Microbial bioprocesses: current states and future prospects

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Section 1: Introduction

Bioprocess: basic unit operations diagram

<table>
<thead>
<tr>
<th>Category of Product</th>
<th>Product</th>
<th>Total revenue</th>
<th>Market value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cells</td>
<td>Bacterial cells</td>
<td>$12 billion</td>
<td>$8 billion</td>
</tr>
<tr>
<td></td>
<td>Yeast and bacteria</td>
<td>$2 billion</td>
<td>$1.5 billion</td>
</tr>
<tr>
<td>Primary metabolism</td>
<td>Biomass</td>
<td>$3 billion</td>
<td>$2 billion</td>
</tr>
<tr>
<td></td>
<td>Biochemicals</td>
<td>$4 billion</td>
<td>$3 billion</td>
</tr>
<tr>
<td></td>
<td>Secondary metabolites</td>
<td>$5 billion</td>
<td>$4 billion</td>
</tr>
</tbody>
</table>

Content

Section 1: introduction
Section 2: Microbial physiology in process conditions
Section 3: Basic bioreactor design
Section 4: new trends in bioreactor design
Section 5: Bioreactor scale-up: chemical engineering or biological issues?
Case study 1: modeling approaches for bioreactor hydrodynamics
Case study 2: whole cell biosensors for the detection of mixing imperfections
Case study 3: modeling in chemical engineering and life science, two applications with Matlab
Flasks

2L bioreactor

20L bioreactor

500 and 2000 L bioreactors

Control system

Centrifugation
Section 2: Microbial physiology in process conditions

- Optimization of microbial growth and product formation
- Importance of response and adaptation to stress conditions

2.1. Microbial growth - basic techniques for the quantification of biomass

- Plate count
- Microscopy (direct count)
- Dry matter
- Spectrophotometer
- Indirect parameters extracted from sensors (direct parameter now possible)

2.2. Interface between cellular components and the extracellular environment

Typical growth curve:

Traditional approach: growth curve

\[ \frac{dX}{dt} = \mu X \]
\( \mu \): specific growth rate (h⁻¹)
Traditional approach: Monod

Microorganisms are considered as a « pool of enzymes »

Theory of a limiting substrate → Monod

\[
\mu = \frac{S}{K_s + S}
\]

First problem

\(\mu\max \) and \(K_s\) varies with pH, T°, nature of the substrate, exposure to stress,...

Can be resolved by one of the numerous Monod modified expression

Second problem: substrate depletion in batch system

\(\mu\max \) and \(K_s\) varies with substrate concentration (simple Monod expression)

BUT ALSO

Substrate concentration can induce a shift from a metabolic pathway to another (homofermentative shift to a heterofermentative pathway)

Solution: fed-batch system (if vessel is considered as perfectly mixed) or structured kinetic model

Different modeling alternatives for microbial growth:

- The simplest way to express microbial growth: Monod type equation (saturation)
- Structured modelling: take into account the internal dynamics of the system to be studied
- Segregated modelling: take into account the heterogeneity of the microbial population

Image analysis
3.2.3. Microbial growth : modelling in different bioreactor configurations

**Mass balance**

\[
\text{Rate of variation of a component with time} = \text{Flow rate IN} - \text{Flow rate OUT} + \text{Production} - \text{Consumption}
\]

- = 0 at steady state
- Depend on bioreactor configuration
- Depend on kinetics of the reaction (in our case: Monod)
Hypothesis:
- Perfectly mixed reactor
- No cellular death
- No metabolite synthesis
- The only limiting substrate is the carbon source
- Dissolved oxygen is not limiting

In these conditions, Monod equation can be applied

\[ \mu = \frac{\mu_{\text{max}} S}{S + K_s} \]

\( V \) = working volume (m³)
\( S \) = substrate concentration (g/l)
\( X \) = biomass concentration (g/l)

\[ V = \frac{X}{S} \]

\[ S \]

\[ X \]

\[ \mu_{\text{max}} \]

\[ K_s \]

\[ r = \frac{X}{S} \cdot \frac{\mu_{\text{max}}}{S + K_s} \]

\( r \) : substrate consumption rate (g/l.h)
\( Y_{x/s} \) : yield coefficient (g biomass per g substrate)
\( m_s \) : maintenance constant (g de substrat / g de cellules . H)

Experimental results: batch culture of Bacillus subtilis
Growth curve: step method for the estimation of the rate of variation with time
Biomass and substrate variation rates: step method
**Computation of $\mu_m$ and $K_s$**

$$\mu = \frac{\text{d}N}{\text{d}t} = \frac{1}{\text{d}N} \frac{1}{N}$$

avec $\text{tg} \theta = \frac{K_s}{\mu_m}$

### Lineweaver-Burke

<table>
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<tr>
<th>$S$ (g/l)</th>
<th>$\mu$ (h$^{-1}$)</th>
<th>$1/\mu$ (s$^{-1}$)</th>
</tr>
</thead>
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<tr>
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<td>1.05</td>
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<tr>
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</tr>
<tr>
<td>9</td>
<td>0.04</td>
<td>0.16</td>
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</table>

$$\mu = \frac{f_x}{X}$$

avec $\text{tg} \beta = \frac{1}{X_{x/s}}$
Productivity associated with batch reactors:

Cultivation timelength + time associated with industrial operations

Total time is calculated by:

\[ t_{\text{total}} = t_{\text{app}} + t_d + t_i + t_r. \]

Amount of biomass produced:

\[ X_f - X_0 = Y_{\text{batch}} S_0. \]

Productivity (batch):

\[ \text{Productivity(batch)} = \frac{\mu_{\text{max}} Y_{\text{batch}} S_0}{\ln \left( \frac{X_f}{X_0} + \mu_{\text{max}} T_0 \right)}. \]

Fed-batch culture: mass balance

Monod hypothesis:
- Perfectly mixed reactor
- No cellular death
- The only limiting substrate is the carbon source
- Dissolved oxygen is provided in excess

First case: metabolites synthesis

1. Growth period: equations identical to those used for batch

\[ \frac{dX}{dt} = \mu X, \quad \frac{dS}{dt} = -\frac{m}{Y} X, \quad \frac{dP}{dt} = 0. \]

2. Metabolite synthesis period (fed-batch):

\[ \frac{dV}{dt} = Q, \quad \frac{dX}{dt} = \mu X, \quad \frac{dS}{dt} = -r_s, \quad \frac{dP}{dt} = r_r X. \]

Variation at the level of the biomass concentration can only be attributed to dilution effect:

\[ \frac{dX}{dt} = \frac{dV}{dt} \frac{X}{V}. \]

Second case: biomass production

1. Growth period: see batch

2. Fed-batch period:

\[ \frac{dX}{dt} = \frac{dV}{dt} \frac{X}{V}, \quad \frac{dS}{dt} = -r_s, \quad \frac{dP}{dt} = r_r X. \]

Hypothesis: added substrate is immediately consumed \((S = 0)\)

\[ \frac{dX}{dt} = \mu X, \quad \frac{dS}{dt} = -r_s, \quad \frac{dP}{dt} = r_r X. \]

\[ r_s = \frac{Q}{V} (S - S_0) \]

If \( S = \text{cst} \) and \( dS/dt = 0 \),

\[ \frac{dV}{dt} = Q, \quad \frac{dX}{dt} = \mu X, \quad \frac{dP}{dt} = 0. \]
Fed-batch culture of S. cerevisiae with Q.R. regulated at a set point of « 1 »

<table>
<thead>
<tr>
<th>Duração (h)</th>
<th>X (g/l)</th>
<th>S (g/l)</th>
<th>Q.R.</th>
<th>Q (l/h)</th>
<th>V (litres)</th>
<th>Sₚₐₜ₋ₘₚₚₚ (g)</th>
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<tr>
<td>4.5</td>
<td>0.58</td>
<td>0.33</td>
<td>1.02</td>
<td>0.604</td>
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<td>8.9</td>
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<td>0.514</td>
<td>10.37</td>
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<td>0.09</td>
<td>1.02</td>
<td>0.529</td>
<td>10.34</td>
<td>7.5</td>
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<td>7.0</td>
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<td>0.99</td>
<td>0.515</td>
<td>10.57</td>
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<td>0.26</td>
<td>1.75</td>
<td>0.128</td>
<td>10.44</td>
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<td>20.03</td>
<td>0.27</td>
<td>0.26</td>
<td>1.03</td>
<td>0.111</td>
<td>12.36</td>
<td>83.9</td>
</tr>
<tr>
<td>20.03</td>
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<td>0.26</td>
<td>1.03</td>
<td>0.111</td>
<td>12.36</td>
<td>576.5</td>
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<td>0.26</td>
<td>0.26</td>
<td>1.03</td>
<td>0.111</td>
<td>12.36</td>
<td>576.5</td>
</tr>
</tbody>
</table>

QR can be measured by gas balance analysis (derived method can also be used to monitor Kᵤᵣ)

Other control procedures:
- Ethanol, glucose sensors
- pHstat, DOstat
- Exponential feeding algorithm (if kinetics parameters are well known)

Continuous reactors: mass balance

Basic principle: maintain µ_max by keeping constant cellular environment during the culture

Parameters:
- dV/dt = 0 ⟷ Qo = Qs = Q
- Dilution rate D = Q/V (h⁻¹)
- Mean residence time ts = V/Q = 1/D (h)
BIOMASS : \[ \frac{dX}{dt} = \mu X - DX = X (\mu - D) \]

SUBSTRATE : \[ \frac{dS}{dt} = DS - DS - D(S - S) \]

METABOLITE : \[ \frac{dP}{dt} = D \cdot P \]

d\(X/dt\), d\(S/dt\) and d\(P/dt\) depend on the value of D:
- Critical dilution rate \(D_c\)
- Maximal dilution rate \(D_{max}\)

1st CASE : \(D > D_c\)
Bioreactor washout \(\rightarrow dX/dt < 0\)

2nd CASE : \(0 < D < D_c\)
Evolution toward a steady state:
- \(X = X_{eq} \rightarrow r_x = \mu X = DX\)
- \(S = S_{eq} \rightarrow r_s = D \cdot (S_a - S)\)
- \(P = P_{eq} \rightarrow r_p = DP\)

3rd CASE : \(D = D_{max}\)
d\(X/dt = X \cdot (\mu_{max} - D) = 0\) \(\rightarrow\) TURBIDOSTAT

Effect of the dilution rate on the biomass \(X\) and substrate \(S\) concentrations (also on generation time \(g\) and productivity \(D \cdot X\))
Chemostat modelling

X and S mass balance equations with Monod kinetics. At steady state, $\mu = D$:

$$S_a = K_S \frac{D}{\mu_{max} - D}$$

$$X_a = \frac{Y_X}{Y_S} D \left(S_a - S_s\right)$$

If we focus our attention on generation time and productivity:

$$\mu = \ln \frac{2}{\ln \frac{2}{D}}$$

Productivity = $X_a D = \frac{Y_X}{Y_S} (S_a - S_s) D$

$$D = D_o \mu_c \left[1 - \frac{K_S}{S_a - S_s}\right]$$

2.4. Impact of process-related stresses on microbial physiology

**Systeme Physique**

- Thermal shock
- Hypoxia
- pH shock
- Carbon starvation
- Carbon excess
- Nitrogen starvation
- Choc osmotique (fed-batch)
- Haute densité cellulaire (fed-batch)
- Turbohypobiosis

**Physiological impact**: Short-term: metabolic shift, Long-term: gene induction/repression Depending also on the intensity and the frequency of stress

**Bioreactor operating mode: synopsis**

System biology

Les différents composants des systèmes (métabolisme, protéome, fluxome) sont organisés en réseaux

Existence d’interactions complexes au sein de ces différents réseaux

Etude des temps caractéristiques du procédé

Temps caractéristiques pour les réactions enzymatiques du métabolisme : peuvent être inférieurs à la seconde (dépendent de la constante cinétique de réaction)

Temps caractéristiques pour la synthèse des ARN : quelques dizaines de secondes (dépendent de la longueur de l’ARN messager)
DNA → mRNA → unfolded protein → folded protein

Example for E. coli:

<table>
<thead>
<tr>
<th>Process</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>mRNA</td>
</tr>
<tr>
<td>mRNA</td>
<td>Unfolded protein</td>
</tr>
<tr>
<td>Unfolded protein</td>
<td>Folded protein</td>
</tr>
</tbody>
</table>

Degradation (half-life time constant)

« Omics » technologies

DNA microarray
2D gel electrophoresis

Overview of the substrate assimilation pathways inside the cell

Analyse des flux métaboliques : méthodologie

Observation de la dynamique interne d’une cellule avec un réseau métabolique simplifié

3 métabolites A, B et C
3 flux externes b1, b2 et b3
3 flux internes v1, v2 et v3

Complexity of the metabolic network : example of central carbon metabolism in E. coli

Holm’s [1996] FEMS microbiology review

Balance de masse dynamique:

S : matrice stœchiométrique
V : vecteur de flux

Balance de masse à l’équilibre : S.v = 0
Réseau métabolique à l'état stationnaire (turnover des intermédiaires) sous forme matricielle :

\[ S \cdot v = 0 \]

On a \( K \) métabolites et \( J \) réactions.

La matrice stœchiométrique \( S \) a donc les dimensions \((K,J)\) et le vecteur de flux \( s \) a la dimension \((J,1)\).

Le modèle revient à un système d'équations linéaires dont le nombre de degré de liberté \( F = J - K \).

3 cas de figure :
- Si \( F = 0 \), le système est déterminé et la solution est unique.
- Si \( F < 0 \), le système est sur-déterminé.
- Si \( F > 0 \), le système est sous-déterminé et il existe plusieurs solutions.

Dans la pratique, pour les réseaux métaboliques les plus complexes, le système est toujours sous-déterminé.

On diminue le nombre de degré de liberté en mesurant certains flux. Le système d'équations linéaires s'écrit alors :

\[ S \cdot v = S_{m} \cdot v_{m} + S_{nm} \cdot v_{nm} = 0 \]

La solution est alors obtenue par :

\[ v_{nm} = -(S_{nm})^{-1} \cdot S_{m} \cdot v_{m} \]

Réseau métabolique pour la croissance de \( E. coli \) sur glucose.

Bascule métabolique déclenchée par :
- Excès de glucose
- Manque d'oxygène (voie des acides mixtes chez certains procaryotes)

Exemples : \( E. coli \)

Section 3 : Basic bioreactor design
Overview of bioreactor operations

- Controller
- Substrate

Cells or metabolites ➔ Heat transfer ➔ By-products ➔ Residual substrates

Bioreactor: system description

Bioreactor design overview
- Mechanical system
- Pneumatic system

Mechanically stirred bioreactor

- Mixing can be provided by:
- Piping: steam, compressed air, thermal regulation
- Valves (manual and/or automatic)

Compressed air

- Compressor
- Oil filter
- Water removal
- Sterilization
- Condenser
- Air dispersion

Filters and holders
Air filter

Scheme of a typical temperature regulation loop

Pressure
Increase the pressure in the vessel in order to:
- Avoid contaminations
- Increase oxygen solubility in water (or aqueous media)

Mass flowmeter

pH management
Relation between voltage and pH:
\[ pH = a + bU \]
Where \( b \) depends on \( T \)

New development: optical system
Liquid electrolyte  Solid electrolyte (gel)

pH probe

computer

pH regulation

Actual technology not very efficient
New development: optical systems

pCO₂

Schéma de la sonde

New development: optical systems

Schéma de pCO₂

La chambre de mesure est séparée du milieu par une membrane en Teflon qui réalise l’étanchéité et est perméable aux gaz. Elle donne une information en mA (4-20 mA)
New trends: mini bioreactors

Basic screening tool (erlenmeyer): 90% of microbial culture experiments (at the academic and private level) are performed in erlenmeyer flasks

Vasala et al. [2006]

Mini-bioreactor development

New development in sensor technology for bioprocess control: optical systems

Principle: collision between oxygen and dye molecules inducing luminescence quenching
2 parameters depending on oxygen concentration

Comparison with polarographic probes:
- No polarization lag time
- No oxygen consumption
- No contamination with other dissolved species
- Non-invasive measurement
- No membrane, no electrolyte required
- Insensitive to electrical Interferences

Microbioreactors

Bioprocess control

Control strategy: closed loop with feedback

Control algorithms
- ON-OFF control
- Proportional (P) control
- Proportional-integral (PI)
- Proportional-integral-differential (PID)
ON-OFF control

- Possibility to introduce a dead zone where controller is never activated.
- Simple system, but tends to lead to oscillations of the controlled variable.

Proportional control

\[ P = P_0 + K_p \cdot \varepsilon \]

- \( P \): signal output (signal envoyé par le contrôleur)
- \( \varepsilon \): error (difference between the output signal, i.e. controller impulse, and the input signal, i.e. the probe signal)
- \( K_p \): proportionnal gain

Proportional-integral control

\[ P = P_0 + K_p \cdot \varepsilon + K_i \cdot \int_0^t \varepsilon \, dt \]

- \( K_i \): integration time constant

- If error is constant, then \( \frac{dP}{dt} = \frac{K_p}{\tau_i} \cdot \varepsilon \cdot t \)

Proportional-differential control

\[ P = P_0 + K_p \cdot \varepsilon + K_d \cdot \frac{d\varepsilon}{dt} \]

- \( K_d \): differential time constant

Proportional-integral-differential control (PID)

\[ P = P_0 + K_p \cdot \varepsilon + K_i \cdot \int_0^t \varepsilon \, dt + K_d \cdot \frac{d\varepsilon}{dt} \]

Practical implementation of PID controller:

- At the level of transmitter
- At the level of central controller
Advanced bioprocess control

Fed-batch control:
- Exponential feed $Q = Q_0 \exp(\mu t)$
- RQ (respiratory quotient)
- Feedback loop (biomass, ethanol or glucose probe)
- pH-stat
- DO-stat

1. Basic bioreactor design: standard geometry
Medium to high viscosity → tangential impeller

Anchor
Ruban hélicoïdal

Dissipated power characteristic curve \( N_p = f(Re) \)

Calculation of power dissipated

Three kinds of variables:
- Geometry
- Nature of the fluid
- Mixing related

Vaschy-Buckingham theorem: from 16 to 13 dimensionless variables
\[ \text{N}_p = k \cdot \text{Re}^x \cdot \text{Fr}^y \cdot \text{We}^z \cdot \left( \frac{H}{d} \right)^\alpha \cdot \left( \frac{D}{d} \right)^\beta \]

- Reynolds number (Re) = \( \frac{\rho N d^2}{\mu} \)
- Power or Newton number (Np) = \( \frac{P}{\rho N^3 d^5} \)
- Weber number (We) = \( \frac{\rho N^2 d^3}{\sigma} \)
- Froude number (Fr) = \( \frac{N^2 d}{g} \)

Fluid flow rates calculation

Pumping:
\[ Q_p = N_q \cdot \frac{d}{\rho} \]
\[ t_p = \frac{V}{Q_p} \]
\[ t_p = 1 / t_L \]

Circulation:
\[ Q_c = Q_e + Q_p = N_{q_c} \cdot \frac{d}{\rho} \]
\[ t_c = \frac{V}{Q_c} \]
### Application

Considering a standard stirred vessel \((D = 0.3 \text{ m})\) filled with water. Mixing is promoted by a TD6 impeller at a stirring rate \(400 \text{ min}^{-1}\).

TD6 dimensionless numbers:
- \(N_p = 5.5\)
- \(N_{qp} = 0.85\)
- \(N_{qc} = 1.51\)

Calculate the power dissipated, peripheral speed, pumping and circulation flow rates, as well as pumping and circulation times.

### Scaling-up basic principle: similarities principle

\[
N_{lab} \cdot (D_{industrial} / D_{lab})^\alpha \cdot (D_{industrial} / D_{lab})^\beta
\]

Avec \(F = D_{industrial} / D_{lab}\)

Example: \(P/V\) as scaling-up criteria

### Application

The culture of an anaerobic microorganism as been set up in a pilot-scale bioreactor with a working volume of 785 liters (standard vessel: \(D = 1\text{ m}\)). Mixing is ensured by a TD6 impeller at a stirring rate of \(180 \text{ min}^{-1}\) in a water-like medium.

Calculate the peripheral speed, pumping and circulation flow rate, and pumping and circulation times.

The culture has to be up-scaled to a \(50 \text{ m}^3\) bioreactor. How does the above mentioned mixing parameters vary if volumetric dissipated power is the scaling factor (i.e., is kept constant during the up-scaling).

### Answer

| Volume (m³) | 0.79  | 50.00 |
| N (s⁻¹)    | 3.00  | 1.16  |
| P(W)       | 610.60| 38891.45 |
| Vitesse périphérique (m/s) | 3.14 | 4.84 |
| Débit de pompage (m³/s) | 0.09 | 2.27 |
| Temps de pompage (s) | 8.52 | 22.07 |

Multiplicative factor = \(f(F)\)

### Transport operations

- Mass transfer
- Heat transfer
- Momentum transfer

### Gas-liquid dispersion and oxygen transfer

Bakker Bakker [2000]
Transition between different gas-liquid flow regimes:
From left to right, the air flow rate is kept constant and stirrer speed is progressively increased.

Flow diagram (dimensionless):
Aeration number (Na) in function of the Froude number (Fr)

Propensity of air flow rate in relation with mechanical stirring intensity

Power dissipated in G-L system: $P_g$ decrease when the air flow rate is increased

Optimization of the impeller geometry

1. Profiled propellers:

2. Hybrid multi-impeller system:

Application:
Consider a standard stirred vessel ($D = 0.1m$). Mixing is ensured by a TD6 impeller ($N = 300 \text{ min}^{-1}$) in a water-like medium. Air is sparged at a flow rate of 0.5 vvm.

The loading curve for the TD6 is:
$Na = 30 \cdot (d/D)^{3.5} \cdot Fr$

What is the maximum flow rate acceptable in order to keep an efficient G-L dispersion (and by keeping a constant stirrer rate)?
Application:

We consider two kinds of impeller: a TD6 and a profiled propeller \((d = 0.5 \text{ m})\). The equations for the loading curve are the following:

**TD6**: \(Na = 30 \times (d/D)^{3.5} \times Fr\)

**Propeller**: \(Na = 6000 \times (d/D)^{1.55} \times Fr^{2.7}\)

Considering the constraints presented in the table, determine the gas-liquid flow regime for each impeller.

<table>
<thead>
<tr>
<th>Impeller</th>
<th>(N_{\text{max}}) for shear ((s^{-1}))</th>
<th>(G_{\text{min}}) for Oxygen transfer ((\text{vvm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD6</td>
<td>0.51</td>
<td>0.13</td>
</tr>
<tr>
<td>Profiled</td>
<td>0.83</td>
<td>0.19</td>
</tr>
</tbody>
</table>

### Résolution:

**Oxygen transfer**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration in liquid phase ((\text{ppm}))</th>
<th>Critical concentration ((\text{ppm}))</th>
<th>Consumption rate ((\text{mmoles/g biomass.h}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10,000</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Oxygen</td>
<td>7</td>
<td>0.8</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\[ q_L = k_L \cdot (C - C_i) \]

Concentrations at the interface are approximated: global exchange coefficients

\[ q_g = K_g \cdot (p_g - p^*) \]

\[ q_i = K_i \cdot (C_g - C_i) \]
30/06/2010

Solubility of oxygen is very low in water, i.e. He is very small.

$q = k \cdot L \cdot S \cdot (C_0 - C_L)$

Amount of oxygen transferred per time:

$Q = k \cdot L \cdot S \cdot (C_0 - C_L)$

General form:

$\frac{dC_L}{dt} = k_La \cdot (C_0 - C_L) - \frac{Q}{V}$(mg/L)

Amount of oxygen transferred per time:

$Q = k_La \cdot (C_0 - C_L)$

General form:

$\frac{dC_L}{dt} = k_La \cdot (C_0 - C_L) - \frac{Q}{V}$

Optimization of the oxygen transfer rate:

- Increase $K_La$ : nature of the medium
- Increase $a$ : play on mixing performances
- Increase $(C_0 - C_L)$ : play on pressure/temperature to modify the GoL equilibrium

Methods for estimating $K_La$ : oxygen probe (indirect)
- Static gassing-in-gassing-out

Methods for estimating $K_La$ : oxygen probe (direct)
- Dynamic gassing-in-gassing-out

Methods for estimating $K_La$ : gas balance (direct)

Application:

Consider a culture of Penicillium sp. The reactor has a volume of 50 L and aeration flow rate is of 0.16vvm (inlet air: 20.94% d’O2 et 79% de N2). Culture is performed at 30°C and at atmospheric pressure ($C_0$ oxygen = 7.6 mg/L).

The data collected from outlet gas analysis and dissolved oxygen probe:

<table>
<thead>
<tr>
<th>Temps (h)</th>
<th>%O2</th>
<th>%CO2</th>
<th>pH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20.26</td>
<td>0.54</td>
<td>7.17</td>
</tr>
<tr>
<td>20</td>
<td>20.1</td>
<td>0.94</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Calculate $K_La$ for the two culture times.
K_\text{La} \text{ calculation:}

Van’t Riet correlations:

- For distilled water (coalescing):
  \[ k_\text{La} = 0.026 \times (P/V)^{0.4} \times (G/S)^{0.5} \]

- For medium containing electrolytes (non-coalescing):
  \[ k_\text{La} = 0.002 \times (P/V)^{0.7} \times (G/S)^{0.2} \]

General form for pneumatic reactor (without mechanical stirring): 
\[ k_\text{La} = C \times (G/S)^{\alpha} \]

Application:

Consider a standard stirred vessel (D = 0.1m). Mixing is ensured by a TD6 impeller (N = 300 min^{-1}) in a water-like medium. Air is sparged at a flow rate of 0.5vvm. Calculate the power dissipated and the K_\text{La} if we consider a non-coalescing medium.

OTR limiting phenomena: foam

Chemical antifoam: silicon backbone

Mechanical defoamer

3.2. Heat transfer

\[ Q = U \times A \left( T_1 - T_2 \right) \]

\[ \frac{1}{U} = \frac{1}{h_1} + \frac{1}{h_2} + \frac{1}{h} \]
Dimensional analysis:

Variables to be considered: D, l, n, p, u, v, C_p et h.
Dimensions to be considered: M, L, T, et t.

→ Nusselt number: \( N_u = \frac{hD}{k} \)
→ Reynolds number: \( Re = \frac{\rho v D}{\mu} \)
→ Prandtl number: \( Pr = \frac{C_p \mu}{\lambda} \)

TD6 with heat jacket:
\( Nu = 0.74 \cdot Re^{0.8} \cdot Pr^{0.33} \cdot V^{0.14} \)

Pitched blade turbine with serpentine:
\( Nu = \frac{0.0075}{\nu} \cdot Re^{0.8} \cdot Pr^{0.33} \cdot V^{0.14} \)

Propeller with serpentine:
\( Nu = 0.011 \cdot Re^{0.8} \cdot Pr^{0.33} \cdot V^{0.14} \)

TD6 with serpentine:
\( Nu = 0.0205 \cdot Re^{0.8} \cdot Pr^{0.33} \cdot V^{0.14} \)

Relation between heat transfer and microbial growth

In intensive aerobic processes, heat released is proportional to the amount of oxygen consumed:

\( q_{\text{gen}} = \frac{\Delta H_p}{\Delta t} \)

Heat extracted by heat exchanger:

\( q_{\text{in}} = h \cdot A \cdot \Delta t \)

At steady-state, heat generated by microbial growth is proportional to heat removed at the level of the exchanger:

\( h \cdot A \cdot \Delta t = \frac{\Delta H_p}{\Delta t} \)

But, scale-up problem, as \( A \sim V^{2/3} \)

Kolmogoroff: eddy scale

Limit of Kolmogoroff → in reality, we have a redistribution of the energy dissipated in the environment due to the agitation of the fluid.
Mixing time measurement

2 phenomena:
- Circulation
- Turbulence

Methods involving probes:

Equations based on circulation

\[ t_m = 3,9 \frac{d}{D}^5 N_1^{-1} \]

Correlation based on turbulence

\[ t_m = 5,9 D^{0.15} \frac{N}{(d/D)^{0.15}} \]

Mixing in function of the number of agitation stages:

Evolution of mixing during scale-up:
Example of the impact of mixing time on process efficiency: concentration gradient during a fed-batch process.

Effect of concentration gradient on cell physiology: example of Lactococcus lactis

Structured model for bioreactor hydrodynamics:
- Compartment model
- Network-of-zones

Mathematical basis:
System of ODEs is numerically resolved by a Runge-Kutta routine (e.g., ode45 routine in MatLab).
Evolution of concentration with time for each compartment:
The evolution of $C_n$ is described by an ordinary differential equation (ODE):
$$ V \frac{dC_n}{dt} = Q_c (C_n - C_j) + Q_t (C_{n+1} + C_{n-1} - 2C_n) $$

Convective fluxes ($Q_c$) are calculated (circulation flow rate) and the turbulence flow rates are estimated on the basis of mixing time experiments.
System of ODEs is numerically resolved by a Runge-Kutta routine (e.g., ode45 routine in MatLab).

Application:
A stirred bioreactor ($V = 500L$) is equipped with a TD6 or a profiled propeller. The hydrodynamics can be modelled by the compartment principle, leading to the model structure shown on the figure.
Write the ODEs system for the evolution of the concentration of a specie $C$ in the compartments.

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Consider a two-staged stirred bioreactor (TD6-TD6) as shown on the figure. If \( q_c = 0.1 \text{ m}^3/\text{s} \) et \( q_e = 0.2 \text{ m}^3/\text{s} \), model and simulate the mixing time when a pulse is added at the level of the first compartment.

Resolving the ODEs system with Matlab:

First file.m:
- Function \( y = f(t,y) \)
- Constant
- Algebraic equations
- Ordinary differential equations, ODEs (in matrix form)

Second file.m:
- Solver ode
- Function plot

Application of structured modelling procedure to oxygen transfer

2 scaling-up criteria:
- \( \rightarrow \) Air flow rate: \( G/V \) (en v.v.m.) ou \( G/S \) (en m/s)
- \( \rightarrow \) Agitation rate : \( \pi Nd \) ou \( P/V \)

But doesn’t necessarily lead to reliable results, considering the spatial distribution of basic hydrodynamic parameter inside the vessel.
4. Bioreactor hydrodynamics : local approach

**FIRST : homogenisation process**

Mechanical constraints → P/V limitation → increase of the mixing time

Consequences : gradients establishment (substrate, pH, O₂...)

**SECOND : circulation process**

Increase of the broth volume → increase of the variability (the randomness) of the circulation paths taken by the microorganisms → this phenomena is not often considered

If we observe three distincts circulation paths followed by a microorganism :

There is not a single value of circulation time, but a CTD

Namdev et al. [1992] Biotechnology and bioengineering
Increase of the randomness at the level of the circulation paths followed by microorganisms

\( \text{Circulation time distribution (CTD)} \)

\[ T = \begin{pmatrix} 1-P & P_1 \\ P_2 & 1-P_2 \end{pmatrix} \]

Don’t keep track of the particle history, but computationally light

Well suited for the simulation of the homogenisation of a solute (large amount of molecules, particles)

Mathematical implementation of stochastic models: Markov chain

Let \( S \) be the discrete state vector of the previous system, the following equation allows us to calculate the discrete time evolution of the system:

\[ S_t = S_0 T^t \]

This equation can be generalised:

\[ T = T_k \]

Stochastic compartment modelling concept

Compartment modelling concept (shift from a black box to a grey box)

Markov chain mixing model

INJECTION
Hydrodynamic modelling of bioreactors

Superimposition of the two stochastic formulations

« Microorganisms view »

5. Microbial growth in bioreactors: physiological variables

Case study: fed-batch culture of S. cerevisiae in large scale bioreactor

Microbial kinetics:

20L stirred bioreactor (RTD6; working volume 10L + glass bulb for SDRs)

Regulation: pH 5.5; T°30°C; pO² 30%; air flow rate (no regulation for the nonmixed part of the SDR)

Exponential feed of glucose (start after 5 hours): \( F = F_0 \exp(\mu t) \)

\( F_0 = 0.086 \text{ ml/min}; \mu = 0.005 \text{ min}^{-1} \)

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Biomass yield ( Y_{ss} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical bioreactor</td>
<td>0.48</td>
</tr>
<tr>
<td>SDR recirculation flow rate ( Q = 18 \text{ l/h} )</td>
<td>0.36</td>
</tr>
<tr>
<td>SDR recirculation flow rate ( Q = 39 \text{ l/h} )</td>
<td>0.45</td>
</tr>
</tbody>
</table>
6. Perspective: modelling the microbial response to bioreactor environmental fluctuations

We have described the physical parameters

BUT

What about the biological implications

What is the microbial response in front of these environmental fluctuations?

TO MAKE THE LINK

What about the environmental sensing capabilities of microorganisms?

In the case of S. cerevisiae:

- Cells react very rapidly in front of glucose fluctuations
- Glucose sensing mechanisms are well documented but unquantified data available
- The cell don’t sense all the environmental fluctuations (some specific metabolic pathways act as intracellular homeostasis devices)

Important in order to make the link between the physical and the biological parameters

Investigation tool: DNA microarray

Transcriptomic response to gradient stress (superimposition of a perfectly mixed small scale bioreactor with a scale-down reactor)

Case study 2: Whole cell biosensors for the detection of mixing imperfections
Background

Bioprocess scale-up – general scheme

- Stirred bioreactor – lab-scale
- Stirred bioreactor – industrial scale

Lack of efficiency compared with stirred reactors:
- Lower transfer efficiency
- No regulation of the main environmental variables (pH, dissolved oxygen)

Drop of mixing efficiency when D↑ at constant P/V

Generation of heterogeneities (substrate, dissolved oxygen, pH, temperature, ...)

Exposure to spatial heterogeneities – hydrodynamic aspects

Cells exposed to local variations in concentrations

Experimental strategy

Fluorescent reporter system

Basic principle:
Using the microbial population as a physiological tracer for the estimation of the bioreactor mixing and transfer efficiency (potentially capturing the stochasticity linked with the CTD)

Extracellular stimuli (S, O2, pH)

Transcriptional network

Transcriptional network – hierarchical classification

Experimental strategy

Choosing the right ORF for my application

E. coli: about 4000 ORFs:

Cultivation in shake flasks on mineral medium

Results

Screening among an E. coli GFP clones library

GFP

Experimental strategy

Flow cytometry – an efficient tool to characterize microbial population heterogeneity

30,000 microbial cells analysed within 30 seconds

E. coli: about 4000 ORFs:

GFP

Transcriptional network

Transcriptional network – hierarchical classification

Results

Screening among an E. coli GFP clones library

GFP

Cultivation in shake flasks on mineral medium

prps::gfp

Experimental strategy

Fluorescent reporter system

Experimental strategy

Choosing the right ORF for my application
**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

Shake flask:
- Easy to handle, well suited to perform parallel cultures, but lack of representativeness compared to the performances of stirred bioreactors.

**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

Intermittent feeding strategy

- OXY-mini
- 4 channels
- IO converter
- Orbital incubator (T° and shaking frequency controls)

**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

Cultures of GFP clones in shaken bioreactors (1L baffled shake flask: initial working volume: 200mL; final working volume: 400 mL)

**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

GFP

GFP

prpoS::gfp

puspA::gfp

**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

Two modes of expression: binary or graded

Inducer Concentration

rpoS

Number of Cells

Gene Expression Level

Zhang et al. (2006) Theoretical biology and medical modelling, 3:18

**Results**

Screening among E. coli GFP clones library

Representativeness of shaken bioreactor

Binary mode of gene expression → sources:
- Short mRNA and protein half-lives
- High sensitivity for the detection of the reporter protein
- Generally not observed for GFP reporter system considering the high protein stability of this system compared with β-galactosidase and luciferase reporters
- This mechanism of gene induction give rise to differentially expressed phenotypes at the protein level. Can potentially be used to gain more sensitivity about the impact of extracellular fluctuations
Results

Regulation of the addition of glucose by the dissolved oxygen level (SP = 30%), PID control.

Results

Regulation of the addition of glucose by the dissolved oxygen level (SP = 30%), ON/OFF control.

Basic observations:
- Binary mode for GFP expression at the end of the batch phase and during the transition from batch to fed-batch phase.
- After the induction of the major part of the population (all the cells are in the GFP+ state), graded mode of GFP expression is observed.
- Successive glucose excess tends to slow down the binary expression phase.

Results

Operating conditions:
- Stirred bioreactor, working volume 10L.
- Mineral medium, glucose as carbon source.
- Fed-batch with exponential feed algorithm.
- Scale-down approaches with DO-controlled fed-batch and partitioned reactor.

Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

Global mixing efficiency

A pcyA::GFPmut2 strain is not influenced by hydrodynamic conditions

Cultures performed under constant glucose feed: pcsiE::gfp strain
**Results**

Cultures performed under constant glucose feed: puspA::gfp strain

To be validated by using a DO-controlled feed

Prytz et al. [2003] Biotech Bioeng. 83:595-603

**Results**

Two main mechanisms proposed to regulate rpoS in high cell density cultures:

- Cell density
  - DeLisa and Bentley [2002] Microbial Cell Factories, 1
- Decreasing growth rate
  - Ihssen and Egli [2004] Microbiology, 150:1637-1648

**Perspectives and conclusion**

ppoS::GFP strains seems to react to the degree of homogeneity inside the bioreactor:

- Homogenous reactor: GFP+
- Inhomogenous reactor: GFP-

Two questions have to be raised:

- Flow cytometry combined with PspA::GFP expression → impact of extrinsic fluctuations
- Characteristic times of hydrodynamic mechanisms compared with those of the biological processes behind GFP synthesis

**Perspectives and conclusion**

Complex phenomena:

- Two sources of noise (extrinsic and intrinsic)
- Very different characteristic time constants (physical and biological processes)
  → A model is required

**Reaction scheme**: 8 rates (including the characteristic time constants) to specify

**ODEs system**: 8 rates (including the characteristic time constants) to specify
Perspectives and conclusion

These equations can be used in the classical deterministic formalism (ODEs solver), but more interestingly in the stochastic formalism:

Probability that reaction \( \mu \) occurs at time \( \tau \) (Gillespie algorithm)

Example: simulation of 30,000 cells after 6 hours of induction