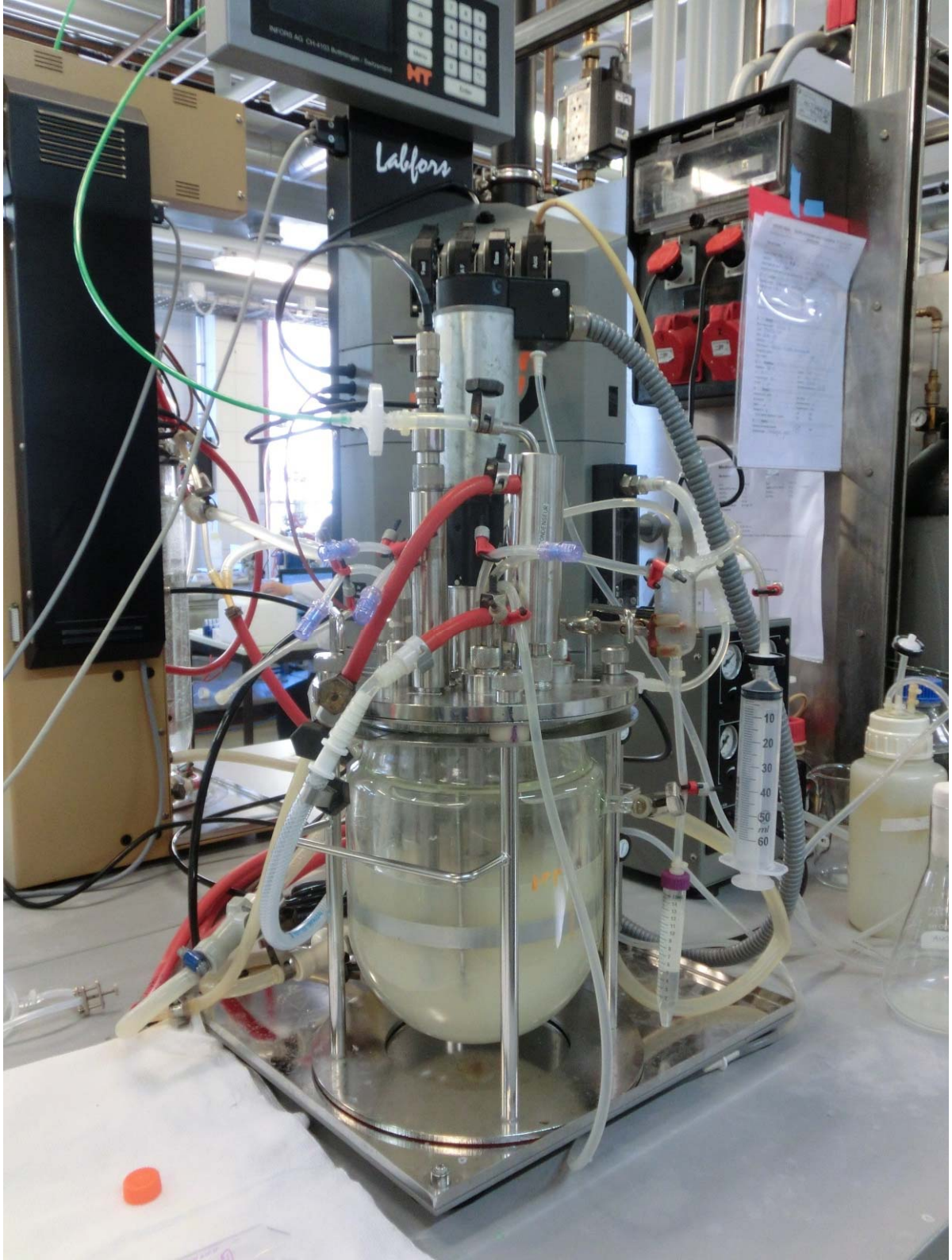


Batch cultivation

Course ChE-311
"Biochemical Engineering"

manfred.zinn@epfl.ch

Agenda

- Requirements for a successful batch
- Growth kinetics
- Monod kinetics
- Stoichiometric model
- Integral medium design
- Growth on defined and complex media
- Substrate inhibition
- Cell physiology of nutrient limited batch cultures

Batch culture

Requirements for successful batch cultures

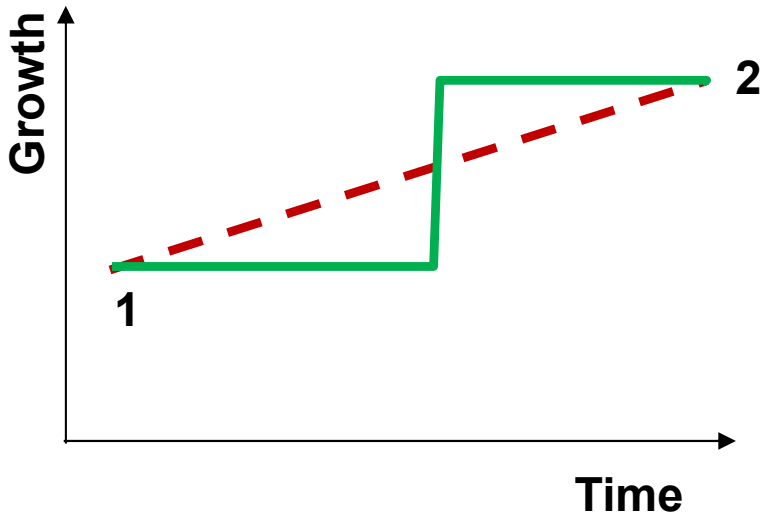
1. Viable inoculum (still growing)
2. Energy source
3. Nutrients to provide the essential materials from which the biomass is synthesized
4. Absence of inhibitors which prevent growth
5. Suitable physicochemical conditions

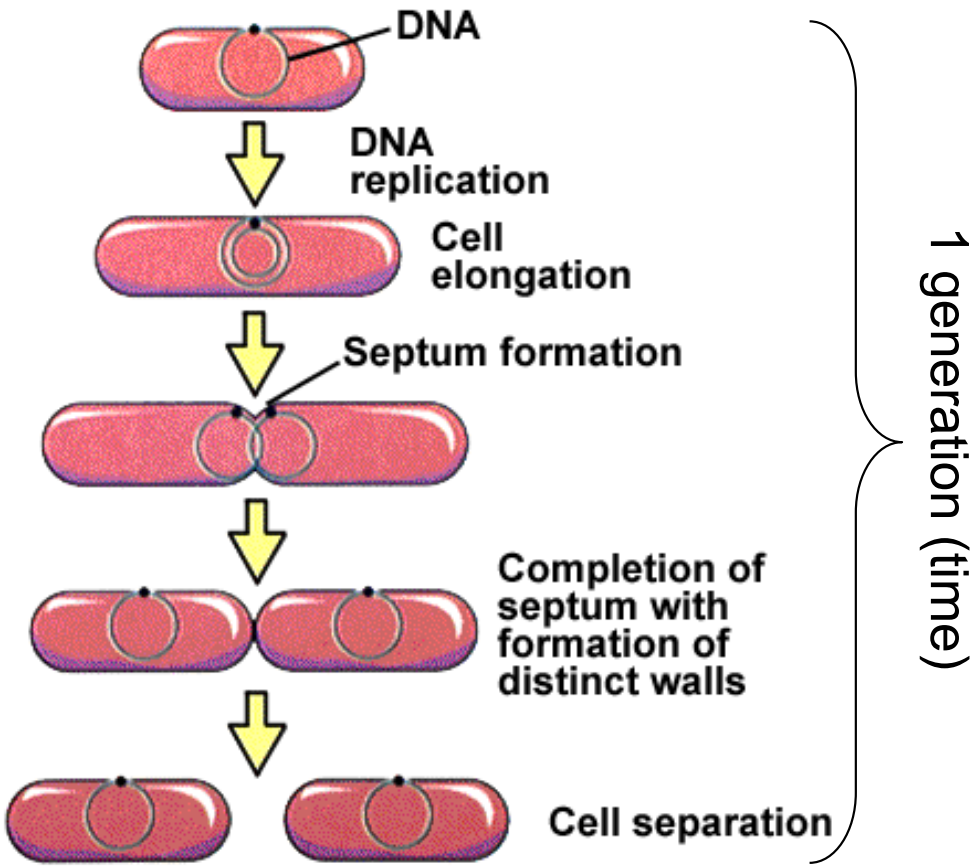
Bacterial growth

Growth = increase in # of cells
(by binary fission)
generation time: 10 min – days

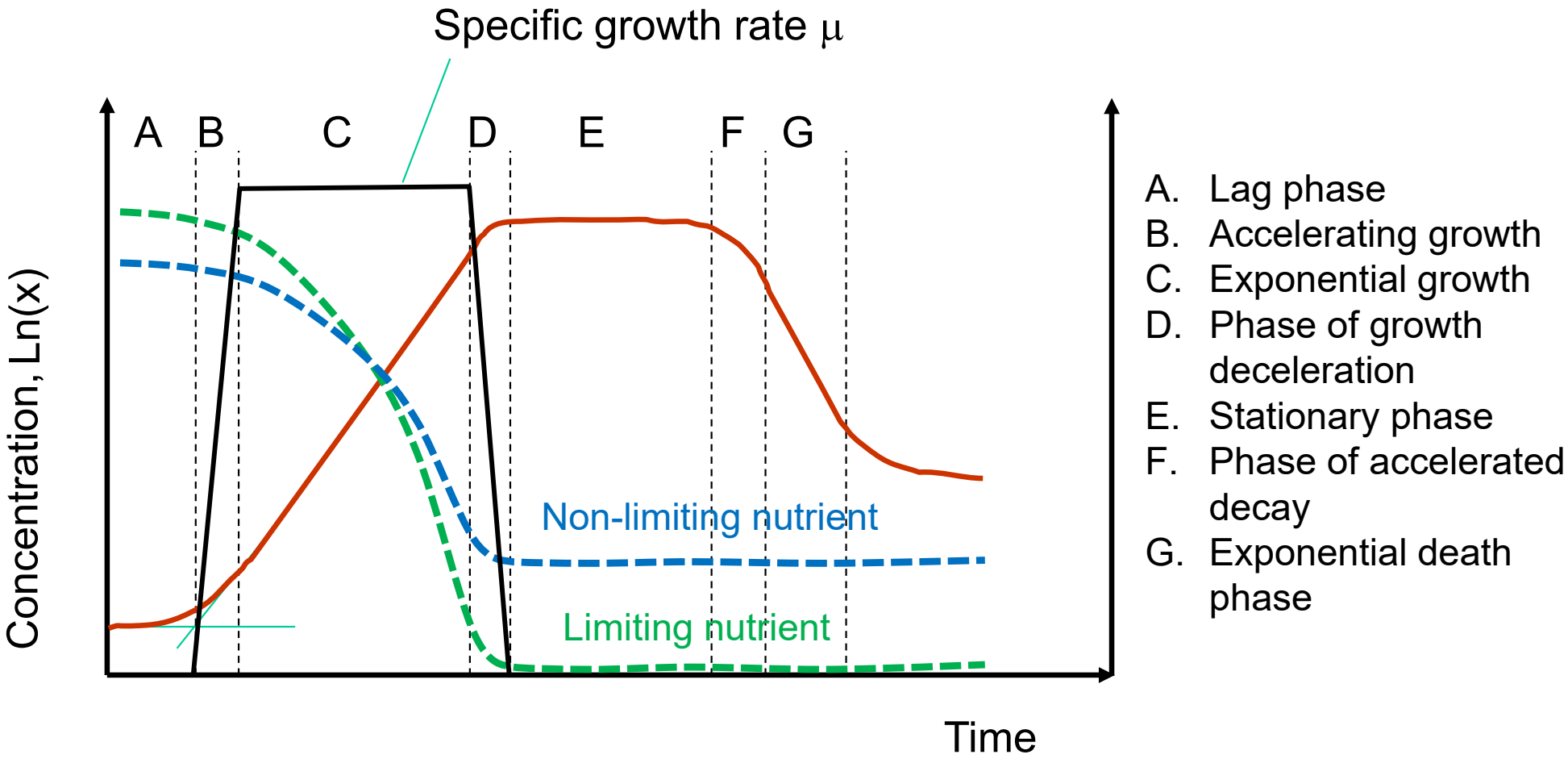
or

Growth = increase in dry biomass





Growth phases in a batch culture



A. Lag phase
B. Accelerating growth
C. Exponential growth
D. Phase of growth deceleration
E. Stationary phase
F. Phase of accelerated decay
G. Exponential death phase

Growth, growth rate, specific growth rate

Growth = increase in # of cells, biomass, protein…

Growth rate (cells h^{-1}, gDW h^{-1}):

Δcell number/Δtime

or: Δdry cell mass/Δtime

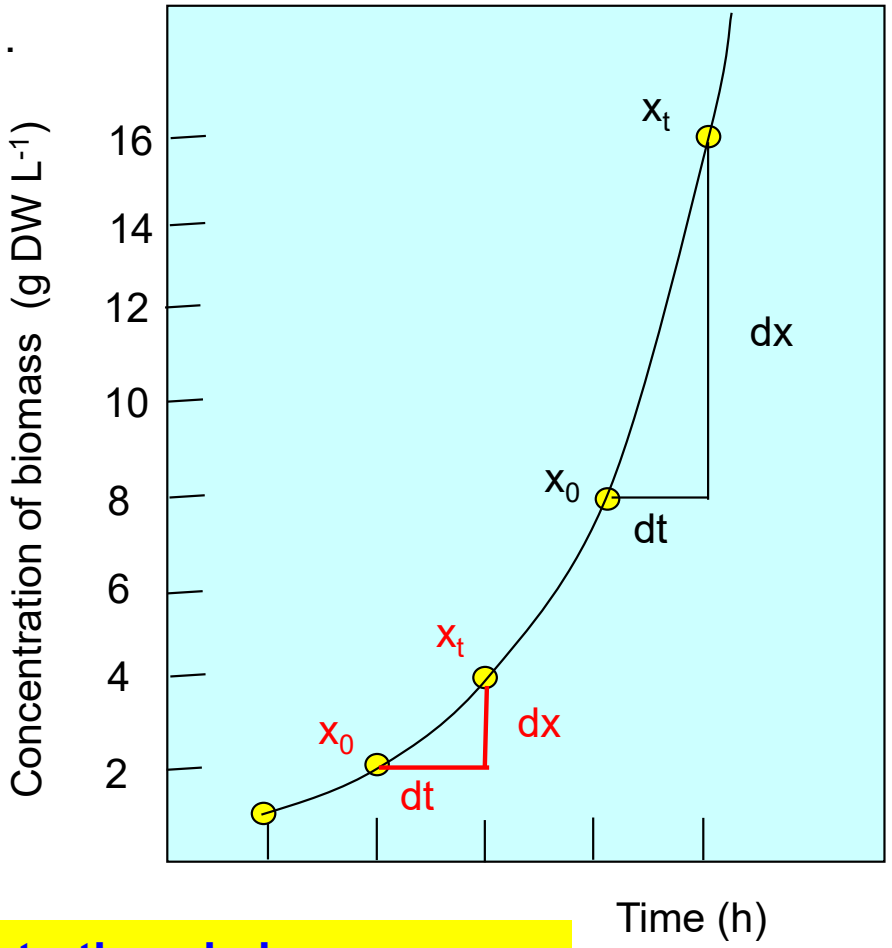
(increases during exponential growth with increasing culture density)

Specific growth rate, μ (h^{-1}):

= Δcell number/Δtime x cell number

or: Δcell mass/Δtime x cell mass

(constant during exponential growth in Brock defined as „k“)



Note: total amounts usually in capitals, concentrations in lower case

Describing exponential growth based on cell number

(1) $$N_t = N_0 2^n$$

(2) $$\log N_t = \log N_0 + n \log 2$$

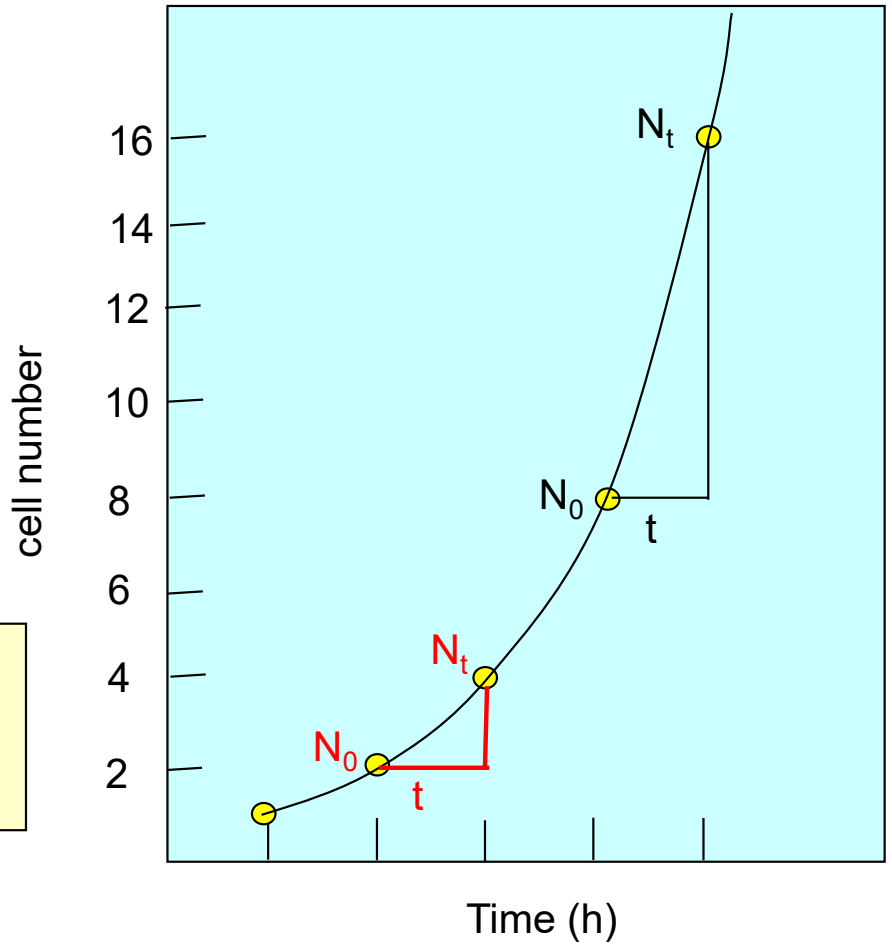
(3) $$n = \frac{\log N_t - \log N_0}{\log 2}$$

(4) $$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{t \log 2}$$

(5) $$t_d = \frac{t}{n} = \frac{1}{k}$$

(6) $$g = \frac{t}{n}$$

N_t or N_0 = cell number at time t or 0
n = number of divisions (generations)
k = divisions (generations) per unit of time
t = time elapsed
t_d = doubling time
g = generation time



Describing exponential growth based on biomass

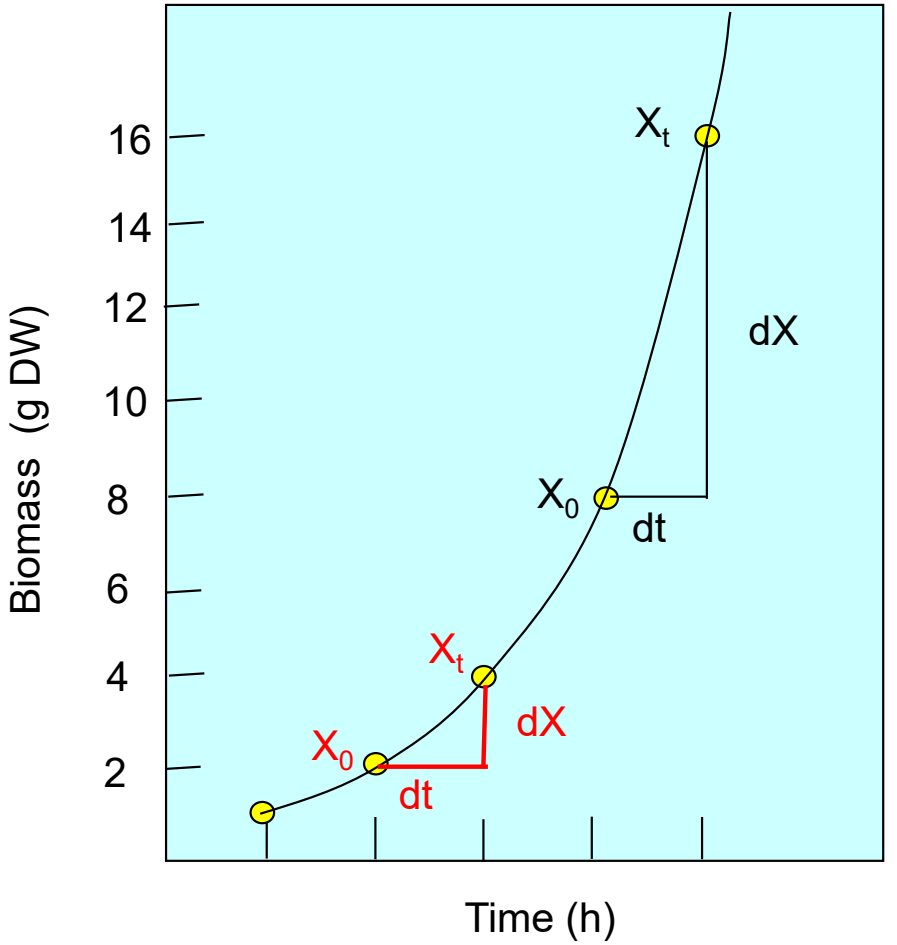
(7) $$\frac{dX}{dt} = \mu X$$

(8) $$\mu = \frac{1}{X} \cdot \frac{dX}{dt}$$

(9) $$X_t = X_0 \cdot e^{\mu t}$$

(10) $$\mu = \frac{\ln X_t - \ln X_0}{t}$$

(11) $$\mu = \frac{\ln 2}{t_d} \qquad (= \ln 2 \cdot k)$$



μ = „specific growth rate“ (usually in h^{-1}); in Brock and also other text books abbreviated as „k“

μ_{max} is the maximum specific growth rate (a constant for the conditions given (T, pH, medium composition, etc.)

The power of exponential growth

Biomass of *E. coli* (cdw): $2.8*10^{-10}$ kg

$$x = x_0 * e^{\mu * t}$$

Maximum growth rate μ_{max}: 1.2 h^{-1}

Time needed to achieve following mass:

Beetle	1 g	13 h
Dog	10 kg	20 h
Earth	$5.97*10^{24}$ kg	66 h
Jupiter	$1.90*10^{27}$ kg	71 h
Sun	$1.99*10^{30}$ kg	77 h
Galaxy	$1.4*10^{11}$ sun	**91 h (less than 4 days!)**

Substrate consumption during batch

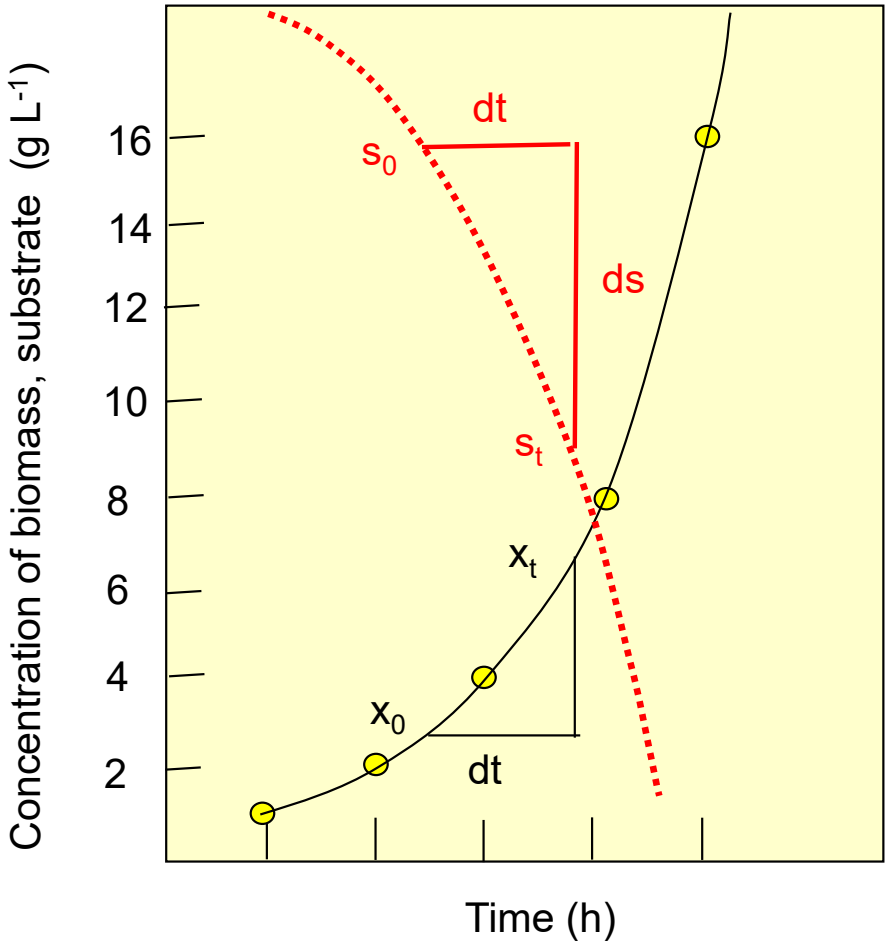
(13) $$Y_{X/S} = \frac{x - x_0}{s_0 - s} = \frac{\Delta x}{\Delta s}$$

(14) $$q_s = \frac{ds}{dt} \cdot \frac{1}{x}$$

(15) $$q_s = \frac{\mu}{Y_{X/S}}$$

(16) $$-\frac{dS}{dt} = q_s \cdot X$$

$Y_{x/s}$ = growth yield
q_s = specific substrate consumption rate



Growth limitations in a batch culture

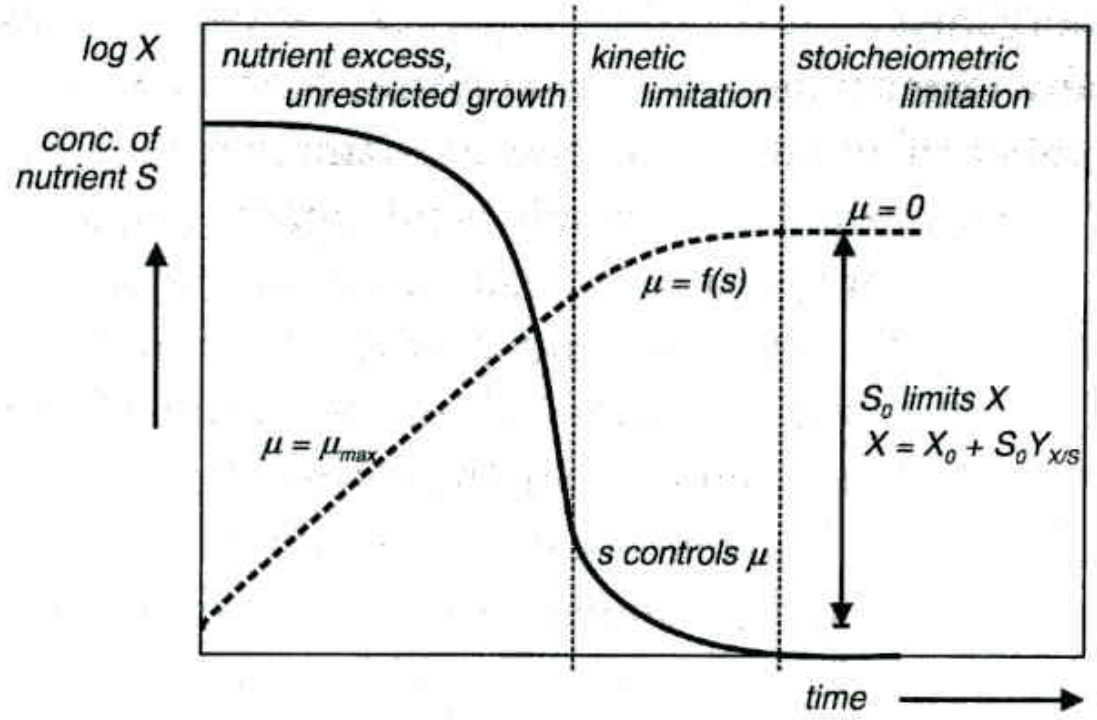


Fig. 3. Kinetic and stoichiometric limitation of microbial growth in a batch culture by the concentration of the limiting nutrient (substrate)*S*. S_0, initial concentration of *S*; *s*, actual concentration of *S*; *X*, biomass concentration; X_0, initial biomass concentration; $Y_{x/s}$, growth yield for nutrient *S*.

Mathematical expression of Monod's modell

1st equation of Monod:

$$\frac{dx}{dt} = \mu \cdot x$$

The speed of growth is proportional to the concentration of the biomass.

2nd equation of Monod:

$$\mu = \mu_{max} \frac{s}{s + K_s}$$

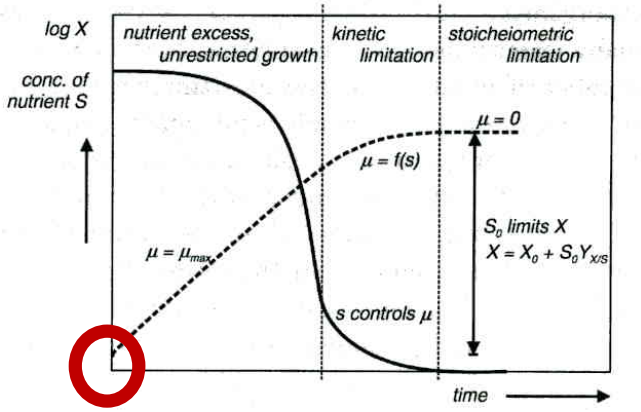
The specific growth rate depends only on the concentration of the limiting substrate according to the Michaelis-Menten kinetics.

3rd equation of Monod:

$$Y_{X/S} = \frac{r_X}{-r_S}$$

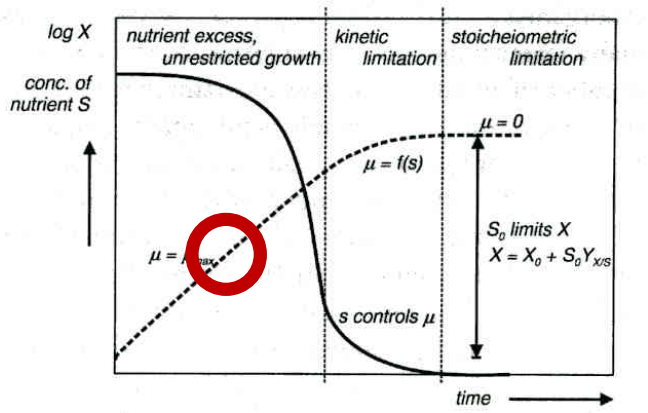
The yield coefficient is constant under exponential growth conditions.

The start of a batch culture in the lab



- A good start of a batch culture is important!
- Lag time is influenced by:
  - Quality of preculture (still growing, density)
  - Medium composition of preculture (change from complex to minimal medium)
  - Growth state of preculture (still growing?)
  - Concentration of medium components (toxic concentration of substrates and inhibiting products in preculture)
  - Oxygen limitation
- Lag time can be determined graphically and mathematically
- Inoculum amount: Ideally 1-2% of batch culture but less than 10%
- Ideal start concentration of a batch culture: OD(600nm) = ca. 0.1

The exponential phase of a batch culture



- The exponential growth phase is an autocatalytic process (no change of growth rate when cells are «unrestricted» in growth)

- During exponential growth phase:
  - Growth rate μ is maximal (= μ_{max}) and is constant
  - Specific uptake rate q_s is maximal and constant
  - Biomass yield $Y_{X/S}$ is constant
  - Average biomass composition is constant
  - Specific CO_2 production rate q_{CO2} is constant

- Average cell size is largest compared to all other growth phases.

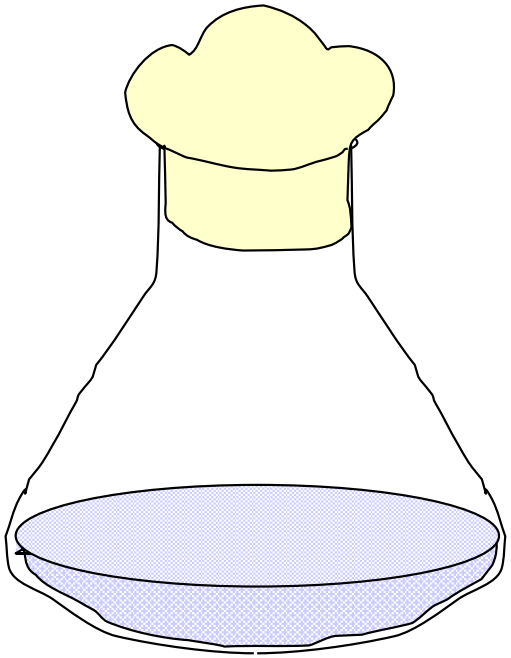
- Duration of exp. growth phase can be controlled by inoculum concentration and available nutrient concentrations.

- Identification of exponential growth phase by linear regression:

$$\ln(x) = \ln(x_0) + \mu * \Delta t$$

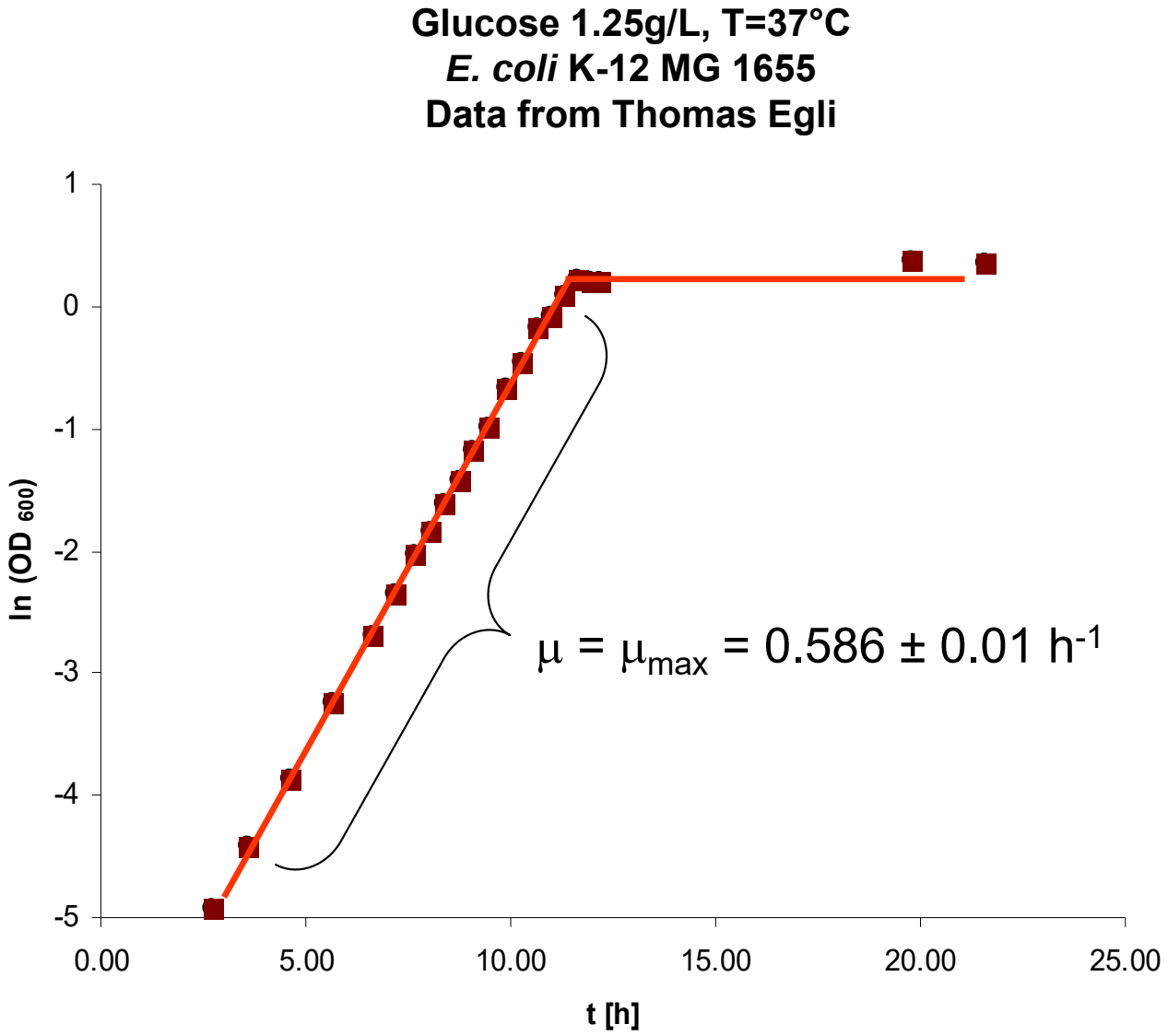
Growth kinetics in mineral medium

Designed medium:



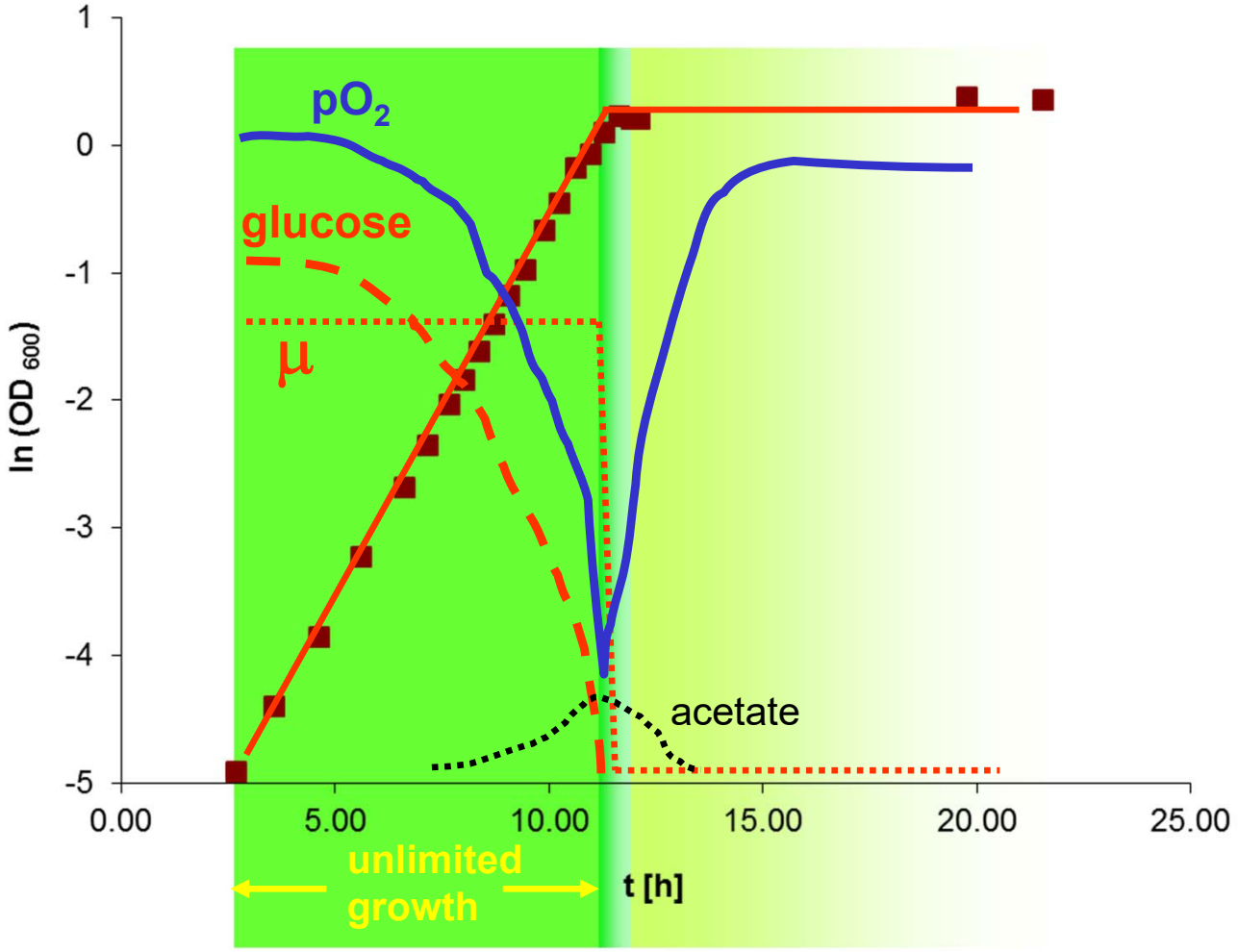
Glucose	**< 4 g**
$Na_2HPO_4 \cdot 2H_2O$	12.8 g
KH_2PO_4	3 g
$(NH_4)_2SO_4$	1.8 g
$CaCO_3$	80 mg
$FeCl_3 \cdot 6\ H_2O$	77 mg
$MnCl_2 \cdot 4\ H_2O$	11.5 mg
$CuSO_4 \cdot 5\ H_2O$	1.5 mg
$CoCl_2 \cdot 6\ H_2O$	1.3 mg
ZnO	4 mg
H_3BO_3	1.2 mg
$NaMoO_4 \cdot 2\ H_2O$	10.4 mg
$MgCl_2 \cdot 6\ H_2O$	113 mg
EDTA $Na_4 \cdot 2\ H_2O$	0.79 g
	per liter

Growth kinetics in mineral medium



Batch growth in mineral medium

More defined than complex medium growth!



Complex media

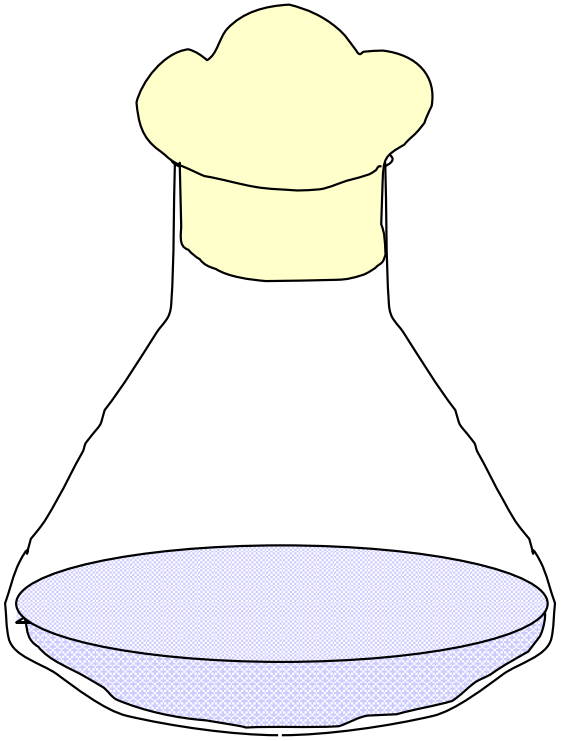
The medium contains ingredients that are not well defined and their composition is not known. Among those there are extracts and hydrolysates (peptones) that can be gained from cheap waste compounds. These media are especially suitable for microorganisms that are difficult to cultivate.

Samples for complex media:

Nutrient broth (peptone from gelatine, beef extract)
Tryptic soy broth (tryptone (pancreatic digestion of casein), peptone from soy beans, glucose, NaCl, K_2HPO_4)

Malt extracts, hay infusions, plum juice, carrot juice. For coprophilic fungi, even horse dung infusions may be used.

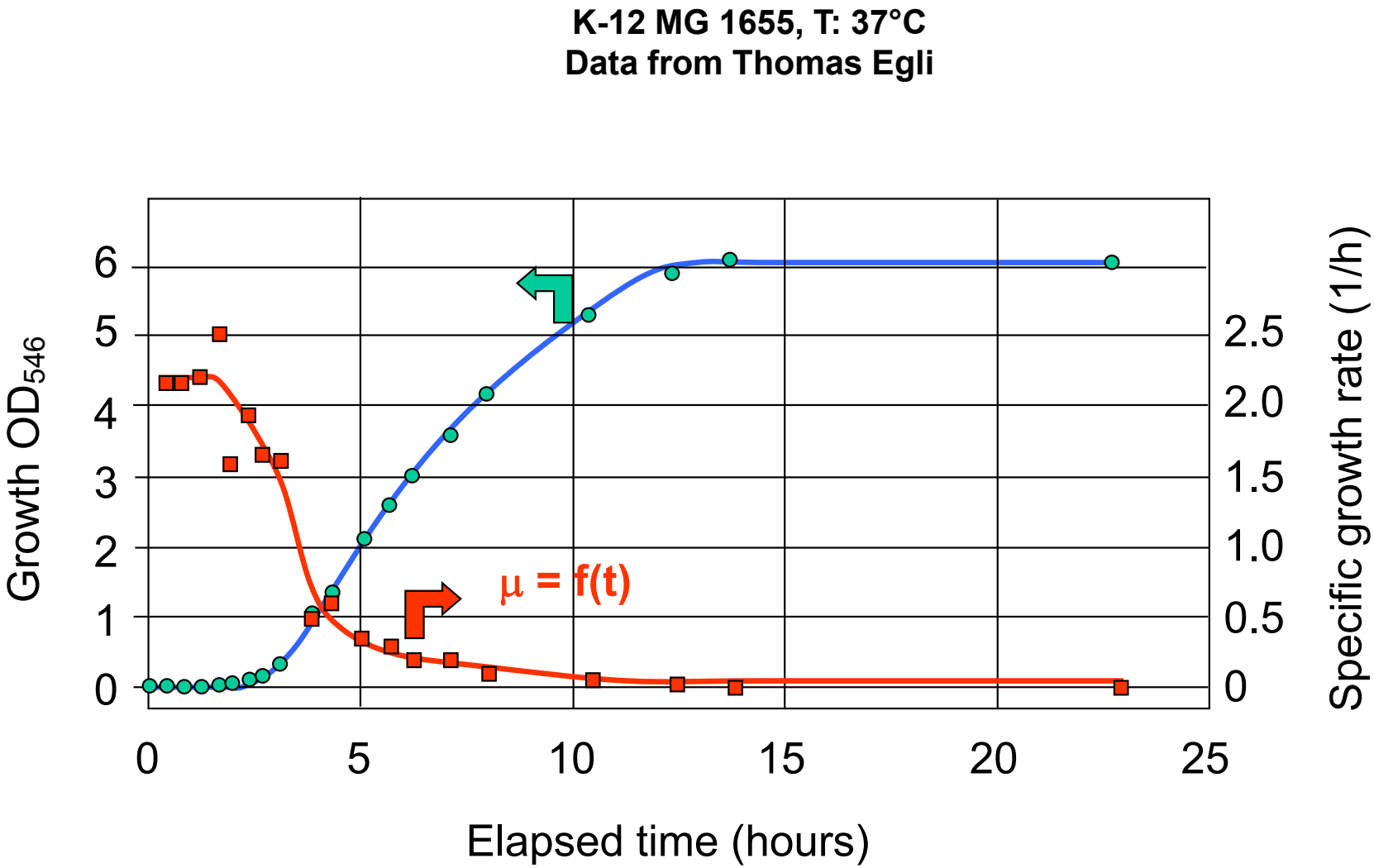
Growth kinetics in complex medium



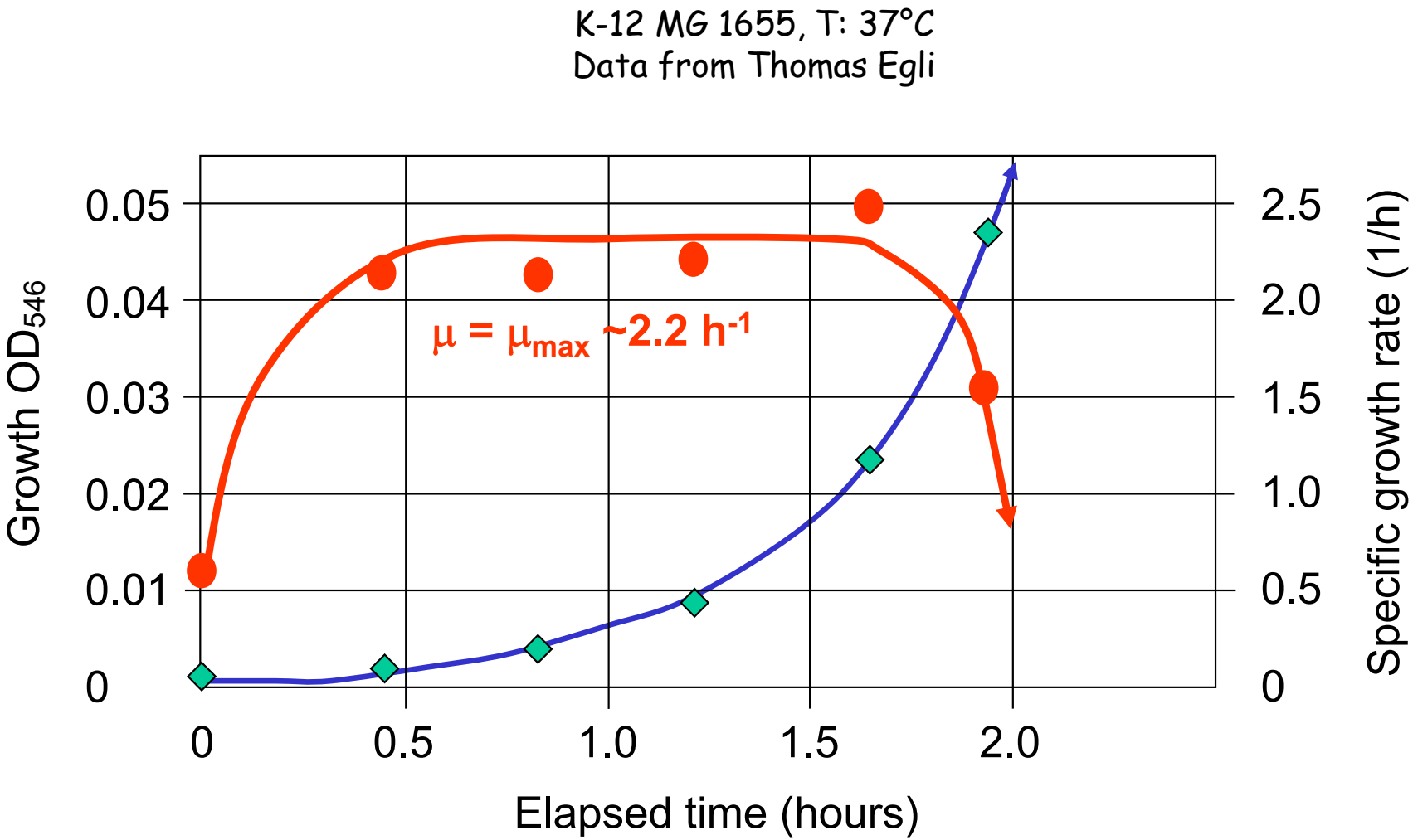
Lysogeny broth (also Luria broth)

Glucose	**0-5 g**
Tryptone	**10 g**
Yeast extract	**5-10 g**
NaCl	**0-10 g**
Distilled water add	**1 Liter**

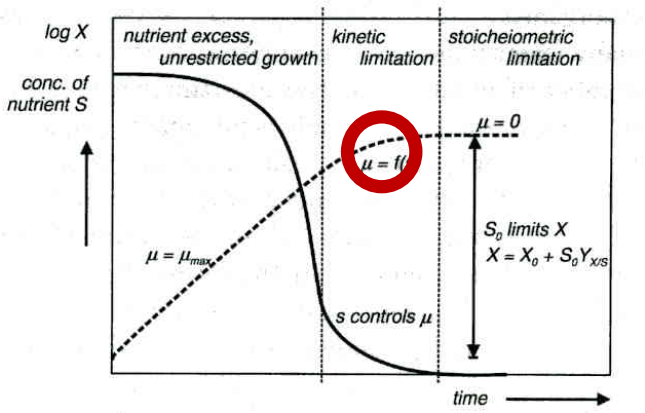
Batch growth of *E. coli* in LB



A quick start!



The decelaration phase of a batch culture



- During the deceleration growth phase the cells are restricted in their growth: One nutrient **kinetically** limits growth.

- During kinetic limitation:
  - Growth rate μ is not maximal (< μ_{max}) and is constantly changing
  - Specific uptake rate q_s is getting smaller
  - Biomass yield $Y_{X/S}$ may get smaller due to maintenance energy consumption
  - Average biomass composition is changing
  - Specific CO_2 production rate q_{CO2} is slowly decreasing

- Average cell size is getting smaller.

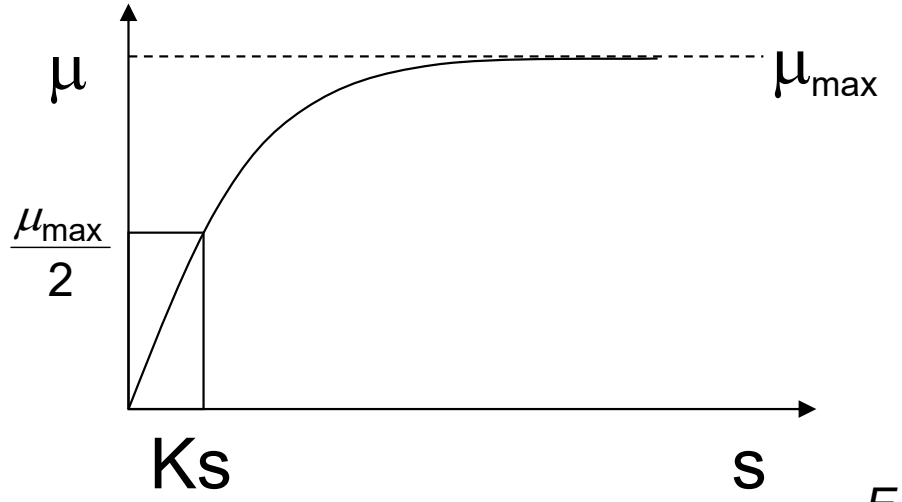
- Cells start to express high affinity uptake systems and start to prepare for stationary phase (synthesis of «alarmones»).

Kinetics according to the Monod model

The growth rate becomes proportional to the concentration of one nutrient when this concentration is getting small. **Only one** nutrient is growth limiting.

$$\mu = \mu_{max} \frac{s}{s + Ks}$$

μ: specific growth rate [h^{-1}]
Ks: saturation or *Monod* constant [mol L^{-1}]
s: actual concentration of substrate s [mol L^{-1}]



$$\mu = \frac{\mu_{max}}{2} = \mu_{max} \frac{s}{s + Ks}$$

$$\Rightarrow \frac{s + Ks}{s} = 2$$

$$\Rightarrow s + Ks = 2s$$

$$\Rightarrow Ks = s$$

E. coli on glucose: Ks: $7*10^{-7}$ - $4*10^{-3}$ g L^{-1}

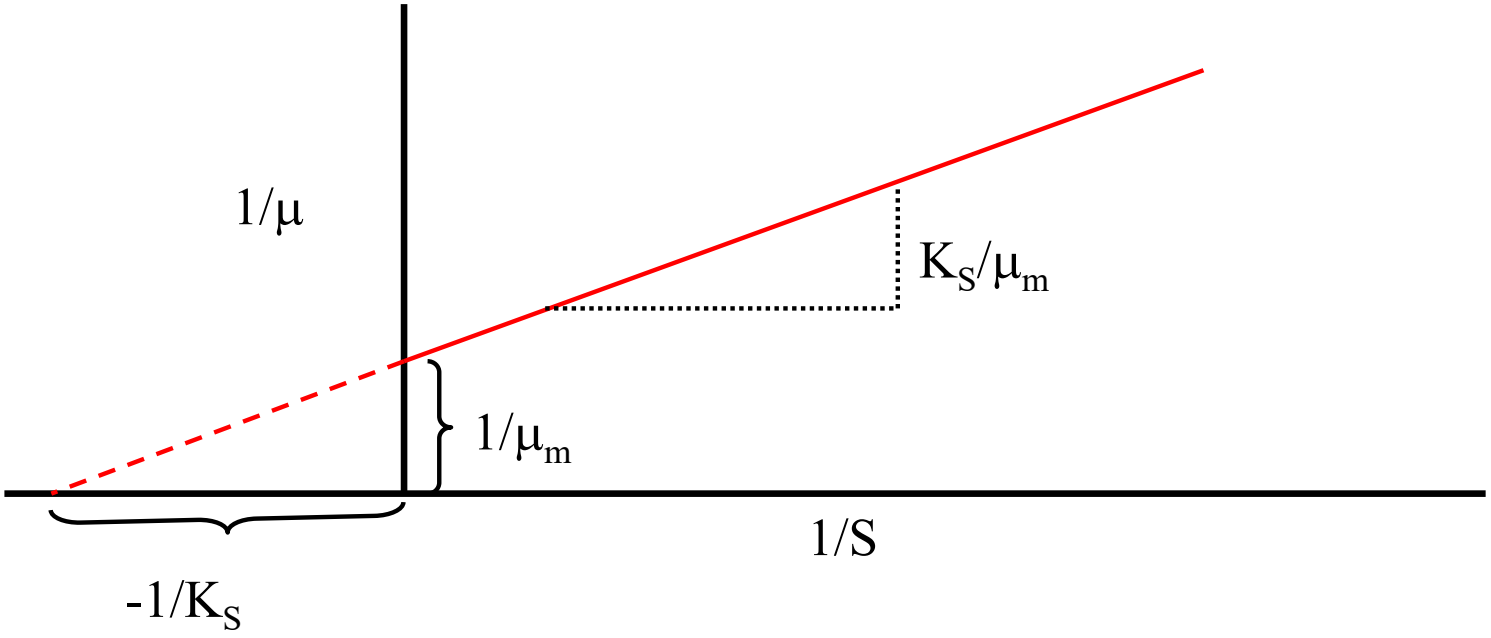
Lineweaver-Burk Plot

Kinetic constants are usually determined in chemostat cultures by varying μ (= D at steady-state) and mesuring s. However, they may also be determined by using several batch cultures with different s_0 concentrations. The Lineweaver- Burk plot (see graph) can be used to determine the growth constants Ks and μ_{max}:

$$\frac{1}{\mu} = \frac{1}{s} \bullet \frac{K_S}{\mu_{max}} + \frac{1}{\mu_{max}}$$

When 1/s ≈ 0 then 1/μ ≈ $1/\mu_m$

When 1/μ ≈ 0 then 1/s ≈ $-1/K_S$



K_s and μ_{max} for different MO

Organism	Substrate	K_s [g L^{-1}]
E. coli	Glucose	0.068
E. coli	Lactose	20.0
E. coli	Mannitol	2.0
S. cerevisiae	Glucose	25.0
Trichoderma viride	Glucose	9300
Xanthomonas campestris	Glucose	1.99 (at 27°C)

Organism	Substrate	μ_{max} [h^{-1}]
Aspergillus niger	Glucose	0.2
S. cerevisiae	Glucose	0.41
Trichoderma viride	Glucose	0.104
Xanthomonas campestris	Glucose	0.29 (at 27°C)

Nutrient uptake

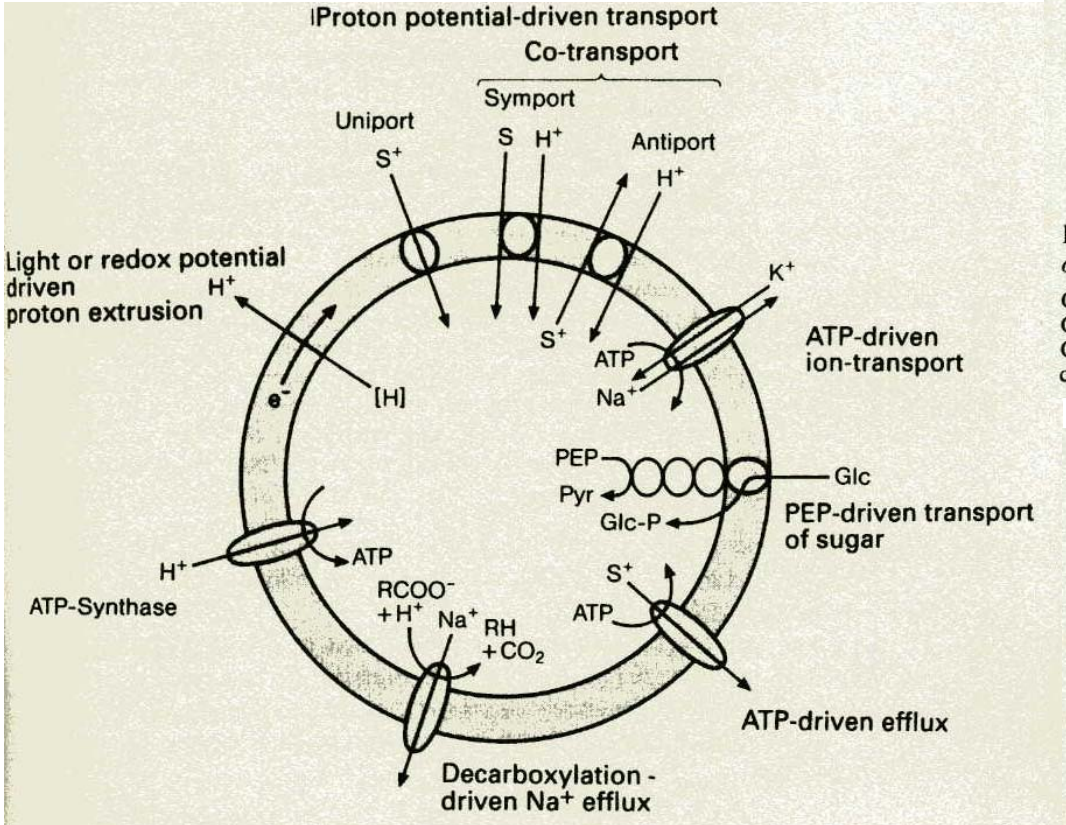


Fig. 7.21. *Summary of systems that transport ions and other metabolites through the cytoplasmic membrane.*

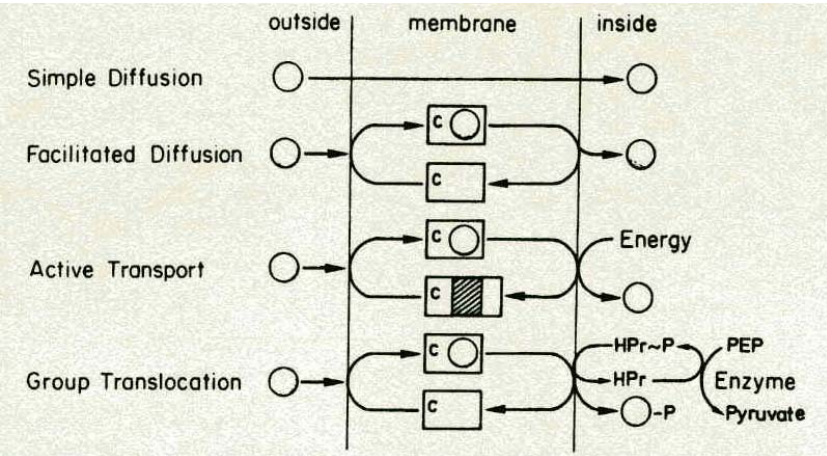


Fig. 7.19. *Schematic representation of the four mechanisms for transport of materials into the cell.*

Circle, substrate to be transported;
C, permease(carrier)-protein;
C with grey hatching, energised carrier;
PEP, phosphoenolpyruvate;
HPr, heat stable protein.
See text for details.

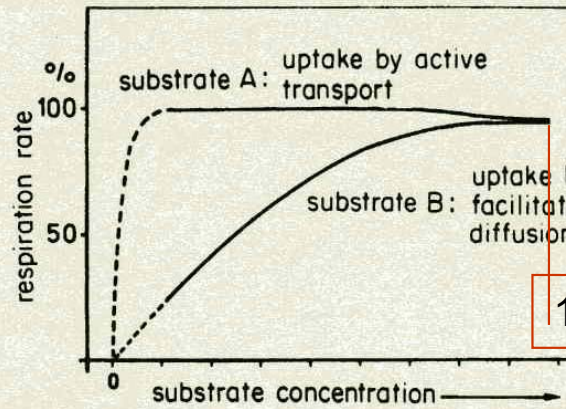


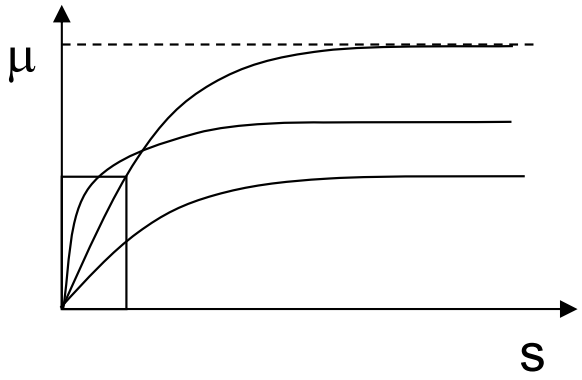
Fig. 7.20. *Substrate saturation curves for the uptake of two substrates by intact bacterial cells measured by oxygen consumption (respiration rate).*

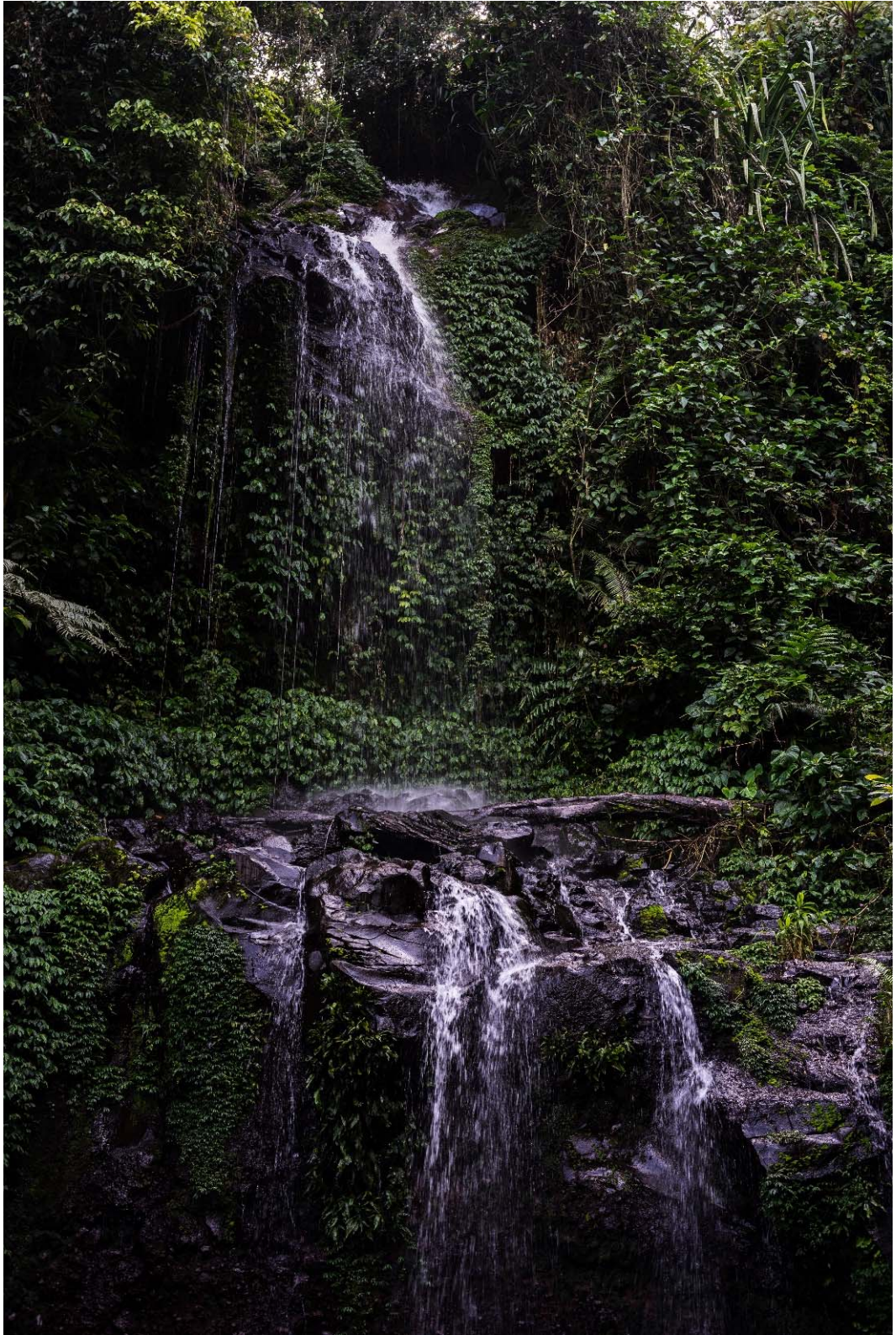
Ks values are important in nature

A low Ks value means:

- High affinity to a particular substrate => faster growth at lower concentration
- Most uptake systems for essential substrates are supported by complex systems (see next slide).

A high μ_{max} is only of competitive advantage in nutrient rich systems (e.g. *E. coli* in bowel)





Factors affecting growth rate

- Temperature
- pH
- Substrate concentration
- Osmotic strength
- Substrate toxicity
- Product inhibition

Effect of temperature on growth rate

Nomenclature of strains:

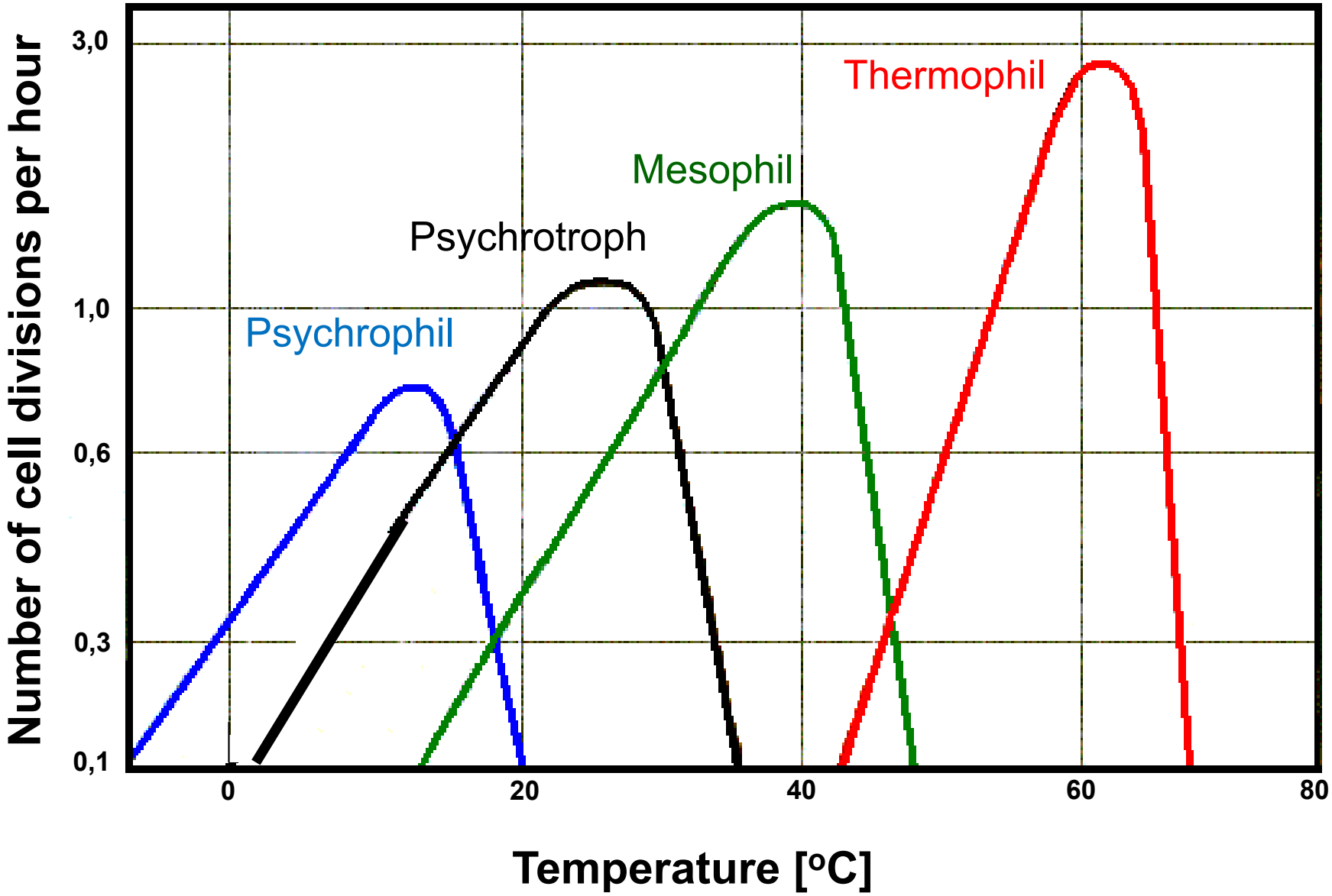
- Psychrophiles (-5°C - 20°C)
- Mesophiles (15°C - 42°C)
- Thermophiles (38°C - 65°C)
- Extremophiles (<5°C or >65°C)

Growth of cells is based on many chemical reactions, therefore the effect of temperature is the same as for chemical reactions

i.e.

reaction rate approx. doubles with a 10°C rise in temperature.

Characterization of cell growth according to temperature



Effect of temperature on μ

Growth can be described by:

$$\frac{dx}{dt} = \mu x - \alpha x$$

Re-arranged:

$$\frac{1}{x} \bullet \frac{dx}{dt} = \mu - \alpha$$

μ = specific growth rate (h^{-1})

α = specific death rate (h^{-1})

Overall growth is a function of growth and death usually with $\mu >> \alpha$

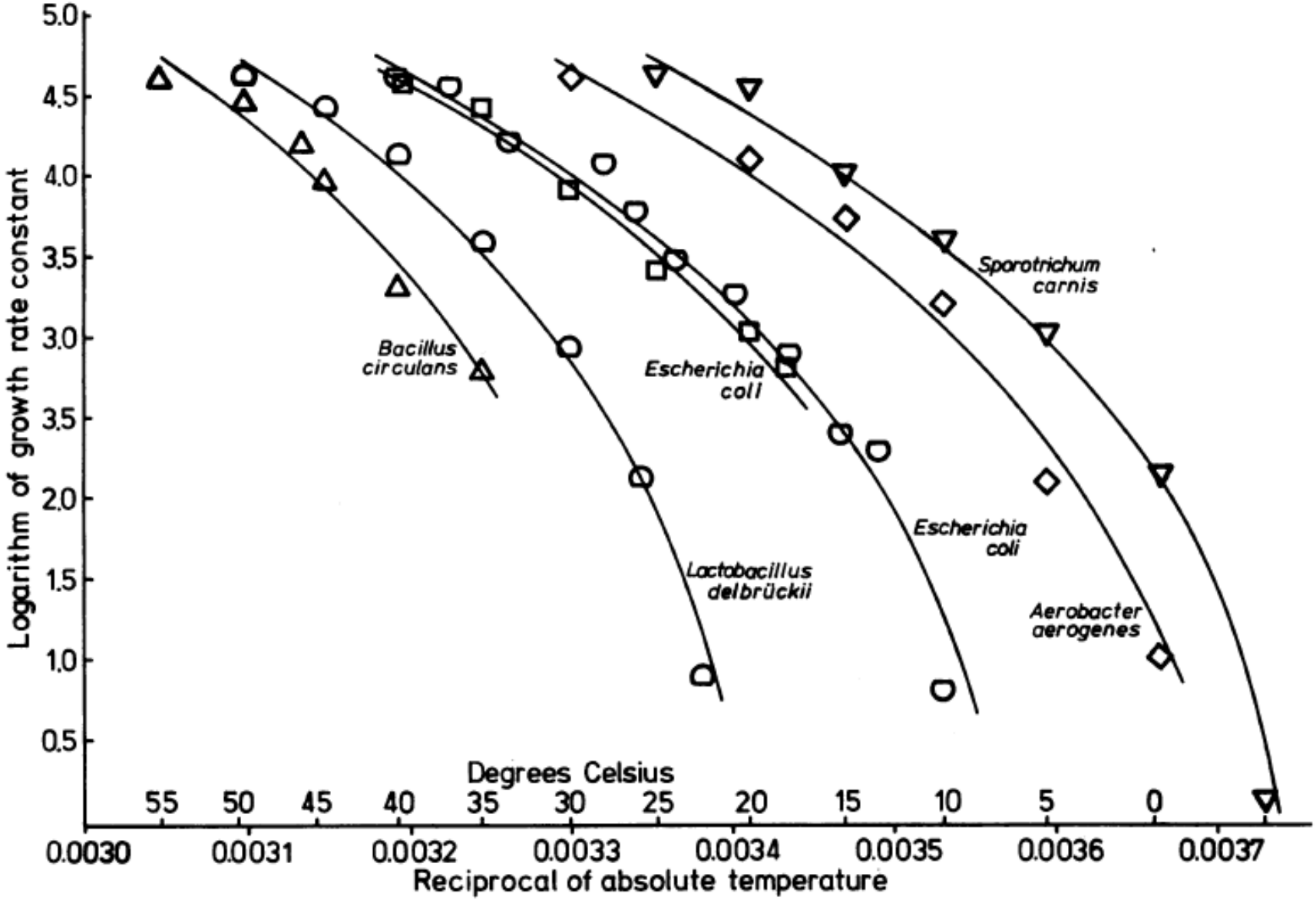
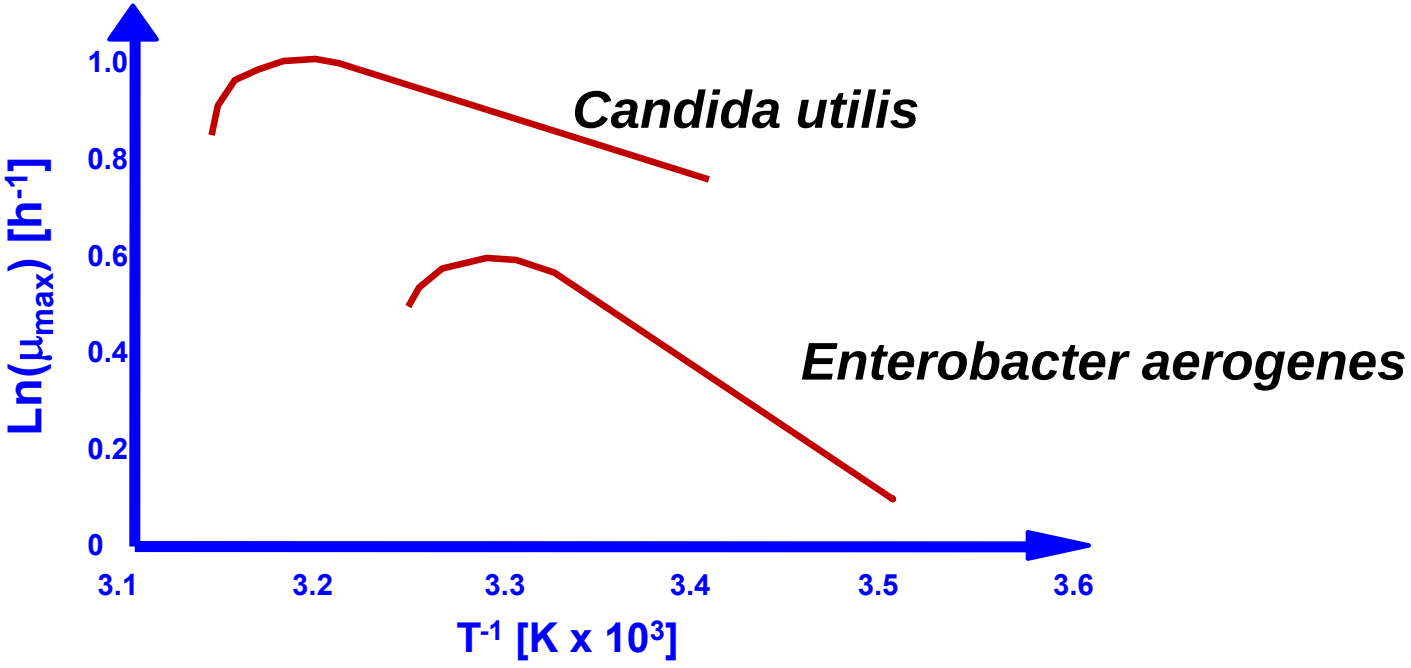


FIG. 1. Arrhenius plot of six sets of data redrawn from Johnson et al. (13). The solid curves correspond to the equation $\sqrt{r} = b\,(T - T_0)$.

D A Ratkowsky, J Olley, T A McMeekin and A Ball
J. Bacteriol. 1982, 149(1):1.

Arrhenius Plot



$$\mu = \overset{\circ}{A}\, e^{-Ea/RT} \qquad \alpha = \overset{\circ}{A'}\, e^{-Ea'/RT}$$

A and A' are constants; Ea and Ea' = activation energies (kJ mol^{-1}); R = Gas constant (8.284 $J^{-1}mol^{-1}K^{-1}$); T= Absolute temperature (K)

Typical Ea and Ea ' values are 62-97 kJ mol^{-1} and 250-293 kJ mol^{-1}, respectively.

Effect of temperature on μ

- Death rate α is more sensitive to temperature than the specific growth rate μ.
- There is an increase of the death rate at higher temperatures due to thermal denaturation of proteins (*e.g.,* uptake enzymes) and increased maintenance energy m.
- The medium requirements may change with higher temperature (more growth factor needed, e.g. *Yersinia pestis* 28°C to 37°C).
- At low temperatures cell regulatory mechanisms are affected and diffusional limitations take place, e.g. substrate transport into and within cells.
- Product formation is usually enhanced at lower temperature (e.g. protein synthesis).
- The RNA content of the cell is enhanced at lower temperature.

pH optimum for growth

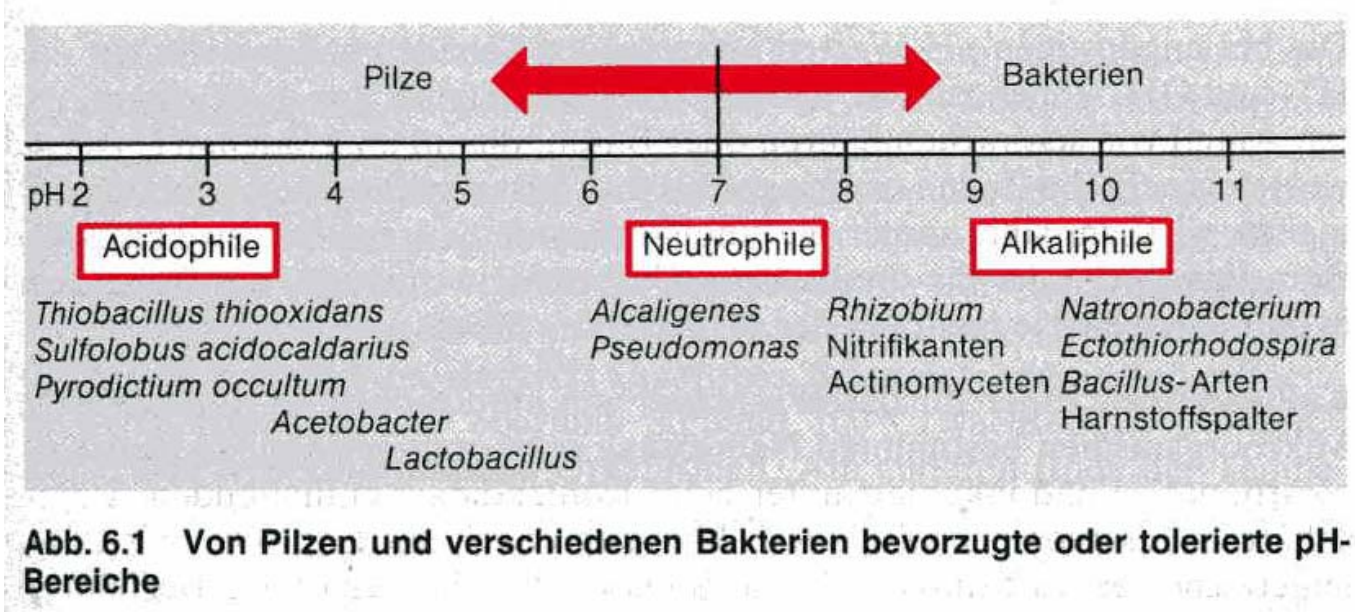


Abb. 6.1 Von Pilzen und verschiedenen Bakterien bevorzugte oder tolerierte pH-Bereiche

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

- Maximum growth rate is maintained over a pH range of 1 to 2 pH units and the full range for growth is 2 to 5 pH units.
- The metabolite product formation can change significantly:
  Aspergillus niger: pH 7: oxalic acid, pH 2: citric acid

Effect of pH and T on μ

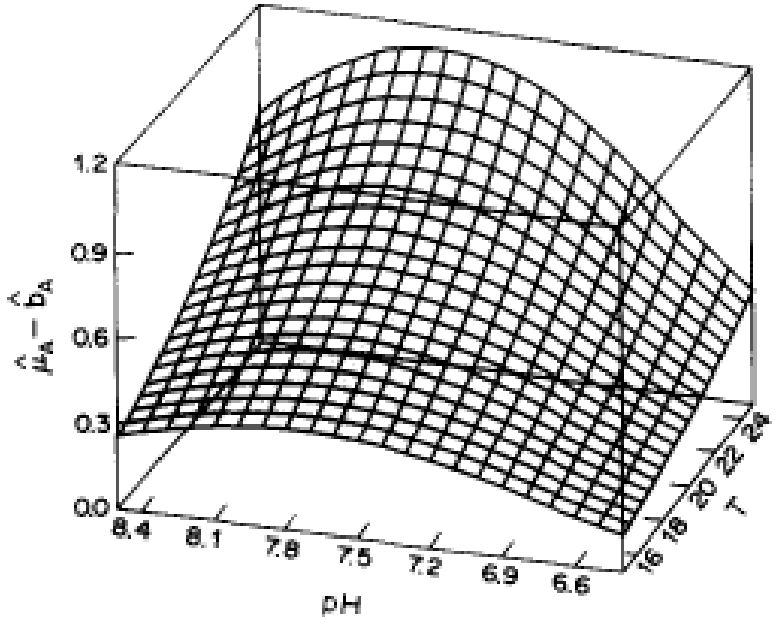


Fig. 4. Dependence of effective maximum specific growth rate on temperature and pH using the obtained functional relationship.

Other factors affecting μ

Osmotic strength	- 330-360 mOsmole (animal cell cultures) - Osmophiles
Substrate toxicity	- Substrate inhibition - Catabolite repression (diauxic growth) - Bottleneck (overflow) metabolism - Osmotic stress
Product inhibition	- End- product inhibition (by metabolites in medium) - Toxic products (NH_4^+, lactate, pyroglutamate etc.)

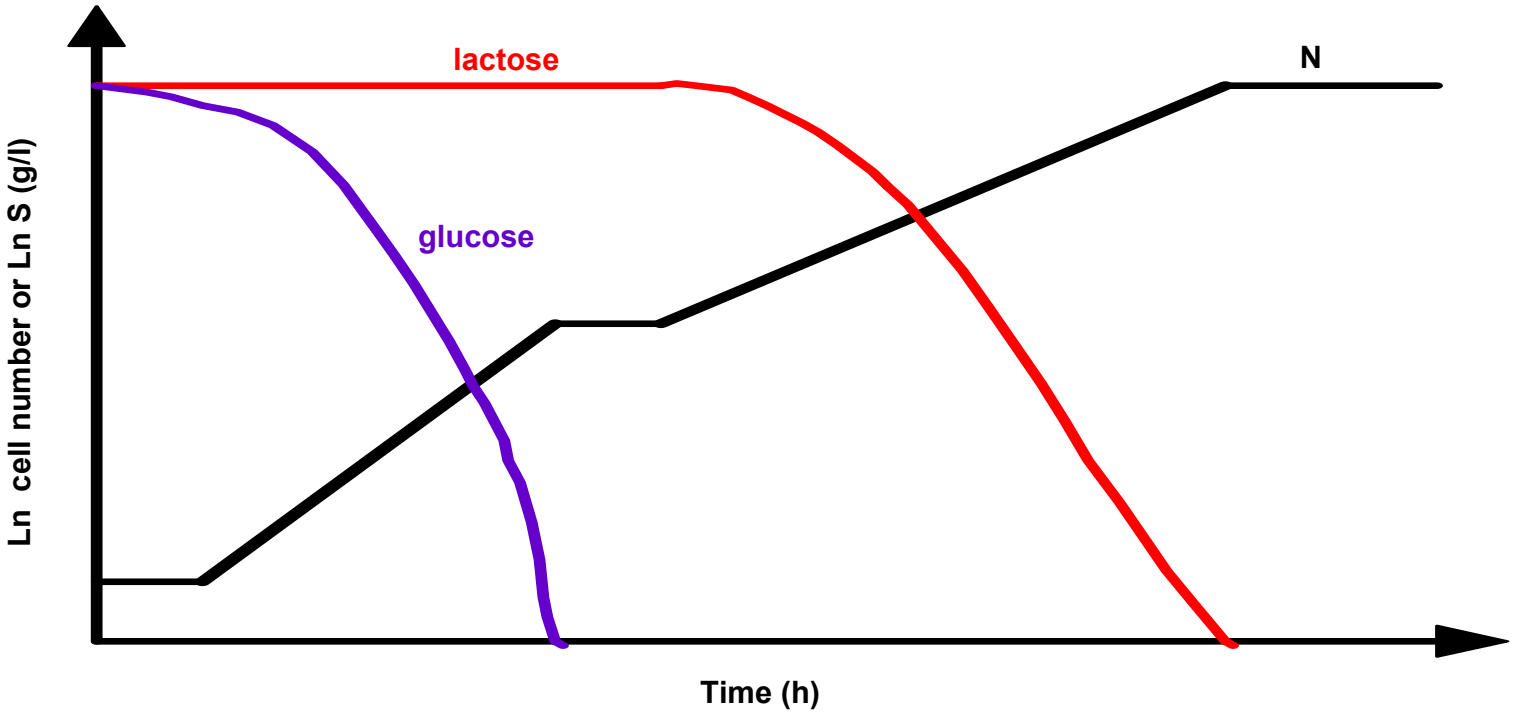
Selecting μ_{max} by medium composition

Composition of a selection of media used to set the maximum specific growth rate (μ_{max}) of *Salmonella typhimurium* in batch culture (from Egli, 2000).

No		Medium	Comments μ_{max} (h^{-1})
1	Brain+heart infusion	full strength	1.94
5	Nutrient broth	diluted 1:2 with medium No 14	1.80
6	Nutrient broth	diluted 1:5 with medium No 14	1.66
7	Casamino acids	1.5%+0.01% tryptophan in medium No 14	1.39
9	20 Amino acids	20 natural amino acids + mineral salt solution[a]	1.27
10	8 Amino acids	natural amino acids + mineral salts solution[a]	1.01
14	Glucose salt	0.2% glucose + mineral salts solution[a]	0.83
15	Succinate salt	0.2% succinate + mineral salts solution[a]	0.66
19	Methionine salt	0.06% methionine + mineral salts solution[a]	0.56
22	Lysine salt	0.014% lysine + mineral salts solution[a]	0.43

[a] Mineral salts solution contained $MgSO_4$, Na_2HPO_4, $Na(NH_4)HPO_4$, KCl and citric acid as chelating agent. It did support no visible growth without addition of a carbon source (...trace elements lacking).

Activators and repressors



Diauxic growth of *E. coli* in aerobic batch culture on mixture of glucose and lactose

Glucose = catabolite repressor of lactose metabolizing enzymes
Constitutive expression = glucose metabolizing enzymes
Inducible expression = lactose metabolizing enzymes

Catabolite repression

Diauxic growth of *E. coli*

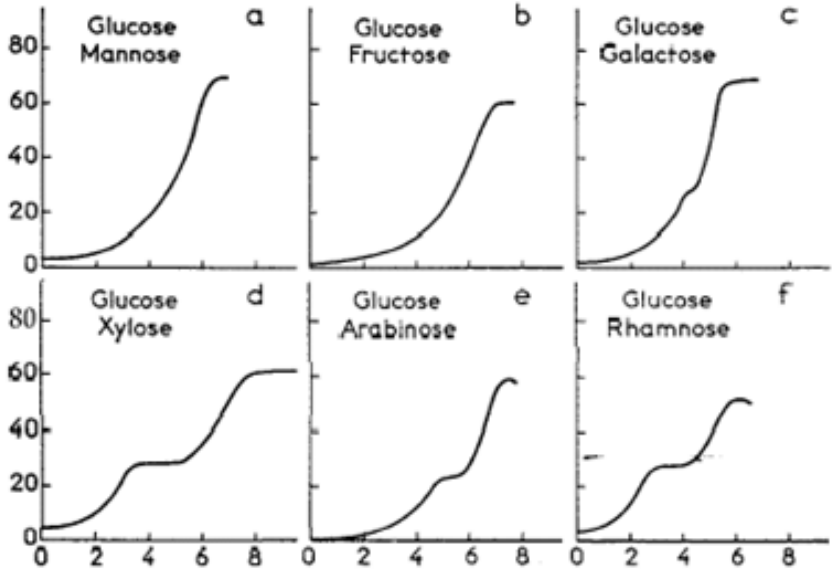
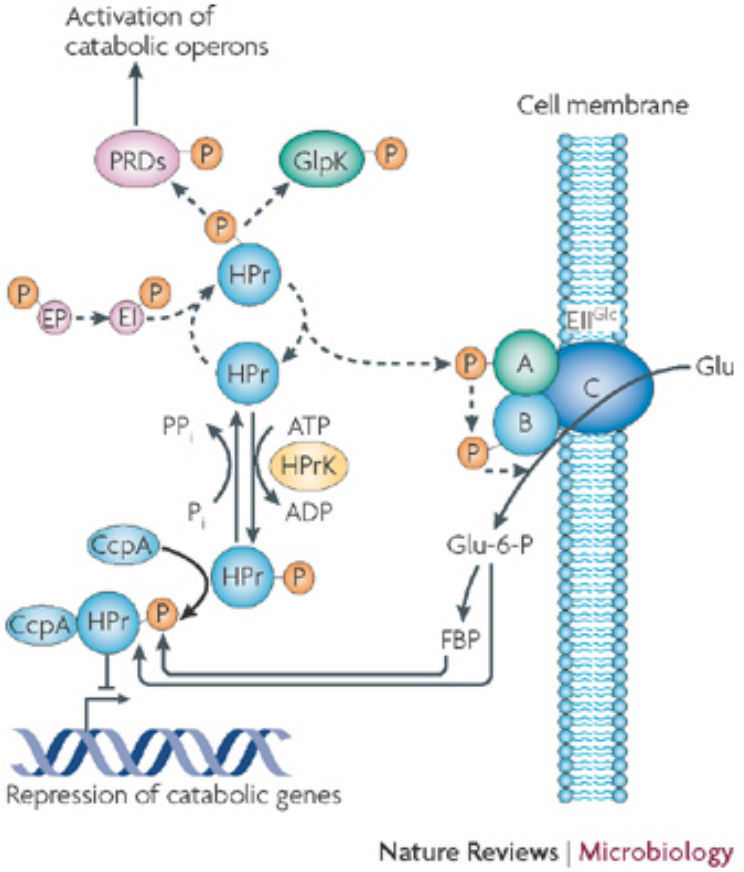
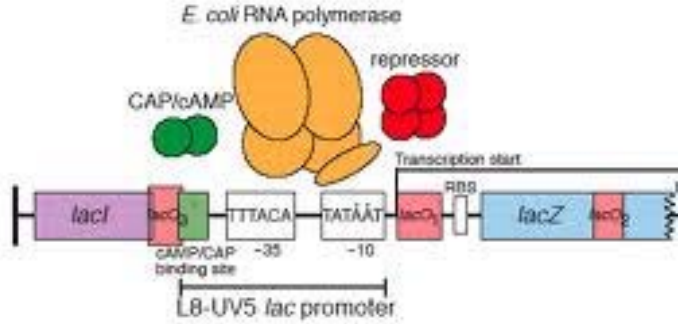


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[30].



Typical example is the lac operon:



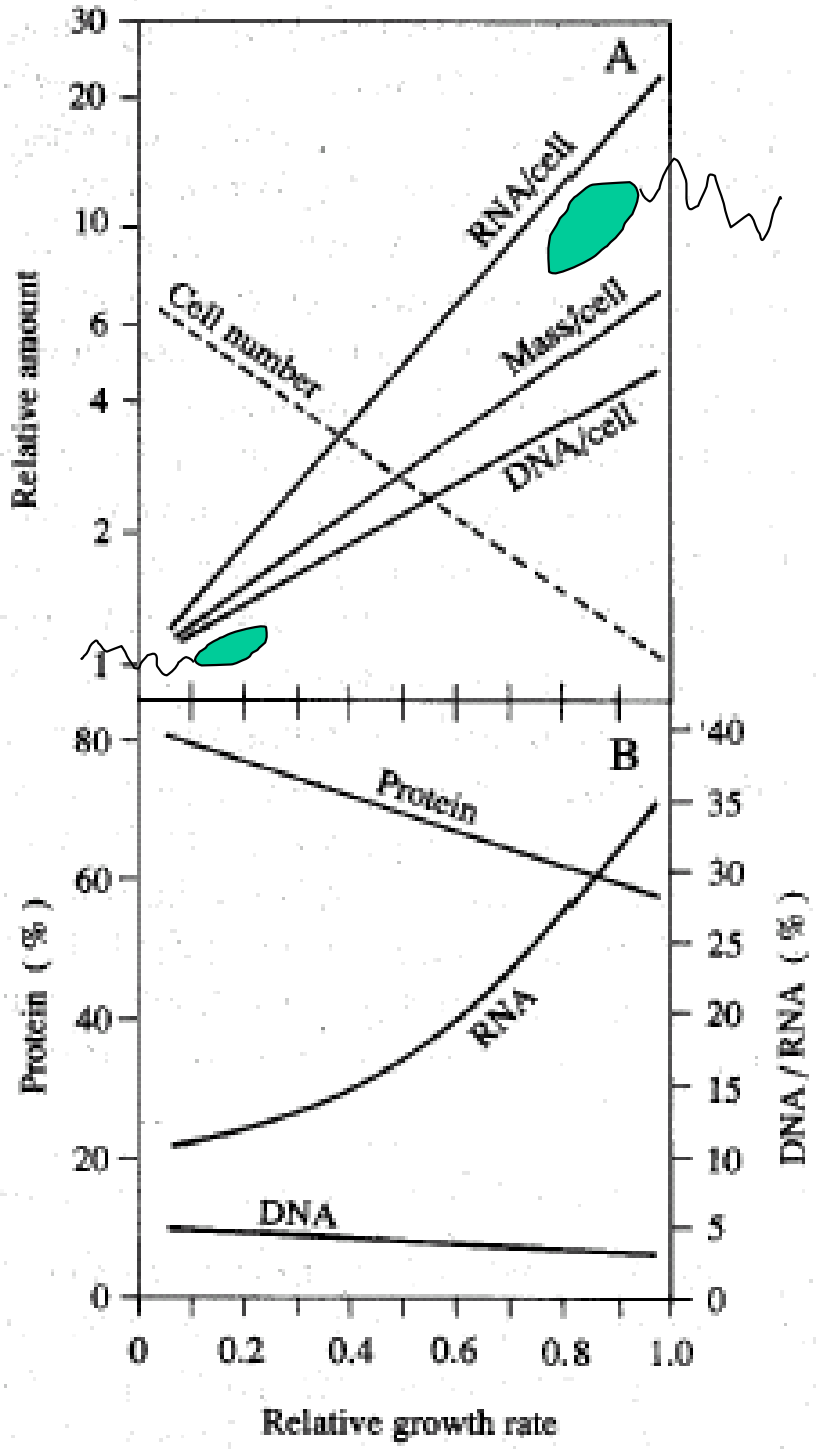
Cell composition depends on μ

These data have been found for unlimited growth in batch and carbon-limited growth in continuous culture (see next lectures)

Comments:

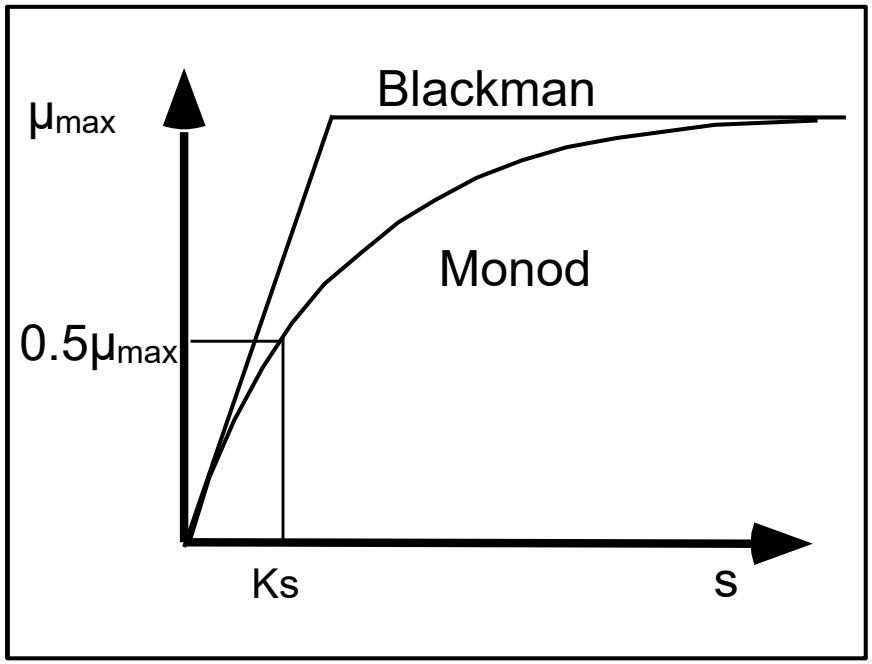
A)
- gives relative amounts per cell
- cell number formed from the same amount of glucose
- note the log-scale of y-axis

B)
- %-age of dry cell weight

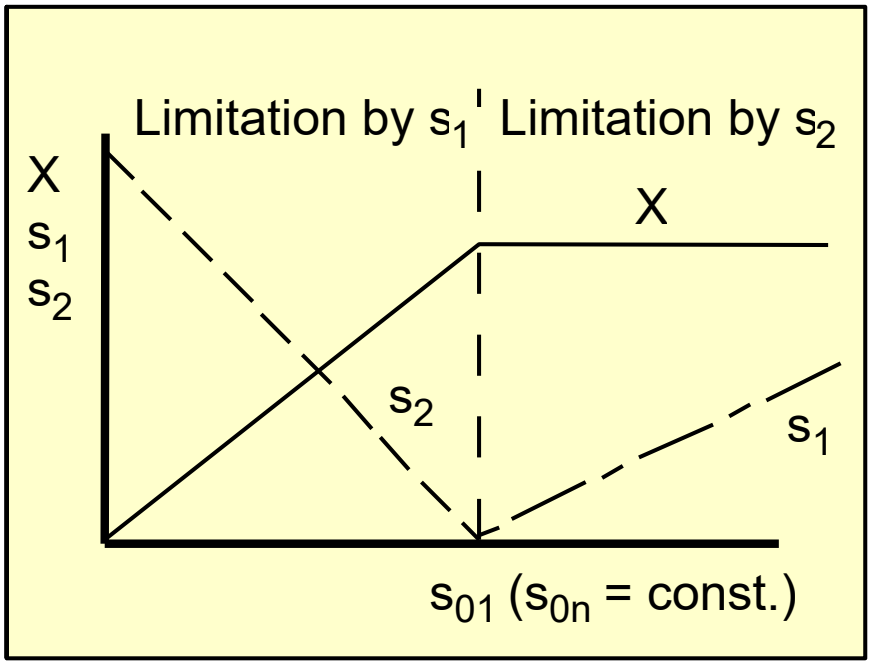


(from Wanner & Egli, 1990)

Growth limitations by nutrients

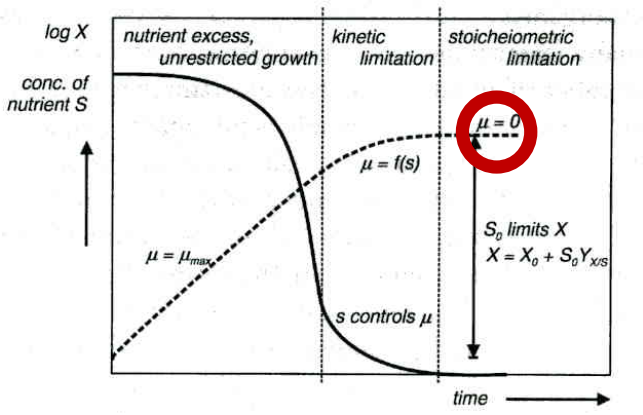


Kinetic limitation



Stoichiometric limitation

The stationary phase of a batch culture



- During the stationary growth phase the cells are not growing anymore.
- During stoichiometric limitation following points are important:
  - Growth (μ) is not measurable ($\mu = 0\ h^{-1}$)
  - Growth and cell lysis are balanced out ($\mu = k_d$)
  - Biomass concentration reached its maximum
  - Average biomass composition is changing (optimization to starvation)
  - Oxygen uptake rate is slowly decreasing
- Average cell size is small, $Gram^+$ strains tend to sporulate.
- Non-carbon limited cultures are synthetizing overflow metabolites (*e.g.*, acetate or EtOH).

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

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

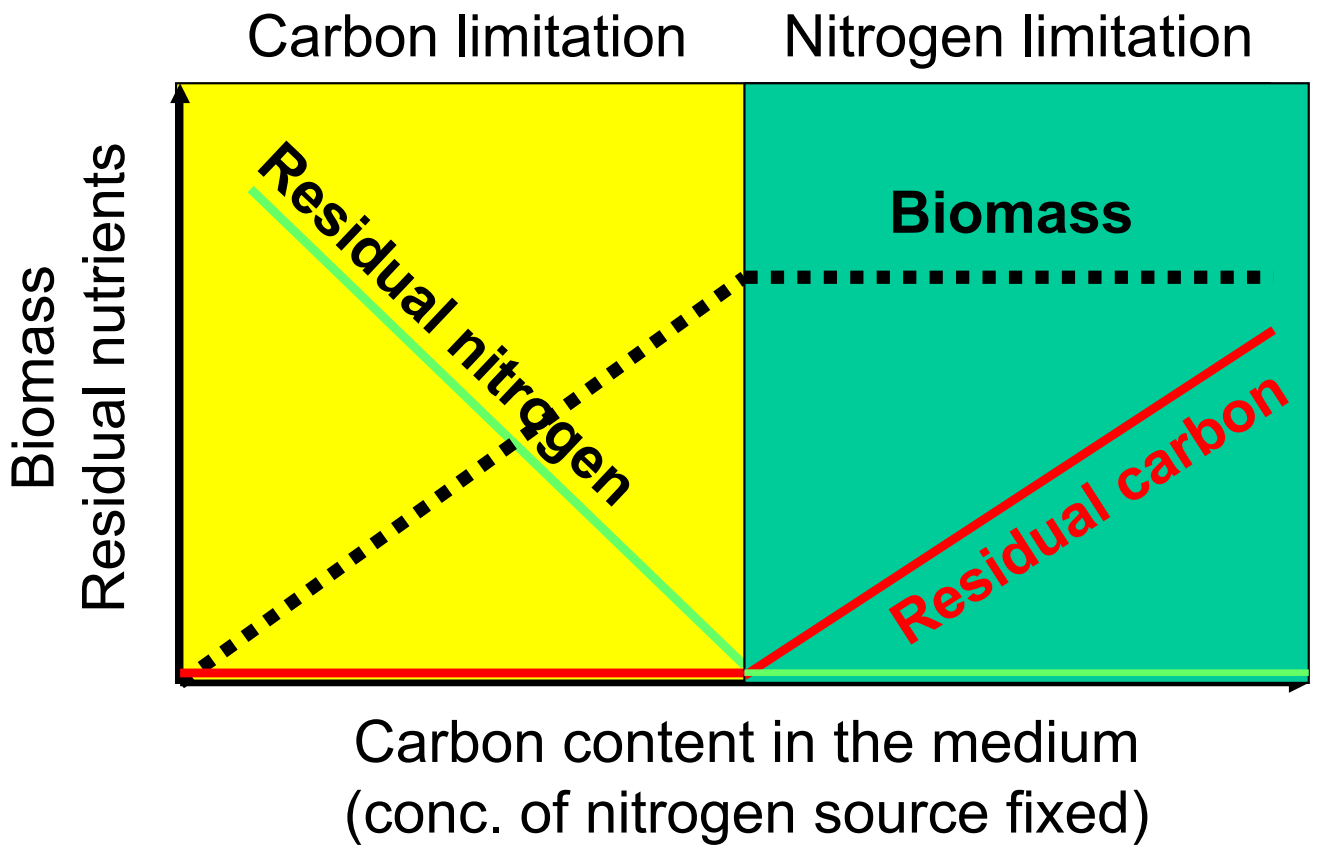
Generally, Yx/s is considered to be a constant value under exponential growth conditions.

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

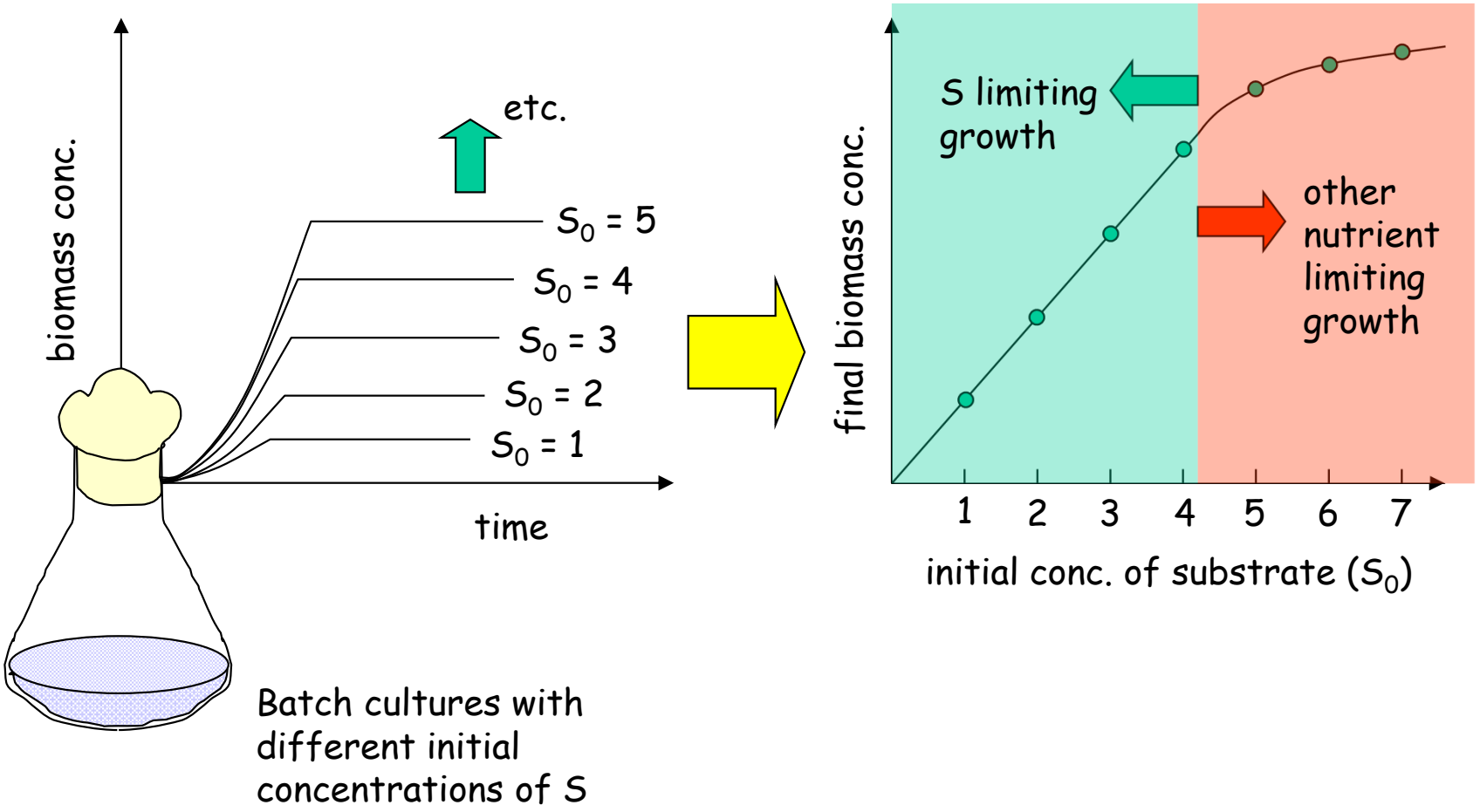
[g g^{-1}] or [g mol^{-1}]

The law of the minimum in the view of a microbiologist

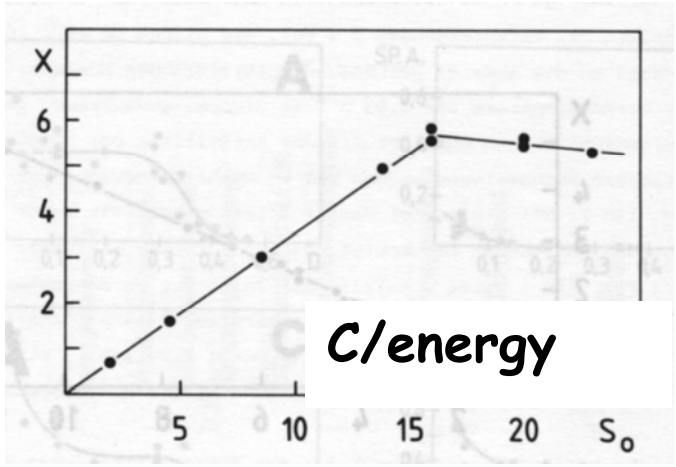
$$X = Y_{X/C} * C \qquad X = Y_{X/N} * N$$

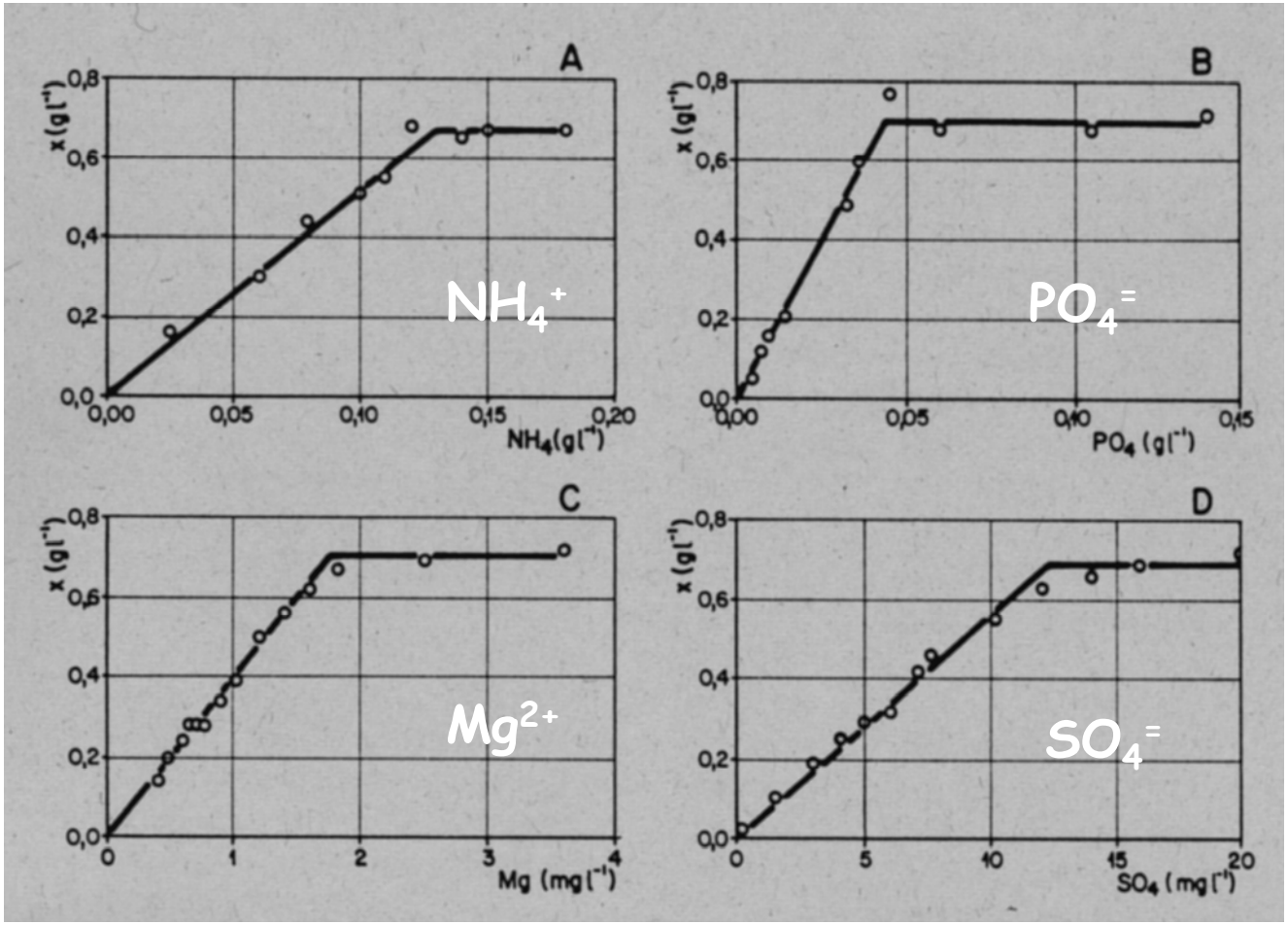


Testing a medium for the growth-limiting substrate



Examples of other nutrient limitations in chemostat cultures





Growth of *Thermus aquaticus* with different conc. of N-, P-, Mg- and S-source in the feed medium, D = 0.63 h^{-1}. (Cometta et al., 1982)

Integral method

1 Choose the growth limiting nutrient

For heterotrophs most often the carbon/energy source because it has to be added in highest quantities

2 Choose the maximum amount of biomass you want to produce in your system

Depends on cultivation system and goal (shake flask or bioreactor; high cell density cultivation or drinking water study, etc.)

3 Choose how much other nutrients should be in excess

Depends on safety factor you want, in biotechnological production you do not want excess because this will be lost

4 Calculate for each nutrient how much you have to add using established average growth yield factors

$Y_{X/S}$ = g biomass formed / g substrate utilized
$Y_{X/C}$ = g biomass formed / g substrate carbon utilized
$Y_{X/S}$ = g biomass formed / mol substrate utilized...

Medium design

How much carbon and nitrogen source do I have to add to a medium to obtain 4 g of dry biomass (CDW)?

$$\text{Gram element consumed} = \frac{\text{Gram dry biomass formed}}{Y_{X/E}}$$

Carbon: 10 gCDW / 1 gCDW/gC = 10 g C

Nitrogen: 10 gCDW / 8 gCDW/gN = 1.25 g N

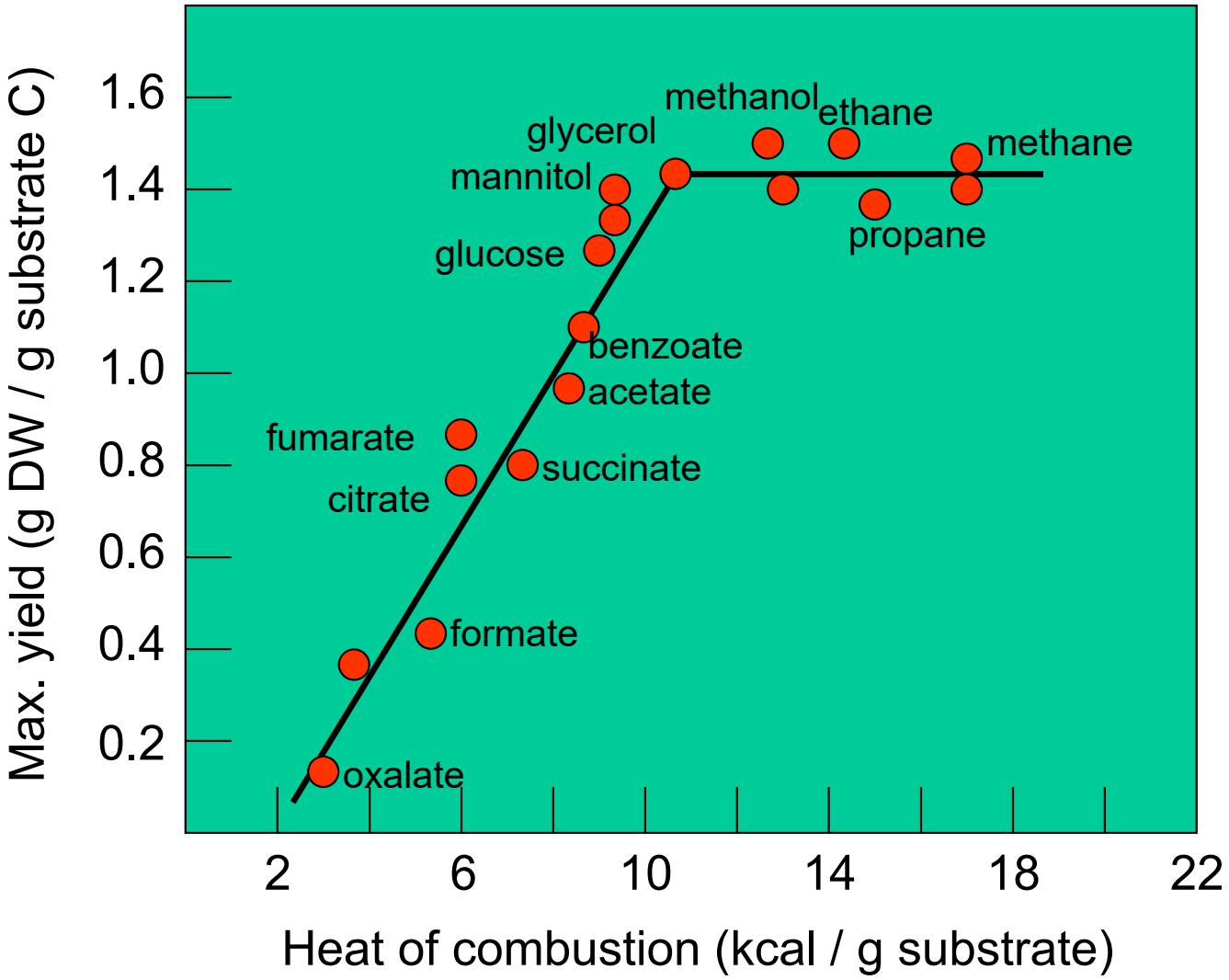
Design of a mineral medium

Design of a carbon-limited minimal medium allowing the production of 10 g L^{-1} of dry biomass, based on elemental growth yields obtained from the composition of dry biomass.

Medium constituent	Source of, function	Growth yield assumed (g dry biomass/g element)	Excess factor assumed with respect to carbon	Mass of element (g/L)	Mass of constituent (g/L)
Glucose	C, energy	1	1	10	25.0
NH_4Cl	N	8	3	3.75	14.33
NaH_2PO_4	P	33	5	1.52	5.88
KCl	K	100	5	0.5	0.95
$NaHSO_4$	S	100	5	0.5	1.87
$MgCl_2$	Mg	200	5	0.25	0.98
$CaCl_2$	Ca	100	10	1.0	2.77
$FeCl_2$	Fe	200	10	0.5	1.13
$MnCl_2$	Mn	10^4	20	0.02	0.046
$ZnCl_2$	Zn	10^4	20	0.02	0.042
$CuCl_2$	Cu	10^5	20	0.002	0.0042
$CoCl_2$	Co	10^5	20	0.002	0.0044

Based on Pirt (1975) and Egli & Fiechter (1981). Elemental growth yields for C and the trace elements Zn, Cu, Mo, and Mn were taken from Pirt (1975). Excess factors were chosen taking into account their variation observed in dry biomass.

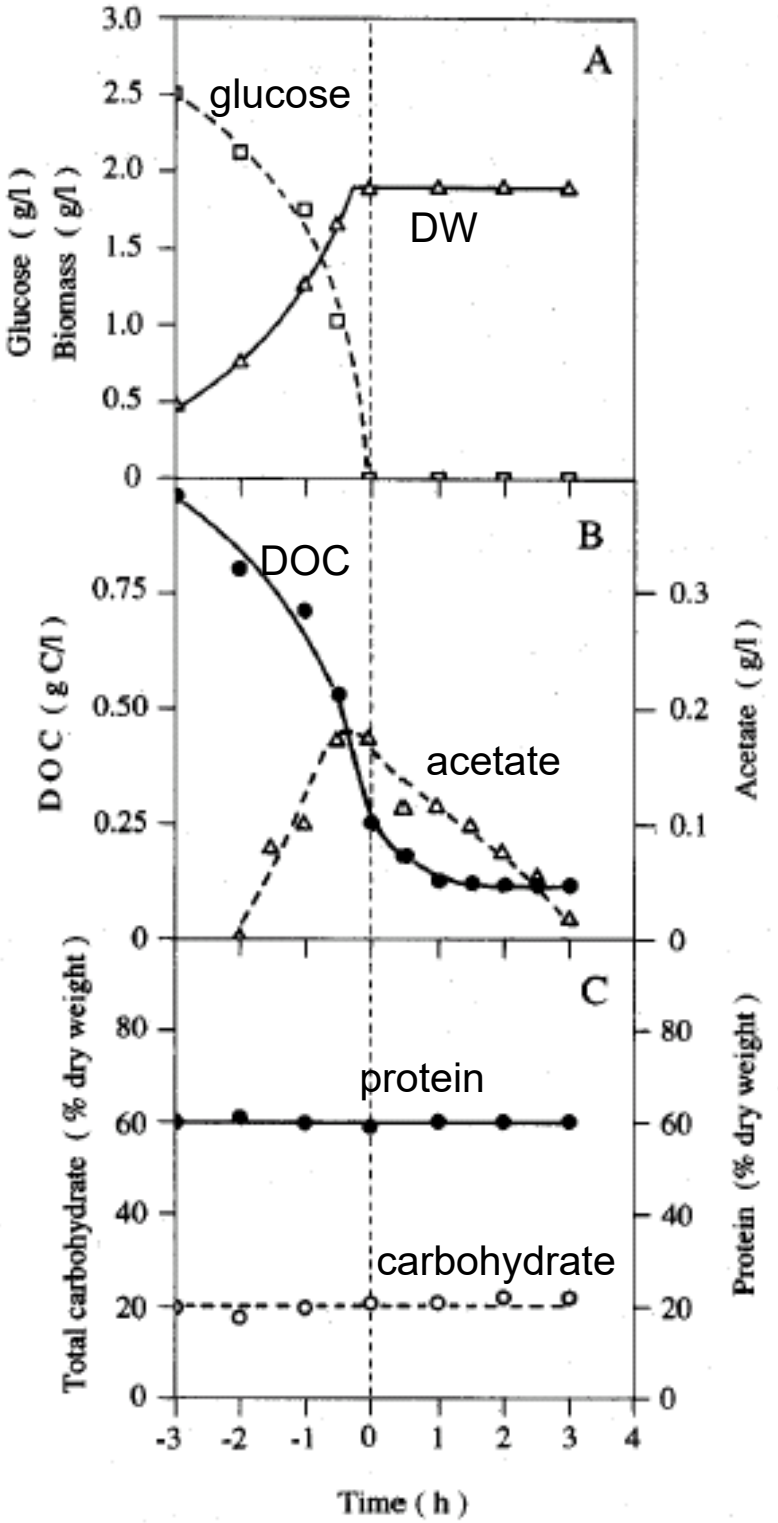
Growth yields for different carbon/energy sources



Carbon-limited batch culture

Comments:

- Acetate is produced and consumed again.
- DOC indicates that this is not the only product and that some carbon remains in the medium.
- “Frozen log cells”



Batch growth of *Klebsiella pneumoniae* in synthetic medium with glucose as limiting substrate.

(from Wanner & Egli, 1990)

Cell rearrangements in C-limited batch culture (1)

Biomass: Usually constant for several hours; exception: cultures that lyse or sporulate, e.g. *Bacillus* spp.

Cell number: Increases, becomes constant after 1-2 hours (DNA replication is finished and cell division is finished whenever possible).

Cell volume: Decreases, frequently also change in morphology observed (e.g., *K. pneumoniae* changes from 0.6 mm rods to 0.2 mm cocci)

Products: Products formed in exponential phase are consumed (for yeast forming ethanol during a second growth phase is observed)

Reserve materials: When accumulated in exp. phase (rarely in large amounts, except *Arthrobacter* spp.) they are consumed.

Cell wall: Low affinity transport systems replaced by high affinity systems (mostly info from chemostat experiments).

Lipid composition of membrane changes; replacement of un saturated fatty acids by saturated ones (tighter lipid layer?).

Peptidoglycane layer turnover stops (in Gram-positives 30-50%/h in exp. phase). Cross-linking and thickness of murein layer increases in Gram-negative cells.

Cell rearrangements in C-limited batch culture (2)

DNA: Rounds of replication are finished; DNA repair activities are much reduced.

RNA: Stable RNA (rRNA) is partly degraded and used as energy and building material for DNA synthesis (after storage and external products have been consumed). Excretion of surplus phosphate and sometimes also RNA bases. Rate of RNA synthesis reduced to ~10%

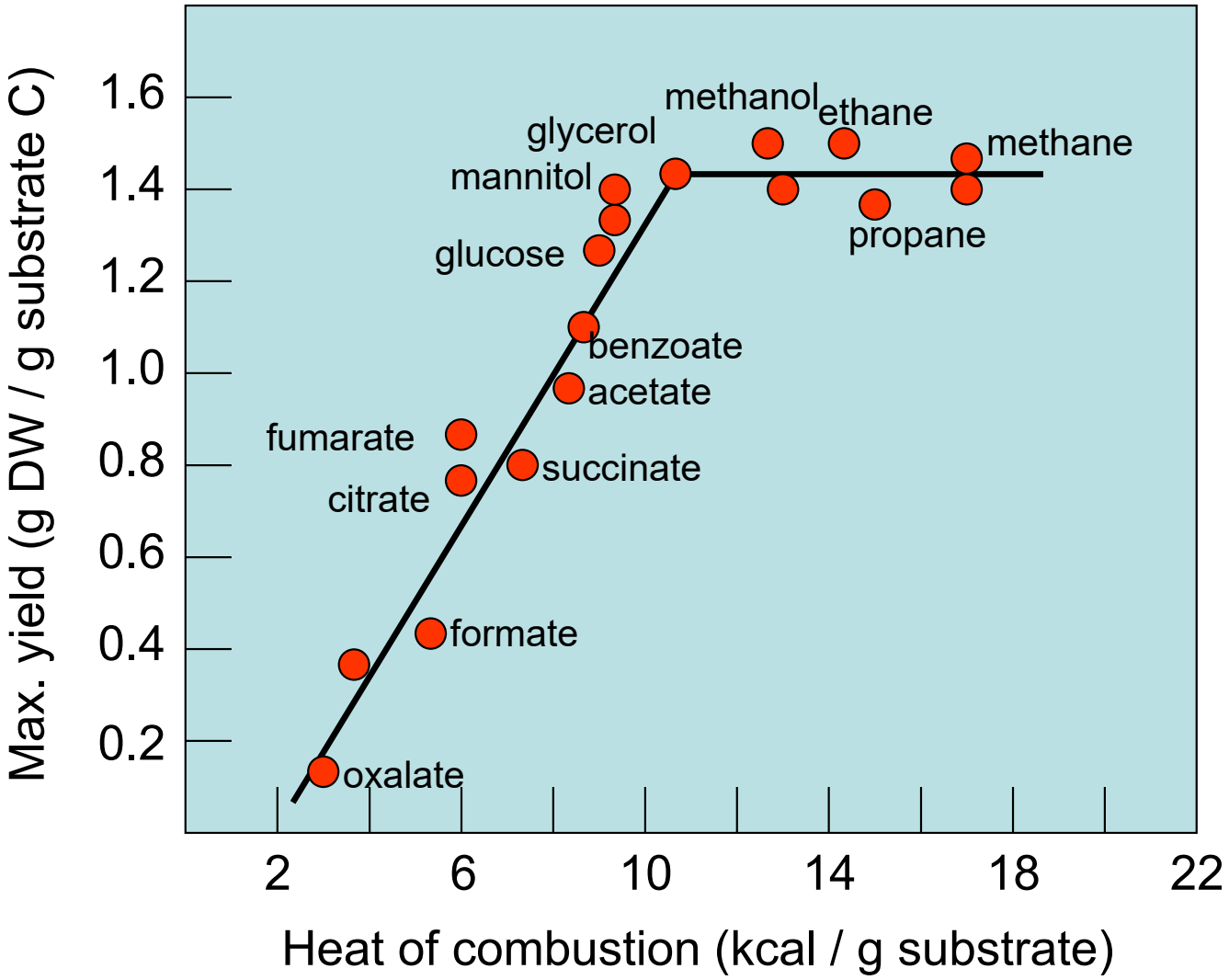
Protein: Protein turnover rates increase from ~2%/h to 5-7%/h
Amino acid pool immediately depleted.

Stringent response induced = ppGpp synthesis, an alarmone that controls expression of many genes; the response is triggered by stalling ribosomes because of unloaded tRNA; the response also includes expression of proteases that are vital for rearrangement.

Protein concentration remains mostly stable, only rearrangement of protein pattern, ca. 30 major new proteins synthesized in *E. coli;* proteins are degraded only after extended starvation periods when rRNA degraded to the minimum required.

ATP: Contradicting results in literature

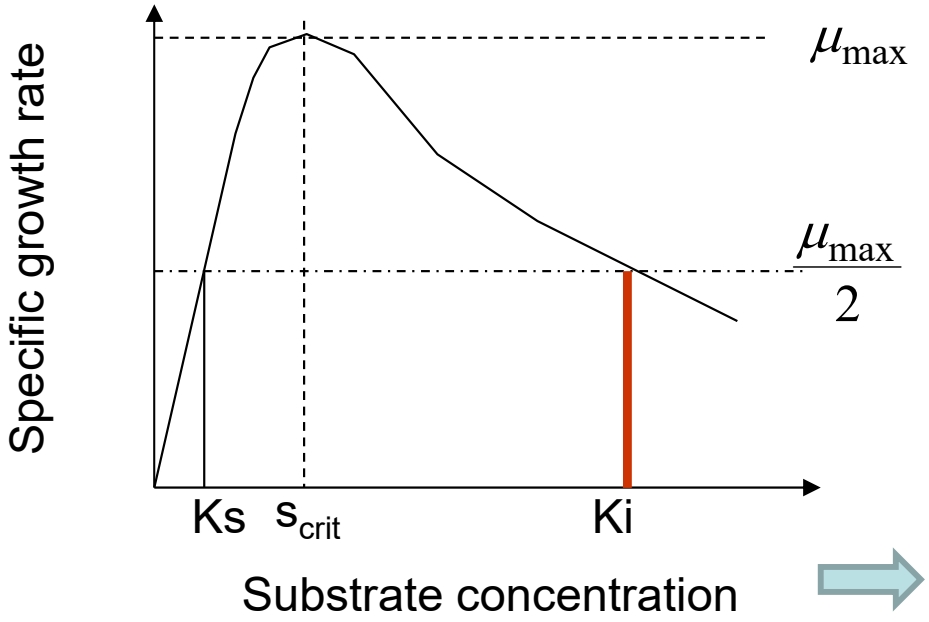
Growth yields for different carbon/energy sources



Too much of the good: Growth inhibition by substrates

Some substrates, for example alcohols, phenol and hydrocarbons, if present in excess, also inhibit growth.

In batch cultures the specific growth rate increases up to a critical substrate concentration s_{crit}, thereafter the inhibitory effect becomes dominant. In case the substrate was initially above s_{crit} but did not result in complete inhibition, the specific growth rate will increase with consumption of S until $s<s_{crit}$.



$$\mu = \mu_{max} \frac{s * K_i}{sK_i + K_s K_i + s^2}$$

K_i is usually in the range of 10x K_s

Too much of the good: Growth inhibition by the substrate

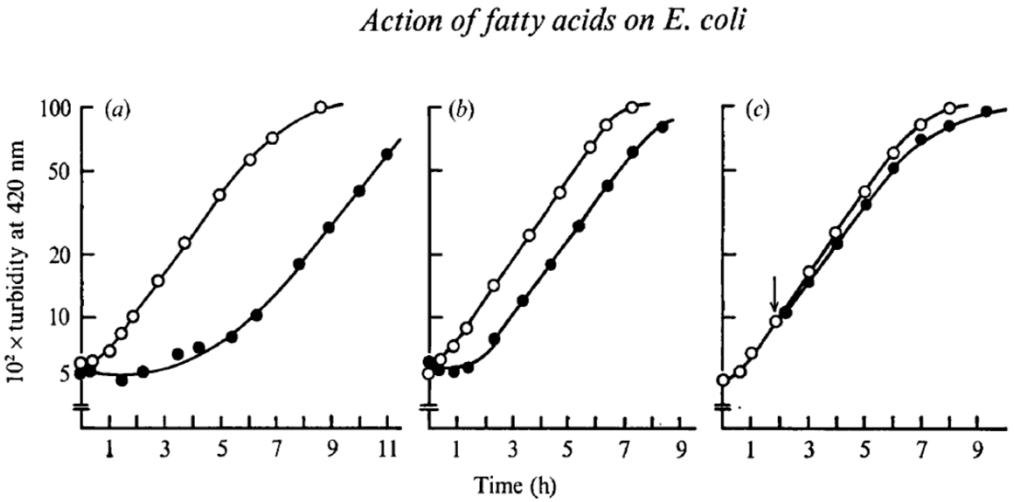


Fig. 1. Effect of lauric acid on the growth of *E. coli* K12/154 in a glucose minimal medium. Growth was measured as turbidity. Media were inoculated with: (*a*) a 2 h stationary-phase culture, (*b*) a mid-exponential phase culture, (*c*) an exponentially-growing culture. In (*c*) 0·1 % lauric acid was added (arrow). ○, Glucose minimal medium; ●, glucose minimal medium+0·1 % lauric acid.

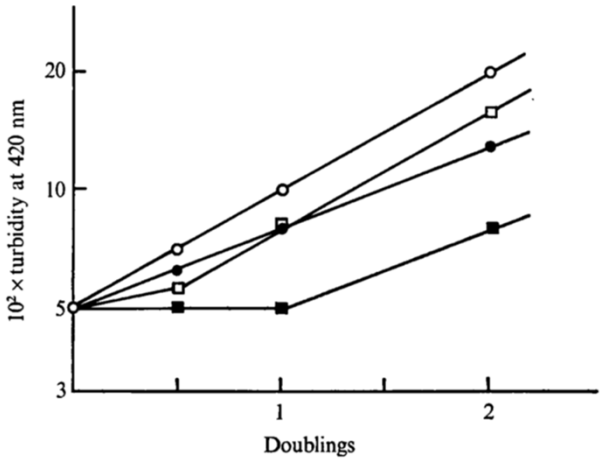


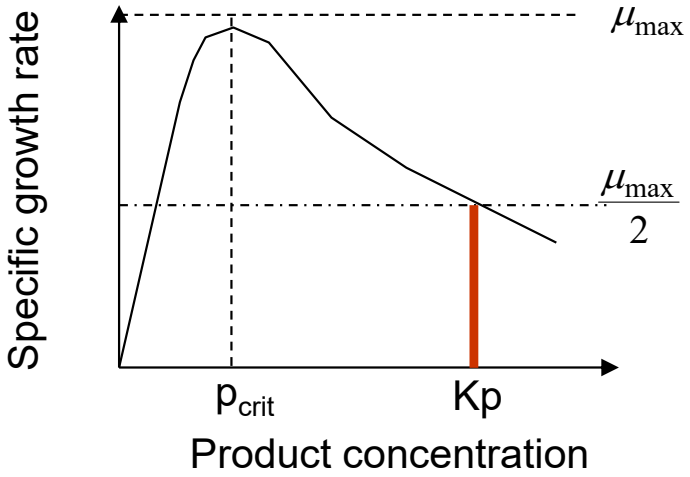
Fig. 2. Effect of a change in carbon source on the growth inhibition of *E. coli* K12/154 by nonanoic acid. Mid-exponential-phase cultures in succinate medium were used as the inoculum for the new media. The dilution was 1:25. The curves from different media were normalized to allow visual comparisons. One doubling time means the time which would be taken for a culture without fatty acid addition to undergo a doubling, whatever the medium. The control curves (open symbols) were theoretically determined from the doubling times of the bacterium in different media and in the absence of fatty acids (for values in minutes see footnote *, Table 1). ○, Succinate; ●, succinate +0·1 % nonanoic acid; □, acetate; ■, acetate+0·1 % nonanoic acid.

Journal of General Microbiology (I975), 91, 233-240

Too much of the good: Growth inhibition by the substrate

Some products, in particular metabolites, such as alcohols, acetate and lactate, inhibit growth.

The effect of product inhibition is a typical effect in wine production!



$$\mu = \mu_{max} * \frac{s_1}{s + K_s} * \frac{K_p}{p + K_p}$$

Aiba (1968)

K_p varies in relation to K_s

Nitrogen (ammonia)-limited batch culture

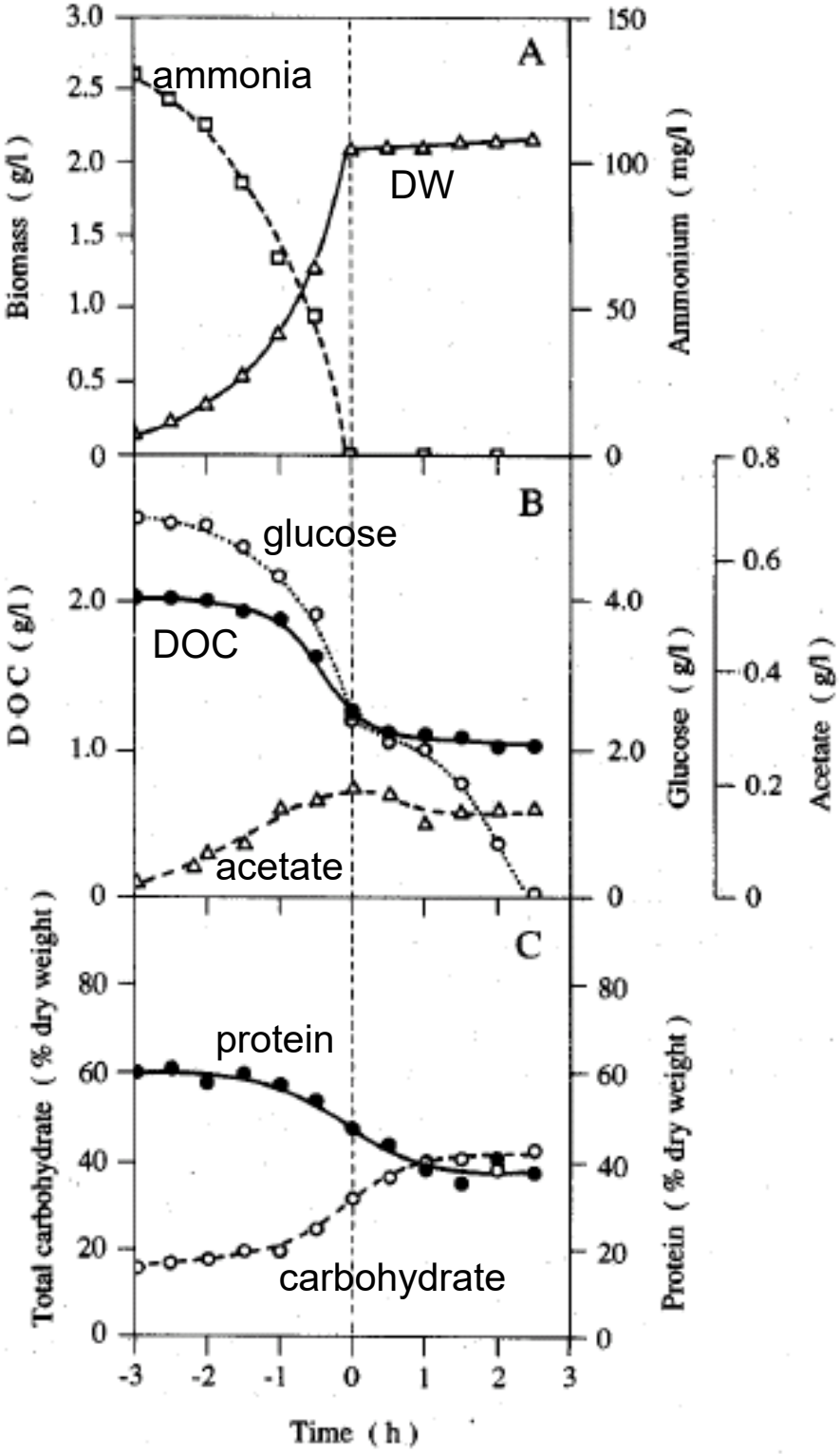
Comments:

- Acetate is produced

- All glucose is consumed and the culture is N- plus glucose-limited (residual carbon?)

- DOC indicates that a lot of carbon remains in the medium as products that are only slowly utilized (or not at all?).

- Cell composition changes slightly before limitation is reached



Batch growth of *Klebsiella pneumoniae* in synthetic medium with ammonia as limiting substrate.

(from Wanner & Egli, 1990)

Cell rearrangements in N-limited batch culture

Biomass:	Increases usually, dependent on organism from 10% to 2-300%
Cell number:	Increases also (not only due to finishing DNA replication rounds), up to 4-fold increase observed (*S.typh*.)
Cell volume:	No clear information (should stay constant or even increase)
Products:	Excretion of metabolites observed (depends on C-source)
Reserve materials:	PHB, lipids, glycogen accumulate, sometimes also polyphosphate
Cell wall:	Expopolysaccharides are formed (slimes, capsules)
DNA:	No clear information
RNA:	RNA degradation was reported but much less than in C-lim cultures.
Protein:	Observed reduction of protein content results from dilution by incorporated storage compounds. Protein turnover increases to 3-5%/h, but no net degradation Expression of enzymes of the N-regulon (30-50 new proteins), including proteases, high affinity amino acid transporter and assimilation systems (GS/GOGAT).
Remarks:	Many experiments in literature probably affected also by running out of carbon source (N and C-limitation at the same time).

Phosphorus (phosphate)-limited batch culture

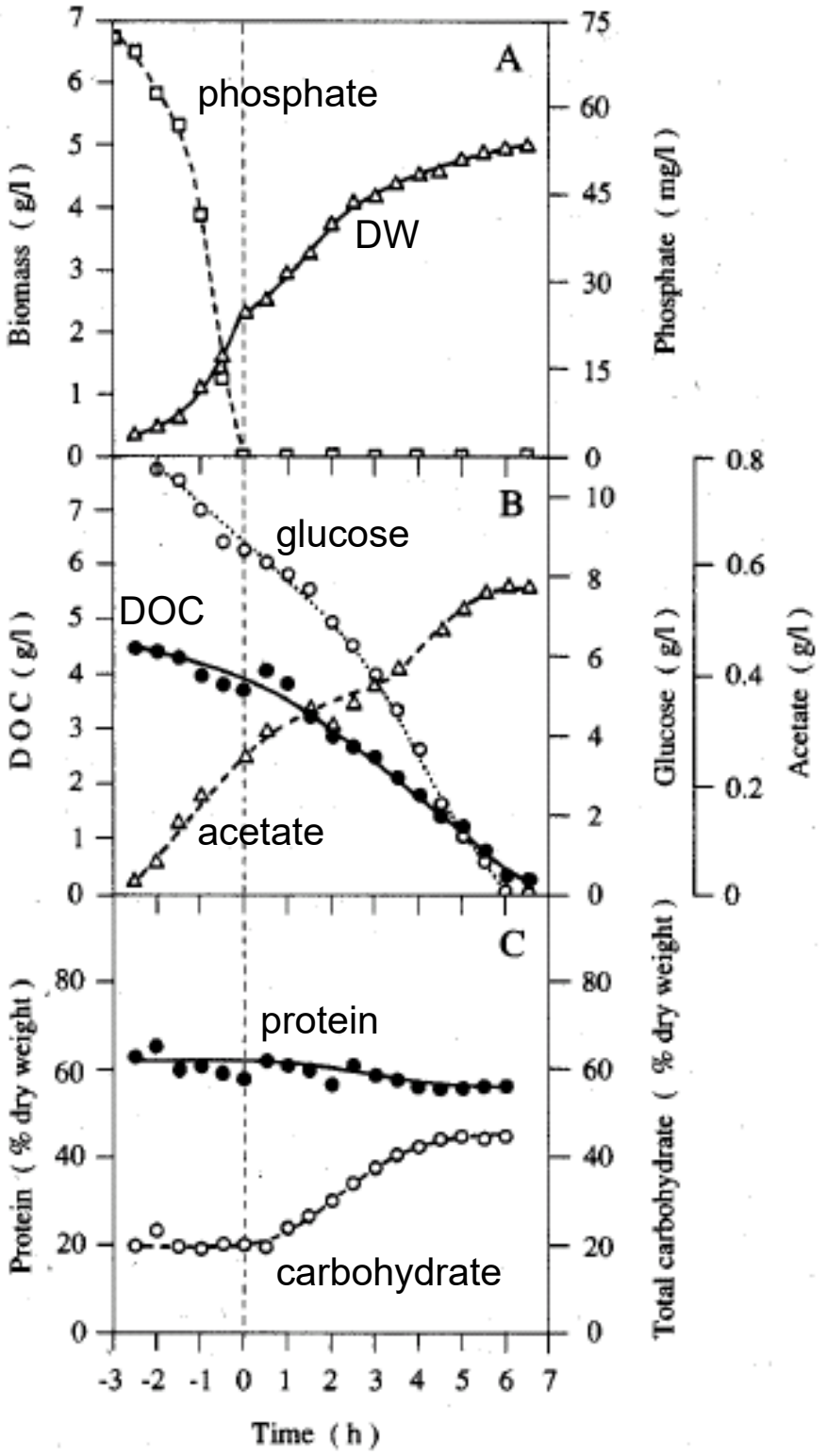
Comments:

- Growth continues after P run out; acetate production also

- All glucose is consumed and the culture is P- plus glucose-limited (residual carbon?)

- DOC indicates that acetate is probably the main excretion product

- Carbohydrate content changes, why not protein (mistake, interference)?



Batch growth of *Klebsiella pneumoniae* in synthetic medium with phosphate as limiting substrate.

(from Wanner & Egli, 1990)

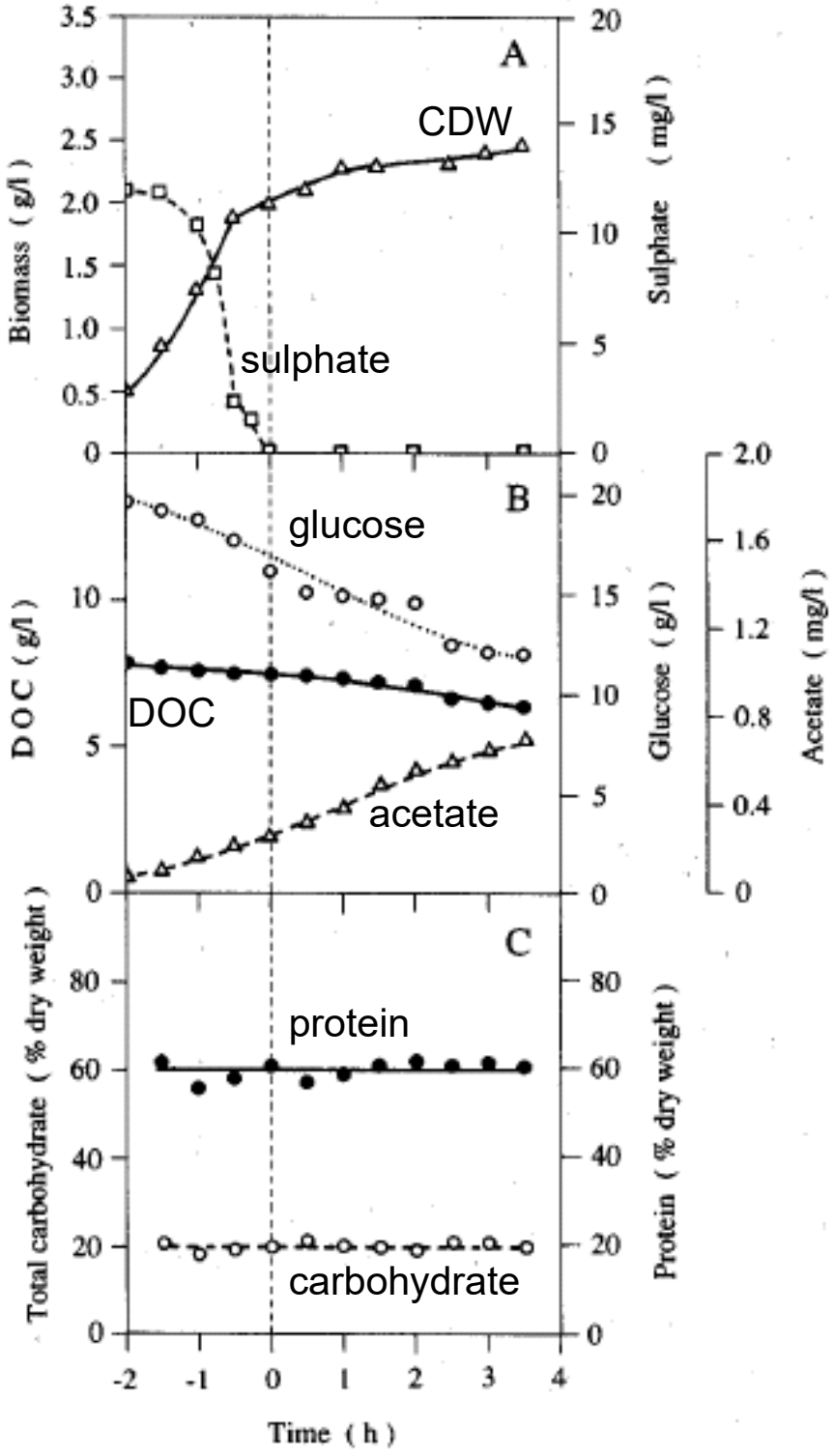
Cell rearrangements in P-limited batch culture

Biomass:	Increases up to 6-fold (in *K. pneumoniae*).
Cell number:	Increases correspondingly to biomass
Cell volume:	No data found (should become smaller due to decrease in μ)
Products:	Metabolites excreted, in *K.pneumoniae* primarily acetate
Reserve materials:	Differing information in literature, probably because the onset of incorporation is sometimes retarded
Cell wall:	Interestingly, in Gram-positives, replacement of P-containing teichonic acids by P-free components
	Also some phospholipids in membrane replaced by P-free analogues. Change of LPS composition and endotoxicity
RNA:	Pi pool immediately reduced to a minimum
	When PP_i present this is used for RNA and DNA synthesis.
	After all external P-sources are used up, degradation of RNA starts (10-25% /h); 60% of RNA-^{32}P reappears in DNA.
DNA:	Synthesis continues, P from all available sources appears in DNA.
Protein:	Expression of P-regulon, 20-30 major proteins including high affinity phosphate transporters, phosphatases, porins.
Remark:	Many experiments in literature probably got affected also by running out of carbon source (P and C-limitation at the same time).

Sulfur (sulphate)-limited batch culture

Comments:

- Growth continues after S runs out; acetate production also
- Glucose consumption continues as does acetate production
- DOC indicates that acetate is the major product
- Protein and carbohydrate content remains stable



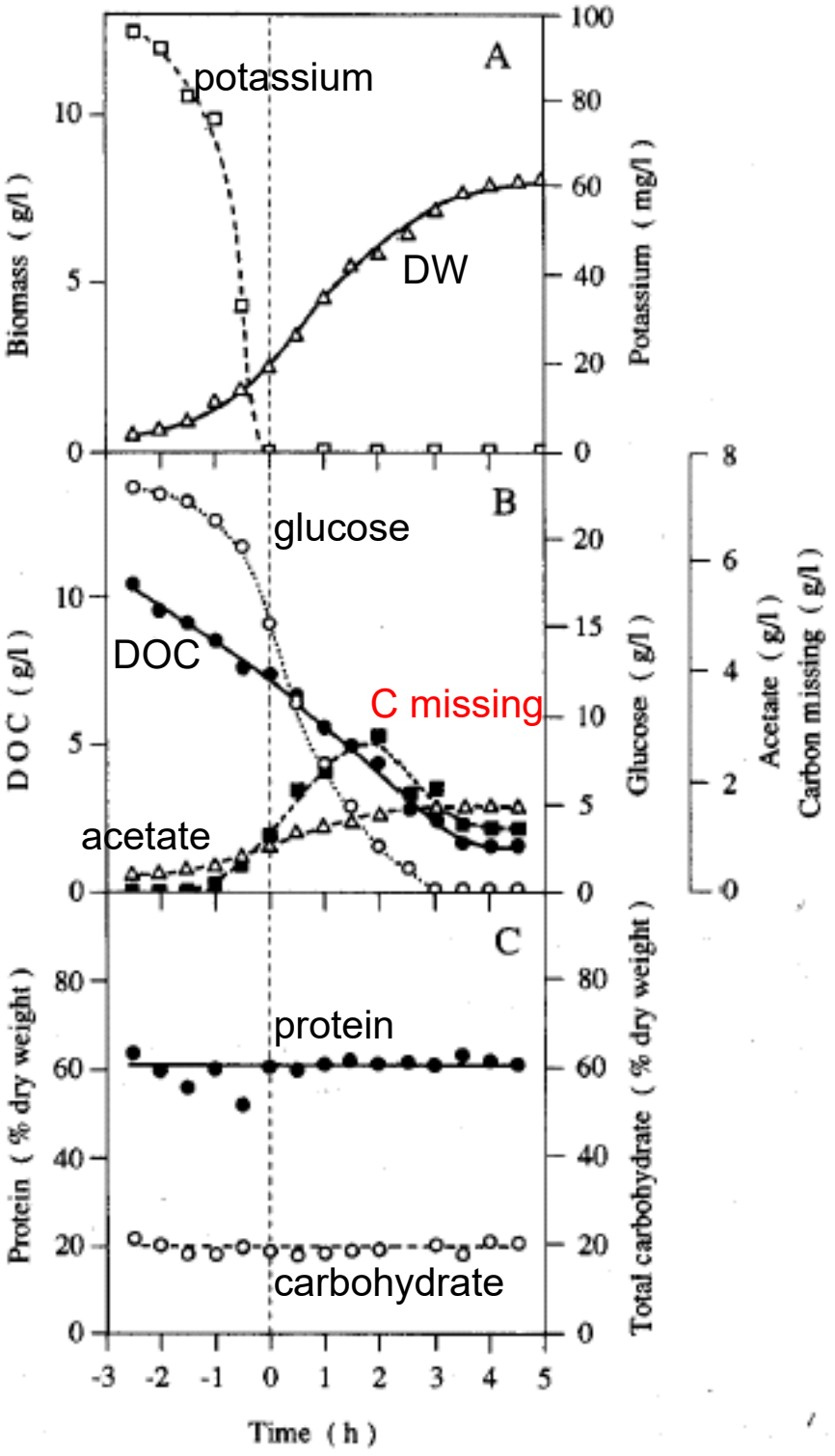
Batch growth of *Klebsiella pneumoniae* in synthetic medium with sulphate as limiting substrate.

(from Wanner & Egli, 1990)

Potassium-limited batch culture

Comments:

- Growth continues after K^+ runs out; acetate production also
- All glucose is consumed and the culture becomes K^+- plus glucose-limited (residual carbon?)
- DOC indicates that not only acetate is excreted, much carbon is missing
- Protein and carbohydrate content remains stable



Batch growth of *Klebsiella pneumoniae* in synthetic medium with potassium as limiting substrate.

(from Wanner & Egli, 1990)

Summary of effects

Changes in parameters of batch culture cells observed within the first few hours after depletion of various nutrients: increase (+), decrease (−), no change (0), no data available in literature (?)

Changes observed after substrate exhaustion	Substrate limiting batch growth:				
	Carbon	Nitrogen	Phosphorus	Sulfur	Potassium
Biomass	0	+	+ +	+	+ +
Cell size	−	?	?	?	?
Cell number	+	+	+ +	+	+ +
Metabolites	−	+ +	+	+ +	+ +
Carbohydrates	0	+ +	+	+	0
Polyphosphate	0	+	−	+ +	0
DNA	+	?	?	?	?
RNA	−	−	−	(0)	?
Protein	0	−	0/−	0	0
ATP pool	−	0/+	?	?	?

(from Wanner & Egli, 1990)

Take home messages

- Growth in a closed system (batch) consists generally of two phases:
  - **1) unlimited growth phase** (not dependent on “limiting” nutrient as all nutrients present in excess; exception non-covalently bound nutrients)
  - **2) phase of limitation**, one nutrient becomes growth limiting (1st kinetic and 2nd stoichiometric limitation), redistribution of this nutrient determines the physiology.
- **Classic (text book) stationary phase** only observed for limitation by carbon/energy source
- **Pattern of biomass in limitation phase** depends on lacking nutrient and the ability to get access to and redistribute this nutrient (usually an element):

  Usually clear-cut distribution pattern for covalently bound nutrients and fuzzy onset of limitation for non-covalently bound nutrients.
- A**vailability of (carbon)energy source** (external, internal) is essential for redistribution, adaptation, survival process.
- **Later phase of limitation** in most experiments affected by multiple nutrient limitations (i.e., mostly cultures become limited by carbon/energy source).
- **Growth inhibition** can be caused by too high concentrations of a substrate.