Biotechnology, Downstream

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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—microbiologists, biochemists...—and process engineers. We have tried to write a book that introduces the subject to both groups, assuming that you belong to one of them. In thirteen lessons the book describes all the 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. Engineers may find the modeling a bit simple, biochemists the description of experiments rather basic.

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 from www.delftacademicpress.nl/d030.php in the form of pdf files. You will also find the PowerPoint files of the illustrations there.

This is the second edition of our *Downstream Processing in Biotechnology*, Delft Academic Press 2013. We have used experience in several courses to rewrite the book. Important changes are:

- a more systematic treatment of balances and units,
- separate lessons on centrifuges and filters,
- the membrane processes microfiltration and reverse osmosis,
- simpler stage calculations for extraction and distillation columns,
- stage calculations with the effect of a bleed in crystallization,
- a complete new explanation of the working of sorption columns,
- a lesson on drying of the product, and finally
- a complete new set of exercises.

We have also shortened the title to *Biotechnology, Downstream*.

Although we have spent much time on this new version, we realize that it 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. We must especially thank Michel Eppink of Synthon, Ton van Boxtel of Wageningen University and Mirjam Oomen of the University of Applied Sciences Utrecht for their comment and encouragement. Also our wives, 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
John Krijgsman

Symbols

Symbols that are only used at one or two points are not listed here. They are explained where they are needed.

A	area	m^2
c	concentration	kg m^{-3}
C	concentration	kg m^{-3}
$ç$	specific heat	J kg^{-1} $^oC^{-1}$
d	diameter (small), distance	m
D	diameter (large)	m
$Đ$	distribution coefficient	-
f	fraction	-
g	gravitational acceleration	m s^{-2}
h	enthalpy	J kg^{-1}, J mol^{-1}
j	(volume) flux	m s^{-1}
k	empirical constant	depends
L	length	m
m	mass	kg
$\dot{m}$	mass flow	kg s^{-1}
$\dot{n}$	molar flow	mol s^{-1}
N	number	-
p	pressure	Pa
$\dot{Q}$	heat flow	W
R	release, removal, retention	-
R	reflux ratio	-
R_G	gas constant	J mol^{-1} K^{-1}
S	separation factor	-
T	temperature	^oC
t	time	s
u	settling velocity	m s^{-1}
u	unknown flow	
V	volume	m^3
$\dot{V}$	volume flow	m^3 s^{-1}
v	velocity ‘along’,	m s^{-1}
w	concentration velocity	m s^{-1}

continued on the next page

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. Production has to be safe and economic; also it should not produce much waste. Finally, you will need to package the product and to 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 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.

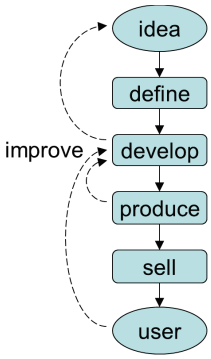

Figure 1-1 Product development cycle

Product development is usually cyclic—you cannot 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 experiments in the lab ('small scale'). A project then needs to become more focused, as less-promising routes are discarded before the '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 version of the large plant. At some point, we will start to organise large scale production: to find an existing plant or to construct a 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 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. In biotechnology it is useful to further divide these blocks in three:

- ferment
- separate
- 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 impurities—that it 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.

'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, process engineers may find our treatment of engineering too 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 (Figure 1-2). The cells might occupy a fraction 0.3 of the volume: 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.)

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

- We are working with live material: organisms that 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 enzymes. As we shall see, 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.

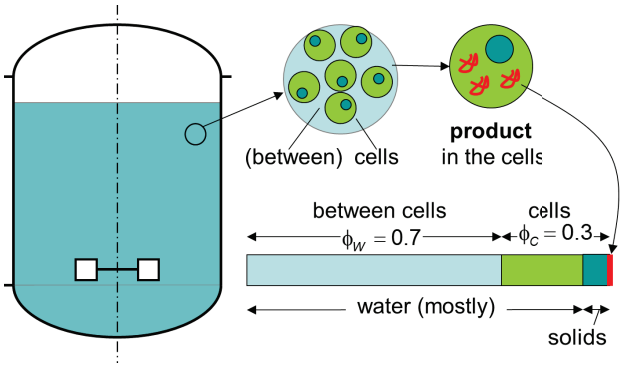

Figure 1-2 Contents of a fermenter
(product inside the cells)

- 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!

Content Description

To design equipment we need to describe the contents quantitatively. Here we use the description shown in Figure 1-3. There are two 'phases':

- the cells and
- the water between the cells

There are three 'components' in the cell phase:

- product
- (other) solids
- (cell) water

We consider the phase between the cells as one component: water.

If the contents have a volume V and the cells a volume V_C then the volume fraction of cells is:

$$\phi_C = \frac{V_C}{V} \qquad 1\text{-}1$$

Note that the fractions of cells and water in between must add up to one.

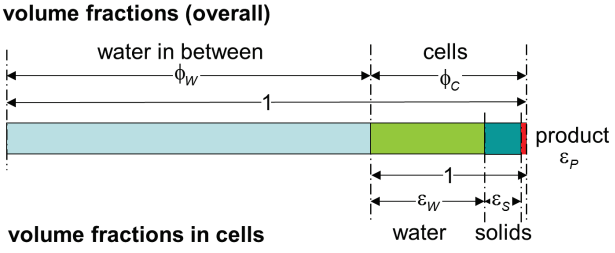

Figure 1-3 Volume fractions in the fermenter