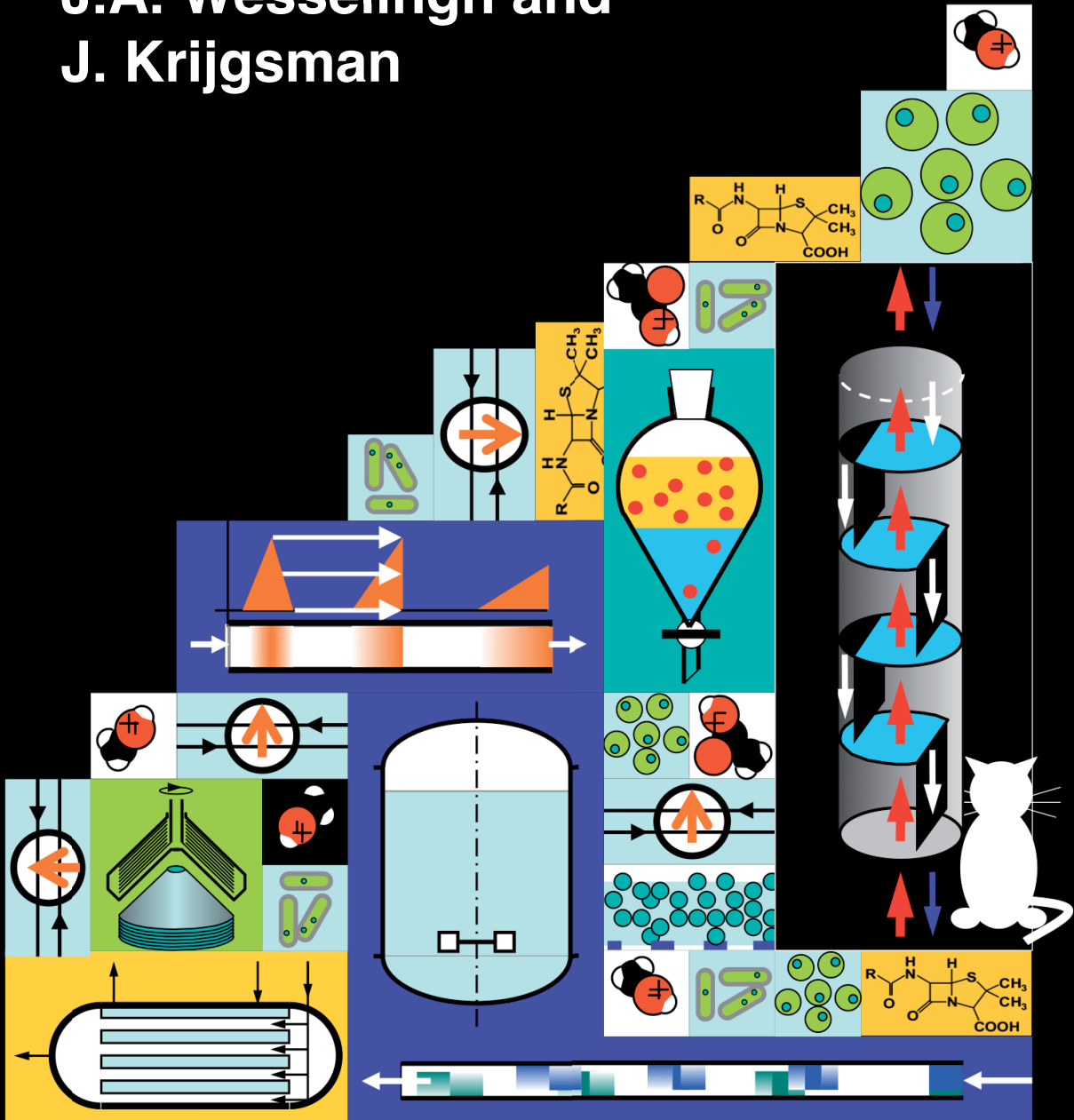


# Downstream Processing in Biotechnology

J.A. Wesselingh and  
J. Krijgsman



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# Contents

## Front

Contents	i
Preface	iii
Symbols	v

## Lessons

1	Ferment	1
2	Release	7
3	Clarify	19
4	Concentrate (1)	35
5	Concentrate (2)	47
6	Extract	59
7	Crystallize	71
8	Distill	81
9	Purify (1)	95
10	Purify (2)	107
11	Process	121

## Back

Derivations	129
Answers	137
Index	143

# Preface

The exciting part of biotechnology is thought to be ‘bio’. That is the place for novel ideas, funding, research, patents... However ‘technology’ comes behind that - without technology, no idea will make it to the market. An important part of technology is downstream processing: the separation and purification of the bio-product. This is usually the most expensive part of a bio-project, and it can require much ingenuity and a huge effort to develop a process that is clean and economic.

The development of a downstream process starts in the lab, but ends with a plant. The plant may have to produce a million times the amounts made in the lab. The challenge is to end with a good plant. To do this two groups of people have to work together. These are the lab people – micro-biologists, biochemists – and the process engineers. We have tried to write a book that introduces the subject to both of you. In eleven lessons it describes all the common steps used in downstream processing, starting in the lab and ending in the plant. The idea is a course of perhaps one or two weeks, depending on your starting knowledge. The level is ‘under-graduate engineering’, perhaps ‘graduate biochemistry’. Engineers will find the modeling a bit simple, biochemists the description of experiments rather basic.

At the end of each lesson you will find a small number of exercises. Use these to find out whether you have absorbed the lesson material. Brief answers are provided at the end of the book. You can download fully worked out exercises from [www.vssd.nl/hlf/d030.htm](http://www.vssd.nl/hlf/d030.htm) in the form of pdf files. If you have access to the program Mathcad Prime 2.0 (or later) you can download the originals and play with them.

In the first version of a technical book it is difficult to avoid errors. (Believe us, we have tried!). We would be pleased to hear mistakes and points that are not clear via [www.vssd.nl/hlf/](http://www.vssd.nl/hlf/).

This is the place to start thanking people, starting with those of you who are going to react. Then the people who have helped us to build up our understanding of the subject: the many students that we have had on ‘Downstream Processing’ and the colleagues of John at Gist Brocades and DSM. It is now time to begin. On to lesson 1...

Hans (J.A.) Wesselingh  
John Krijgsman

# Symbols

$A$	area	$\text{m}^2$
$c$	concentration	$\text{kg m}^{-3}$
$C$	concentration	$\text{kg m}^{-3}$
$C_R$	cake ratio	-
$\zeta$	specific heat	$\text{J kg}^{-1} \text{ }^\circ\text{C}^{-1}$
$d$	diameter (small, particle)	m
$D$	diameter (large)	m
$\mathcal{D}$	distribution coefficient	-
$\partial$	slope of equilibrium line	-
$E$	extraction factor	-
$f$	fraction	-
$g$	gravitational acceleration	$\text{m s}^{-2}$
$h$	mass enthalpy	$\text{J kg}^{-1}$
$H$	molar enthalpy	$\text{J mol}^{-1}$
$j$	(volume) flux	$\text{m s}^{-1}$
$k$	empirical constant	depends
$K$	mole fraction distribution coefficient	-
$l$	length	m
$m$	mass	kg
$\dot{m}$	mass flow	$\text{kg s}^{-1}$
$M$	molar mass	$\text{kg mol}^{-1}$
$n$	(molar) amount	mol
$\dot{n}$	molar flow	$\text{mol s}^{-1}$
$p$	pressure	Pa
$\dot{Q}$	heat flow	W
$r$	radius	m
$R$	release, removal, retention	-
$R_M$	medium resistance	
$r_C$	cake resistance per height	$\text{m}^{-2}$
$S$	settling ratio	-
$T$	temperature	$^\circ\text{C}$
$t$	time	s
$u$	velocity 'across', particle velocity	$\text{m s}^{-1}$
$u$	unknown flow	
$V$	volume	$\text{m}^3$
$\dot{V}$	volume flow	$\text{m}^3 \text{ s}^{-1}$

$v$	velocity 'along',	$\text{m s}^{-1}$
$w$	concentration velocity	$\text{m s}^{-1}$
$\dot{W}$	work flow (power)	W
$x$	mole fraction in liquid	-
$y$	mole fraction in vapor	-
$z$	height, small	m
$Z$	height, large	m

## Greek Symbols

$\alpha$	concentration factor	-
$\Delta$	change	-
$\varepsilon$	void fraction in particle	-
$\phi$	void fraction	-
$\eta$	viscosity	Pa s
$\rho$	density	$\text{kg m}^{-3}$
$\Sigma$	'sigma' of a centrifuge	$\text{m}^2$
$\omega$	angular velocity	$\text{s}^{-1}$

## Subscripts

0	initial
0,1	inlet, outlet of a single stage
0,1,2,..	stage number
A,B	components
C	cake, concentrate, cycle
E	solvent ('extractant')
F	feed
G	gel
L	liquid
M	maximum
P	permeate
S	solid, saturation
W	water

## Superscript

'	other phase
---	-------------



# 1 Ferment

*You have managed to get micro-organisms to make the right product in a 200 mL laboratory flask. So the job is finished... or is it? No! You still have to get the product out of the cells, to remove cell debris and other contaminants, to concentrate, purify and test the product. Then you have to scale up to production. Production has to be safe and economic; also it should not produce too much waste. Finally, you will need to package the product and to get it sold. You will have to keep in touch with your customers, and improve the product. There is far more work ahead, most of it outside the laboratory...*

## Develop a Product

To see where we are, and what this course is about, let us stand back for a moment. The subject is part of the development of a product (here a bio-product) that is to help a user or customer. Such a development is a huge effort, involving many people and large amounts of resources. The steps in product development are shown in Figure 1-1.

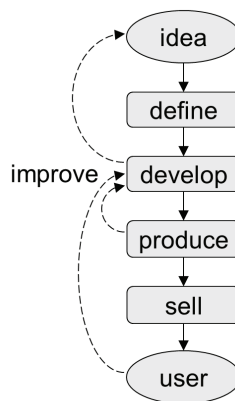


Figure 1-1 Product development cycle

Product development is usually cyclic – you cannot really say where it begins. Even so, it is commonly taken to begin with an ‘idea’. This might come from the market (‘market pull’) or from the developer (‘market push’). The first steps are exploring or defining – briefly looking at, and evaluating many possibilities. For the kind of products we consider here (chemicals, food, pharmaceuticals...) this leads to experimentation in the lab (‘small scale’). A project then needs to become more focused, as less-promising routes are discarded before the strict ‘develop’ stage. This is where the number of people involved becomes large – as do the costs. This step usually requires a pilot plant – a small scale version of the large plant. At some point, we will start to organise large scale production: to find existing plant or to construct new plant. We begin to produce. While we are trying out production, we are already starting to develop a market – a difficult part in many projects. Hopefully sales will take off – and we will be able to get back our investment. Even at this point, we cannot sit

## Downstream Processing in Biotechnology

back. We will have to keep on improving the product, the manufacturing process and our marketing. The cycle should keep on turning with new projects...

Penicillin and insulin are products where the development has gone through a large number of cycles, during several decades. These cycles often involve the introduction of better micro-organisms that improve the yield or quality of the product. These are first tested in the lab, then in the pilot plant, and finally in production.

This book does not cover the whole of product development. It focuses on the 'develop' and 'produce' blocks in our scheme (Figure 1-2). In each block you will see the sub-blocks 'ferment', 'separate' and 'formulate'. The fermenter contains the micro-organisms making the product. It is the core of the bio-process. Even so, we only discuss it in so far as it interacts with the 'separate' step. Here we separate the product from the many contaminants – that is what this book is about. This is 'downstream' from the fermenter – hence the name. The 'formulate' step is to bring the product in the form required by the customer – as a liquid, powder, granulate or whatever, with its packaging. We do not discuss formulation in this book.

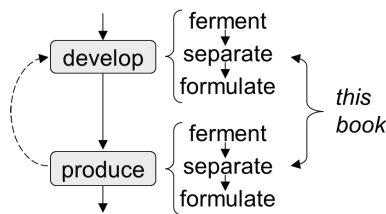


Figure 1-2 Subdivision of 'develop' and 'produce'

'Develop' has connections with idea formation and full scale production. Forming ideas is often the world of laboratory people such as microbiologists and biochemists – full scale production that of process operators and engineers. The book has to bridge these worlds. The microbiologist may find our treatment of microbiology very simple – and that of engineering daunting. On the other hand, some process engineers may find our treatment of engineering simple... (Just in case you wonder: your authors are process engineers, but they have extensive experience in biotechnology.)

### Ferment (using Cells)

Bio-products are made inside the cells of micro-organisms. These are usually kept as a suspension ('broth') in a fermenter or bio-reactor (Figure 1-3). It is quite difficult to process the products from a broth. The reasons are:

1. The product may be excreted by the cells to give an 'extra-cellular product'. This is the case for a bio-product such as citric acid, antibiotics and enzymes. As we shall see, this is a desirable situation. However, not all products are excreted – for example many proteins

## 1 Ferment

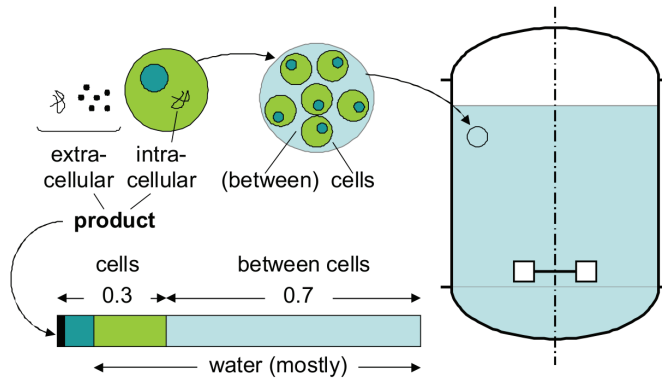


Figure 1-3 Contents of a fermenter

are retained in the cell. These 'intracellular products' can be very valuable, but they have to be released from the cells before further processing.

2. The broth contains a lot of water, both inside and outside the cells. This might be 80% in the production of a bulk chemical such as ethanol, or up to 95% for pharmaceutical proteins. All this water usually has to be removed.
3. The broth will contain a large number of components. Some of these may closely resemble the product. The product may be less than one per cent of the broth. Even so, you may need a high purification – up to 99.9999% for some applications in pharmaceuticals!
4. The broth can be viscous, especially if cell and product concentrations are high. This makes for difficult handling in the first stages of the separation process.
5. Finally, we are working with live material: organisms that are continuously adapting and evolving. So fermentations are variable – we have

## Process Steps

The characteristics above determine the usual sequence of downstream process steps (Figure 1-4):

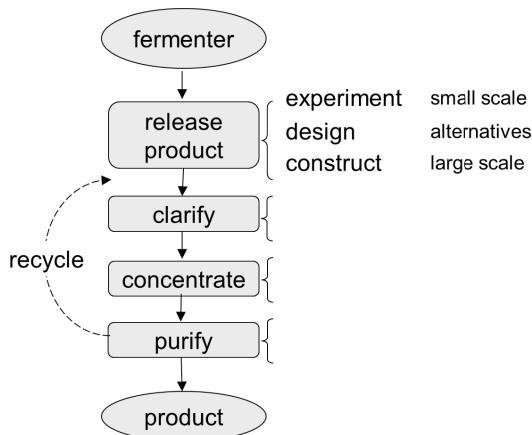


Figure 1-4 Sequence of process steps

## Downstream Processing in Biotechnology

- Intracellular products are released from the cells (lesson 2)
- Cells and debris are separated to clarify the broth (lesson 3).
- The product is concentrated by removing water (lessons 4 and 5).
- Further separations then follow to purify the product (lessons 6 to 10).

Bio processes form large flows of waste water. You can reduce these waste flows – and also product losses – by a good choice of the separations and their sequence. You will often have to recycle solvent streams. We discuss recycling and the whole of sequencing in lesson 11.

The development of each step usually starts with lab experiments – by lab people, not by process people. These experiments form the base of all development work, and the scaling up to and construction of large installations. So it is important that also lab people have an idea of how their experiments will be used by process engineers – and that the process engineers have some idea of experimentation.

Each separation step requires equipment. We will show the important pieces, say a bit about their sizing and operation, and discuss their use of energy and chemicals. We often need many separation steps. There are losses in each step, and these accumulate rapidly. Suppose that each step gives a loss of five percent (which is not much). The yield of the process is then:

$$0.95^1 = 0.95 \text{ after one step}$$

$$0.95^2 = 0.90 \text{ after two steps}$$

$$0.95^3 = 0.86 \text{ after three steps}$$

...

$$0.95^{10} = 0.60 \text{ after ten steps.}$$

So we need to design and operate carefully to get a reasonable yield.

### A Warning

We end this introduction with a warning. This book uses models (and simple equations) to help you understand how different pieces of equipment work. You might get the impression that these would allow you to design downstream processes from scratch. This would be a mistake: the models help to interpret and extend experiments, but on their own they are seldom sufficiently accurate. You cannot design a downstream process for a new product without experimenting.

### Further Reading

J.A. Wesselingh, Søren Kiil and Martin E. Vigild *Design & Development of Biological, Chemical, Food and Pharmaceutical Products*, John Wiley & Sons 2007.

Pauline M. Doran *Bioprocess Engineering Principles*, 2<sup>nd</sup> ed., Academic Press 2012

## Exercises

After each lesson you will find a few exercises. Most of these are small and meant to get you to think about what you have just read.

### 1-1 Fermenter Contents

Yeast cells are almost spherical. They have a diameter  $d = 10 \mu\text{m}$ . The number of cells in a suspension is  $N = 5 \cdot 10^8 \text{ mL}^{-1}$ . The volume fraction of water in the cell is  $\varepsilon_W = 70\%$  and the densities of water and the solids in the cell are  $\rho_W = 1000 \text{ kg m}^{-3}$  and  $\rho_S = 1400 \text{ kg m}^{-3}$ .

Calculate the volume fraction occupied by the cells and the fraction of solids ('dry mass fraction') in the suspension.

**Hint:** calculate everything for one cubic metre.

### 1-2 Yield and Profit

A bio process has the following steps with their yields on the most important raw material:

fermentation	0.85
product release	0.95
filtration	0.95
concentration	0.95
purification (1)	0.95
purification (2)	0.95

The initial production after losses is 1000 ton per year; this is sold for 10 million Euros per year. The cost of the process is 8 million Euros per year giving a profit of 2 million Euros per year.

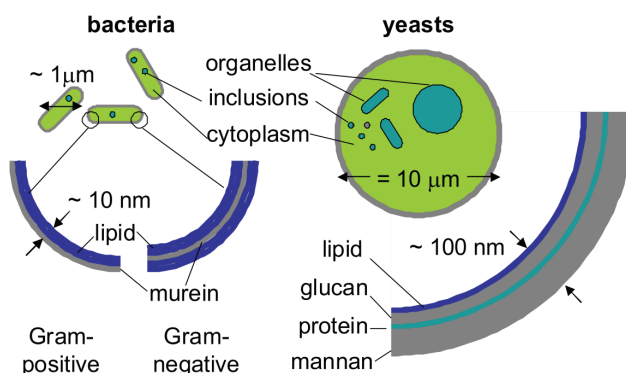
- Calculate the overall yield of the process.
- Assume that you can shave off 0.01 of each of the losses (so that the yields become 0.86, 0.96, 0.96... and so on.) Calculate the new process yield.
- By how much will your production increase, assuming that you process the same amount of raw material?
- If you can sell the increase for the same price (a big if!), by how much will your profit increase? By which percentage?

## 2 Release

Bio-products are formed inside cells. They are often released naturally to the surrounding liquid. If this does not happen, geneticists may be able to modify the cell so that it does release the product. Separation of whole cells from a suspension is relatively easy – and the whole cells can sometimes be put to use. So ‘extracellular’ products are preferred. However, many products are retained in the cell; especially those obtained by genetic manipulation, for example in *E.coli*. These intracellular products have to be released for further processing. Before considering the different methods for doing that, we first look briefly at the structure of cells.

### Micro-organisms

Two important groups of micro-organisms used in biotechnology are the bacteria and the yeasts (Figure 2-1).



2-1 Micro-organisms and their walls

Bacteria have dimensions of the order of a micrometre. Their walls are thin – about ten nanometres thick – and often fragile. The two main groups are those of the Gram-positive and the Gram-negative bacteria. (The name Gram is from the Danish scientist who made this classification, it has nothing to do with the mass unit gram.) In the Gram-positive bacteria, the cell wall consists of two layers: the inner one consisting mainly of phospholipids, with a thin outer coating of protein or polysaccharide. The walls of the Gram-negative bacteria have two phospholipid layers. The oily liquid films formed by phospholipids are hydrophobic, so they form a barrier for water. They can be disrupted by surfactants.

Yeasts are larger than bacteria: they might have diameters up to ten micrometres. The walls are much thicker – typically about a hundred nanometres – and contain layers that mainly consist of the polysaccharides glucan and mannan. As a result the walls are stronger than those of bacteria and more difficult to disrupt. Polysaccharide and protein layers can be weakened or disrupted by enzymes.