Biotechnology, Downstream

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@Delft Academic Press / VSSD
Third edition 2019

Published by Delft Academic Press / VSSD
Leeghwaterstraat 42, 2628 CA Delft, The Netherlands
tel. +31 15 27 82124
dap@vssd.nl

www.delftacademicpress.nl
About this book
www.delftacademicpress.nl/d030.php

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Printed version:
ISBN 97890-6562-4390

NUR 952

Key words: biotechnology

Content

The lessons have a verb as title. Such a title is short and clear – and it conveys what has to be done.

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Preface

The word biotechnology consists of two parts. The first – bio – is associated with excitement: novel ideas, funding, research, patents… The second – technology – has a more staid image. However, it is just as important: without technology, no idea will make it to the market. A large part of technology is downstream processing: the separation and purification of the bio-product. This is often 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 amount made in the lab. The challenge is to make a good design. To do this two groups of people have to work together. These are lab people such as microbiologists and bio-chemists – and process engineers. If you belong to either group, read on.

The thirteen lessons introduce common steps used in downstream processing. The idea is a course of one or two weeks, depending on your starting knowledge. The level is 'undergraduate engineering' or 'graduate biochemistry'; we have found that people working on this subject in industry also appreciate the text.

At the end of each lesson you will find a 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 worked exercises in the form of pdf files from www.delftacademicpress.nl/d030.php. You will also find the PowerPoint files of the illustrations there.

This is the third edition of this book. The important changes are in extraction and distillation, where we now use a graphical method. We have also changed the layout to make it more readable, and modified the text where things were wrong or not too clear. We realize that even this version will not be perfect. Please tell us about errors and points that are not clear via www.delftacademicpress.nl

This is the place to thank those who have helped us: the many students that we have had on 'Downstream Processing' and the colleagues of John at Gist Brocades and DSM. And our families, who have had the patience to endure the writing, rewriting and occasional spat. It is now time to begin. On to lesson 1…

Hans (J.A.) Wesselingh and John Krijgsman

Symbols

This list only contains symbols that are used througout the book. Symbols that are used occasionally, are explained there and not listed here.

A	area	m^2
c	concentration	$\mathrm{kg\ m^{-3}}$
C	concentration	$\mathrm{kg\ m^{-3}}$
$\mathcal{C}$	specific heat	$\mathrm{J\ kg^{-1}\ {}^\circ C^{-1}}$
d	diameter (small), distance	m
D	diameter (large)	m
$\mathcal{D}$	distribution coefficient	$-$
g	gravitational acceleration	$\mathrm{m\ s^{-2}}$
h	enthalpy	$\mathrm{J\ kg^{-1}, J\ mol^{-1}}$
j	(volume) flux	$\mathrm{m\ s^{-1}}$
k	empirical constant	depends
L	length	m
m	mass	kg
$\dot{m}$	mass flow	$\mathrm{kg\ s^{-1}}$
p	pressure	Pa
$\dot{Q}$	heat flow	W
R	release, retention, reflux ratio	$-$
T	temperature	$\mathrm{{}^\circ C, K}$
t	time	s
u	velocity ‘across’	$\mathrm{m\ s^{-1}}$
v	velocity ‘along’	$\mathrm{m\ s^{-1}}$
V	volume	m^3
$\dot{V}$	volume flow	$\mathrm{m^3\ s^{-1}}$
w	concentration velocity	$\mathrm{m\ s^{-1}}$
W	width	m
$\dot{W}$	work flow (power)	W
x	mole fraction in liquid	$-$
y	mole fraction in vapour	$-$
z	depth, height	m
Z	depth, height	m

Greek Symbols

α	angle	$\circ$
Δ	difference	$-$
∂	slope of isotherm	$-$
ε	volume fraction in a phase	$-$
η	viscosity	$\mathrm{Pa\ s}$
ρ	density	$\mathrm{kg\ m^{-3}}$
ϕ	volume fraction	$-$
ω	angular velocity	$\mathrm{s^{-1}}$

Subscripts

$\vdots_{\odot}$	slurry (particles in liquid)
$\vdots_{\downarrow}$	settling, precipitant (going down)
$\vdots_{0,1,2}$	positions, times
$\vdots_{0}$	initial, reference, ambient
$\vdots_{A}$	product A, air
$\vdots_{B}$	product B, bottoms
$\vdots_{C}$	cell, cake, crystal
$\vdots_{D}$	distillate, dissolved
$\vdots_{E}$	solvent ('extractant')
$\vdots_{F}$	feed
$\vdots_{L}$	liquid
$\vdots_{max}$	maximum
$\vdots_{P}$	product, protein
$\vdots_{S}$	solid
$\vdots_{V}$	vapour
$\vdots_{W}$	water

Superscript

$\vdots'$	other phase

1 Begin

Your micro-organisms are making the right product in a 200 mL laboratory flask. So the job is finished… NO! You 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. Production has to be safe and economic, and what are you going to do with the waste? You have to package the product and sell it. You will have to keep in touch with your customers to improve the product. There is more work ahead, much of it outside the laboratory...

Develop a Product

To see what this course is about, let us stand back for a moment. You are developing a bio-product that is to help a user or customer. This is a huge effort, involving many people and large amounts of resources. Steps in product development are shown in Figure 1-1.

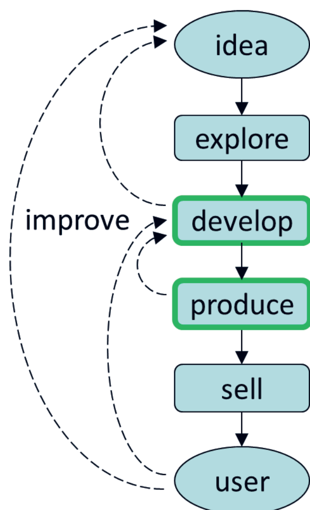
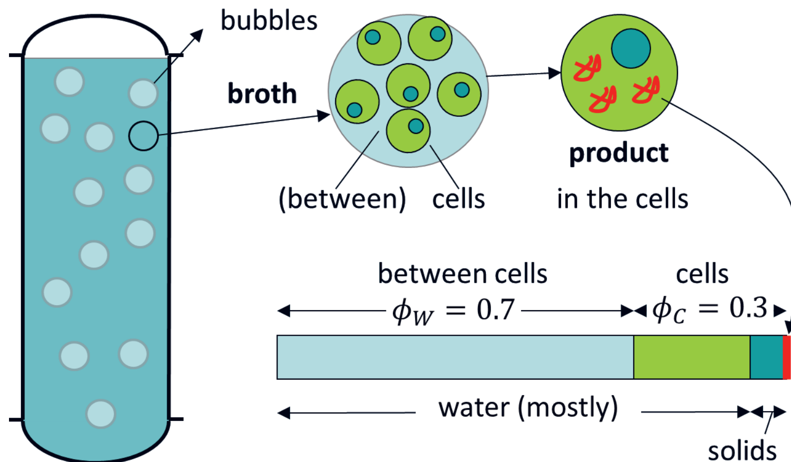


Figure 1-1 Product development cycle

Product development begins with an idea. This might come from the market ('market pull') or from the developer ('market push'). The first step is to explore – to briefly look at and evaluate many possibilities. For the kind of products we consider here (chemicals, food, pharmaceuticals…) this leads to small scale experiments in the lab. A project then needs to become more focused, as less-promising routes are discarded before the 'develop' stage. This requires many

organisms form a gas. These bubbles are easily removed, and we do not further consider them. The cells might occupy a fraction 0.3 of the volume of the broth: the rest is mostly water. The cells also contain water. The product can be



either inside the cells ('intracellular') or outside ('extracellular'). The fraction of the volume occupied by the product is small: often in the range of 0.01..0.1. (For pharmaceutical proteins this could be as low as 0.001.)

Figure 1-2 Broth from a fermenter
(product inside the cells)

It is quite difficult to process the products from a broth. The reasons are:

- Our organisms are continuously adapting and evolving. So fermentations are variable – we have to allow for this in design and development.
- The product may be excreted by the cells. This is the case for bio-products such as citric acid, antibiotics and many enzymes. This is a desirable situation. However, not all products are excreted – for example many proteins are retained in the cell. These intracellular products can be valuable, but they have to be released from the cells before further processing.
- 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. This water usually has to be removed before the final purification.
- 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!

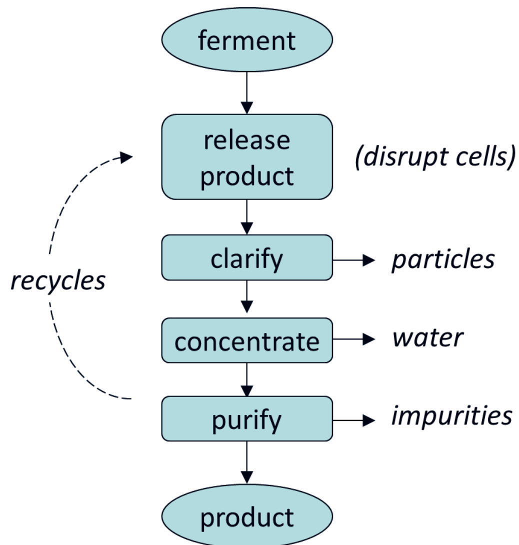


Figure 1-4 Sequence of process steps

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 streams. We discuss sequencing and recycling in lessons 7..13.

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 of 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:

$0.95^1 = 0.95$ after one step
$0.95^2 = 0.90$ after two steps
$0.95^3 = 0.86$ after three steps
…
$0.95^{10} = 0.60$ after ten steps.

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

1-4 Number of Components (2) @

In Figure 1-2 we consider three components in the cell phase and one in the water phase. In the drawing of an ethanol fermenter below we consider two components in the water phase and two components in the cell phase. Can you understand why we do this? Do you see the kind of rule that we apply?

1-5 Number of Phases (1)

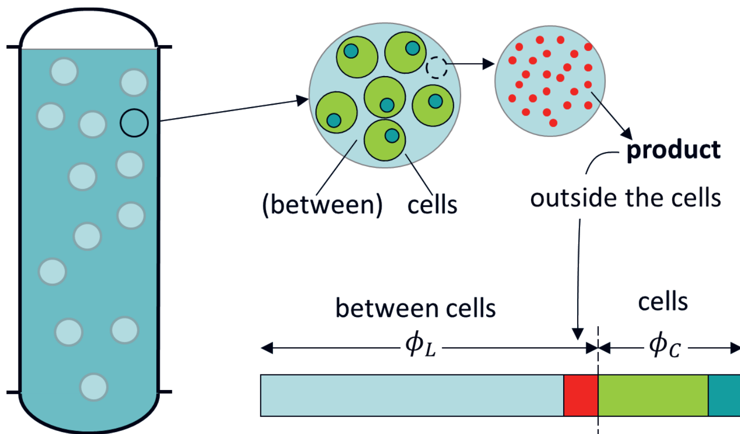
Is a cell a gas, a liquid or a solid? We regard the cells as a 'phase'. Does the word phase have the same meaning here as in physical chemistry?

1-6 Number of Phases (2) @

When designing a pump to empty a fermenter we might regard the broth as a single phase. If the broth contains gas bubbles that have to be removed before the pump, we would regard the contents as two phases: broth and bubbles. If we also want to separate the cells, we would regard them as three phases. Do you see the kind of rule that we apply?

1-7 Volumes in a Fermenter @

The following exercises consider the fermentation of ethanol. This is excreted and ends up in the liquid between the cells. In this system we again assume two phases: the cells and the liquid between the cells. However, the liquid now contains a substantial volume of product.



We take the broth to form a cylinder with a diameter $D = 2$ m and height $Z = 5$ m. The volume is given by:

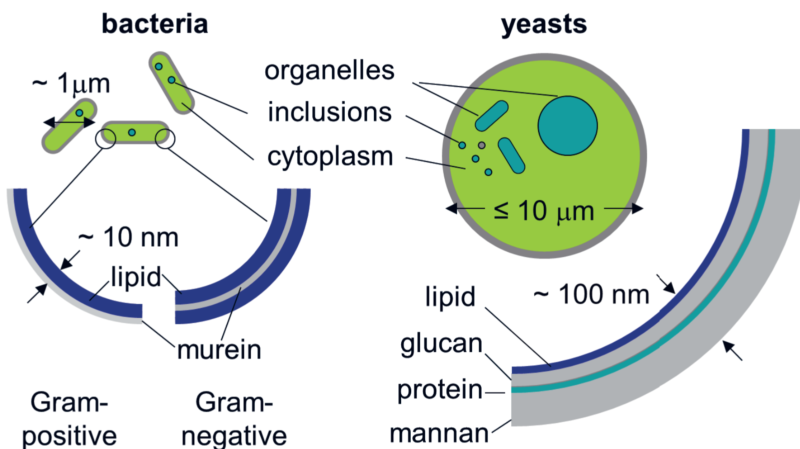
$$V = \frac{\pi}{4} D^2 Z$$

2 Release

Bio-products are formed inside cells, so we may need to get them out. They are sometimes released naturally to the surrounding liquid. However, many products are retained in the cell (especially those obtained by genetic manipulation, for example in *E. coli*). These have to be released for further processing. Before considering the different methods for doing that, we first look briefly at the structure of cells.

Look at the Cells

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



2-1 Micro-organisms and their walls

Bacteria have dimensions of around 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.) In the Gram-positive bacteria, the cell wall has two layers: the inner one of phospholipids, and a thin outer coating of protein or polysaccharide. The walls of the Gram-negative bacteria have two phospholipid layers. These oily liquid films are hydrophobic, so they form a barrier for water. They can be disrupted by surfactants.

4 Filter

In a filter we clarify by retaining particles behind a fine ‘medium’ or membrane.

In the Lab

Figure 4-1 shows the principle. The medium is usually a specially woven cloth with small openings. The feed – with particles – is first circulated over the filter so that the large particles bridge and form a ‘cake’ which stops the smaller particles. So the holes in the medium can be larger than the particles. Liquid is forced through the cake and the medium by a pressure difference. Clear filtrate is collected behind the medium.

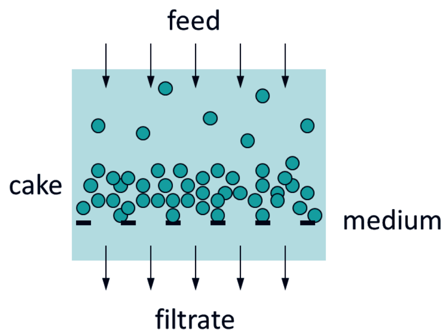


Figure 4-1 Principle of filtration

Figure 4-2 shows a funnel filter used in the lab. It can provide data for large installations, as we discuss below. The medium covers the coarse pores of a support. The feed is poured into the upper part of the funnel and pulled by vacuum into the flask below. Initially the flow is only limited by the resistance of the medium. However, the flow goes down as a cake forms – especially when the feed contains fine particles. The right side of the figure shows how the volume V of filtrate increases in time.

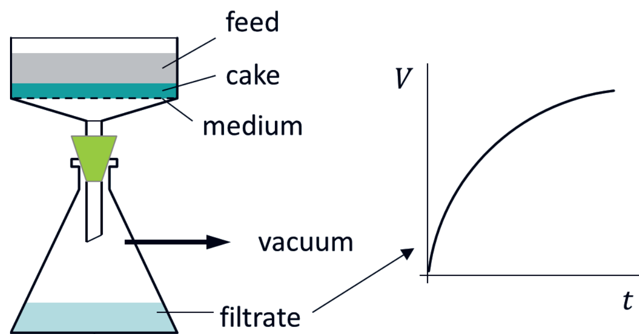


Figure 4-2 Funnel filter

7 Extract

Extraction is a process that uses two non-mixing liquids such as water and a hydrocarbon. It can greatly increase the purity of dissolved products. Most bio-products do not dissolve in a hydrocarbon, but can sometimes be made to do so with suitable additives.

In the Lab

To extract, we form a 'dispersion' of the two liquids by shaking or agitating the two together. In the lab this can be done in a separation funnel (Figure 7-1).

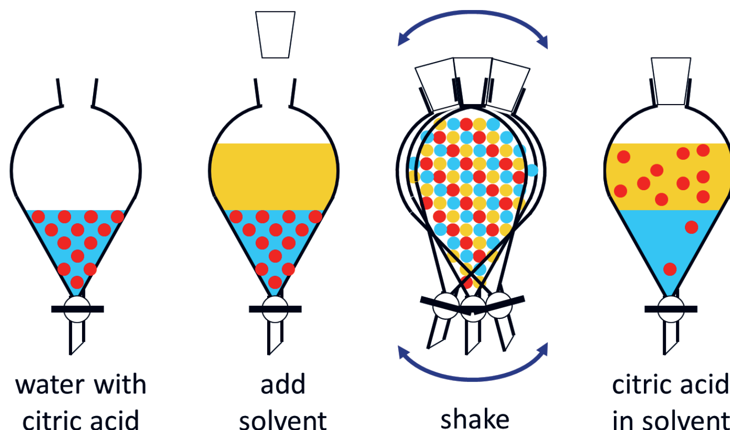


Figure 7-1 A separation funnel

The product (here citric acid) moves from water into the solvent (here an apolar amine dissolved in a hydrocarbon); the contaminants stay in the water. This might take a minute. We carefully remove the lower layer using the valve and analyze both layers to determine the concentrations of citric acid. That in the water has a value c; that in the solvent a value C. These are 'equilibrium' values that we have obtained after shaking so long that there are no further changes.

Modern lab equipment has a different look and more capabilities than a separation funnel, but the idea is the same.

Equilibrium

The equilibrium diagram for our system at $25\ ^{\circ}\mathrm{C}$ is shown in Figure 7-2. We have left out the units of the concentrations; throughout this lesson they are in $\mathrm{kg\ m^{-3}}$. The concentration C first rises with a slope $\mathcal{D}_0$, then levels off to a maximum C_{max}.