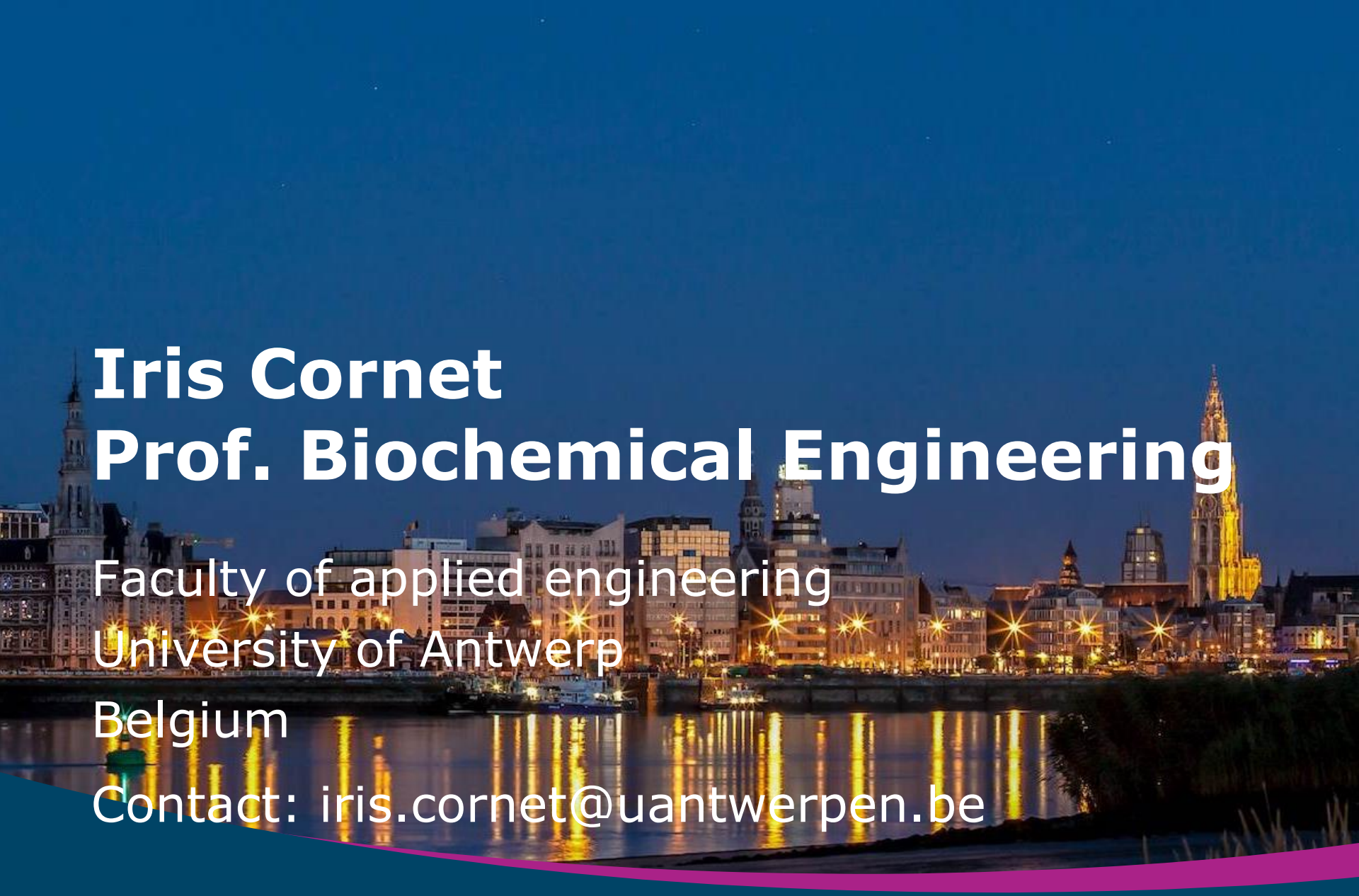


Iris Cornet
Prof. Biochemical Engineering

Faculty of applied engineering
University of Antwerp
Belgium

Contact: iris.cornet@uantwerpen.be

BIT
Biochemical Engineering Technology
University of Antwerp

Bioreactors

Solid state fermentation

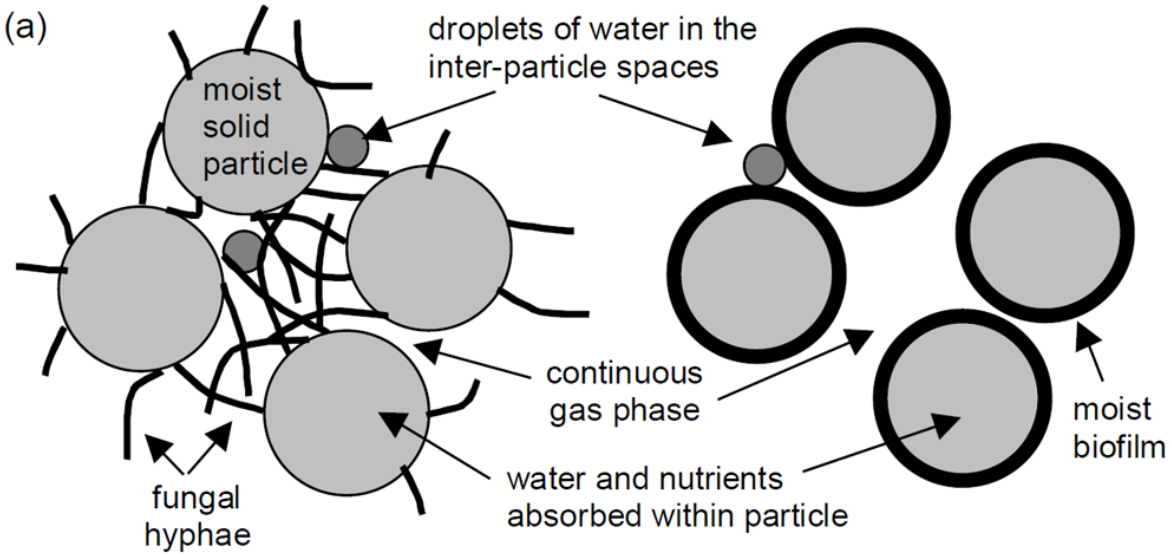
What do you know about SSF?

Solid state fermentation
Content

- What is solid state fermentation (SSF)?
- Advantages of SSF
- Problem statement SSF
- SSF reactor design
- Process modeling
- SSF reactor control
- Conclusions

What is SSF?

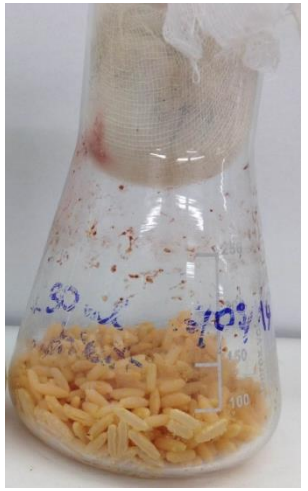
- Bioprocess in absence or near-absence of free water
- Heterogeneous process with 3 phases:
  - Solid = substrate or support
  - Liquid = moisture in substrate and aqueous film
  - Gas = continuous gas phase – oxygen supply



What is SSF?
Two types of carriers

- Solid substrate
  - Crops: wheat bran, soybean meal, rice, …
  - Agricultural or forestry waste: straw, bagasse, sawdust, …

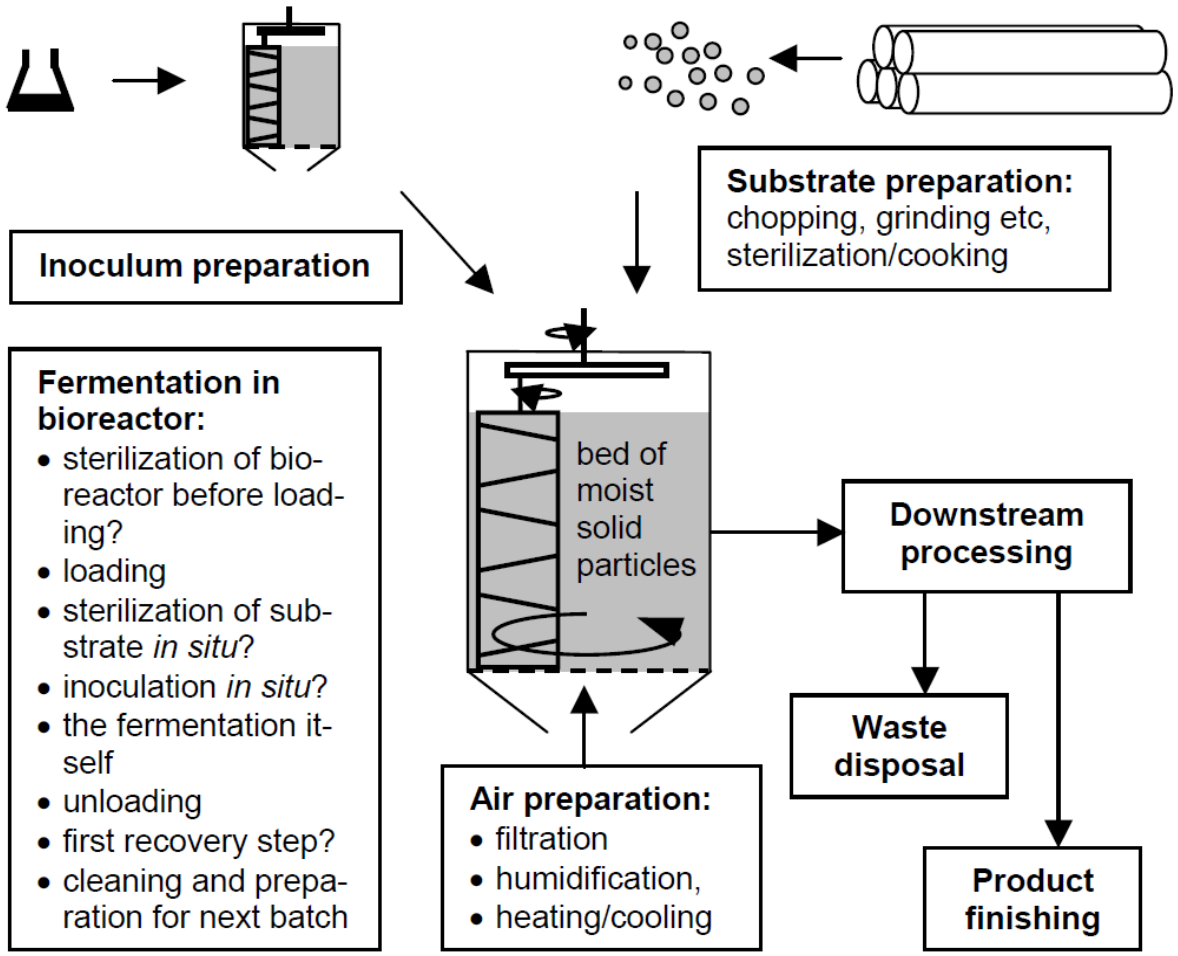
→ physical support for microorganisms & provides carbon source, nitrogen source, growth factors



- Inert carrier
  - Porous chemically inert material: PUR foam, macroporous resin, …

→only support for microorganisms and liquid culture is medium kept in pore structure

What is SSF?
General process steps



What is SSF?
History

- Traditionally used for fermented foods
- 1900 production of enzymes
- 1940 production of penicillin
- Advances in submerged fermentation (SmF)
- 1970s renewed interest → Reuse of organic wastes from agriculture and food processing
- 1990 Theoretical base of SSF bioreactor technology

What is SSF?
History

Time	Products
2000 B.C.	Bread, vinegar
1000 B.C.	Sauce, koji
550 B.C.	Kojic acid
7th century	Kojic acid in Japan
16th centrury	Tea
18th century	Vinegar
1860-1900	Sewage treatment
1900-1920	Enzyme
1920-1940	Gluconic acid, citric acid
1940-1950	Penicillin
1950-1960	Steroid
1960-1980	Protein feed
1990	Bioremediation, biological detoxification, biotransformation, biopulping, alfatoxin, ochratoxin, endotoxin, gibberelic acid, zearalenone, cephamycin

What is SSF?
Applications

- Biological detoxification of agro-industrial residues
- Bioconversion of biomass and production of high value chemicals such as antibiotics, alkaloids, growth factors, enzymes, organic acids, biopesticides, biosurfactants, biofuels, aroma compounds, etc.

What is SSF?
Applications

- Chemistry
- Food industry
- Pharma
- Energy
- Environmental field

Advantages and disadvantages of SSF over SmF?

Advantages SSF

SmF (submerged fermentation)	SSF (solid state fermentation)
High energy consumption	*Energy saving*
High water polution	*Water saving*
Oxygen limitation	*Sufficient oxygen*
Expensive substrates or pretreatment	*Use of low-cost residues*
	High product yield

SSF is even more sustainable than SmF

→HOT SPOT in recent research

Problem statement SSF

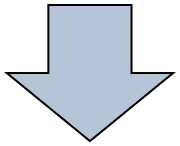
SmF (submerged fermentation)	SSF (solid state fermentation)
Easy mixing	*Mixing is difficult, growth is dependent on nutrient diffusion*
Temperature control is easy	*Removal of metabolic heat is difficult*
Homogeneity	*Heterogeneity*
Easy on-line control of process	*Difficult on-line control*

Use of solid matrix has big implications on engineering aspect of bioreactor design and operation

Problem statement SSF
Temperature

Absence of free water

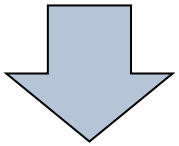
Low thermal conductivity of solid substrates



Problems with removal of metabolic heat

Problem statement SSF
Humidity

Humidity

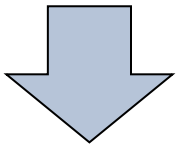


Problems to keep humidity due to removal by evaporation

Problem statement SSF
Oxygen

Difficult mixing → not the same oxygen concentration

Mixing to improve mass and heat transfer



Damaging the fungal mycelia

Problem statement SSF
Nutrients

Substrates can differ in

- Composition
- Mechanical properties
- Porosity (inter and intra particle space)
- Water holding capacity
- Specific surface area
- Etc.

General goal of engineering in fermentation?

Problem statement SSF

Maximization of

- Rate of formation (productivity)

$$\Pr\left[\frac{kg}{h.m^3}\right] = \frac{X_{harvest} - X_{initial}}{t_{process} \cdot V_{bioreactor}}$$

- Yield of product

SSF bioreactor has not yet reached a high degree of development

Problem statement SSF

In general: fermentation research elements

- Desired product
- Producing strain
- Desired environment
  - Nutrients
  - Temperature
  - Oxygen
  - Humidity!
- Reactor design

} Additional for SSF

SSF Reactor design
Basic designs

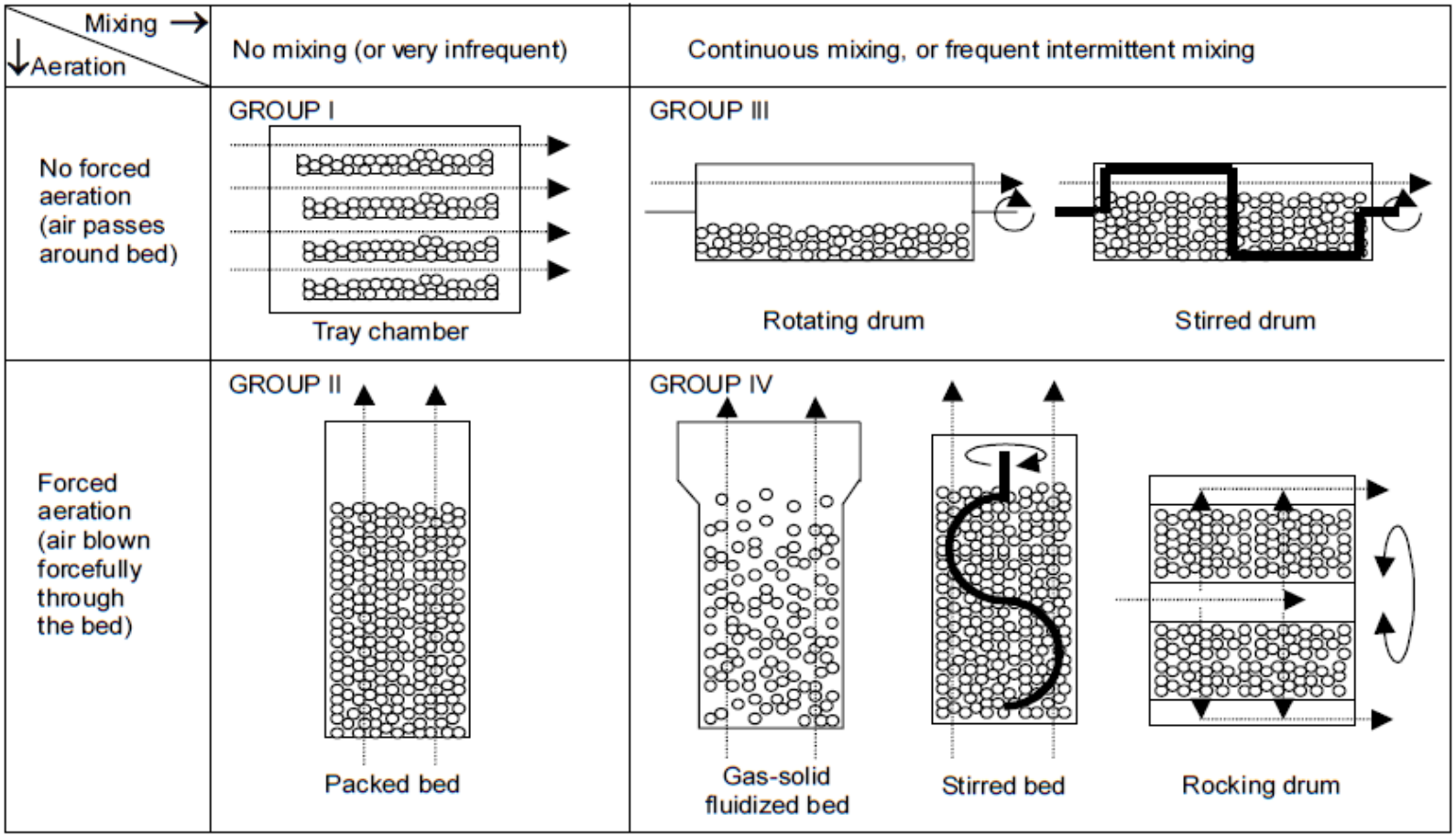


Fig. 3.2. Basic design features of the various SSF bioreactors, showing how they can be classified into four groups on the basis of how they are mixed and aerated. From Mitchell et al. (2000) with kind permission from Springer Science and Business Media

SSF Reactor design
Basic designs

Three types of industrial SSF reactors

- Tray bioreactors (TB)
- Packed-bed bioreactors (PBR)
- Rotating drum bioreactors (RDB)

SSF Reactor design
Basic designs

Tray bioreactors

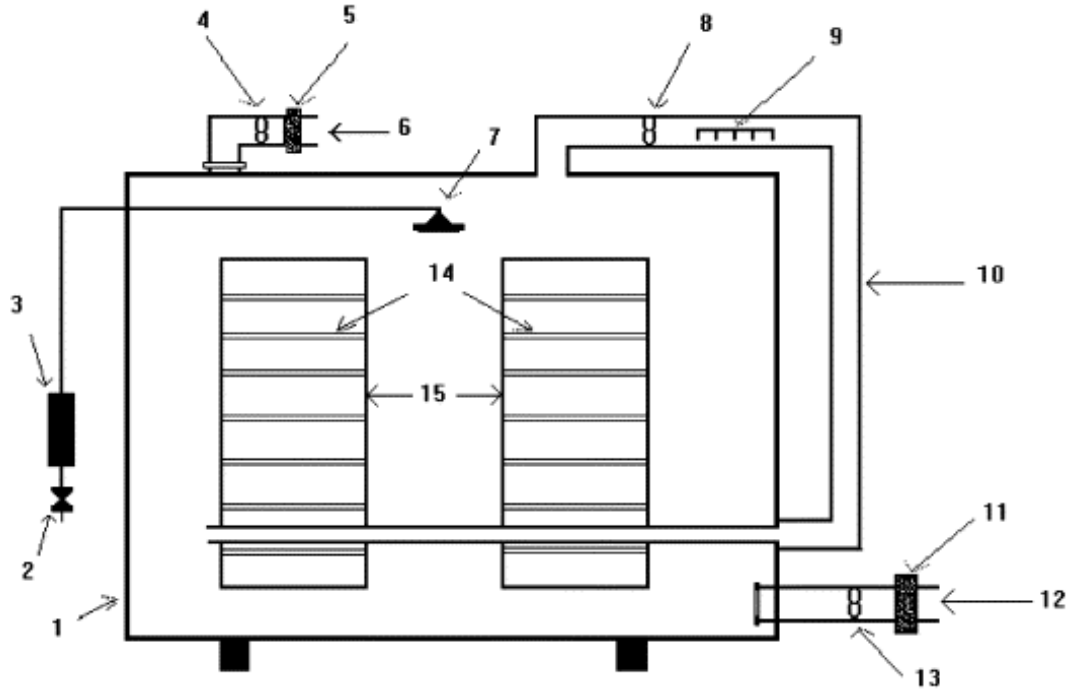
Packed-bed bioreactor

Rotary drum bioreactor

SSF Reactor design
Tray bioreactors

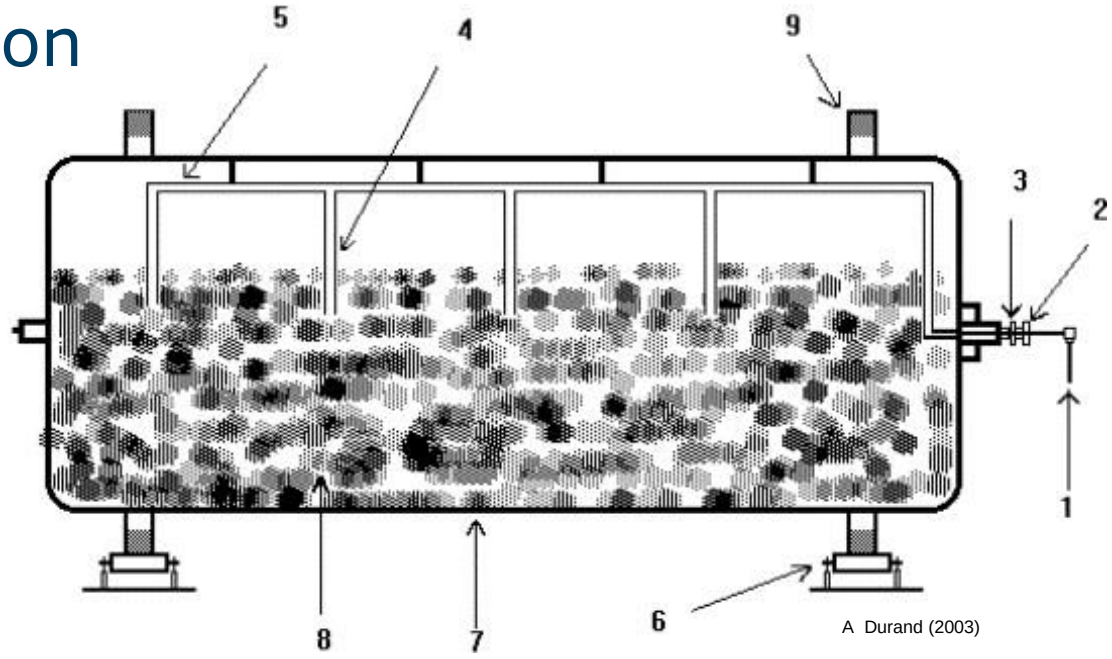
- Simple use, low cost, easy operation

→ problem: temperature



SSF Reactor design
Rotary drum bioreactors

- Mixing by rotation
- Internal or external cooling
- Aeration



A Durand (2003)

SSF Reactor design
Packed-bed bioreactors

- Advantages
  - High substrate loading possible
  - Cooling via evaporation by forced aeration
- Essential
  - Substrate with a sufficiently high interparticle volume → sufficient aeration of the column
- Control of the process parameters
  - Flow rate air
  - Temperature of air
  - Water saturated air

SSF Reactor design
Selecting the right reactor

Critical questions for choosing the right reactor

- In what degree is the microorganism affected by agitation?
- What is the influence of temperature and temperature increase on the microorganism?
- What are the aeration requirements?

SSF Reactor design
Selecting the right reactor

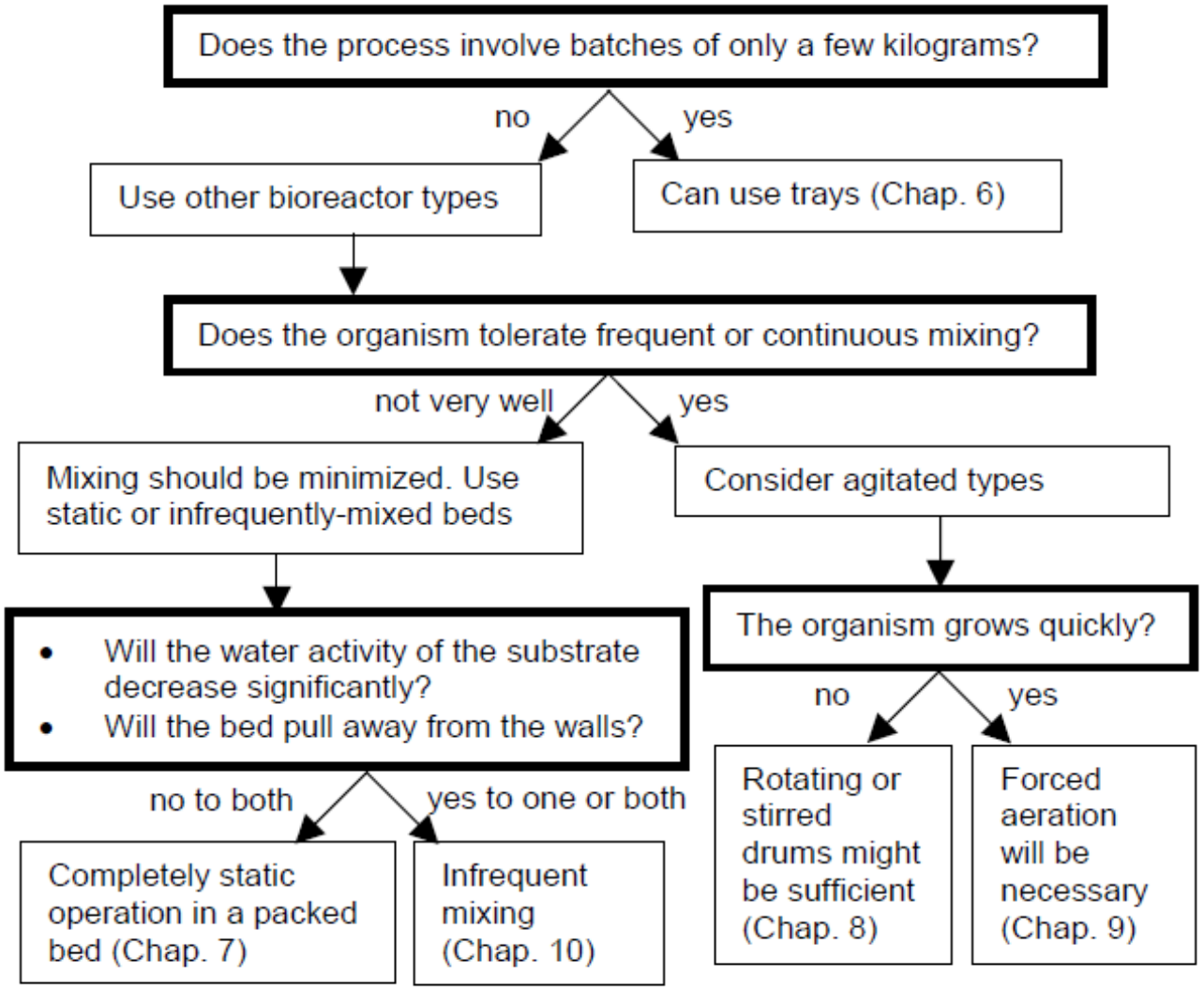
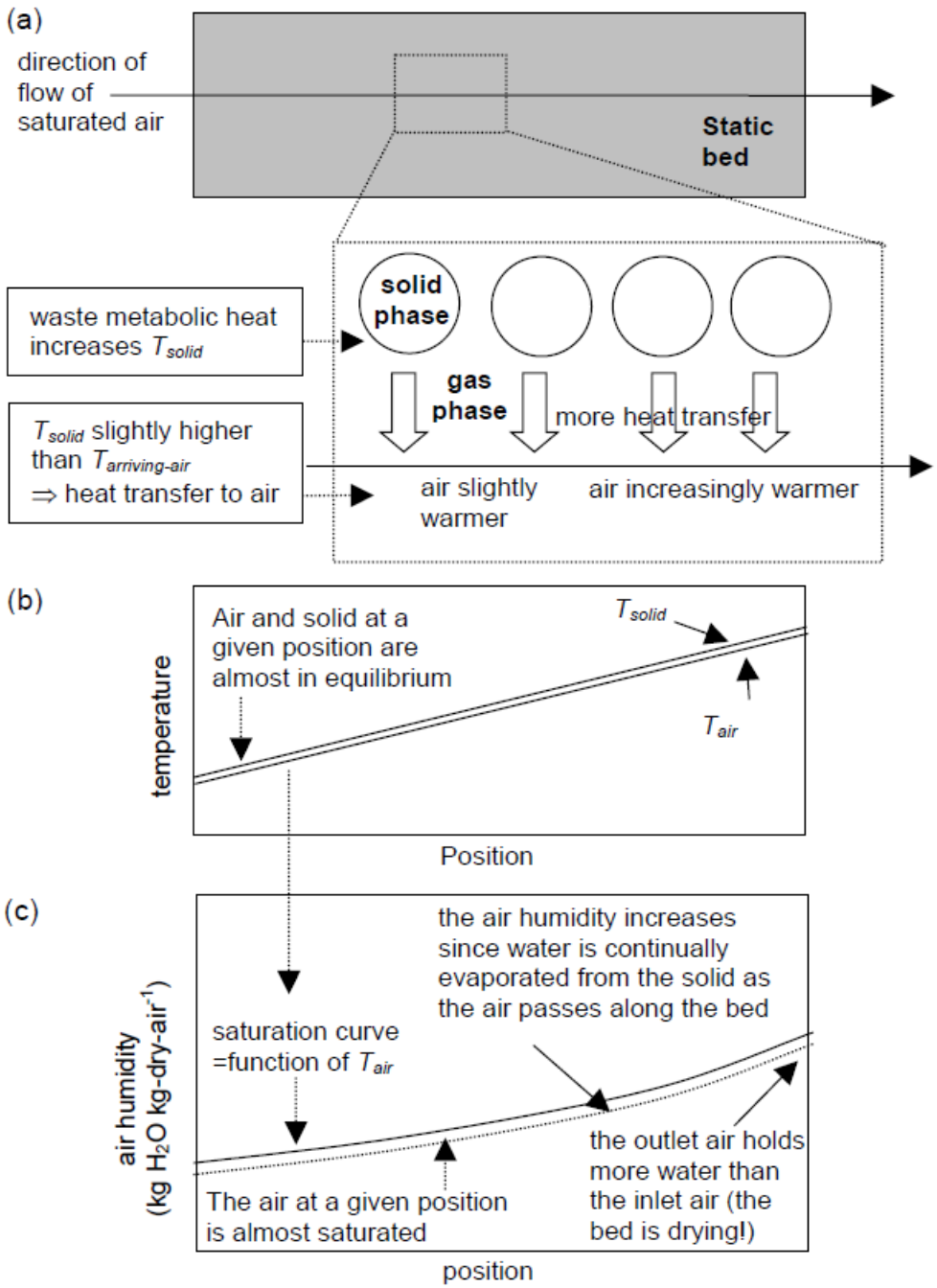


Fig. 3.3. A suggested key for SSF bioreactor selection

Looking closer at some selected reactor aspects

SSF reactor design
Convective flow



Saturated air at low velocity

Consequences:

(a) Mechanism of formation of axial T-gradient

(b) Axial T-gradient

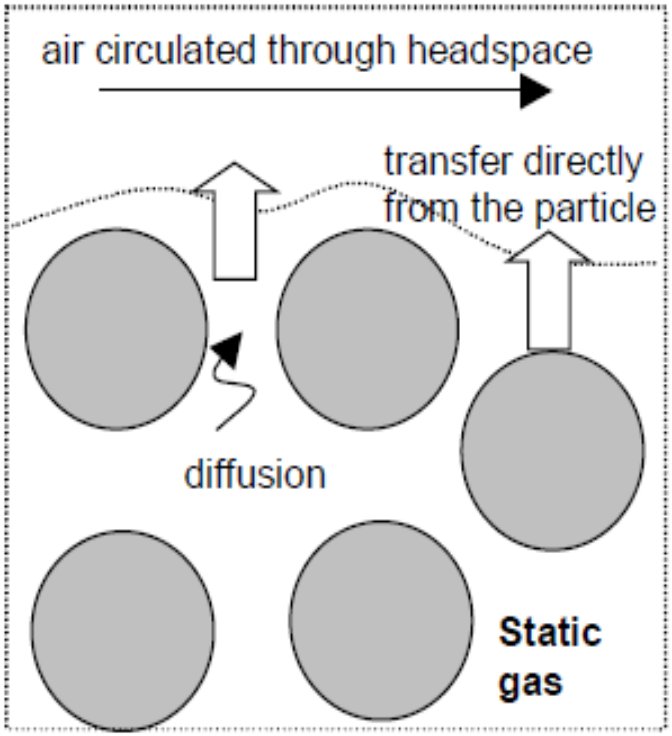
(c) Influence of (b) on evaporation

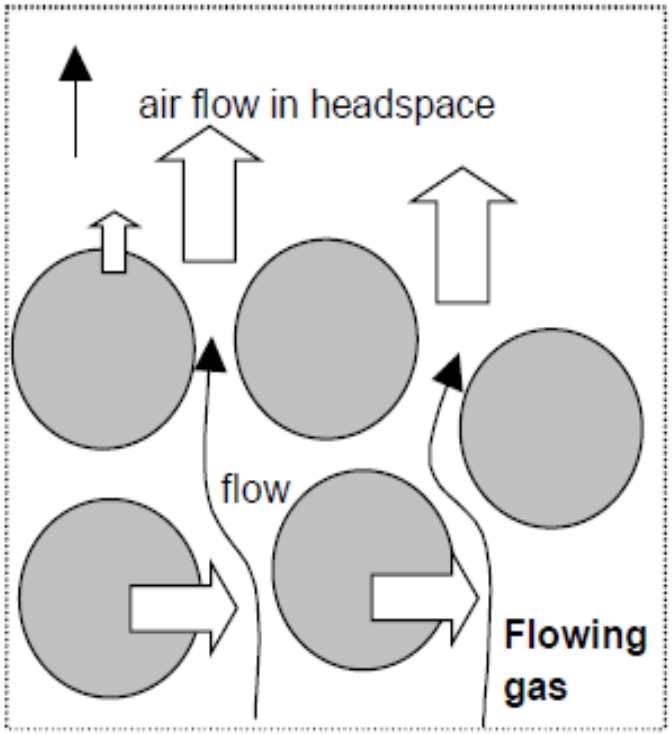
SSF reactor design

Bed-to-headspace heat and mass transfer

Unaerated <-> Forcefully aerated bed

Conduction and diffusion <-> Convection





in this case much of the transfer from the solids occurs within the bed

SSF reactor design
Convective flow

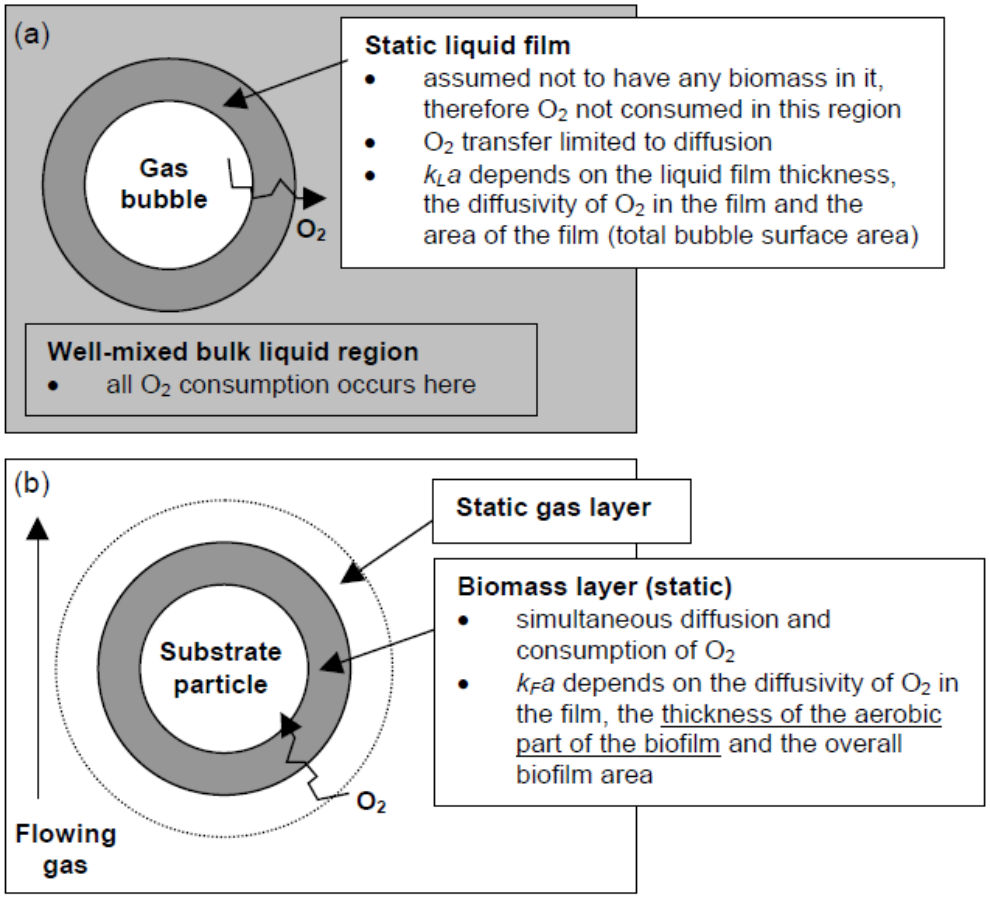


Fig. 4.5. Comparison of the situations for O_2 transfer in **(a)** SLF and **(b)** SSF

Comparing Oxygen transfer in SmF and SSF

What will happen when scaling-up the SSF reactor?

SSF reactor design
Scaling-up

- Increase of temperature, pH, O2, substrate, moisture gradients
- Scale-up usually based on empirical criteria related to transport processes
- Basis for most significant improvements is the application of mathematical modeling techniques

Process modeling
Microorganisms

Critical parameters

- Particle size: compromise
  - High surface area for microbial attack
  - Lower microbial respiration/aeration
- Moisture level/water activity
  - Mass transfer of water and solute across cell membrane
  - Water activity = relative humidity of the aqueous atmosphere in equilibrium with the substrate
    - a_w = 1.00 for pure water
    - a_w < 1.00 for solutions

Process modeling
Microorganisms

Moisture level/water activity

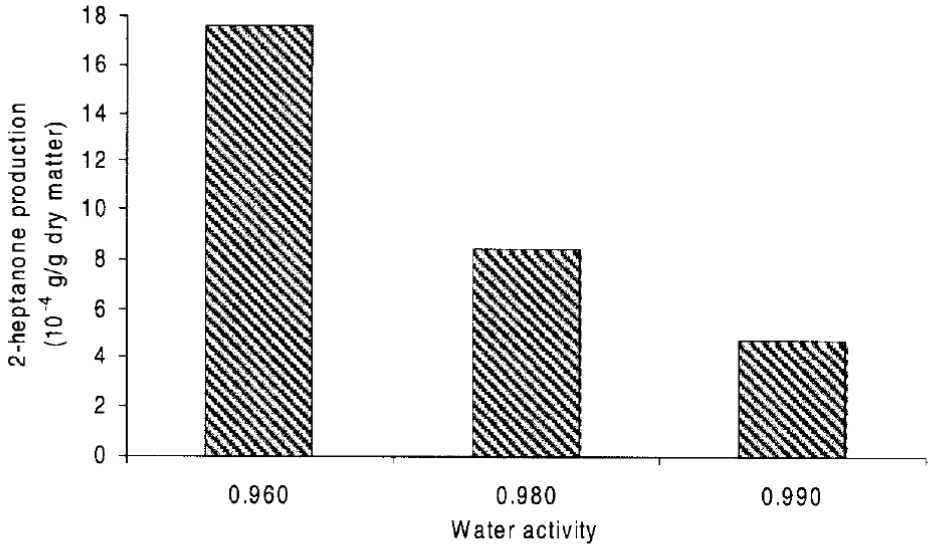


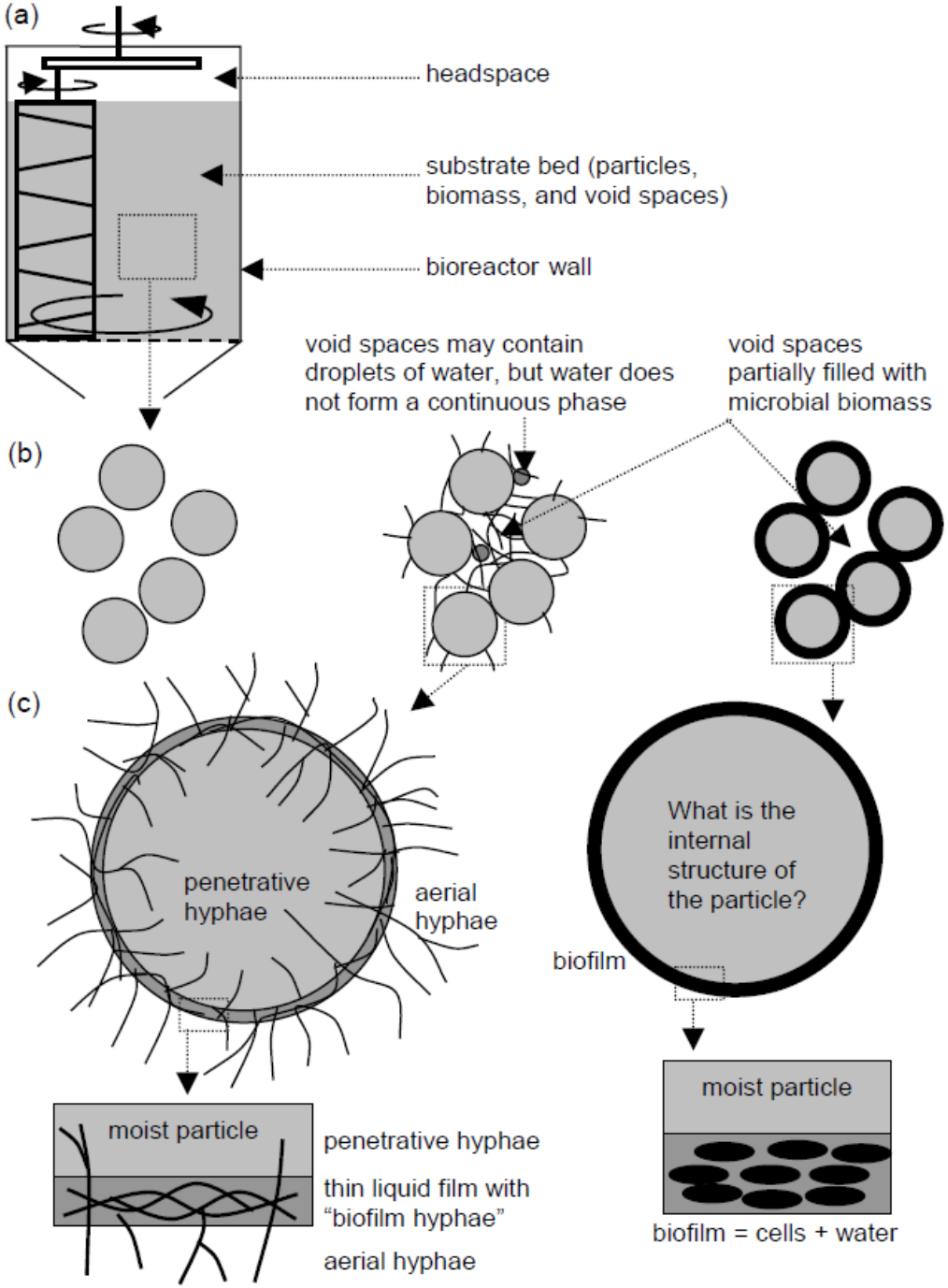
Figure 6: Influence of water activity on aroma production by *T. viride* TS cultivated for 5 days on a solid medium.

Process modeling

Two levels

- Macro scale = reactor level
  - Static SSF: tray, packed bed
  - Dynamic SSF: rotating drum, stirring
- Micro level: mathematical modeling of
  - Substrate particle digestion
  - Microbial growth
  - Enzymatic kinetics to explain microscopic fermentation steps

Process modeling
Micro scale

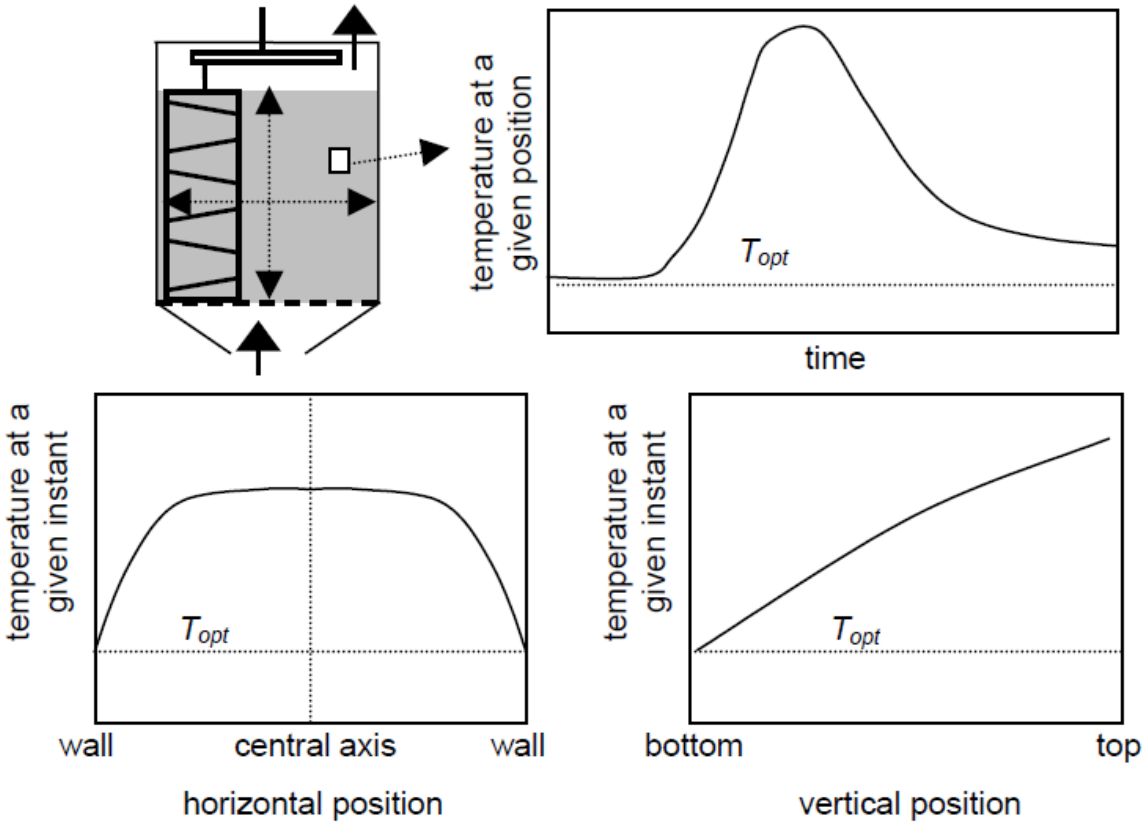


Biomass

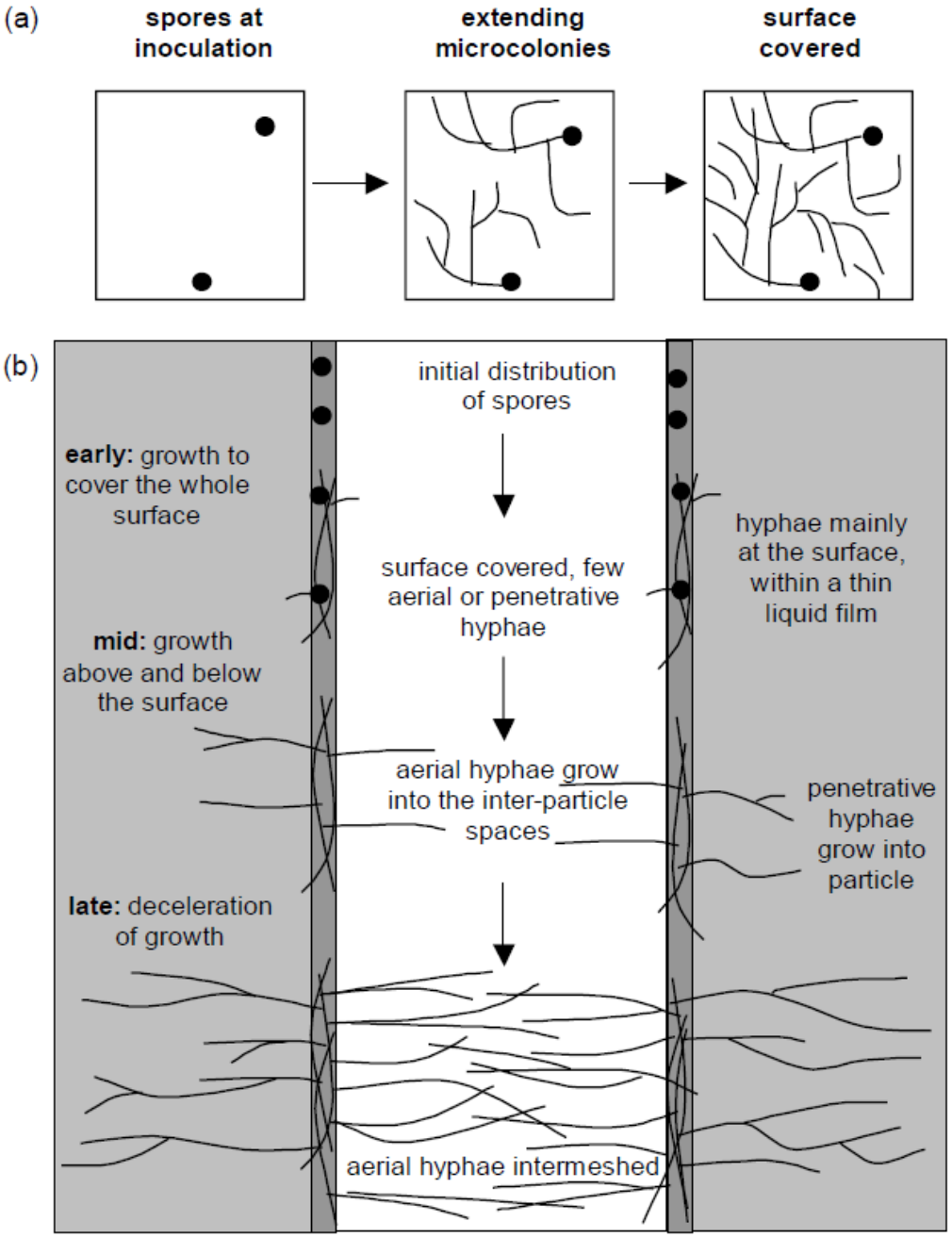
- Microbial cells stay attached to substrate
- Fungal mycelia penetrate into substrate

Process modeling
Micro scale

- Substrate bed

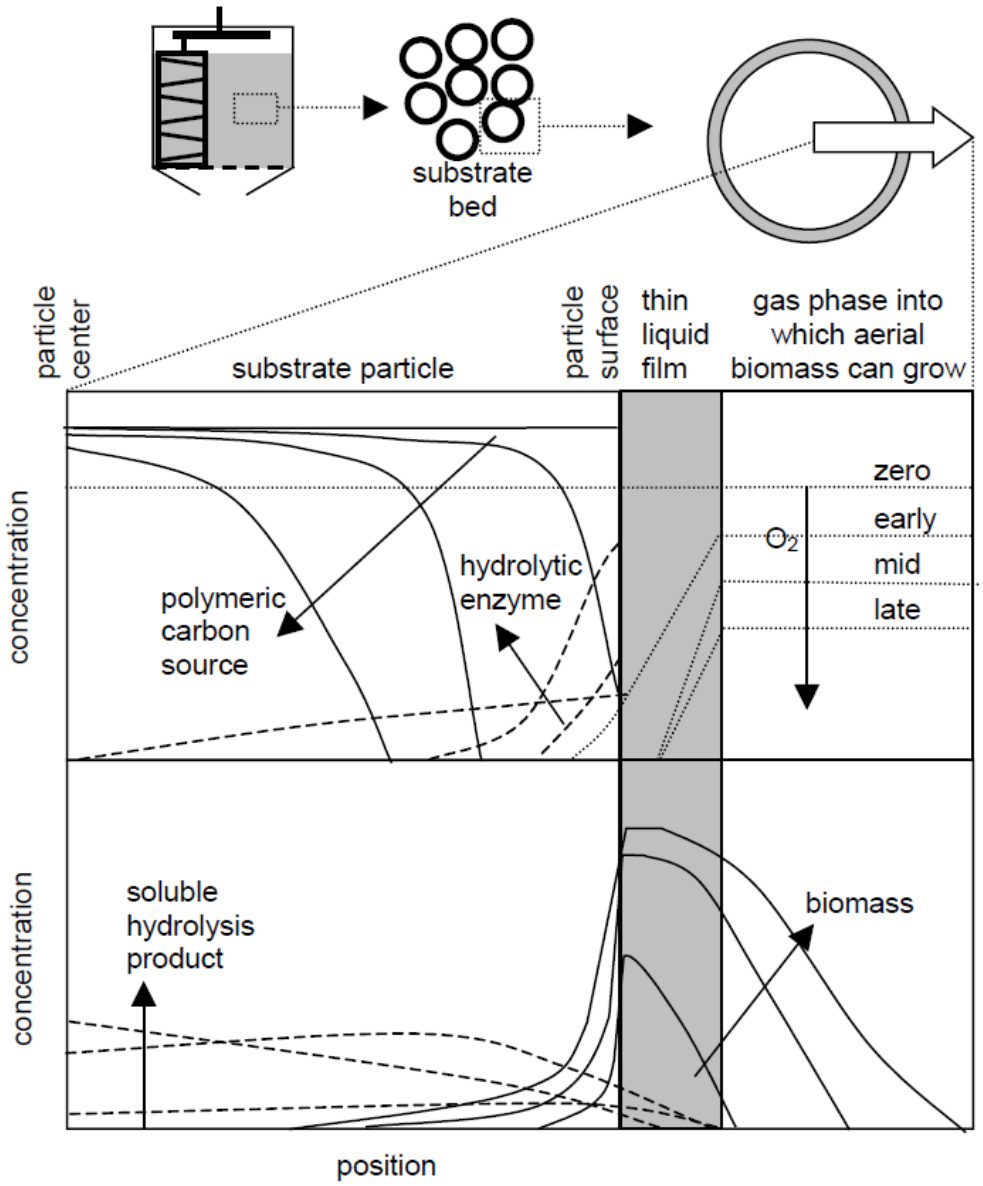


Process modeling
Micro scale



- Biomass distribution

Process modeling
Micro scale

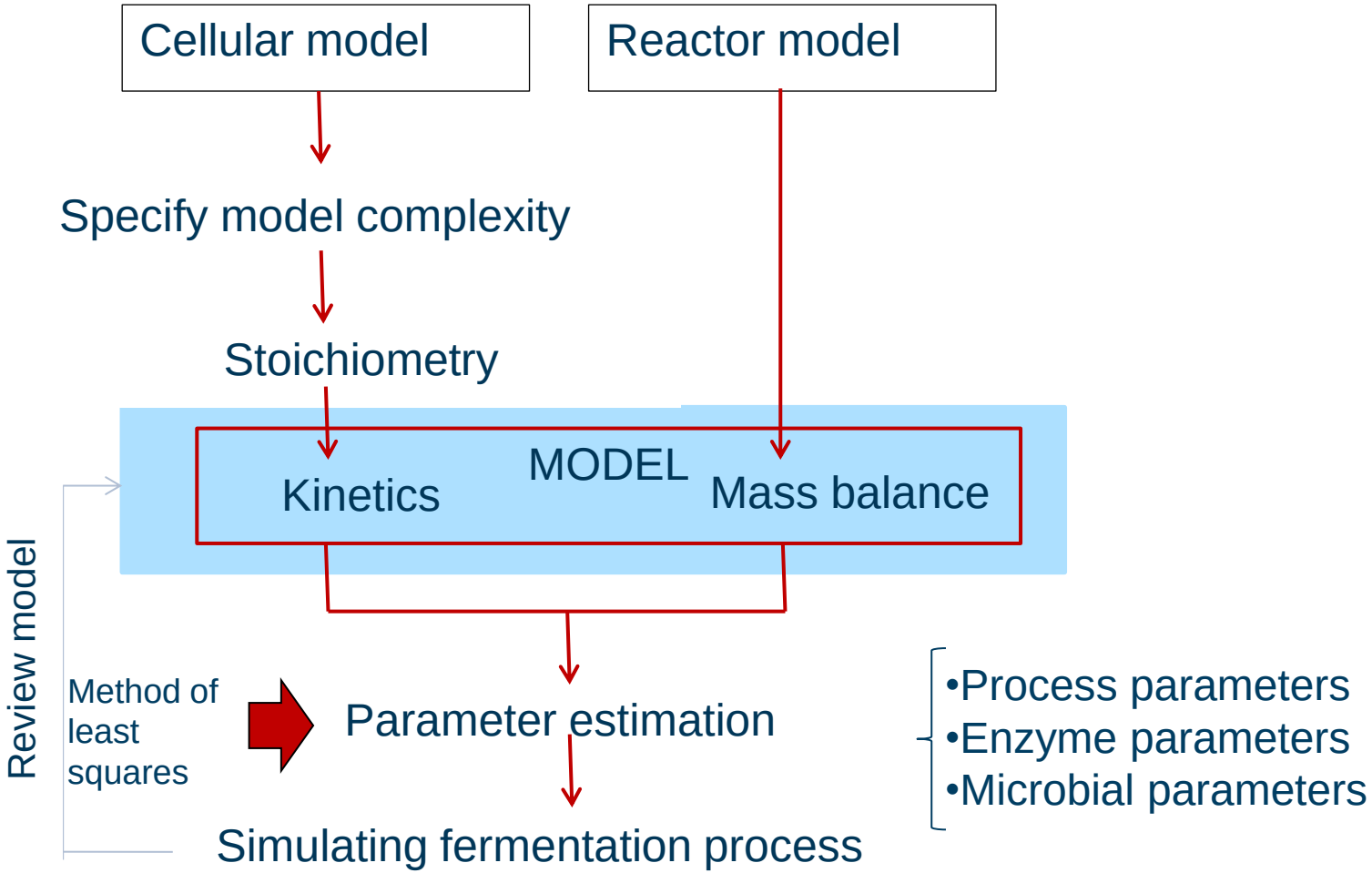


Changing concentration profiles

- Growth of a biofilm. on and in the particle
- Particle = polymeric carbon source

(Also shrinking of particle)

Process modeling



Process modeling
Kinetics

Biomass estimation

Two problems

- Measuring separately from substrate
- Homogeneous sampling for off-line measurement

Process modeling
Kinetics

Biomass estimation

- Indirect methods
  - DNA, **glucosamine**, ergosterol, protein (Kjeldahl), metabolic activity (respirometry)
- Model studies, e.g. growth on gelatine and melting afterwards and recovery by centrifugation
- Recent methods: OUR and CER

Process modeling
Kinetics

Biomass estimation by

Metabolic gas balance method

- On-line
- Fast

Measuring evolution of

- Oxygen uptake rate (OUR)
- Carbon evolution rate (CER)

→ linear related to biomass evolution

Process modeling
Kinetics

O2 consumed = O2 out – O2 in (1)

Volumetric flow at fermentor entrance:

$V_{O2e} = (\frac{20.9}{100})F_e$ (2)

$V_{N2e} = (\frac{79.1}{100})F_e$ (3)

F_e air flow at the fermentor entrance (/h)

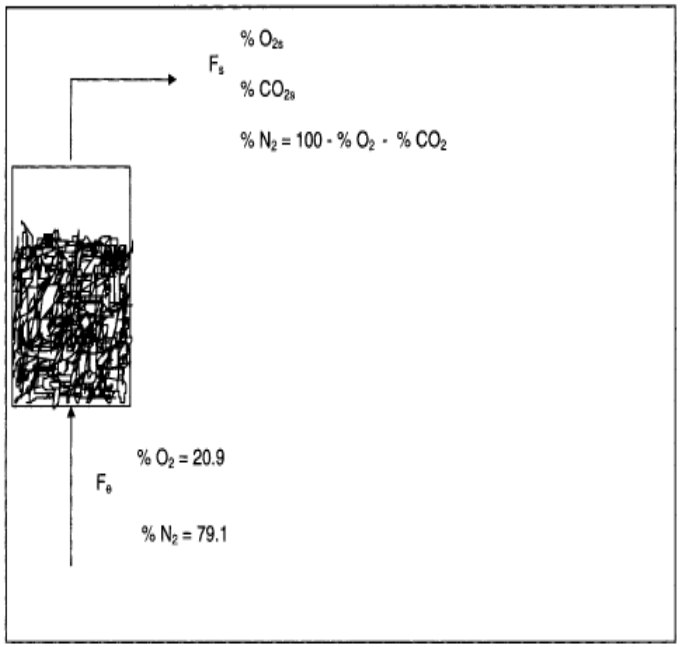


Figure 4.3. Volumetric composition of air in the flow at entrance and exit from a solid-state fermenter

Process modeling
Kinetics

Volumetric flow at fermentor exit:

$$V_{O2s} = \left(\frac{\%O_{2s}}{100}\right) F_s \qquad (4)$$

$$V_{CO2s} = \left(\frac{\%CO_{2s}}{100}\right) F_s$$

$$V_{N2s} = \left(\frac{100-\%O_{2s}-\%CO_{2s}}{100}\right) F_s \quad (5)$$

F_s air flow at the fermentor exit (/h)

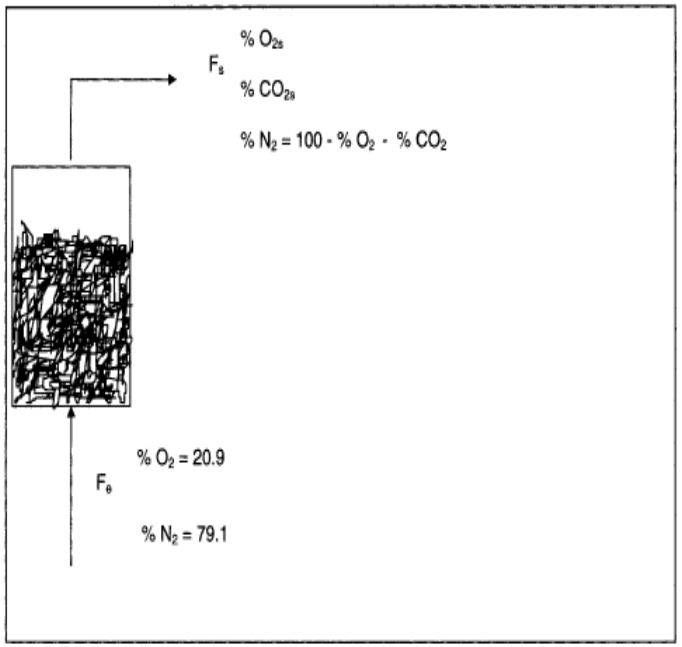


Figure 4.3. Volumetric composition of air in the flow at entrance and exit from a solid-state fermenter

Process modeling
Kinetics

Volumetric oxygen consumption (Eq. (1), (2) & (4))

$$VO_{2cons} = \left(\frac{20.9}{100}\right) F_e - (\%O_{2s}/100) F_s \quad (6)$$

Air is compressible fluid → relation between F_e and F_s

$$VN_{2e} = VN_{2s} \quad (7) \text{ (Volume } N_2 \text{ = cte) (7)}$$

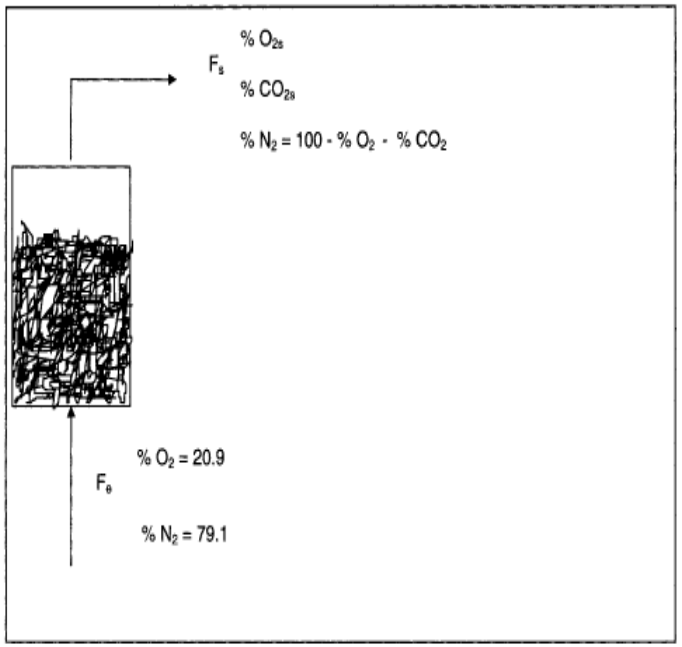


Figure 4.3. Volumetric composition of air in the flow at entrance and exit from a solid-state fermenter

Process modeling
Kinetics

Relation between air flow at entrance and exit (Eq. (3), (5) &(7))

$$F_s = \frac{79.1F_e}{(100-\%O2-\%CO2)} \quad (8)$$

Volumetric oxygen consumed (Eq. (6)&(8))

$$VO_{2cons} = \left(0.209 - \frac{0.791\%O2}{(100-\%O2-\%CO2)}\right)F_e \quad (9)$$

Assuming no CO2 in entrance gas.
Volumetric CO2 produced

$$VCO_{2prod} = \left(\frac{0.791\%CO2}{(100-\%O2-\%CO2)}\right)F_e$$

Process modeling
Kinetics

Oxygen balance during microbial growth

Fermentation: which part of the substrate (e.g. oxygen) is used for

- Maintenance (endogeneous process)
- Biomass growth
- Product production

O2 consumed = O2 applied for biomass growth + O2 applied for maintenance + O2 applied for product formation

OUR = oxygen consumed in time interval Δt = $\frac{\Delta O_2}{\Delta t}$ (rate of O2 consumption)

Process modeling
Kinetics

Oxygen balance during microbial growth

O2 consumption rate $\frac{dO}{dt} = mX$ (Maintenance)+ $\frac{1}{Y_{xo}} \cdot \frac{dX}{dt}$ (Biomass growth) + $\frac{1}{Y_{po}} \cdot \frac{dP}{dt}$ (Product formation)

Process modeling
Kinetics

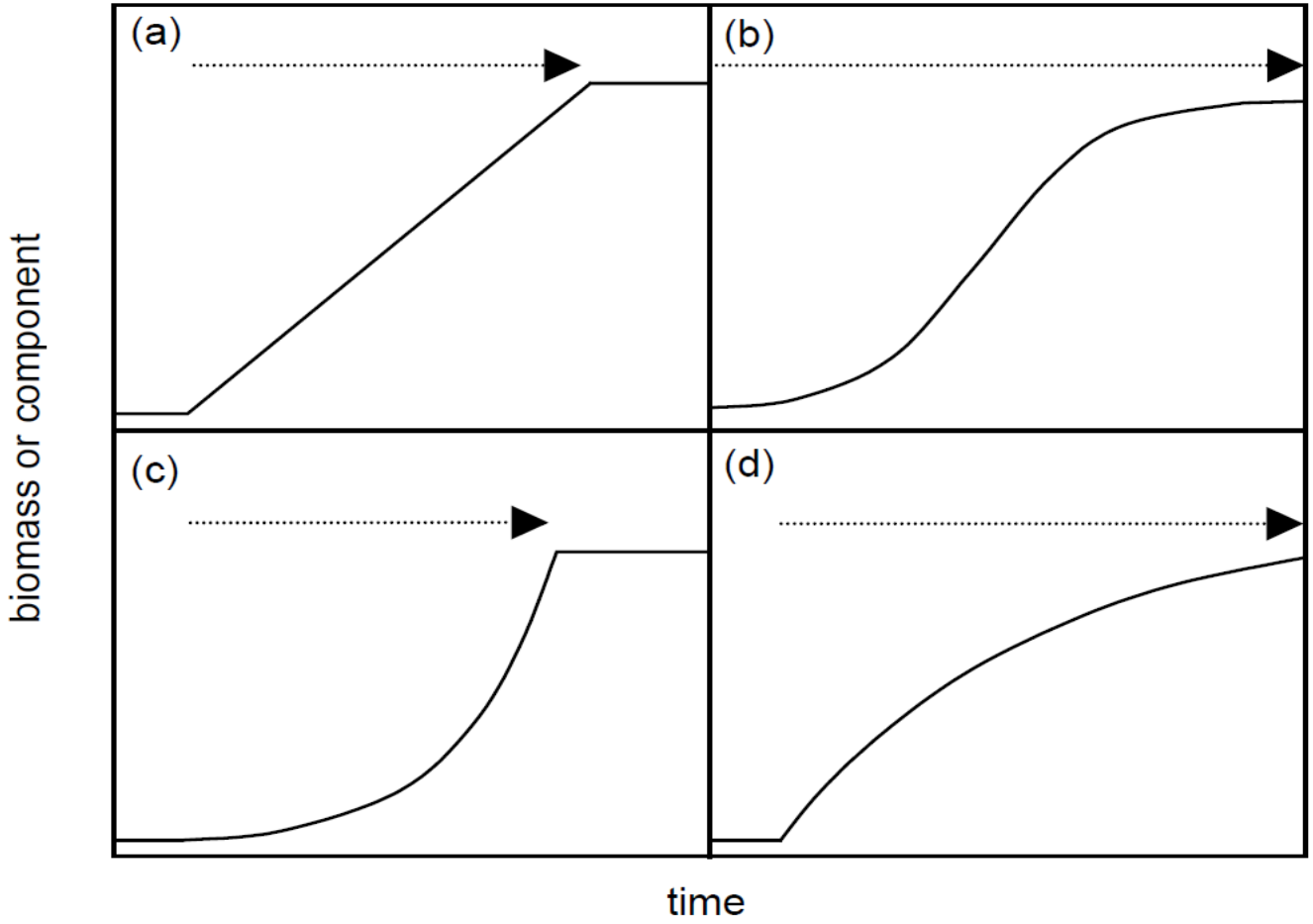


Fig. 14.5. Various types of kinetic profiles that have been found in SSF. The arrows indicate the parts of the profile that correspond to the kinetic type. **(a)** linear; **(b)** logistic; **(c)** exponential; **(d)** deceleration

Process modeling
Kinetics

Table 14.1. Equations that have been used to describe growth profiles or parts of growth profiles in SSF systems[a]

Name	Equation	Equation number	Parameters to be found by regression
Linear	$C = C_o + kt$	(14.4)	C_o, k
Exponential	$C = C_o e^{-\mu t}$	(14.5)	C_o, μ
Logistic	$C = \dfrac{C_m}{1 + \left(\dfrac{C_m}{C_o} - 1\right) e^{-\mu t}}$	(14.6)	C_o, C_m, μ
Deceleration	$C = C_o \exp\left(A(1 - e^{-kt})\right)$	(14.7)	C_o, A, k

75% of the cases

[a] In the past these equations have been used for biomass concentrations expressed on both absolute and relative bases.

Process modeling
Kinetics

Table 16.1. Differential forms of the equations that have been used to describe growth profiles or parts of growth profiles in SSF systems

Name	Equation[a]	Equation number	Parameters[b]
Linear	$\frac{dC_{XA}}{dt} = k$	(16.1)	k
Exponential	$\frac{dC_{XA}}{dt} = \mu C_{XA}$	(16.2)	μ
Logistic	$\frac{dC_{XA}}{dt} = \mu C_{XA}\left(1 - \frac{C_{XA}}{C_{XAM}}\right)$	(16.3)	C_{XAM}, μ
Deceleration	$\frac{dC_{XA}}{dt} = kAC_{XA}e^{-kt}$	(16.4)	k, A

75% of the cases

[a] The integrated form of these equations are given in Table 14.1. These equations are expressed in terms of absolute biomass concentration (e.g., g-dry-biomass g-IDS^{-1}).
[b] These parameters may later be expressed as functions of the environmental conditions.

Process modeling
Heat and mass transfer

Mathematical modeling of transport and thermodynamics in an SSF reactor

- Mass balance [kg/h]
- Energy balance [J/h]

Process modeling
Heat and mass transfer

Energy balance [J/h]

$$m_{bed} C_{Pbed} \frac{dT_{bed}}{dt} = \pm Q_A \pm Q_B \pm Q_C + \ldots + r_Q,$$

m_{bed} mass of the bed [kg]

C_{pbed} overall heat capacity of the bed [J/(kg.°C)]

T_{bed} temperature of the bed [°C]

r_Q rate of metabolic heat production [J/h]

Q_A, Q_B, Q_C rates at which different heat transport phenomena occur [J/h]

Process modeling
Heat and mass transfer

Mass balance of water [kg/h]

$$\frac{dM_{water}}{dt} = \pm R_A \pm R_B \pm R_C + \ldots + r_W ,$$

M_{bed} overall mass of water in the bed [kg]

r_W rate of metabolic water production [kg/h]

R_A, R_B, R_C rates of different mass transfert phenomena involving water [kg/h]

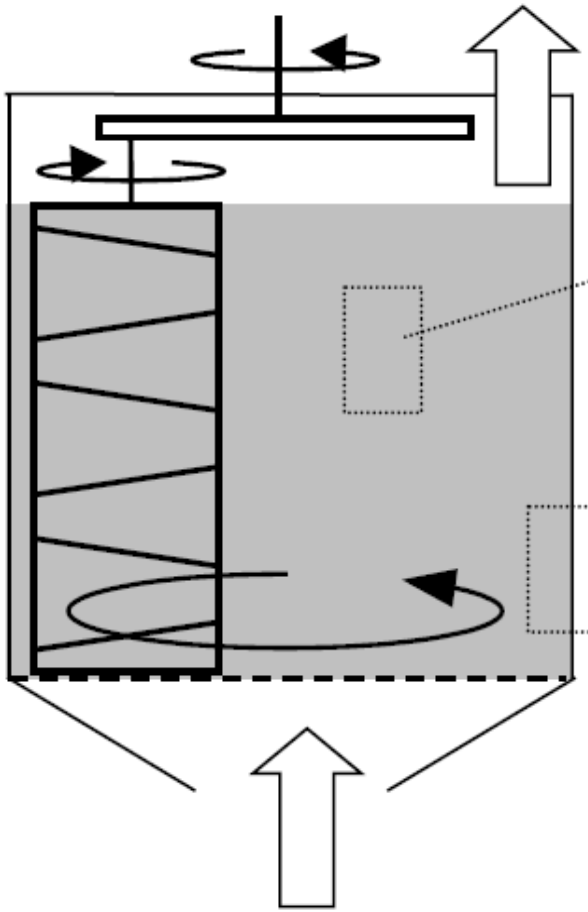
Process modeling
Heat and mass transfer

Energy and mass balances [J/h]

In the substrate bed:

- Metabolic heat production
- Conduction: in response to temperature gradient
- Diffusion: in response to concentration gradients
- Convective heat transfer: in case of forceful aeration
- Evaporation: from solid into air phase
- Convective mass transfer: in case of forceful aeration

Process modeling
Heat and mass transfer



T = temperature of outlet air
H = humidity of outlet air
F = air flow rate (dry basis)

Microbial variables and parameters
X = biomass
μ = specific growth rate constant
X_{max} = maximum biomass
Y_Q = yield of metabolic heat

Bed variables and parameters
T = bed temperature
C_{PB} = overall bed heat capacity
M = total bed mass

Thermodynamic constants
C_{Pair} = heat capacity of dry air
ΔH_{vap} = heat of vaporization of water
C_{Pvapor} = heat capacity of water vapor

T_{in} = temperature of inlet air
H_{in} = humidity of inlet air
F = air flow rate (dry basis)

Associated with heat transfer through the bioreactor wall
h = heat transfer coefficient
A = area across which heat transfer takes place
T_{surr} = temperature of the surroundings

Process modeling *Heat and mass transfer*

KINETIC SUB-MODEL

parameters within the kinetic equation are expressed as functions of the environmental conditions.

e.g., $\mu = \dfrac{a.e^{-Ea1/(R(T+273))}}{1+b.e^{-Ea2/(R(T+273))}}$

Growth kinetic equation

$$\frac{dX}{dt} = \mu X\left(1-\frac{X}{X_{max}}\right)$$

BALANCE/TRANSPORT SUB-MODEL

terms within the balance equations describe how growth affects the environmental variables

Energy balance (each term has the units of power, i.e., Watts)

$MC_{PB}\dfrac{dT}{dt} = Y_Q\dfrac{dX}{dt}$	metabolic heat production
$-h.A.(T\text{-}T_{surr})$	heat removal through the wall
$-FC_{Pair}(T-T_{in})$	heat removal by the dry air
$-F\Delta H_{vap}(H-H_{in})$	latent heat removal by evaporation
$-FC_{Pvapor}(HT\text{-}H_{in}T_{in})$	sensible heat removal by water vapor

many of the terms within the balance equations describe transport phenomena and these equations include the various operating variables

Operating variables

Variables, related with the aeration, agitation, and cooling systems, that can be manipulated by the operator. In this case the conditions of the inlet air (F, H_{in}, and T_{in}) and the temperature of the surroundings T_{surr} (which could be water in a cooling jacket)

SSF reactor control

Monitoring

- Measurement of environmental parameters (temperature, pH, water content and activity)
- Measurement of carbon cycle (biomass, substrate cencentrations, CO2)

Difficult due to heterogeneity

SSF reactor control

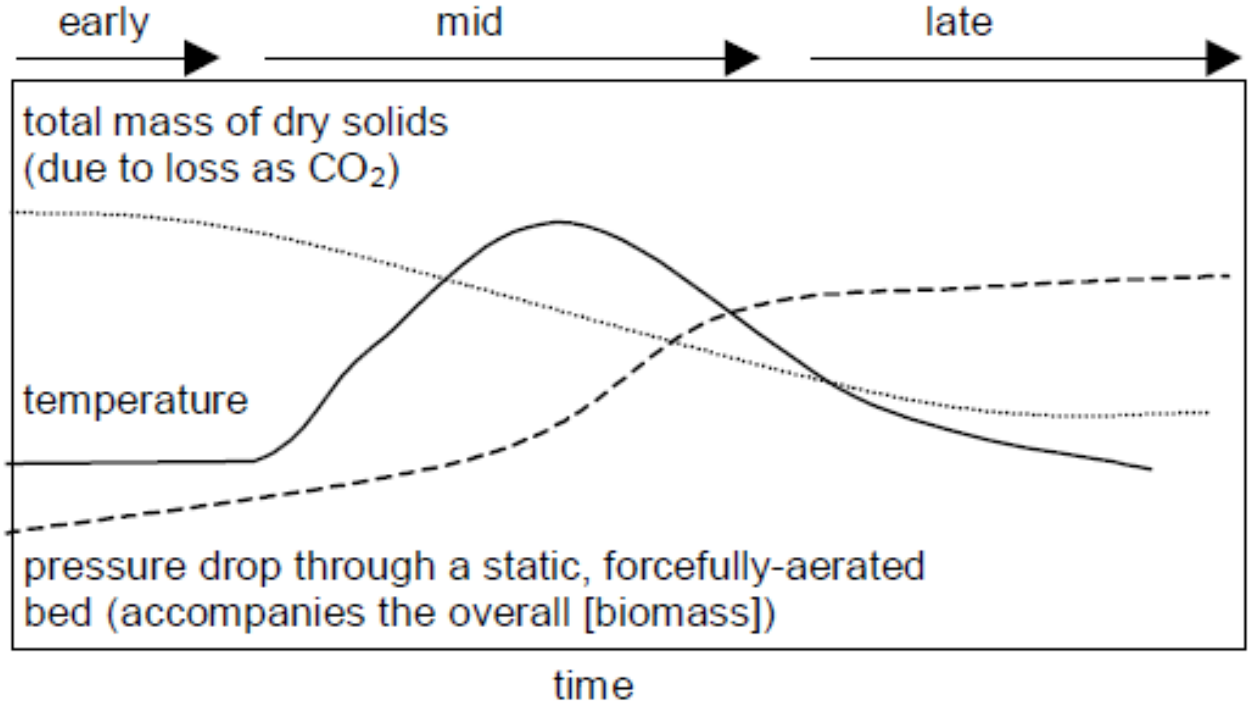
Direct measurements: classical sensors

- Temperature sensors
  - at various distances from the centre of the fermentor
  - Linked to control systems for moisture content
- pH
- Water content

SSF reactor control

Indirect measurements of biomass

- Respirometry
- Pressure drop (PD)



SSF reactor control

Recent measurement methods

- Aroma sensing
- Infrared spectrometry
- Artificial vision
- Tomographic techniques (X-rays, MRI)

Measurement techniques are important to improve performance of SSF bioreactors

Conclusion

SSF is not a simple technology

To deal with the complexity

- Scaling-up SSF needs to be based on engineering principles
- Mathematical models of bioreactor operation will be important tools in the design and optimization SSF bioreactors
- Process control theory should be extended

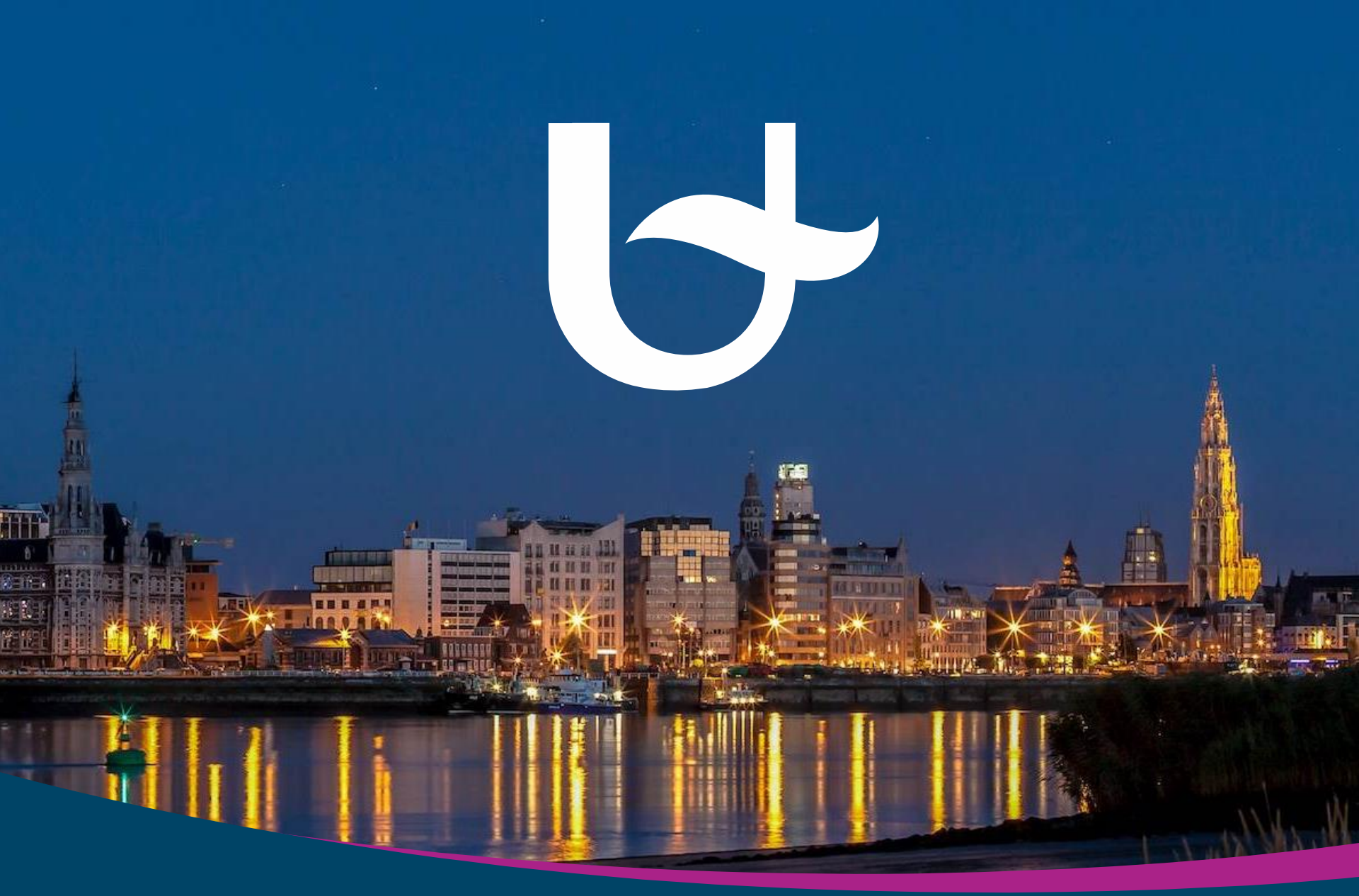
Further reading

Mitchell et al. (2006) *Solid state fermentation bioreactors*. Springer

Pandey et al. (2008) *Current developments in solid-state fermentation.* Springer

Questions

- What is solid state fermentation?
- Why is scaling-up of an SSF bioreactor more difficult than an SmF reactor?
- Describe the three basic types of SSF bioreactors and what criteria are used to choose the right reactor.
- Describe the way to follow-up the biomass concentration on-line in an SSF reactor.
- Give an overview of the modeling of an SSF bioreactor.



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Biochemical Engineering Technology
University of Antwerp