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Development of an Industrial Biotechnology Process

Stirred Tank Bioreactors/Process integration/ Scale-up

Kurt Eyer

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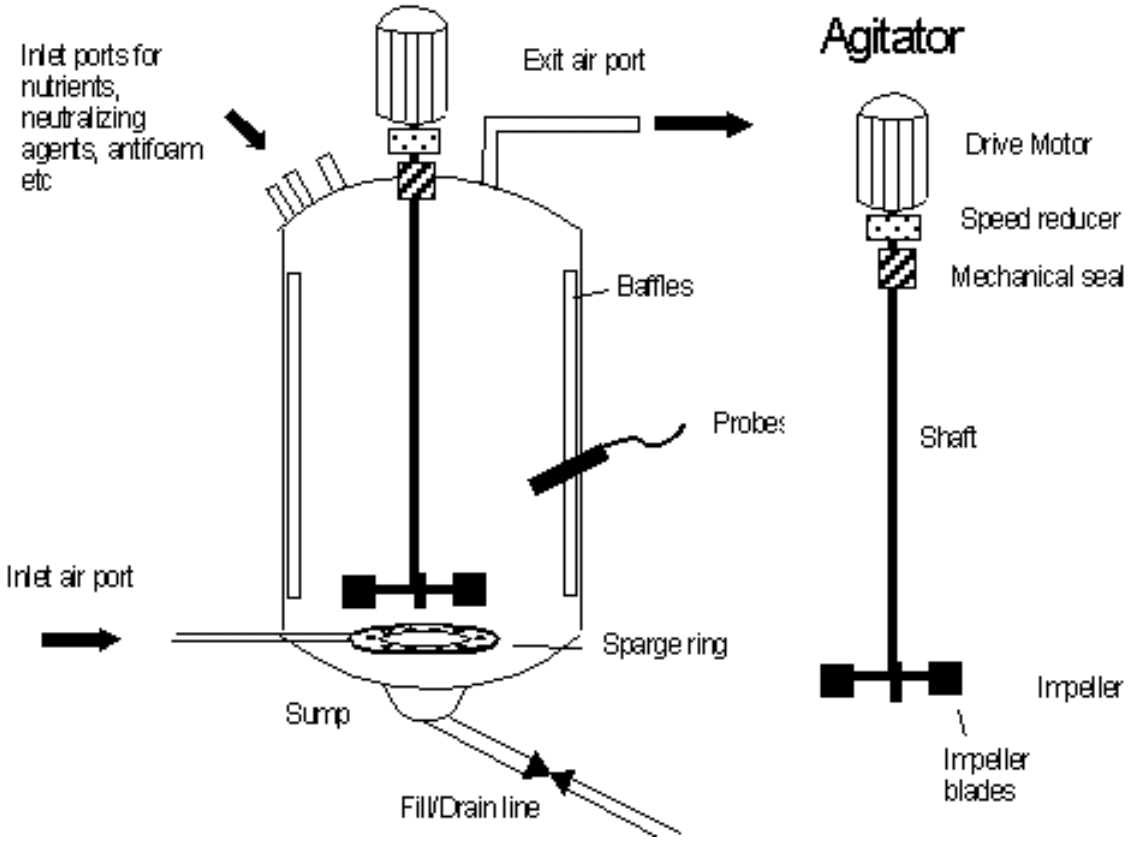
The stirred tank bioreactor (STR)

A typical bioreactor used for microbial fermentations is shown in the following figure:



Laboratory scale bioreactors with liquid volumes of less than 10 litres are constructed out of Pyrex glass. For larger reactors, stainless steel (V4A, 316L) is used.

The stirred tank bioreactor (STR)

Stainless steel

Stainless steel refers to various alloys of primarily iron, nickel and chromium. Molybdenum may also be added to increase the resistance of the steel to corrosion. Stainless steels come in different grades. The commonly encountered grades are designated by standard codes, for example:

- 302
- 304
- 316
- 318

In general, the higher the number, then the greater the resilience of the steel.
The grade of stainless steel most widely used in the construction of bioreactors is ***316L***. The "L" indicates the steel has a low carbon content.
Stainless steels used in bioreactors are often polished to a mirror finish.This finish makes cleaning and sterilization easier. Stainless steel components used in the construction of bioreactors are joined in an oxygen-free environment using a special technique known as TIG welding.TIG stands for Total Inert Gas and the technique involves the use of argon to displace the air.
The presence of oxygen in the welds can cause corrosion at the weld.

The stirred tank bioreactor (STR)

Standard geometry

A stirred tank reactor will either be approximately cylindrical or have a curved base. A curved base assists in the mixing of the reactor contents.

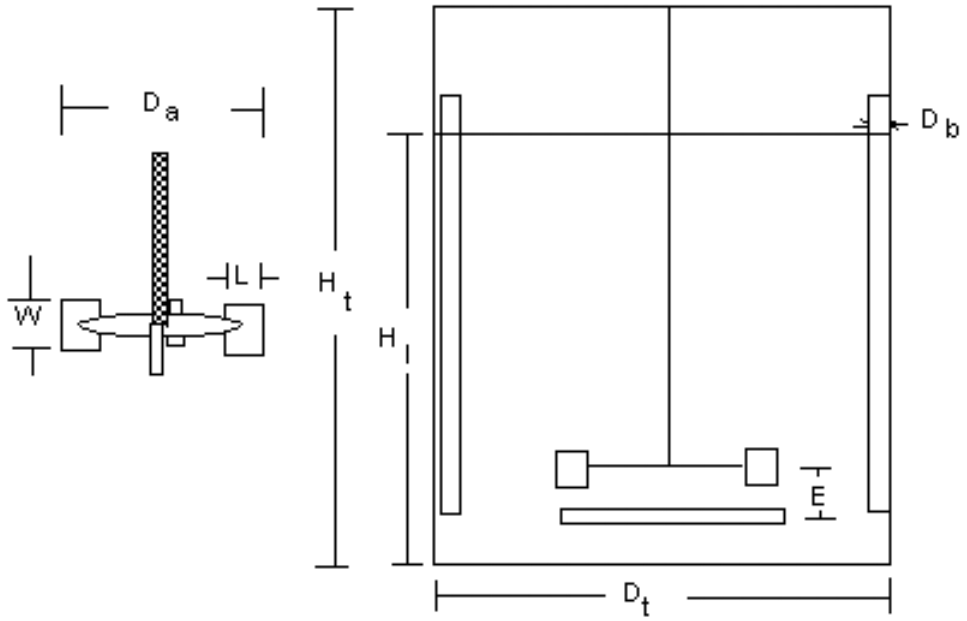
Stirred tank bioreactors are generally constructed to standard dimensions.

That is, they are constructed according to recognized standards such as those published by the International Standards Organisation and the British Standards Institution.

These dimensions take into account both mixing effectiveness and structural considerations.

The stirred tank bioreactor (STR)

Standard geometry



A mechanically stirred tank bioreactor fitted with

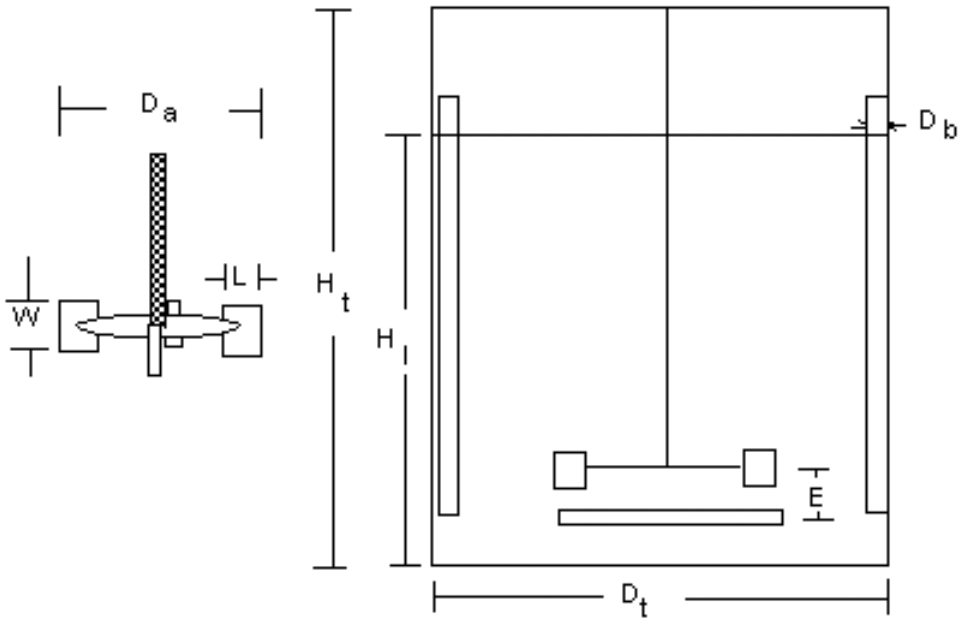
- a sparger and
- a Rushton turbine

will typically have the following relative dimensions:

Ratio		Typical values	Remarks
Height of liquid in reactor to height of reactor	H_L/H_t	~0.7-0.8	Depends on the level of foaming produced during the fermentation
Height of reactor to diameter of tank	H_t/D_t	~1 - 2	European reactors tend to be taller than those designed in the USA
Diameter of impeller to diameter if tank	D_a/D_t	1/3 - 1/2	Rushton Turbine reactors are generally 1/3 of the tank diameter. Axial flow impellers are larger.
Diameter of baffles to diameter of tank	D_b/D_t	~0.08 - 0.1	
Impeller blade height to diameter of impeller	W/D_a	0.2	
Impeller blade width to diameter of impeller	L/D_a	0.25	
Distance between middle of impeller blade and impeller blade height	E/W	1	

A tank's height:diameter ratio is often referred to as its aspect ratio.

Example 1: Calculate the dimensions of the reactor



A stirred tank bioreactor is approximately cylindrical in shape. It has a total volume (V_t) of 100,000 litres.

The geometry of the reactor is defined by the following ratios

$D_t:H_t$	0.50
$D_a:D_t$	0.33
$D_b:D_t$	0.10

Example 1: Calculate the dimensions of the reactor

Convert the volume to SI units.

The volume of the reactor in SI units is 100 m^3

(This is a very important step - Always use SI units!!!!)

Use the equation describing the volume of a cylinder

Since $H_t = 2 \times D_t$

Our equation becomes

Example 1: Calculate the dimensions of the reactor

Substituting in our value of V_t, we get D_t, H_t, D_a, D_b

$D_t =$

$H_t = 2 \times D_t =$

$D_a = D_t / 3 =$

$D_b = D_t / 10 =$

Example 2: Calculate the dimensions of the reactor

The geometry of a cylindrical tank with a volume of 120,000 litres is described by the following ratios:

$H_l = 1.5 \times D_t$
$D_a = 1/3 \times D_t$
$H_t = 1.4 \times H_l$

Calculate the dimensions of the tank: D_t, H_t, H_l, D_a

D_t = m

H_t = m

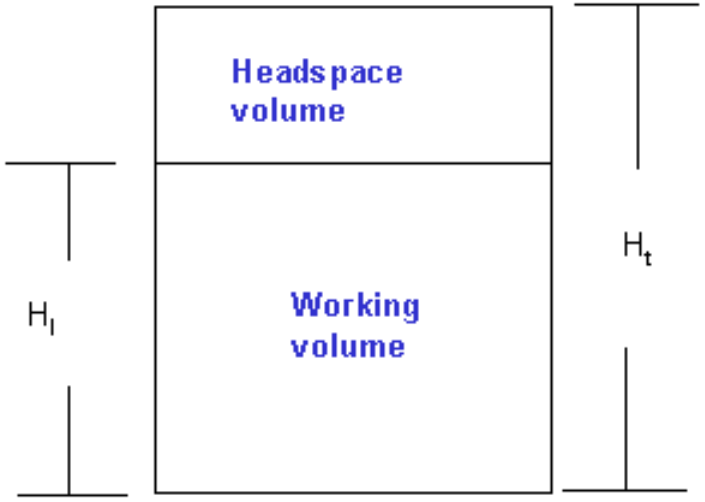
H_l = m

D_a = m

The stirred tank bioreactor (STR)

Headspace volume

A bioreactor is divided in a working volume and a head-space volume. The working volume is the fraction of the total volume taken up by the medium, microbes, and gas bubbles.
The remaining volume is calles the headspace.

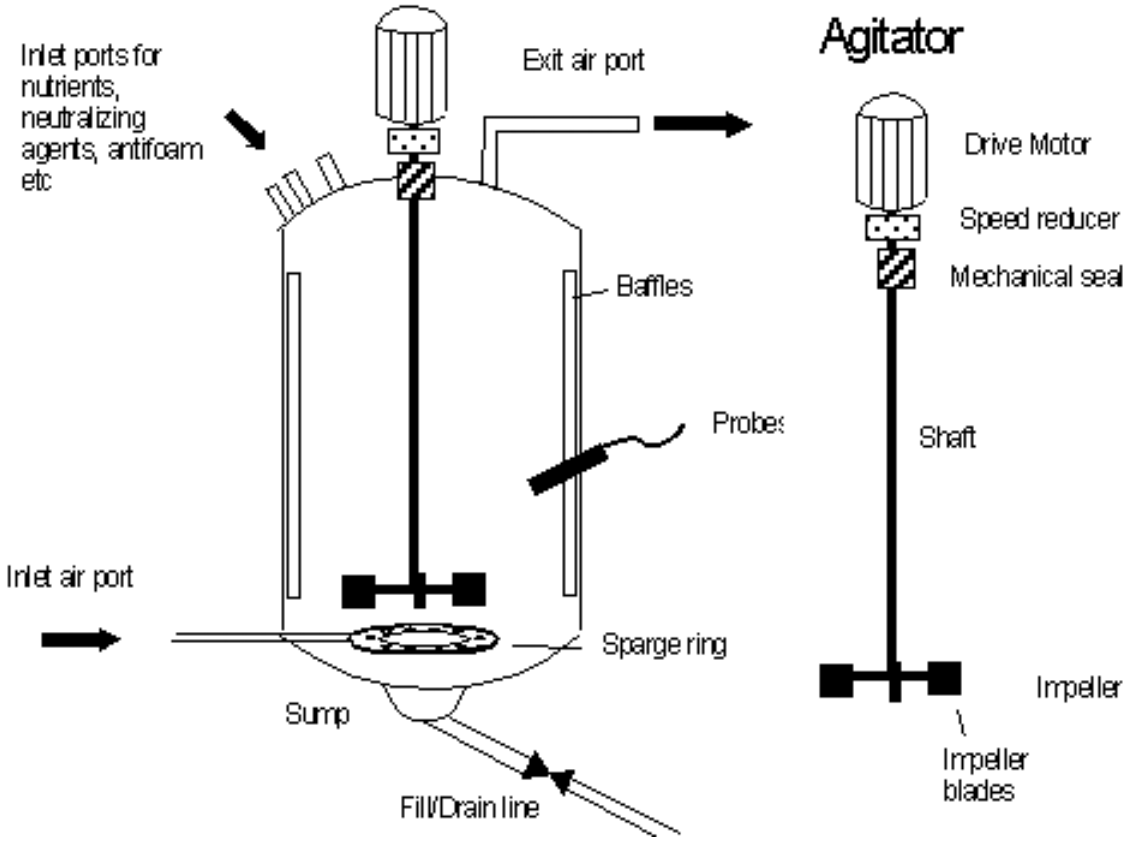


Typically, the working volume will be 70-80% of the total fermenter volume.
This value will however depend on the rate of foam formation during the reactor. If the medium or the fermentation has a tendency to foam, then a larger headspace and smaller working volume will need to be used.

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

A modern mechanically agitated bioreactor will contain:



- An agitator system
- An oxygen delivery system

- A foam control system
- A temperature control system
- A pH control system
- Sampling ports
- A cleaning and sterilization system.
- A sump and dump line for emptying of the reactor.

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

Agitation system

The function of the agitation system is to

- provide good mixing and thus increase mass transfer rates through the bulk liquid and bubble boundary layers.
- provide the appropriate shear conditions required for the breaking up of bubbles.

The agitation system consists of the agitator and the baffles.
The baffles are used to break the liquid flow to increase turbulence and mixing efficiency.

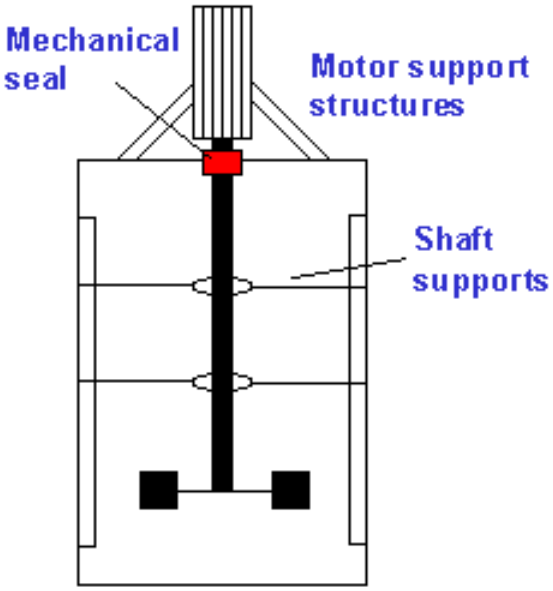
The number of impellers will depend on the height of the liquid in the reactor. Each impeller will have between 2 and 6 blades. Most microbial fermentations use a Rushton turbine impeller.

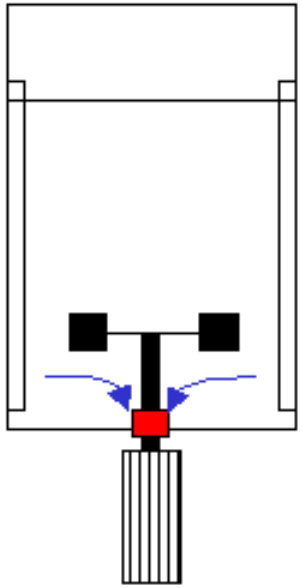
A single phase (ie. 240 V) drive motor can be used with small reactors. However for large reactors, a 3 phase motor (ie 430 V) should be used. The latter will tend to require less current and therefore generate less heat.

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

Agitation system - Top entry and bottom entry impellers





Oxygen delivery system.

The oxygen delivery system consists of

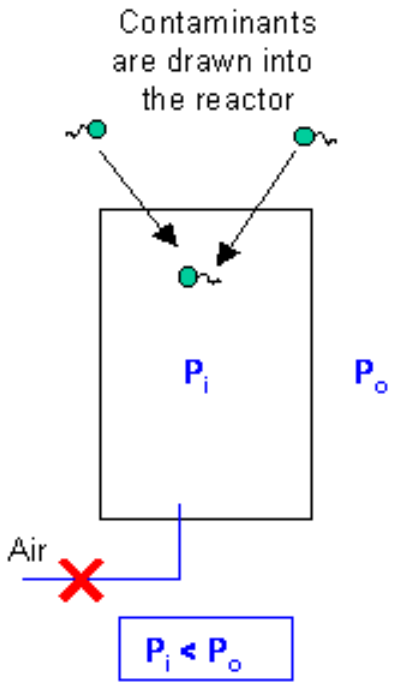
- a compressor
- inlet air sterilization system
- an air sparger
- exit air sterilization system

The stirred tank bioreactor (STR)

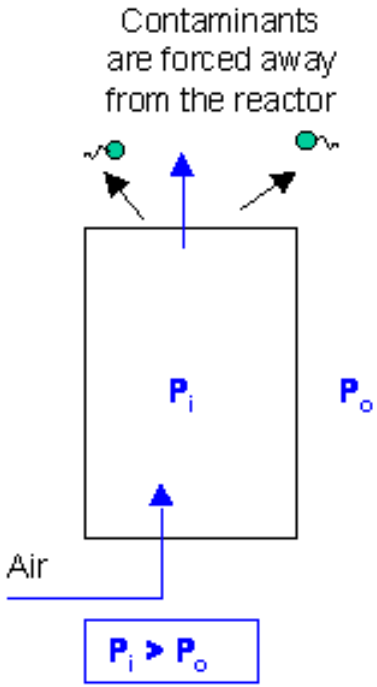
Basic features of a stirred tank bioreactor

Oxygen delivery system - Air sterilization system

Positive pressure



Without aeration, a vacuum forms as the reactor cools.



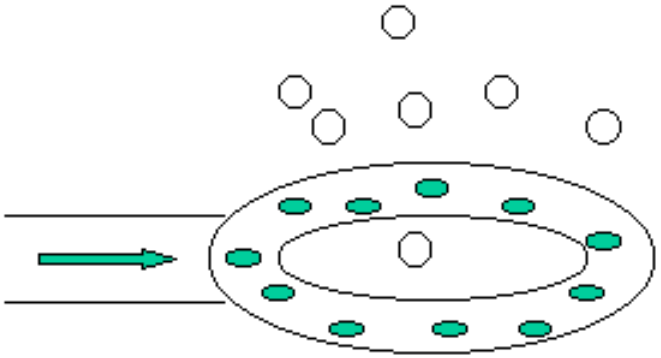
With aeration, positive pressure is always maintained and contaminants are pushed away from the reactor

The stirred tank bioreactor (STR)

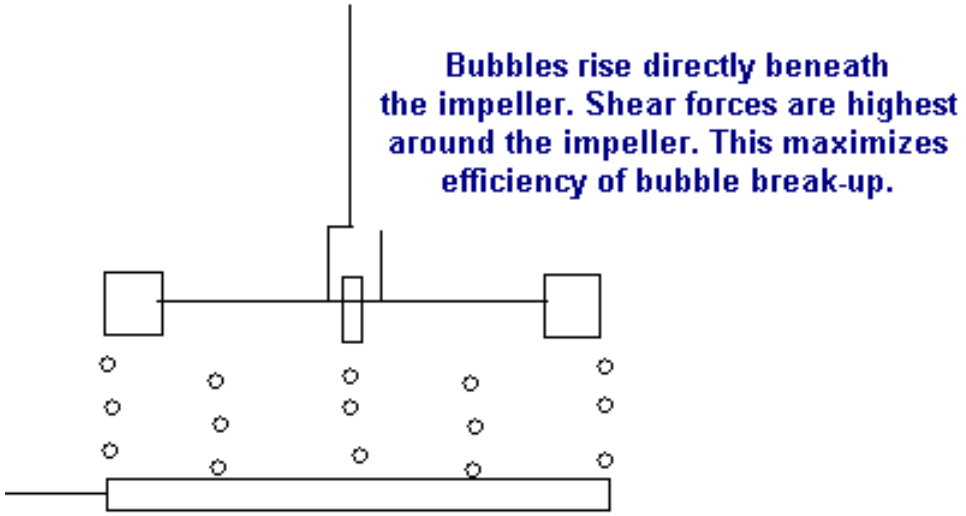
Basic features of a stirred tank bioreactor

Oxygen delivery system - Sparger

The air sparger breaks the incoming air into small bubbles.
Various designs can be used such as porous materials made of glass or metal. However, the most commonly used type of sparger used in modern bioreactors is the sparge ring:



The sparge ring must be located below the agitator and be approximately the same diameter as the impeller.



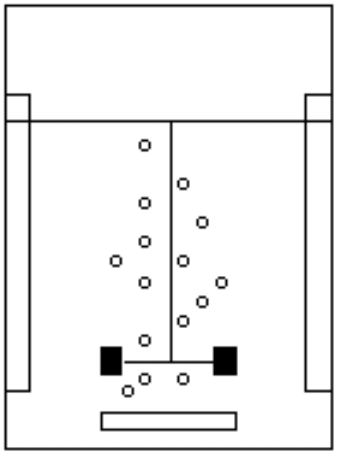
Thus, the bubbles rise directly into the impeller blades, facilitating bubble break up.

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

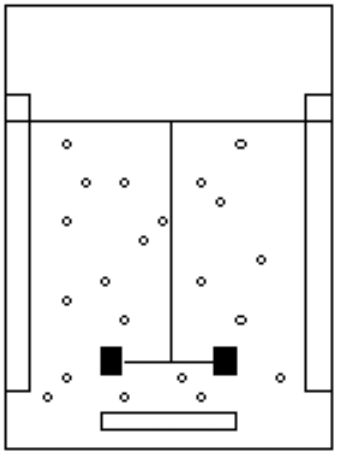
Oxygen delivery system - Effect of impeller speed

As discussed earlier, the shear forces that an impeller generates play a major role in determining bubble size. If the impeller speed is to slow then the bubbles will not be broken down. In addition, if the impeller speed is too slow, then the bubbles will tend to rise directly to the surface due to their bouyancy.



Slow impeller speed

The bubbles will not be sheared into smaller bubbles and will tend to rise directly towards the surface



Fast impeller speed

Smaller bubbles will be generated and these bubbles will move with throughout the reactor increasing the gas hold up and bubble residence time

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

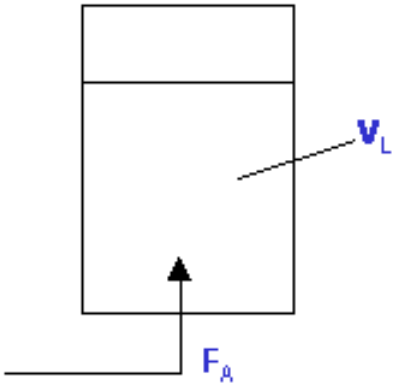
Oxygen delivery system - Air flow rates

Air flow rates are typically reported in terms of volume per volume per minute

or

vvm

$$vvm = \frac{Volumetric\ air\ flow\ rate}{Liquid\ volume}$$



$$vvm = \frac{F_A\ (litres.min^{-1})}{V_L\ (litres)}$$

The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

Oxygen delivery system – Foam control

Foam control is an essential element of the operation of a sparged bioreactor. The following photograph shows the accumulation of foam in a 2 litre laboratory reactor.

Excessive foam formation can lead to blocked air exit filters and to pressure build up in the reactor. The latter can lead to a loss of medium, damage to the reactor and even injury to operating personnel.

Foam is typically controlled with aid of antifoaming agents based on silicone or on vegetable oils. Excessive antifoam addition can however result in poor oxygen transfer rates.

Factors affecting antifoam requirements

The following factors affect the foam formation and the requirement for antifoam addition.

- the fermentation medium
- products produced during the fermentation
- the aeration rate and stirrer speed.
- the use of mechanical foam breakers
- the head space volume
- condenser temperature

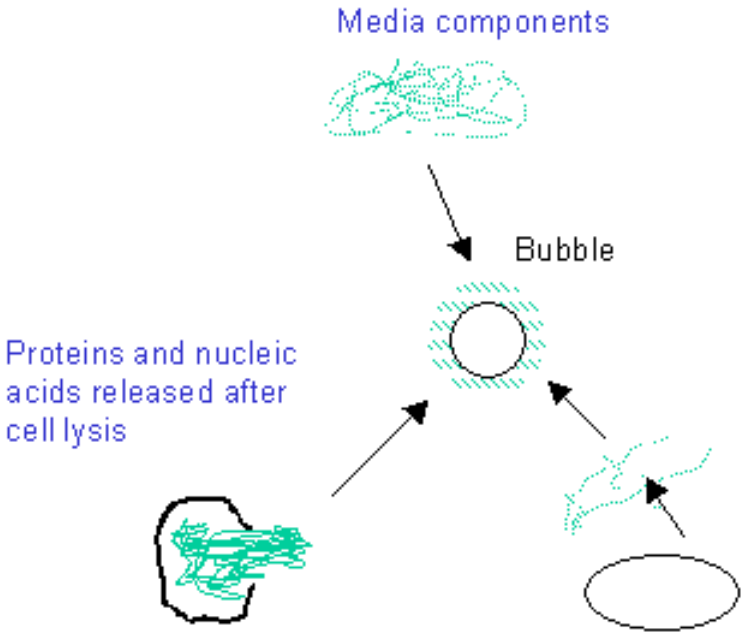
The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

Oxygen delivery system – Foam control

Factors affecting antifoam requirements - Medium and cells

Media rich in proteins will tend to foam more readily than simple media. For example, the use of whey powder and corn steep liquor, two common nitrogen sources will contribute significantly to rate of foam formation and the antifoam requirement.



Many cells also produce detergent-like molecules. These molecules can be nucleic acids and proteins released upon the death of the cells or proteins and lipid compounds produced during the growth of the cells.

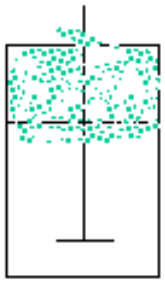
The stirred tank bioreactor (STR)

Basic features of a stirred tank bioreactor

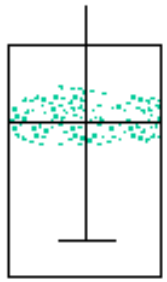
Oxygen delivery system – Foam control

Factors affecting antifoam requirements - Aeration rate and stirrer speed.

Higher stirrer speeds and higher aeration rates increase foaming problems. These problems can in fact be so significant that they limit the stirrer speeds or aeration rates that can be used in process.



Fast stirring speed



Slower stirring speed

A fast stirrer speed will lead to the faster formation of foam.

The stirred tank bioreactor (STR)

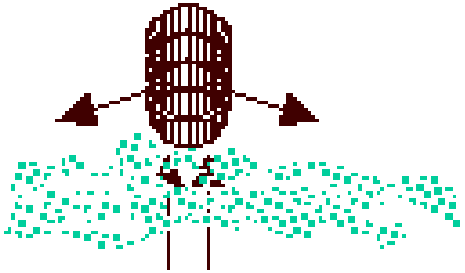
Basic features of a stirred tank bioreactor

Oxygen delivery system – Foam control

Factors affecting antifoam requirements - Mechanical foam breakers

Mechanical foam breakers can eliminate or at least reduce the antifoam requirement.

These devices generate sit above the liquid and generate high shear forces which break the bubbles in the foam. High shear agitators and nozzles connected to high shear pumps are often used.
For small scale reactor systems such as those used in the culture of animal cells, ultrasonic foam breakers are sometimes used. These generate high frequency vibrations which break the bubbles in the foam.



The foam is sucked into a high shear device and in the process is broken up.

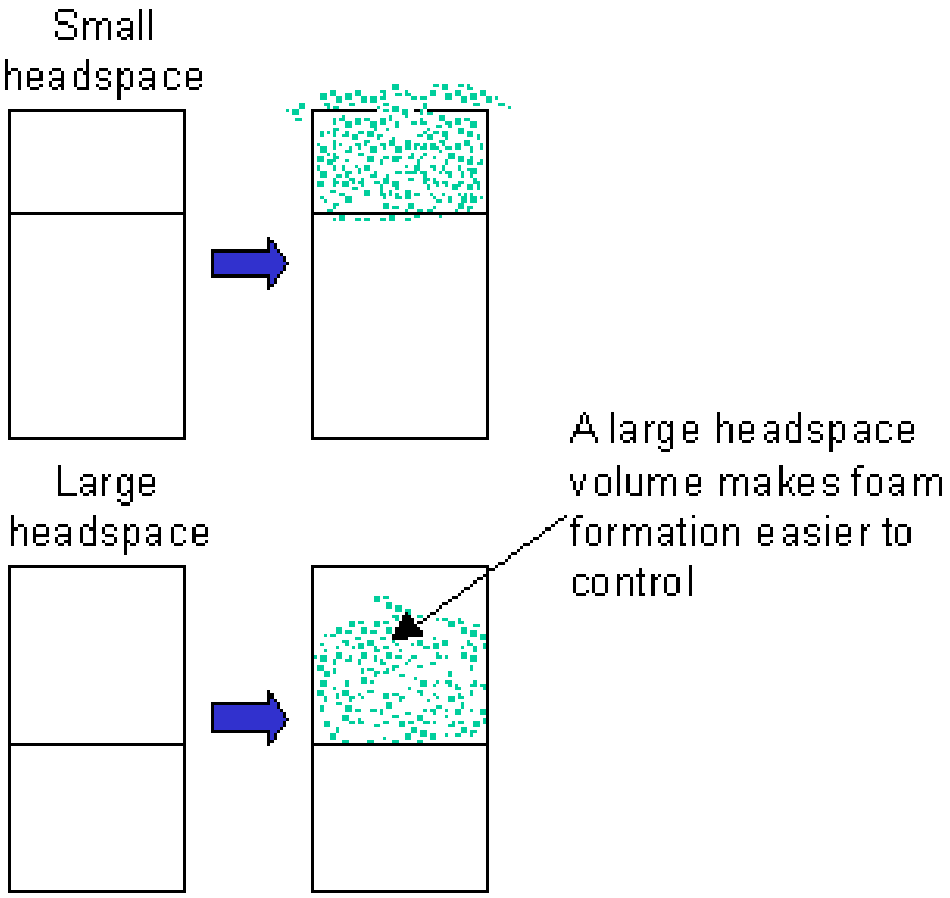
For small scale reactor systems such as those used in the culture of animal cells, ultrasonic foam breakers are sometimes used. These generate high frequency vibrations which break the bubbles in the foam

The stirred tank bioreactor (STR)

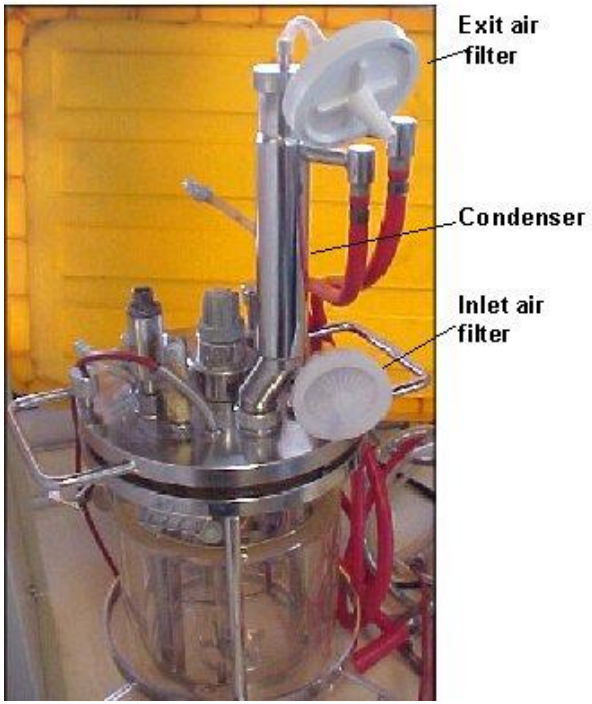
Basic features of a stirred tank bioreactor

Oxygen delivery system – Foam control

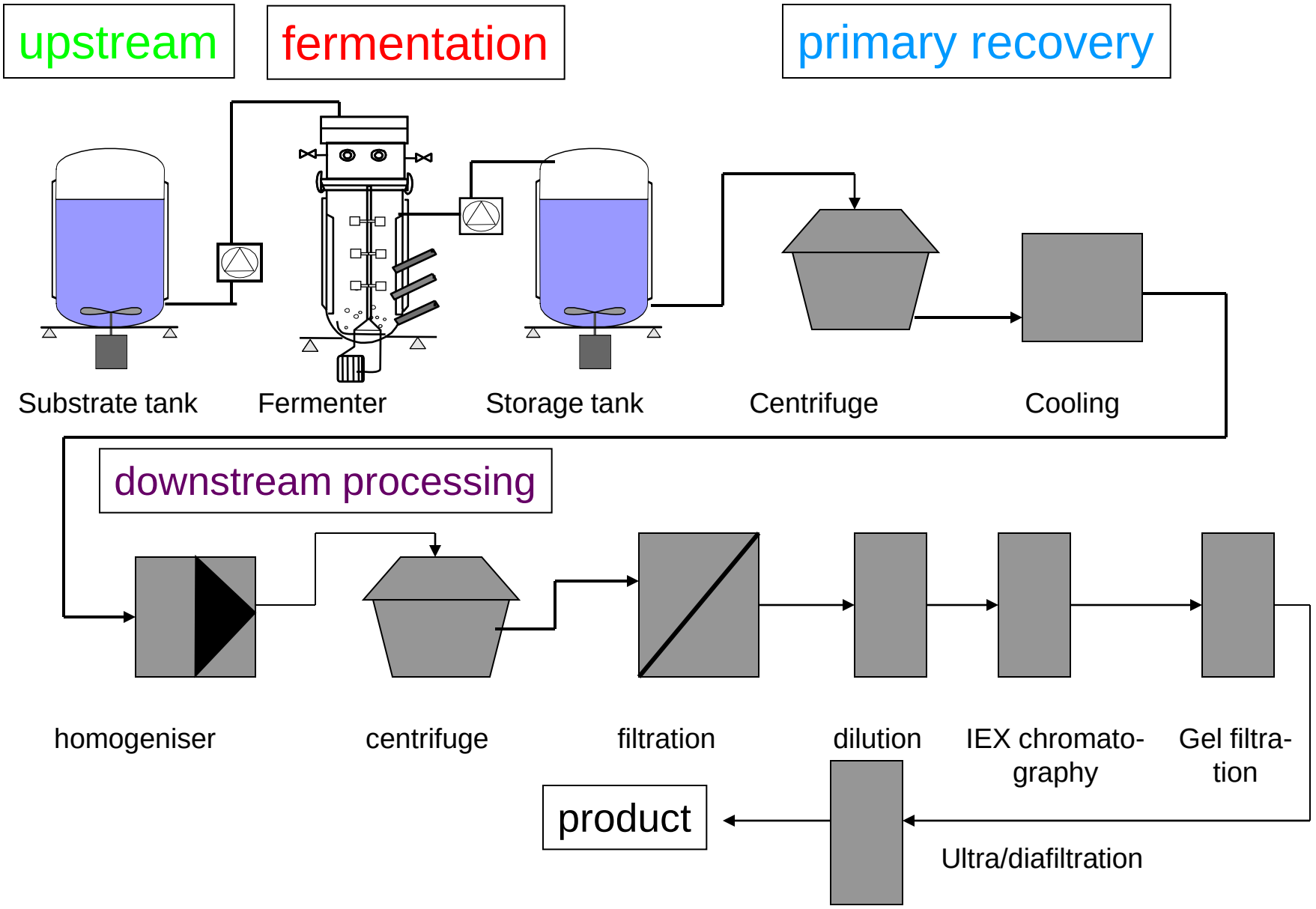
Headspace volume

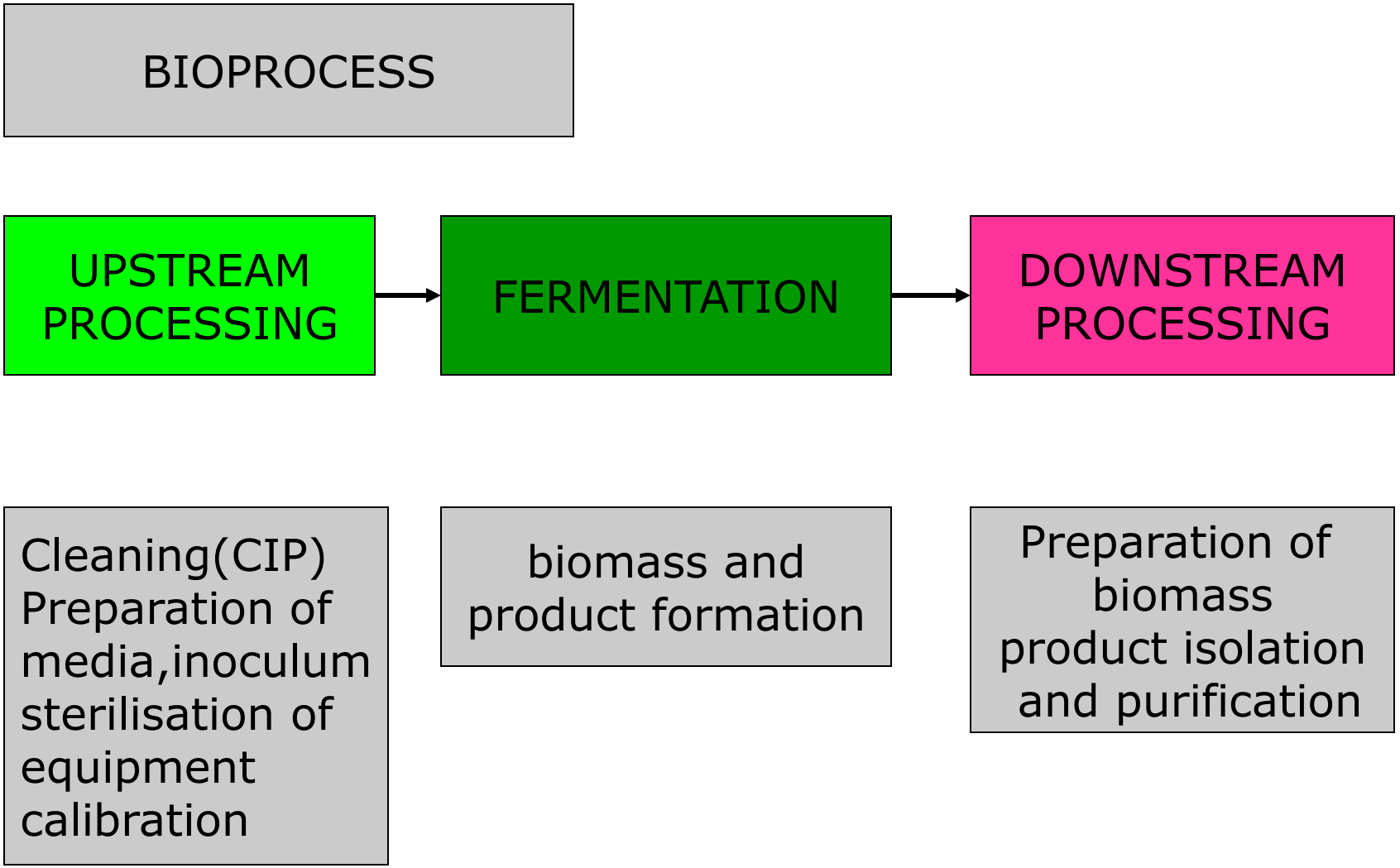


In laboratory scale reactors, a cold **condenser temperature** can help to control the foam. The density of the foam increases when it moves from the warm headspace volume to the cold condenser region. This causes the foam to collapse



Principle configuration of a bioprocess (example)




BIOPROCESS
UPSTREAM PROCESSING
FERMENTATION
DOWNSTREAM PROCESSING
Cleaning(CIP)
Preparation of media,inoculum
sterilisation of equipment
calibration
biomass and product formation
Preparation of biomass
product isolation and purification

Process integration

- Once a process has been defined, either a new process or an existing one, the next step is to define how to integrate each part of the process to make the most efficient and cost effective production process.
- Ideally process integration should not be something done after each part of the process has been optimized and defined but should start from the outset of any new process

Integration of USP and DSP

- USP involves all stages upto and including the cell culture and production phase
- DSP involves all stages from the product harvesting from the bioreactor, through cell separation and chromatographic steps to final product formulation
- The aim of any production process should be to facilitate the integration of the USP and DSP

Process integration

- Medium supply
- Medium preparation and feeding (continuous sterilization)
- USP- CIP and SIP
- Batch, fed- batch, continuous culture
- Single stage or multiple stage culture
- Cell separation
- Product concentration (capture)
- Intermediate purification
- Polishing
- Formulation
- Analytics- QA and QC

Process integration

When a cell line/organism is initially chosen it should be with respect to:

- FDA approval (retroviruses etc.)
- stability
- glycosylation profile (??)
- high productivity
- whether it is suspension (or anchorage dependent)
- whether it can be grown to high density or requires immobilzation
- whether batch, fed- batch or continuous process will be supported
- shear sensitivity and growth on defined medium

USP

- Choose system based on concentration and product stability
- Avoid high perfusion rates- productivity high but concentration low-large volumes
- Define system which is scalable
- Define system with lowest labour costs- roller bottles to be avoided
- Define seed train

USP

- Define kinetics
- If production continuous will DSP be continuous or as series of batches (stability dependent)?
- If continuous how long can one run be operated with consistency of product?
- Risk of contamination or mutation higher with continuous

DSP

- All processes require the prior removal of cells from the product- containing medium
- Cells should be removed rapidly (even from continuous processes) to avoid cell lysis and product contamination by host cell proteins and proteases
- Viability within USP should be maintained high to avoid cell lysis
- Medium should be chosen to have the minimum of protein contaminants, while maintaining high cell viability

Scale-up

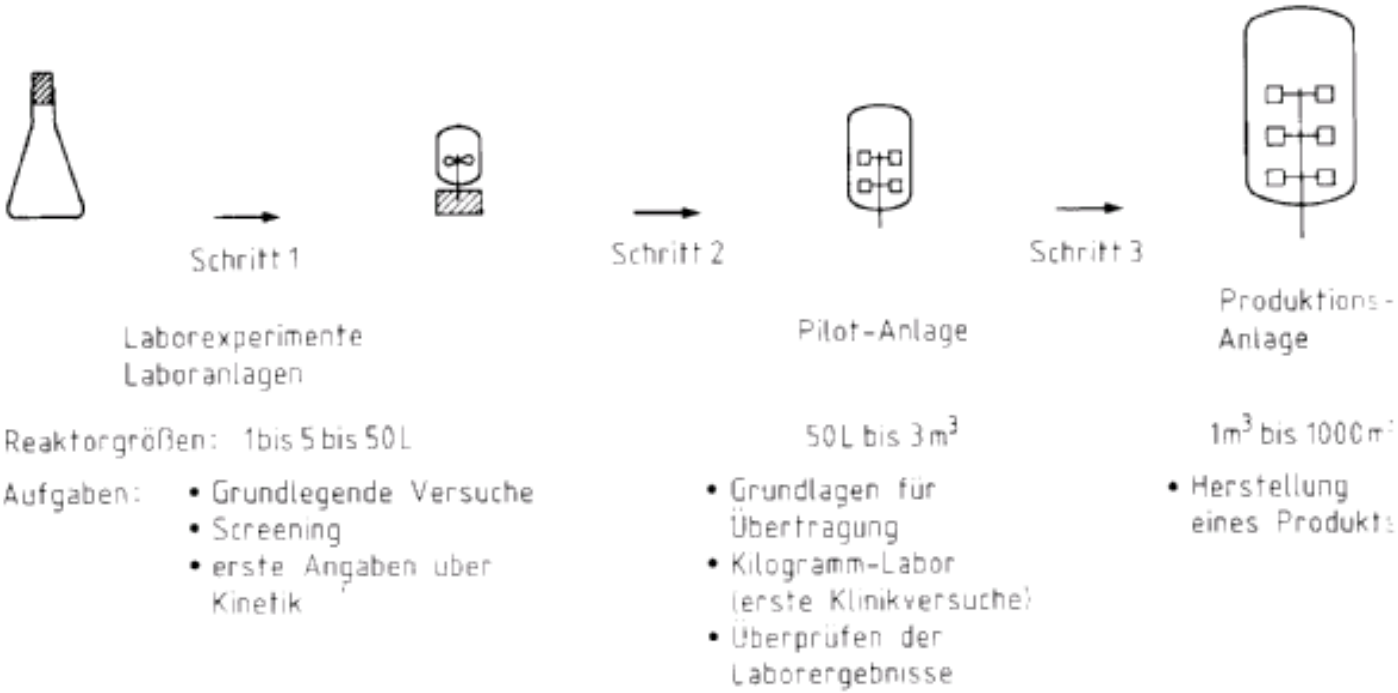
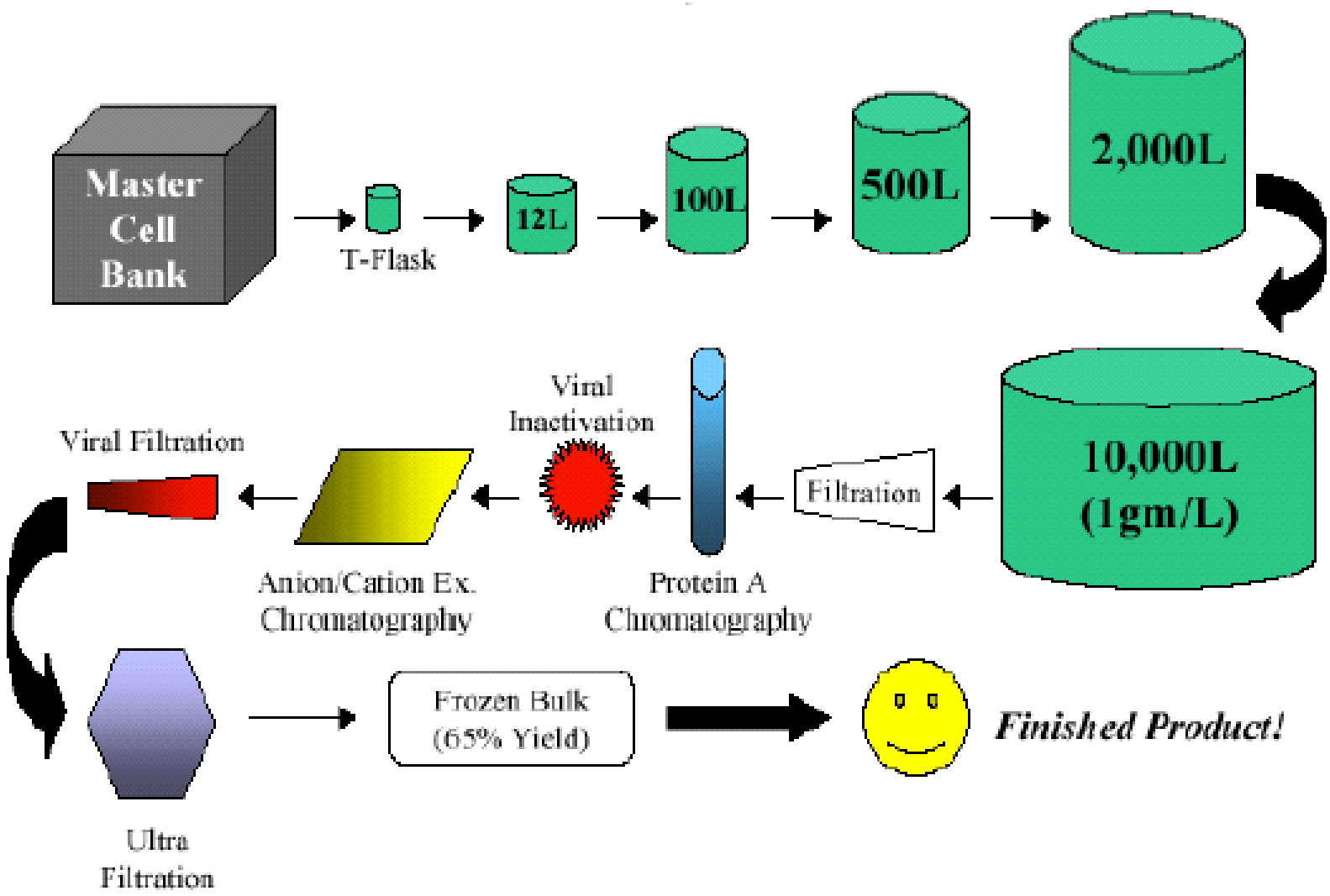


Abb. 6-15. Darstellung der Schritte bei der biologischen Maßstabsvergrößerung.


Master Cell Bank
T-Flask
12L
100L
500L
2,000L
10,000L (1gm/L)
Filtration
Protein A Chromatography
Viral Inactivation
Anion/Cation Ex. Chromatography
Viral Filtration
Ultra Filtration
Frozen Bulk (65% Yield)
Finished Product!

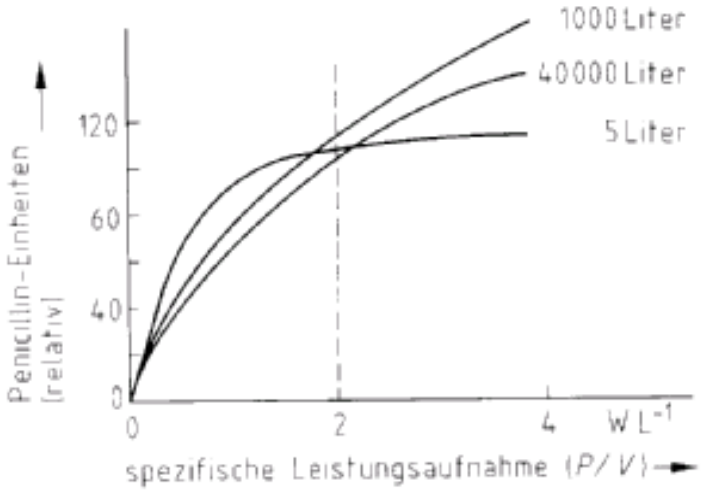
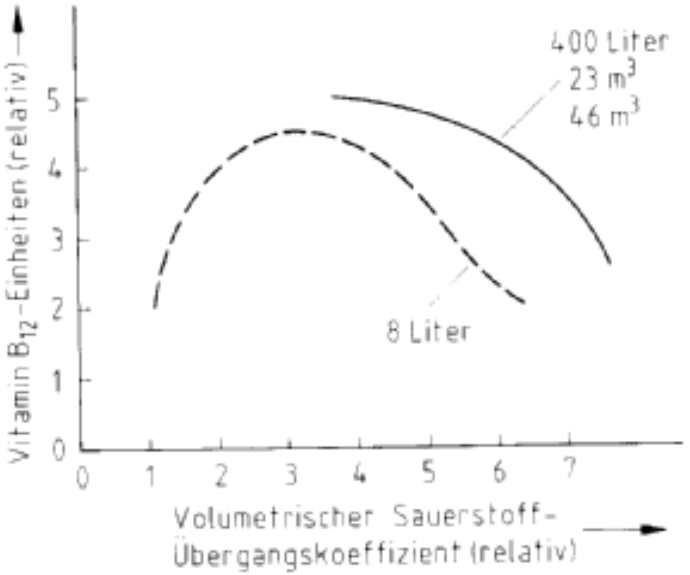


Abb. 6-17. Beispiel 1: Zusammenhang zwischen der Penicillin-Ausbeute und der spezifischen Leistungsaufnahme (für verschiedene Bioreaktorgrößen).



Ab. 6-18. Beispiel 2: Zusammenhang zwischen der Vitamin B_{12}-Ausbeute und dem volumetrischen Sauerstoff-Übergangskoeffizienten (für verschiedene Bioreaktorgrößen).

Basics for scaling-up

- from chemistry
- Based on similarity theory

Assumptions:

geometric similarity remains

Nutrient composition and also cultivation parameters keep constant during scale-up (T, pH, …..)

Homogeneity over the whole reactor

Basics for scaling-up

Based on this assumptions:

$P_0 \sim N^3 D^5$

$V \sim D^3$

$F \sim N D^3$

P_0 : Stirring power
F: pump capacity of stirrer

Scale-up criteria:

a) $P_0/V \sim N^3 D^2$

b) $F/V \sim N$

c) $v \sim N D$ (velocity of stirrer: tip speed)

d) $Re \sim N D^2$

e) $k_L a \sim (N^3 D^2)^{0.4\text{-}0.7} \sim (N^3 D^2)^{0.5}$

f) $t_m \sim 1/N$

Basics for scaling-up

Comparison of different strategies for up-scaling with factor 125

Strategy	**P**	**P/V**	**$k_L a$**	**N**	**t_m**	**N D**	**N D^2**
P/V =const	125	1	1	0,34	2.9	1,7	8,5
N = const	3125	25	5	1	1	5	25
v = const	25	0,2	0,4	0,2	5	1	5
Re = const	0,2	0,002	0,04	0,04	25	25	1

Increase of volume: factor 125 (at constant geometric ratio)

Scale-up

Generally bioreactors maintain height to diameter (H/D) of 2:1 or 3:1 (note for STR ideal is 1:1 with respect to liquid height)

If H/D maintained constant during scale-up- surface to volume ratio decreases dramatically (i.e. m^2/m^3)

Result: less important effect of surface aeration, lower heat transfer surface etc.

Wall growth: becomes very important, since at small scale, cells with altered metabolism are common, whereas at larger scale smaller surface area means less important effect, but productivity lower.

If geometrical similarity is maintained then physical conditions must change since N and D_i define all quantities (cf.Table)

Interdependence of scale-up parameters

TABLE 10.2 Interdependence of Scale-up Parameters

Scale-up criterion	Designation	Small fermenter, 80 l	Production fermenter, 10,000:l			
			Constant, P_0/V	Constant, N	Constant, $N \cdot D_i$	Constant, Re
Energy input	P_0	1.0	125	3125	25	0.2
Energy input/volume	P_0/V	1.0	1.0	25	0.2	0.0016
Impeller rotation number	N	1.0	0.34	1.0	0.2	0.04
Impeller diameter	D_i	1.0	5.0	5.0	5.0	5.0
Pump rate of impeller	Q	1.0	42.5	125	25	5.0
Pump rate of impeller/volume	Q/V	1.0	0.34	1.0	0.2	0.04
Maximum impeller speed (max. shearing rate)	$N \cdot D_i$	1.0	1.7	5.0	1.0	0.2
Reynolds number	$ND_i^2\rho/\mu$	1.0	8.5	25.0	5.0	1.0

With permission, from J. Y. Oldshue, *Biotechnol. Bioeng. 8*:3–24 (1996) John Wiley & Sons, Inc.

Scale-up

Different scale- up rules can give different results:

Constant P_0/V provides constant OTR
Constant Re provides similar flow patterns
Constant N gives constant mixing times
Constant tip speed gives constant shear

All scale- up problems are linked to transport processes

The relative time scales for reaction are important for defining the homogeneity in the bioreactor.

Scaling- up involves moving from the process being controlled by cell kinetics at lab scale to control by transport limitations at large scale.

Therefore results at small scale may be unreliable at large scale.

To facilitate prediction of limitations can use time constants for conversion and transport processes (Table).

Processes with small time constants relative to the main process are approx. at equilibrium e.g. if $1/k_La << t_{O2}$ conversion (time constant for oxygen consumption) then supply is greater than demand and medium will be saturated with O_2.

Equations describing some time constants

TABLE 10.3 Some Time Constants (Equations)[a]

Transport process	Equation
Flow	L/v or V/Q
Diffusion	L^2/D
Oxygen transfer	$1/k_L a$
Heat transfer	$V\rho C_p/UA$
Mixing	$t_m = 4V/(1.5ND^3)$. stirred vessel
Conversion processes:	
Growth	$1/\mu$
Chemical reaction	C/r
Substrate consumption	C_s/r_{max} $(C_s >> K_s)$
	K_s/r_{max} $(C_s << K_s)$
Heat production	$\rho C_p \Delta_k T/r\Delta H$

[a]With permission, from N. W. F. Kossen, in T. K. Ghose, ed., *Biotechnology and Bioprocess Engineering*, United India Press Link House, New Delhi, 1985, pp. 365–380.

If consumption is same order of magnitude as oxygen supply ($1/k_La \sim t_{O2}$) the pO_2 will be very low (see this case in results from 20 m^3 reactor and diagram of reactor). Cells therefore pass through regions of low oxygen concentration, and essentially anaerobic, resulting in changed cell metabolism.

Therefore scale- up is empirical; or scale- down and study factors having major effects, then maintain these constant

Time constants for 20m^3 bioreactor

TABLE 10.4 Time Constants, 20-m^3 Fermenter[a]

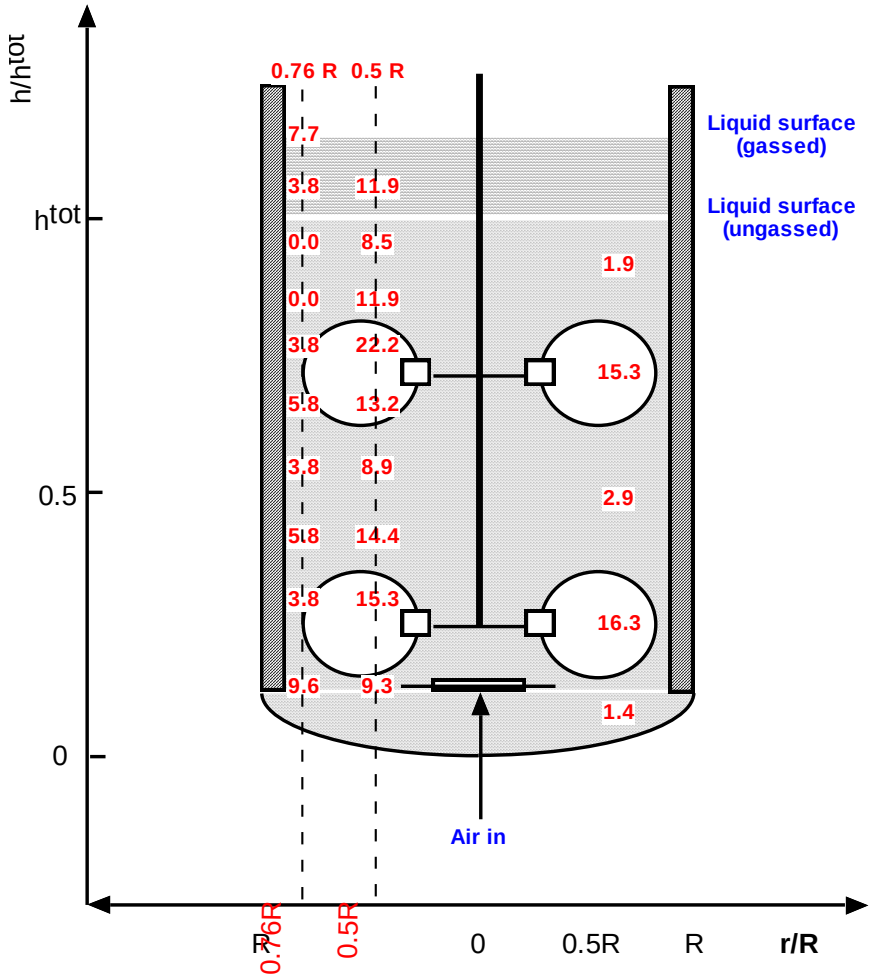
Transport phenomenon	Time constant(s)
Oxygen transfer	5.5 (noncoal.)–11.2 (coal.)[b]
Circulation of the liquid	12.3
Gas residence	20.6
Transfer of oxygen from a gas bubble	290 (noncoal.)–593 (coal.)
Heat transfer	330–650
Conversion	
Oxygen consumption, zero order	16
First order	0.7
Substrate consumption	$5.5 * 10^4$
Growth	$1.2 * 10^4$
Heat production	350

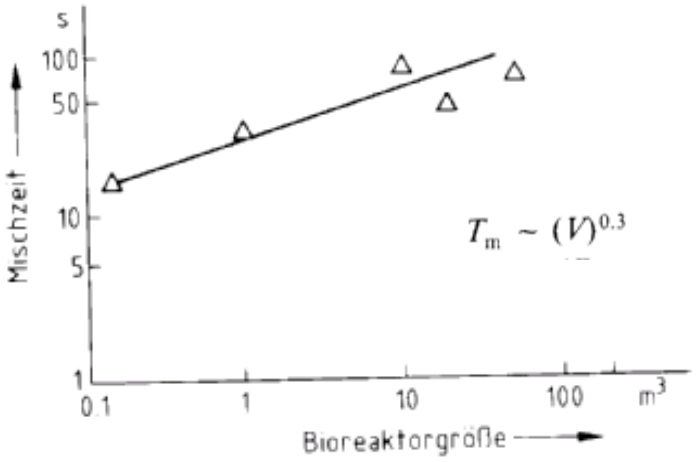
[a]With permission, from N. W. F. Kossen, in T. K. Ghose, ed., *Biotechnology and Bioprocess Engineering*, United Press Link House, New Delhi, 1985, pp. 365–380.

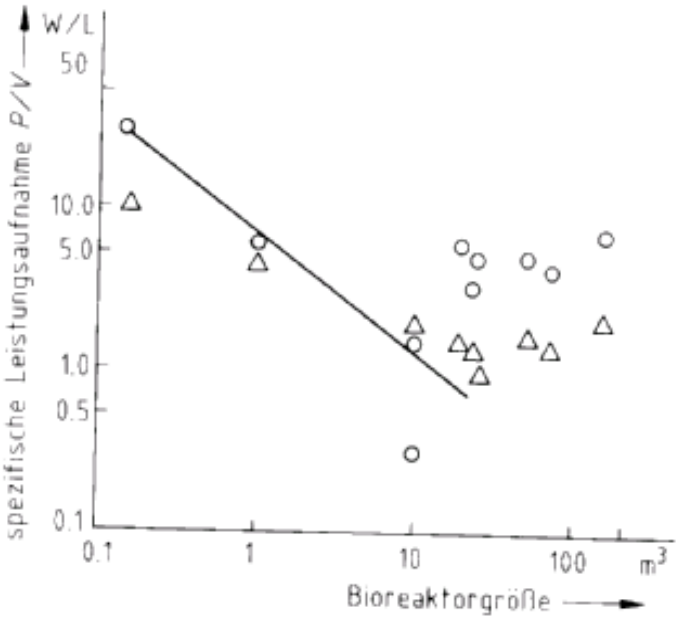
[b]Coal. = coalescing air bubbles.

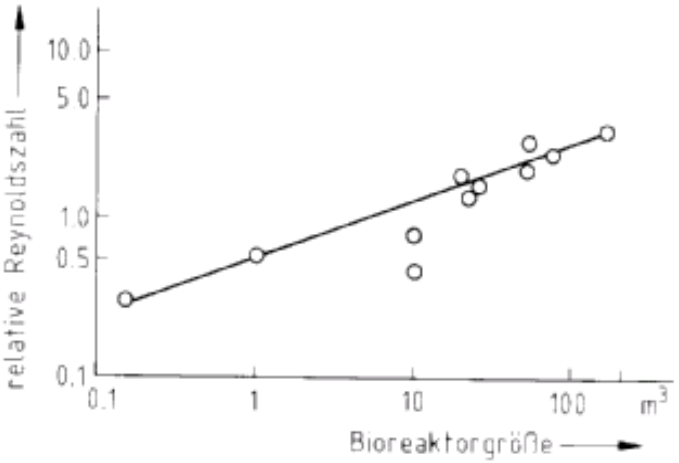
Scale-up

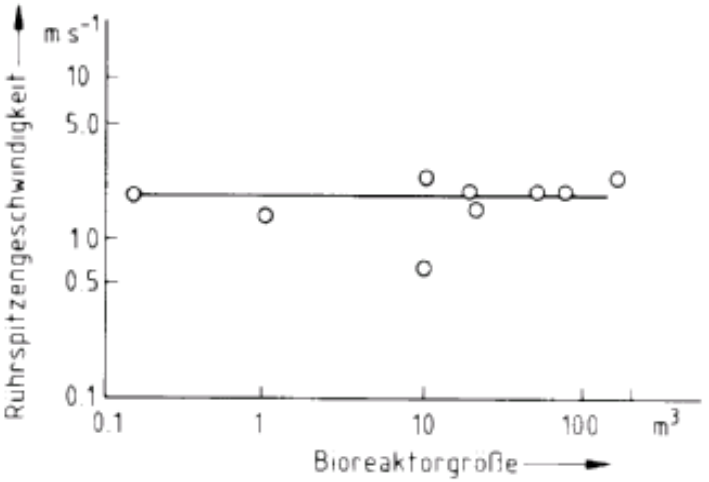
Measured oxygen concentrations in a 20 m^3 bioreactor




s
100
50
10
5
1
Mischzeit
$T_m \sim (V)^{0.3}$
0.1
1
10
100
m³
Bioreaktorgröße


W/L
50
10.0
5.0
1.0
0.5
0.1
spezifische Leistungsaufnahme P/V
0.1
1
10
100
m³
Bioreaktorgröße


10.0
5.0
1.0
0.5
0.1
relative Reynoldszahl
0.1
1
10
100
m³
Bioreaktorgröße


m s⁻¹
10
5.0
1.0
0.5
0.1
Ruhrspitzengeschwindigkeit
0.1
1
10
100
m³
Bioreaktorgröße

Scale- up of DSP

Rule:

Maintain column height constant, vary diameter to maintain constant linear flow rate

e.g. 10 cm/h

Example 1: Scale - up

After a batch fermentation, the system is dismantled and approx. 75% of the cell mass is suspended in the liquid phase (2 l), while 25% is attached to the reactor walls and internals in a thick film (ca. 0.3 cm). Work with radioactive tracers shows that 50% of the target product (intracellular) is associated with each cell fraction. The productivity of this reactor is 2 g product / L at the 2 L scale.

What would be the productivity at 20000 L scale if both reactors had a heigh-to-diameter ratio of 2 to 1?

Example 2: Scale - up

Consider the scale-up of a fermentation from 10 L to 10000 L vessel. The small fermenter has a height-to-diameter ratio of 3. The impeller diameter is 30% of the tank diameter. Agitator speed is 500 rpm and three Rusthon impellers are used.

Determine the dimensions of the large fermenter and agitator speed for:

a) Constant P/V
b) Constant impeller tip speed
c) Constant Reynold number

Assume geometric similarity and use table 10.2 / Folie 42

Example 3: Scale - up

Ein mikrobiologischer Prozess ergab in einem 10 L –Bioreaktor die besten Resultate bei einer Drehzahl N_1 = 500 rpm. Die Belüftungsmenge betrug 1 vvm. Unter Beibehaltung von Stamm, Nährlösung sowie Belüftungsrate soll dieses Verfahren auf einen Bioreaktor von 10000 L übertragen werden.

Frage: Wie gross wird die Drehzahl N_2 im grossen Bioreaktor sein unter der folgenden Annahmen:

a) P_0/V bleibt konstant
b) Die Mischzeit bleibt konstant
c) Die Rührerspitzengeschwindigkeit, v, bleibt konstant
d) Die Reynoldszahl bliebt konstant

Example 4: Scale - up

In the small bioreactor of 10 l, air is supplied at 10 l per minute [thus at 1 vvm (volume/volume – minute)]. The air inlet and outlet oxygen concentration is 21% and 19% respectively. In scaling up to 1000 l, the superficial velocity is kept constant. Assuming the oxygen demand of the culture remains constant, what will the outlet oxygen concentration be? If the respiratory quotient is one, compare the CO_2 concentration at the outlet of the gas stream in the small scale and large scale. You can neglect the evaporation of water and the effect of hydrostatic pressure.

La **coalescence** est un phénomène par lequel deux substances identiques, mais dispersées, ont tendance à se réunir. On peut citer à titre d'exemple les gouttes de mercure qui lorsqu'elles viennent à se toucher se rassemblent subitement pour ne faire qu'une seule goutte. On peut citer aussi l'huile que l'on a mélangée à l'eau et secouée énergiquement. On observe alors que les petites gouttes d'huile fusionnent entre elles progressivement jusqu'a ne former qu'une seule grande goutte traduisant la séparation finale entre l'huile et l'eau.