Principles of
Downstream Techniques in Biological and Chemical Processes

Mukesh Doble, PhD, FRSC

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LIST OF ABBREVIATIONS

2D	two-dimensional
ATZ	alumina, titania and zirconia
BSA	bovine serum albumin
CA	cellulose acetate
CSTR	continuous stirred tank reactors
DCF	discounted cash flow
ED	electrodialysis
ERR	economic rate of return
GE	General Electric
GPC	gel permeation chromatography
HETP	height equivalent of a theoretical plate
HIC	hydrophobic-interaction chromatography
HTU	height of transfer units
ICI	imperial chemical industries
IEC	ion exchange chromatography
IEF	isoelectric focusing
IgG	immunoglobulin G
IP	isoelectric point
IRR	internal rate of return
MF	microfiltration
NF	nanofiltration
NPV	net present value
NTU	number of transfer units
PET	polyethylene terephthalate
PSO	polysulphone
PVDF	polyvinylidene difluoride
RO	reverse osmosis
ROI	return on investment
TLC	thin layer chromatography
TPC	total plant cost

TPDC	total plant direct cost
TPIC	total plant indirect cost
UF	ultrafiltration

LIST OF SYMBOLS

[C]	amount of soluble protein released as a function of time, t
$[C]_f$	amount of active protein finally recovered
$[C]_o$	maximum amount of soluble protein
$[S_H]$	concentration of solute in the heavy
$[S_L]$	concentration of solute in the light
A	area of contact
A	overall contact area
a	activity
A	cross sectional area of the tube
A	filtration area
A	membrane surface area (cm^2)
a	packing area per bed volume
a	surface area of adsorbent per tank volume
A_h and A_c	area on the hot and cold sides, respectively
b_d	bed thickness
b_m	membrane thickness
C	concentration
c	solute concentration
c	total molar concentration
c_0	maximum concentration
C_b	bulk mass concentration of foulant
C_i	molar concentration of species i
C_m	solute concentration in the mobile phase
c_n	solute concentration in the liquid in stage *n* and also flowing out
C_{n-1}	concentration entering stage n
C_s	solute concentration in the stationary phase
c_s	concentration of the solute that is being filtered near the surface of the membrane
C_T	sum of concentration of all the species
D	agitator diameter

d	degrees of freedom
d	diameter of the spherical particle
D	diffusion coefficient
D	effective diffusion coefficient in the pores
d	particle size or pore diameter in the filter cake
d	wall thickness
D_{AB}	diffusivity of A in B
D_d	dispersion coefficient
D_e	diffusion coefficient of the protein
e	washing efficiency
E	activation energy
E	current efficiency
E_d	activation energy for deactivation of the protein
F	Faraday's constant
F	feed flow rate
F	feed quantity
f	fraction of the bulk concentration that contributes to deposit growth
F1 and F2	streams entering the unit
F3 and F4	streams leaving the unit
*f*c	crushing strength of the material
Fs	volumetric flow rates
G	crystal growth rate
G	free energy
g_c	driving force
h	heat transfer coefficient
I	current
J_i	flux of component i (moles/h cm^2)
k	constant
K	equilibrium constant
k	first order rate constant
K	partitioning coefficient
K	proportionality constant
k	thermal conductivity of the material
K_d	distribution coefficient
K_H	Henry's law constant
K_K	Kick's constant
k_L	gas to liquid mass transfer coefficient

k_L	mass transfer coefficient
K_R	constant
L	length of the packed chromatographic bed
n	constant (if the adsorption is favorable, then n < 1; if it is unfavorable, then n > 1)
n	moles of solute
n	number of cells
N	number of components
n	number of disks
N	number of stages
N	rpm
N	solution normality
n	volume of wash liquid divided by the volume of liquid retained in the cake
N_{Re}	Reynolds number
Nu	Nusselt number
P	operating pressure
P	number of phases
P_0	atmospheric pressure (kg/ cm^2 a)
P_1	initial pressure (kg/ cm^2 a) after bleeding
P_2	final pressure after filling (kg/cm^2 a)
p_A and p_B	partial vapor pressures of component A and B, respectively
$P_{L,i}$	permeability coefficient of component i;
P_o	permeability constant
P_{os}	osmotic pressure
Pr	Prandtl number
Q	rate of heat transfer
q	adsorbed solute concentration
q	amount of solute adsorbed per amount of adsorbent (gm/gm)
Q	flow rate, m^3/s
q	heat transfer per unit time (W)
Q	solvent flow
Q	volumetric flow rate
q and q_F	the final and feed concentrations of the solute in the adsorbent, respectively
Q_i	moles of component i permeated in time t

Q_i and Q_o	blood flow at the inlet and outlet of the dialyzer, respectively
q_n	solute concentration in the adsorbent
Q_o	initial flow rate through the unfouled membrane
q_o and K_e	constants (q_o maximum adsorption capacity and *Ke* adsorption constant)
r	distance from the axis of rotation
r	distance of the solid particle from the center of the axis of the centrifuge
R	gas constant
r	rate of reaction
R	resistance
R_0 and R_1	the outer and inner radius of the bowl
r_{ads}	rate of adsorption per volume of tank
Re	Reynolds number
R_m	clean membrane hydraulic resistance
R_p	time-dependent resistance of the growing cake
R_{po}	resistance of the initial foulant deposit
s	cake compressibility
T	absolute temperature Kelvin (K)
t	filtration time
T	operating temperature
T	temperature in K
t	time
t_0	time at which this concentration exits
$t_0\sigma$	standard deviation of the peak
t_f	cake formation time
t_w	time required for the washing
u	fluid circulation velocity
U	fluid velocity
U	overall heat transfer coefficient between the air and the solids
v	velocity
V	bed volume
V	cumulative filtrate volume
v	terminal settling velocity of the solid in a dilute solution
V	total volume of filtrate

v	velocity of liquid through the bed of solid
v	velocity of the slurry flowing
V	volume fraction of each component
V	volume of the tank
V_0	volume required to elute the maximum concentration c_0
$V_0\sigma$	standard deviation
V_f	volume of filtrate collected during that period
V_m	volume of mobile phase
V_r	volume eluted from the start of sample injection to the peak maximum
V_s	volume of stationary phase
Vs	single stage volume (=V/N)
V_w	volume of wash water required
$W_{1/2}$	peak width measured at half peak height
$W_{1/2av}$	average half width of the two peaks
x	concentrations in mol per volume in each of the stream
x	direction along the flow
x	solute concentration in solution (gm/mL)
X	weight fraction of each component
x and x_F	the concentration of the solute in the final and feed solution, respectively
x and x_F	the solute concentrations in the outlet and the feed, respectively
x^*	concentration in the solution which would be in equilibrium with the adsorbent
X_A and X_B	mole fractions of component A and B, respectively
X_f, X_r, Y_0, and Y_1	the weight fractions of solute in the feed, raffinate/ solvent and extract, respectively
$X_{f,i}$	mole fraction of component i in the feed liquid
y_A	mole fraction of A
$Y_{p,i}$	mole fraction of component i in the permeate

Greek Symbols

α	area blocked per mass of deposit
α_s	specific resistance of the foulant cake
β	fraction of time that the filter is submerged

Δp	pressure drop across the bed
ΔP	change in partial pressure of pure component i across the membrane
ΔP	pressure applied
Δt_{ij}	separation between peaks
ε	voidage
ε	volume fraction of liquid (or void space) in the stage
ε_b	void fraction in the bed
ε_c	void fraction in the cake
η	removal efficiency
θ	angle the disk makes with the vertical axis
μ	fluid viscosity
μ	viscosity of liquid
ρ	density
ρ	liquid density
ρ_o	mass of cake solids per volume of filtrate
υ (=F/A)	superficial velocity

PREFACE

In 2012 the world chemical and pharma market was € 4068 billion (http://www.statista.com/statistics/263111/revenue-of-the-chemical-industry-worldwide-and-in-the-eu/). The US market revenue of organic chemical industries is about $147 billion (2009) with an annual growth expected to be 11.2%. The contribution of chemical and pharma industries to the overall US economy is about 8.7%. The global biotech market is $289 billion, with annual growth of 10.8% (2009) (http://www.ibisworld.com/industry/global/global-biotechnology.html). The United States is the leading biotech player, with more than 60 billion US dollars of revenue. In 2006, biopharmaceuticals generated approximately 150 billion US dollars of revenue worldwide (2011). Biotechnology products represented 21% of the total $714 billion global market for prescription drugs in 2012, equivalent to $150 billion of sales. In 2010, the sales of industrial chemicals created by using biotechnology in at least one step of the production process equalled €92 billion globally, and this is expected to increase to €228 billion by 2015. The total biotech industry size in 2013 in India is US $4.3 billion (biopharma taking up 64% of the share). It ranks second in Asia and 12th globally (http://www.ibef.org/industry/biotechnology-india.aspx). The above statistics emphasize the fact that chemical/pharmaceutical and biochemical/biotechnology industries play a very important role in the global economy, contributing sizably to the GDP, and are expected to grow in a healthy manner for a long time to come.

While the product is manufactured in a reactor/bioreactor/fermentor, it is recovered and purified in subsequent unit operations, which could be several in numbers. The economy of a manufacturing process is determined by the cost effectiveness of the downstream operations. The downstream processing steps will vary depending upon whether the product is a bulk or a high value chemical. There is some overlap between the downstream processing steps of a chemical and a biochemical process, although there are a few steps that are unique to the biochemical process, such as chromatography.

This book discusses downstream and unit operations practiced in chemical and biochemical industries. The commercial scale of operation of these two industries, as indicated above from the financial, is very large, and so the efficiency and cost involved in downstream processing determines the profitability of these industries.

Chapter 1 introduces the various principles involved in downstream, cost factors, and other issues. Chapter 2 discusses diverse chemical and biochemical industrial processes with special focus on downstream unit operations. Chapter

3 focuses on particle size reduction, bacterial cell breakage, and recovery of intracellular material. The latter is not relevant if the product is released by the microorganisms into the extracellular medium. Chapter 4 deals with the isolation or removal of solids from a solution. Here the solids could be dead biomass or impurities. Chapter 5 discusses various product recovery techniques while chapter 6 deals with product purification/enrichment techniques. Product polishing, stabilisation, and finishing techniques are discussed in chapter 7. A manufacturing plant has several utility services, which are discussed in chapter 8. The future trends and research opportunities in the area of downstream are discussed in chapter 9. A few basic fundamental concepts of chemical and biochemical engineering are discussed in chapter 10. The book includes several problems at the end of each chapter, which will help the reader to assimilate the material. The book contains several line diagrams and mathematical formulae that could be used for design purposes.

—Mukesh Doble
October 15, 2015

ABOUT THE AUTHOR

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Mukesh Doble, PhD, is a Professor and Head of the Department of Biotechnology at IIT Madras in Chennai, India. He has previously worked for 20 years at Imperial Chemical Industries (ICI) and General Electric (GE) Technology centers. His areas of interest are drug design, biomaterials, bioreactors, and bioremediation. He holds BTech and MTech degrees in chemical engineering from IIT, Madras, India, and a PhD from the University of Aston, Birmingham, UK, and his postdoctoral work was performed at the University of Cambridge, UK, and Texas A&M, Texas, USA. He has authored or coauthored 240 technical papers, seven books, including the books *Cyclic Beta-Glucans from Microorganisms: Production, Properties and Applications; Drug Design, Basics and Applications; Green Chemistry and Processes; Biochemical Engineering; Biotreatment of Industrial Effluents; Biotransformations and Bioprocesses*; and *Homogeneous Catalysis: Mechnanisms and Industrial Applications*. He has filed six patents. He is a fellow of the Royal Society of Chemistry, London, and a recipient of the Herdillia Award for Excellence in Basic Research from the Indian Institute of Chemical Engineers and was named the Dow Professor M. M. Sharma Distinguished Visiting Professorship in Chemical Engineering at Institute of Chemical Technology, Mumbai, India. He is on the editorial board of the journal *Chemical Engineering* and a member of the American and Indian Institutes of Chemical Engineers.

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CHAPTER 1

DOWNSTREAM PROCESSING PRINCIPLES, ECONOMICS AND ISSUES

CONTENTS

1.1 INTRODUCTION

A chemical (Figure 1.1a) or a biochemical (Figure 1.1b) process consists of an upstream, reactor and downstream sections. The upstream section in a chemical plant will consist of the raw materials preparation. Whereas, the upstream section in a biochemical process consists of facilities for the preparation of micro-organism and media, sterilization of raw materials

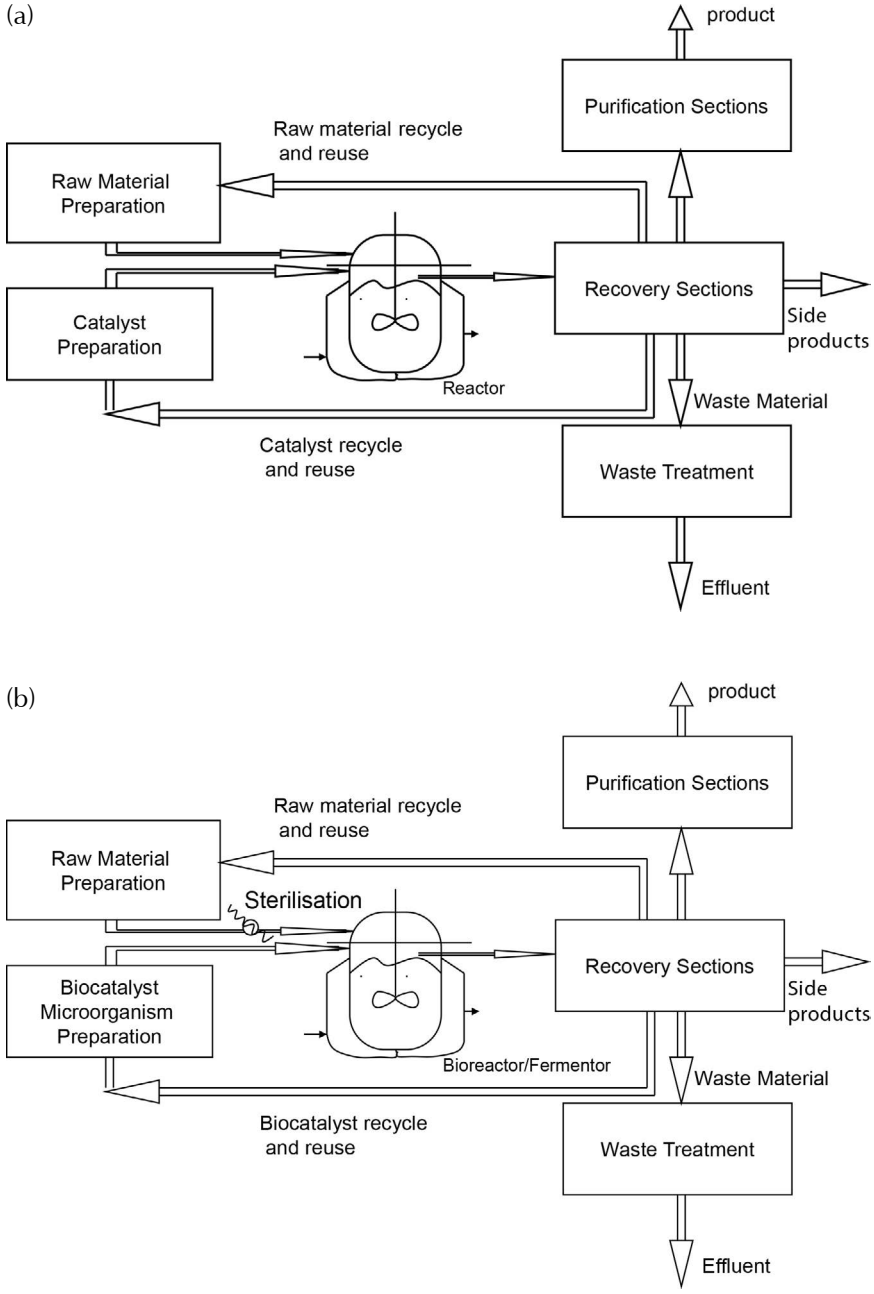

FIGURE 1.1 (a) Chemical process flow sheet; (b) Biochemical process flow sheet.

and inoculation of the micro-organism. The reactor section is the fermentor or a reactor/bioreactor and the associated machinery. An aerobic fermentor may contain air purification, compression and injection systems. The product is prepared in the reactor section. The downstream in a biochemical or chemical process section may consists of the following:

1. separation, recovery, and recycle of micro-organisms, enzymes, catalysts (metal, heterogeneous or homogeneous catalyst, if present) or raw materials,
2. recovery of the product,
3. purification of the product to the required degree, and
4. effluent treatment and disposal.

Downstream is where the product is recovered, purified, and stabilized. Recovery of side product is also carried out in the downstream section.

In addition utilities such as, air, steam, coolant, and hot oil are the other sections, which are also associated with a manufacturing facility. Figures 1.1a and 1.1b show a typical process flow sheet. Sections 1.2, 1.3, and 1.4 may be called the downstream processing section and effluent treatment is generally considered as a separate unit.

Downstream plays a crucial role in arriving at a pure product in an economical way. Purity becomes very crucial for pharmaceutical and health care products. A complex downstream section adds to the final selling price of the product and hence, its competitive nature in the market. Currently, bulk chemicals, pharmaceuticals, antibiotics and food products are manufactured using biochemical route. The process could be based on fermentation or a biocatalyst such as, a pure enzyme or a whole cell. Chemical route is still competitive than biochemical route for bulk chemicals, but the advantages offered by the latter over the former is tremendous in several areas (such as, milder conditions, better product quality, green approach and less effluents, etc.).

Chapter 2 introduces several products manufactured through chemical and biochemical routes, with main focus on the downstream. A typical downstream consists of removal of insolubles, isolation of product, its purification and polishing. The initial removal of insolubles from the mother liquor includes filtration and centrifugation. Isolation of products involves removing the desired product from a very dilute product liquid

(may be from 5 to 15%) and it includes adsorption, solvent extraction, distillation, etc. This step is meant to concentrate the product. The third step involves the purification of the product and the operations include chromatography, electrophoresis, and crystallization (or distillation if the product is stable at high temperature). Depending upon the product purity desired these operations are repeated many times. The final step will include crystallization, lyophilization, stabilization of the product and drying. Steps such as, crystallization, lyophilization, and drying are required if the desired product is in the solid form.

After fermentation, if the product is extracellular in nature (i.e., if the desired metabolite diffuses out and accumulates in the fermentation broth), then the biomass is collected after the reaction and is disposed and the liquid will be processed for the product. Whereas, if the desired product is intracellular in nature (i.e., if the product accumulates inside the cell), then the biomass has to be collected after the reaction, the cells have to be broken and the intracellular material needs to be extracted from this liquor. So the intracellular products will include few more downstream steps such as, cell harvesting, cell breakage and removal of cell debris and other unwanted proteins. This issue is not relevant in a chemical process.

1.2 VARIOUS DOWNSTREAM OPERATIONS

Figures 1.2a and 1.2b show the possible set of unit operations involved in the downstream for recovery of extra and intracellular product respectively from a biochemical reaction. If the product is an intracellular material then the cells have to be harvested and they have to be disrupted to liberate the product. This disruption includes mechanical, chemical and biochemical methods. Removal of cell debris is also a difficult step. Several primary, secondary and final recovery and purification steps can be adopted depending upon the concentration of the product, purity desired, its physico-chemical properties, and properties of the other impurities in the medium and cost of the recovery operation.

The various unit operations in the downstream can be listed based on the physical–chemical principles as shown in Table 1.1.

A typical design of downstream unit includes determining (i) the cycle time for the operation, (ii) the deciding on the operating parameters such as,