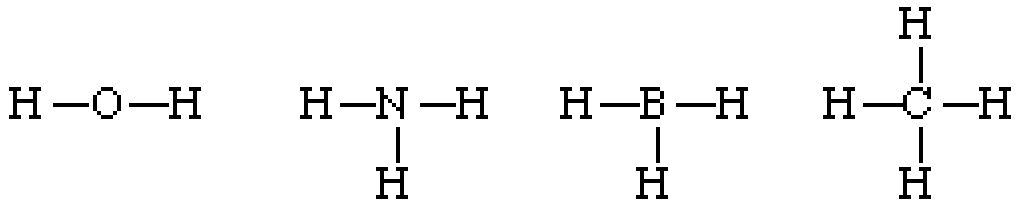
A high degree of reduction indicates a low degree of oxidation.

Degree of reduction

The number of available electrons found in organic material is calculated from the ***valence*** of the various elements:

The valence of an atom is the number of bonds that it can do. To be clearer: a valence of 1 means that an atom can bound only 1 atom which is also termed monovalent. In analogy there are atoms that are divalent, trivalent or tetravalent.

The number of atoms which are typically bonded to a given atom is termed the valence of that atom. Thus, in the examples shown below, hydrogen would have a valence of one, oxygen would have a valence of two, nitrogen and boron would have a valence of three, and carbon would have a valence of four.



Degree of reduction

Degree of reduction of important elements:

C = +4
H = +1
N = -3 *
O = -2
P = +5
S = +6

The degree of reduction, γ, of any element in a compound is equivalent to its valency e.g. γ_C in CO_2 = +4 but γ_{CO2} = 0

Calculation of reduction degree of glucose ($\gamma_{glucose}$):

Glucose = $C_6H_{12}O_6$
C-mole formula = CH_2O
$\gamma_{glucose}$ = 4 + 2 –2 = 4

* Number of available electrons for N depends on reference state: -3 for NH_3, 0 for molecular N (N_2), 3 for HNO_2, and 5 for HNO_3.

Degree of reduction

Calculation of reduction degree of biomass:

biomass formula = $CH_{1.82}N_{0.19}O_{0.47}$

$\gamma_{biomass} = 4 + 1.82 - (0.19 \times 3) - (0.47 \times 2) = 4.31$

Degree of reduction of important compounds:

Calculation of reduction degree of glutamine:

Glutamine = $C_5H_{10}O_3N_2$

C-mole formula = $CH_2O_{0.6}N_{0.4}$

$\gamma_{glutamine} = 4 + 2 - 1.2 - 1.2 = 3.6$

Degree of reduction

General calculation of reduction degree of a substrate:

Substrate formula = $C_wH_xO_yN_z$

Number of available électrons n_e = $4w + x - 2y - 3z$

$\gamma_s = (4w + x - 2y - 3z) / w$

Degree of reduction for CO_2, H_2O and NH_3 is Zero (=0)

Electrons available for transfer to oxygen are conserved during metabolism.

Degree of reduction

Exercises

Calculate reduction degrees of :

1. Ethanol (C_2H_6O)
2. D(+)-Xylose ($C_5H_{10}O_5$)
3. Lactic acid ($C_3H_6O_3$)
4. n-Butanol ($C_4H_{10}O$)
5. Palmitic acid ($C_{16}H_{32}O_2$)
6. Other compounds of your choice

And calculate the C-molar formula and CMFW for each compound

Compound	C-mole formula	γ_s	CMFW
Biomass	$CH_{1.82}N_{0.19}O_{0.47}$	4.31	24
Methane	CH_4	8.00	16
Methanol	CH_3OH	6.00	32
Ethanol	*$CH_3O_{0.5}$*	*6.00*	*23*
Glycerol	$CH_{2.67}O$	4.67	30.67
Acetic acid	CH_2O	4.00	30
Lactic acid	*CH_2O*	*4.00*	*30*
Glucose	CH_2O	4.00	30
Formaldehyde	CH_2O	4.00	30
Gluconic acid	$CH_2O_{1.17}$	3.67	32.72
Glutamine	$CH_2O_{0.6}N_{0.4}$	3.60	29.20
Succinic acid	$CH_{1.5}O$	3.50	29.50
Citric acid	$CH_{1.3}O_{1.17}$	2.96	32
Formic acid	CH_2O_2	2.00	46

Energy balance

$$CH_{sH}O_{sO} + Y_{O/S}\,O_2 + Y_{N/S}NH_3 \longrightarrow$$

$$Y_{X/S}CH_{xH}N_{xN}O_{xO} + Y_{P/S}CH_{pH}N_{pN}O_{pO} + Y_{C/S}CO_2 + Y_{W/S}H_2O$$

Degree of reduction balance:

$$\gamma_s = 4 + s_H - 2s_O$$
$$\gamma_X = 4 + x_H - 3x_N - 2x_O$$
$$\gamma_P = 4 + p_H - 3p_N - 2p_O$$
$$\gamma_{CO2} = 0$$
$$\gamma_{H2O} = 0$$

The stoichiometry can be used to generate an elemental balance (for the elements C,H,N,O and available electrons), mass balance, available electron balance and energy balance.

Energy balance

Of the equations only 5 are independent. However, the more equations that are written, and the more the measurements are made, the more redundancy has the system.
This redundancy may be used to test for data consistency.

Since it is impossible to measure the amount of water formed in the bioprocess it is unwise to make a balance on oxygen or hydrogen.

Generally one would choose to make balance on carbon, nitrogen and available electrons:

$$\mathbf{C:} \quad 1 = Y_{X/S} + Y_{P/S} + Y_{C/S}$$

$$\mathbf{N:} \quad Y_{N/S} = Y_{X/S} x_N + Y_{P/S} p_N$$

$$\gamma: \quad \gamma_s - 4\, Y_{O/S} = Y_{X/S}\, \gamma_x + Y_{P/S}\, \gamma_p$$

Note: Degree of reduction of NH_3, CO_2 and H_2O = 0

Theoretical oxygen demand

$$C_wH_xO_yN_z + a\ O_2 + b\ N_3 \longrightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O + f\ C_jH_kO_zN_m$$

Electron balance: $\boxed{w\ \gamma_s - 4\ a = c\ \gamma_x + j\ f\ \gamma_p}$

This equation can be re-arranged:

$$\boxed{Y_{O/S} = a = ¼\ (w\gamma_s - c\gamma_x - jf\gamma_p)}$$

Oxygen demand can be calculated withouth knowing the CO_2 prodcution!

Energy balance

$$C_wH_xO_yN_z + a\ O_2 + b\ NH_3 \longrightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O + f\ C_jH_kO_zN_m$$

$$w\gamma_s - 4a = c\ \gamma_x + f\ j\gamma_p$$

This equation can be re-arranged to determine the fraction of available electrons in the substrate which have been conserved in each species:

$$1 = \frac{c\gamma_X}{w\gamma_S} + \frac{f\,j\gamma_P}{w\gamma_S} + \frac{4a}{w\gamma_S}$$

$$1 = \xi_X + \xi_P + \varepsilon$$

ξ_X = **fraction of available electrons incorporated into biomass**, or the energy yield coefficient (ratio of the heats of oxidation of biomass produced to the substrate consumed.

ξ_P = **fraction of available electrons incorporated into extracellular products** (fraction of energy of the substrate contained in the product).

ε = **fraction of available electrons transferred to oxygen** (ratio of heat evolved from S to heat of combustion of S (C-moles)).

Maximum possible yield

In the absence of product formation, if all electrons were used for biomass synthesis ($\mathbf{1 = \xi_X}$; $\gamma_x = 4.2$ if composition of cells is unknown)

$$C_{max} = \quad \mathbf{Y_{X/S\ max}} = \frac{\mathbf{w\gamma_S}}{\gamma_x} \qquad [\text{mol mol}^{-1}]$$

The maximum possible product yield in the absence of biomass synthesis can be determined:

$$f_{max} = \quad \mathbf{Y_{P/S\ max}} = \frac{\mathbf{w\gamma_S}}{\mathbf{j\gamma_P}} \qquad [\text{mol mol}^{-1}]$$

Maximum Biomass yield can be expressed in terms of mass or as number of C-atoms in the biomass ***per substrate C atom*** consumed. These quantities are sometimes known as ***thermodynamic maximum biomass yields:***

$$\mathbf{Y_{X/S\ max}} = \frac{\dfrac{\mathbf{w\gamma_S}}{\gamma_x}\ (\text{MW cells})}{(\text{MW substrate})} \qquad [\text{g g}^{-1}]$$

Maximum possible yield

Carbon yield (C_{max}/w)

Substrate	Formula	γ_s	Thermodynamic maximum yield corresponding to $1 = \xi_X$	
			Carbon yield	Mass Yield
Alkanes				Y_{XS}, max
Hexadecane (n)	$C_{16}H_{34}$	6.1	1.5	2.5
Alcohols				
Methanol	CH_4O	6.0	1.4	1.1
Ethanol	C_2H_6O	6.0	1.4	1.5
Glycerol	$C_3H_8O_3$	4.7	1.1	0.9
Carbohydrates				
Glucose	$C_6H_{12}O_6$	**4.0**	**0.95**	**0.8**
Sucrose	$C_{12}H_{22}O_{11}$	4.0	0.95	0.8
Starch	$(C_6H_{10}O_5)_x$	4.0	0.95	0.9
Organic acids				
Formic acid	CH_2O_2	2.0	0.5	0.3
Acetic acid	$C_2H_4O_2$	4.0	0.95	0.8
Lactic acid	$C_3H_6O_3$	4.0	0.95	0.8
Fumaric acid	$C_4H_4O_4$	3.0	0.7	0.6

C_{max} /w: Number of C-atoms in the biomass per substrate C atom consumed

High g_s : high energy content

Theoretical predictions of yield coefficients

In aerobic fermentations:

growth yield per available electron in oxygen molecules is approx. 3.14 $\pm$ 0.11 gdw cells / electron when ammonia is used as nitrogen source.
The number of available electrons per oxygen molecule (O_2) is four.

e.g: $C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$

the total number of available electrons in 1 mole glucose is 24

$\Rightarrow Y_{x/s} = 24\ (3.14) = 76$ gdw cells / mol
or Yx/s = 76/180 = 0.4 gdw cells / g glucose

Most measured values of Yx/s (aerobic growth on glucose) = 0.38 – 0.51 g/g

TABLE 6.1 Summary of Yield Factors for Aerobic Growth of Different Microorganisms on Various Carbon Sources

Organism	Substrate	Y_{XS} g/g	Y_{XS} g/mol	Y_{XS} g/g-C	Y_{XO_2}[a] g/g
Enterobacter aerogenes	Maltose	0.46	149.2	1.03	1.50
	Mannitol	0.52	95.2	1.32	1.18
	Fructose	0.42	76.1	1.05	1.46
	Glucose	0.40	72.7	1.01	1.11
Candida utilis	Glucose	0.51	91.8	1.28	1.32
Penicillium chrysogenum	Glucose	0.43	77.4	1.08	1.35
Pseudomonas fluorescens	Glucose	0.38	68.4	0.95	0.85
Rhodopseudomonas spheroides	Glucose	0.45	81.0	1.12	1.46
Saccharomyces cerevisiae	Glucose	0.50	90.0	1.25	0.97
Enterobacter aerogenes	Ribose	0.35	53.2	0.88	0.98
	Succinate	0.25	29.7	0.62	0.62
	Glycerol	0.45	41.8	1.16	0.97
	Lactate	0.18	16.6	0.46	0.37
	Pyruvate	0.20	17.9	0.49	0.48
	Acetate	0.18	10.5	0.43	0.31
Candida utilis	Acetate	0.36	21.0	0.90	0.70
Pseudomonas fluorescens	Acetate	0.28	16.8	0.70	0.46
Candida utilis	Ethanol	0.68	31.2	1.30	0.61
Pseudomonas fluorescens	Ethanol	0.49	22.5	0.93	0.42
Klebsiella sp.	Methanol	0.38	12.2	1.01	0.56
Methylomonas sp.	Methanol	0.48	15.4	1.28	0.53
Pseudomonas sp.	Methanol	0.41	13.1	1.09	0.44
Methylococcus sp.	Methane	1.01	16.2	1.34	0.29
Pseudomonas sp.	Methane	0.80	12.8	1.06	0.20
Pseudomonas sp.	Methane	0.60	9.6	0.80	0.19
Pseudomonas methanica	Methane	0.56	9.0	0.75	0.17

[a] Y_{XO_2} is the yield factor relating grams of cells formed per gram of O_2 consumed.

With permission, from S. Nagai in *Advances in Biochemical Engineering*, vol. 11, T. K. Ghose, A. Fiechter, and N. Blakebrough, eds., Springer-Verlag, New York, p. 53, 1979.

Theoretical predictions of yield coefficients

ATP Yields:

anaerobic fermentation: $Y_{X/ATP}$ is approx. 10.5± 2 gdw cells / mol ATP

aerobic fermentation: $Y_{X/ATP}$ varies between 6 - 29 gdw cells / mol ATP

When the energy yield of a metabolic pathway is known (N moles of ATP produced per gram of substrate consumed):

$$Y_{X/S} = Y_{X/ATP}\, N$$

e.g yeast: $C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$

Glycolysis: 2 ATP/ mol glucose (in yeast)

$Y_{X/S}$ ~ 0.117 gdw /g glucose, $Y_{P/S}$ = 0.51 g ethanol / g glucose

Heat of reaction for processes with biomass production

How can we estimate the heat of reaction associated with cell metabolism and growth?

$$C_wH_xO_yN_z + a\ O_2 + b\ H_gO_hN_i \longrightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O$$

Heats of reaction for cell growth can be estimated using microbial stoichiometry and the concept of available electrons:

$$\Delta h_c^0 = -\ Q_o\ \gamma\ x_C$$

Q : heat evolved per mole of available electrons transferred to oxygen during combustion
γ: degree of reduction of the compound with respect to N_2
X_c: number of carbon atoms in the molecular formula

Energy balance

The **calo-respirometric quotient (Q_O)** is a constant:

Q_O = 26.95 kcal/ g equivalent available electrons

or

Q_O = 112.76 kJ/ g equivalent available electrons

(1 calorie = 4.184 Joule)

This means that an energy balance may be determined simply by multiplying the number of available electrons of each species in the balance equation by Q_O:

$$Q_O(Y_{X/S}\,\gamma_x) + Q_O(Y_{P/S}\,\gamma_p) = Q_O\,\gamma_s - Q_O\,4Y_{O/S}$$

Thus:

450- 460 kJ energy are liberated as heat for every mole oxygen consumed in the process.

Energy balance

The more reduced is the substrate (the higher the degree of reduction) and the larger the amount of heat liberated during the process.

Methane $\gamma_s = 8$

Heat liberated upon oxidation of methane to CO_2 and H_2O = **112.76 x 8** = 902.08 kJ/ C-mole

Glucose $\gamma_s = 4$

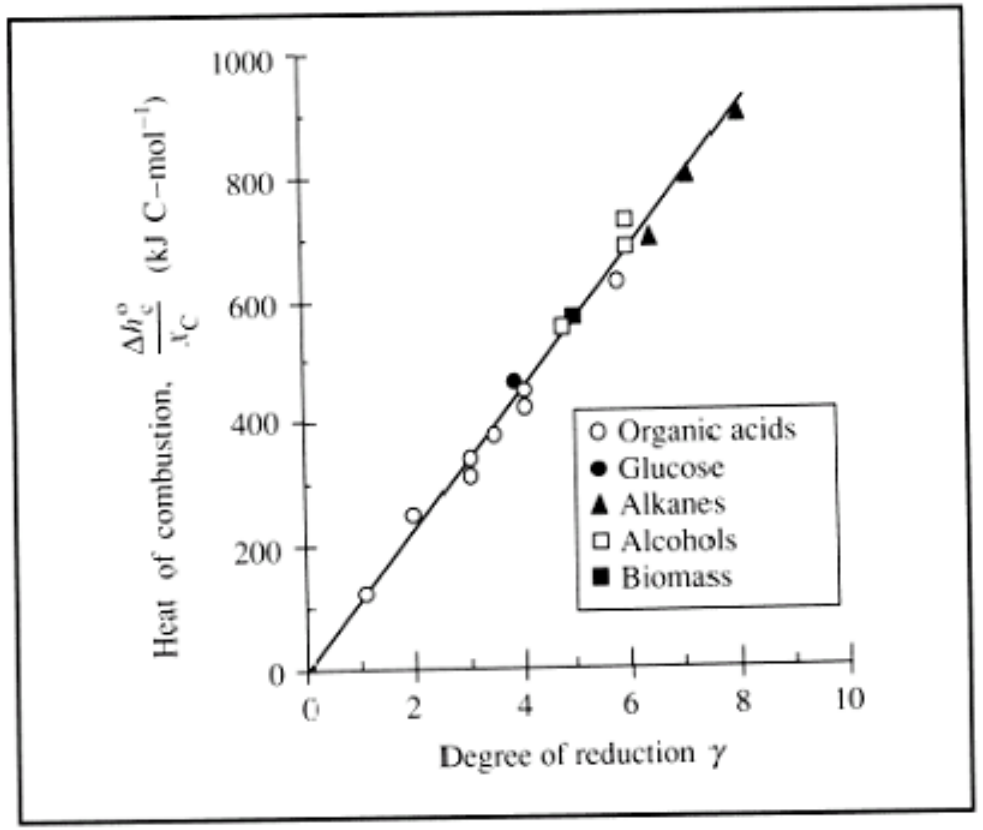
Heat liberated upon oxidation of glucose to CO_2 and H_2O= **112.76 x 4**= 451.04 kJ/ C-mole

i.e. twice as much heat is liberated per C- mole methane as for glucose

These figures represent the **total amount of heat liberated during the bioprocess**, but do not give an indication of the **rate at which the heat is evolved**- and therefore the cooling rate required to maintain a constant temperature.

Rate of heat liberation in bioprocesses

Figure 5.5 Relationship between degree of reduction and heat of combustion for various organic compounds. (From J.A. Roels, 1987, Thermodynamics of growth. In: J. Bu'Lock and B. Kristiansen, Eds, *Basic Biotechnology*, Academic Press, London.)



Energy balance

Exercises

Calculate the amount of heat liberated during aerobic bioprocesses in which the carbon and energy source is:

1. Glucose
2. Ethanol
3. Xylose
4. Lactic acid
5. Butanol
6. Palmitic acid
7. Other compounds of your choice

Assume that all of the substrate is oxidized to CO_2 and H_2O

Medium as a source of chemical energy

- Cells metabolize substrate for two reasons (ideas?):
  1. Convert the substrate to biomass
  2. Power the cellular machinery
- As for any chemical reaction, the heat of reaction can be calculated if the enthalpy of the reactants and products are known.

$$\Delta H = \sum nh_{products} - \sum nh_{reactants}$$

- For power generation in the presence of oxygen, the substrate is oxidized to CO_2, H_2O (and potentially N_2).
- The macroscopic stoichiometry of the aerobic reaction is

$$\underbrace{C_wH_xO_yN_z}_{\text{C source}} + aO_2 + \underbrace{bH_gO_hN_i}_{\text{N source}} \rightarrow \underbrace{cCH_\alpha O_\beta N_\gamma}_{\text{Biomass}} + dCO_2 + eH_2O$$

Medium as a source of chemical energy

- For aerobic processes, most of the heat generated is due to the reduction of O_2.
- The heat production in the bioreactor $\dot{Q}$ can thus be estimated based on the oxygen consumption rate with $\boldsymbol{\Delta H_{O_2} = 460\ \mathbf{kJ/mol}}$:

$$\dot{Q} = \left(\dot{V}_{O_2,in} - \dot{V}_{O_2,out}\right) * \Delta H_{O_2}$$

$\dot{Q}$: generated heat in kJ per mole
$\dot{V}_{O_2}$: molar O_2 flow in/out of the bioreactor

- If the yield coefficients for the C and N source are known, the oxygen consumption per biomass may be estimated and consequently the expected total heat production calculated.

Rate of heat liberation in bioprocesses

If 450- 460 kJ heat evolved per mole oxygen consumed then rate of heat production can be determined from measurement of rate of oxygen consumption (OUR; mole O_2/L/h) or rate of substrate consumption (r_s).

$$\mathbf{OUR = qO_2 \cdot X = \mu X / Y_{X/O2}}$$

Where:

qO_2 = specific oxygen uptake rate (mole O_2 consumed/C-mole biomass/h)

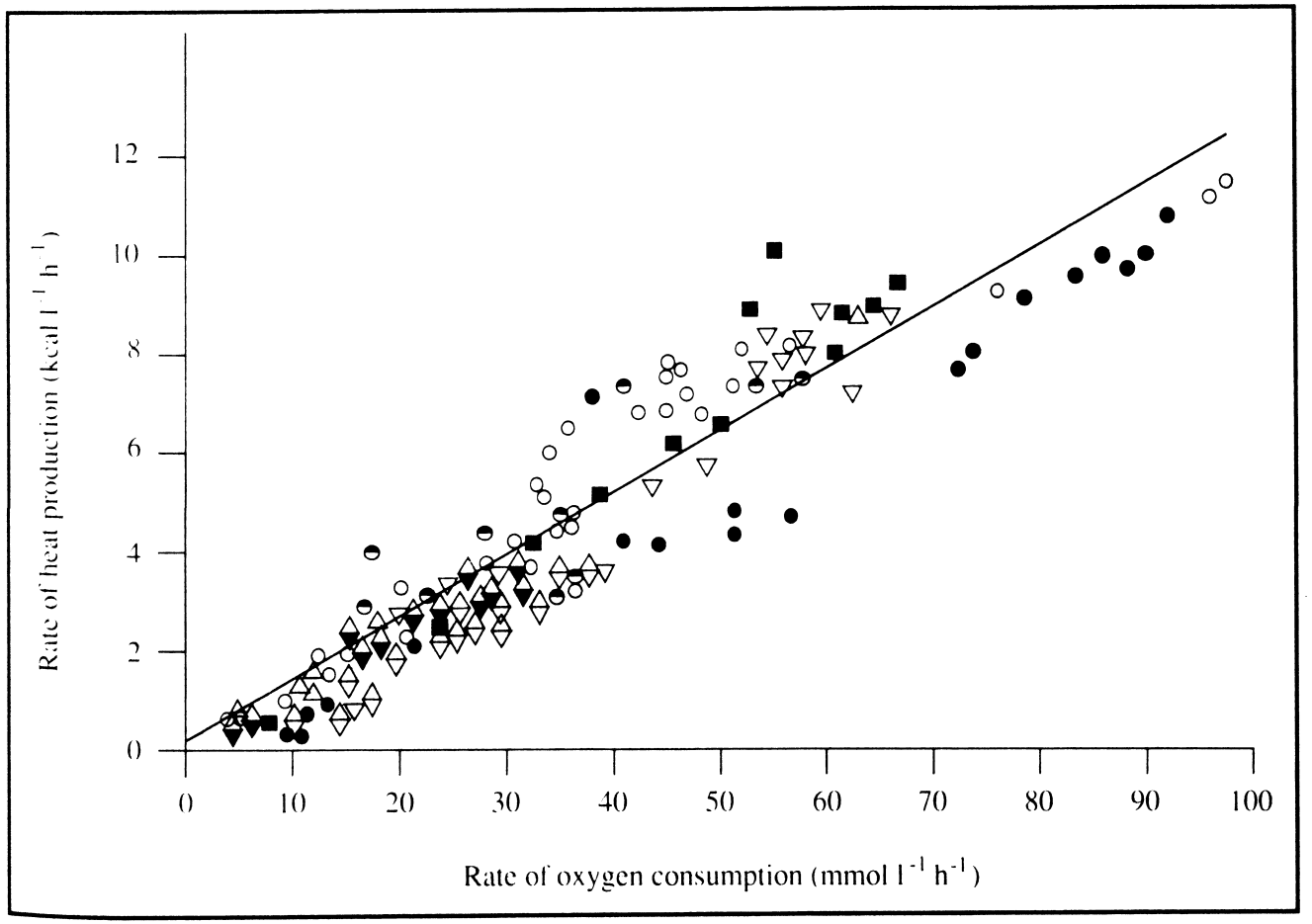
X = C-mole biomass/L

$Y_{X/O2}$ = C-mole biomass formed/ mole O_2 consumed

Therefore:

OUR x 460 = kJ heat evolved/L/h

Figure 3.2 Correlation between rate of heat evolution and rate of oxygen consumption for a variety of microbial fermentations. (○) *Escherichia coli*, glucose medium; (◈) *Candida intermedia*, glucose medium; (△) *C. intermedia*, molasses medium; (▽) *Bacillus subtilis*, glucose medium; (■) *B. subtilis*, molasses medium; (◓) *B. subtilis*, soybean-meal medium; (◈) *Aspergillus niger*, glucose medium; (●) *Asp. niger*, molasses medium. (From C.L. Cooney, D.I.C. Wang and R.I. Mateles, Measurement of heat evolution and correlation with oxygen consumption during microbial growth, *Biotechnol. Bioeng.* **11**, 269–281; Copyright © 1968. Reprinted by permission of John Wiley and Sons, Inc.)



Summary

- Second to the cells, the medium is an important part in biotechnology. Industry tends to use standard media including complex compounds, whereas in research clearly well-defined media are used.

- A well designed medium has a clear nutrient limitation and other medium components are in excess.

- Elemental electron balances are important tools to predict also scaleability of the process (e.g. minimal oxygen transfer rate and corresponding cooling required).

Happy medium preparations!