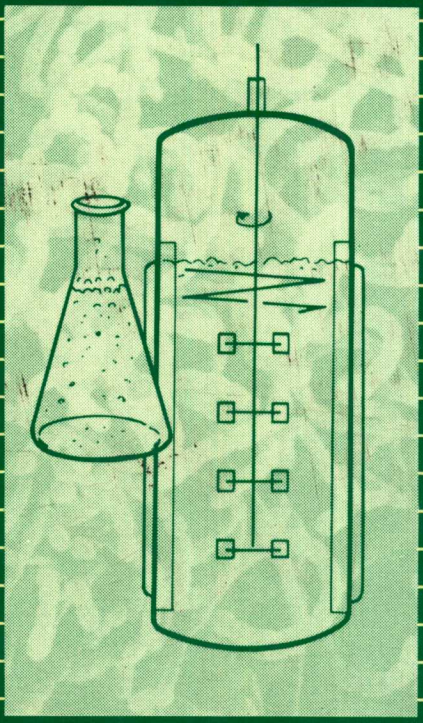
Critical Reports on Applied Chemistry Volume 29

Chemical Engineering Problems in Biotechnology

Edited by M. A. WINKLER



Published for the Society of Chemical Industry by ELSEVIER APPLIED SCIENCE SCI

Critical Reports on Applied Chemistry Volume 29

Chemical Engineering Problems in Biotechnology

Edited by M. A. Winkler

SCI
FOR THE APPLICATION OF CHEMISTRY AND RELATED SCIENCES

Published for the
SOCIETY OF CHEMICAL INDUSTRY
by
ELSEVIER APPLIED SCIENCE
LONDON and NEW YORK

ELSEVIER SCIENCE PUBLISHERS LTD
Crown House, Linton Road, Barking, Essex IG11 8JU, England

Sole distributor in the USA and Canada
ELSEVIER SCIENCE PUBLISHING CO., INC.
655 Avenue of the Americas, New York, NY 10010, USA

WITH 26 TABLES AND 73 ILLUSTRATIONS

British Library Cataloguing in Publication Data

Chemical engineering problems in biotechnology.
1. Chemical engineering. Applications in biotechnology
I. Winkler, M. A. (Michael A.) II. Society of Chemical Industry III. Series
660

ISBN 1-85166-454-8

Library of Congress Cataloging-in-Publication Data

Chemical engineering problems in biotechnology/edited by M. A. Winkler.
p. cm.—(Critical reports on applied chemistry; v. 29)
Includes bibliographical references.
ISBN 1-85166-454-8
1. Biochemical engineering. 2. Biotechnology. I. Winkler, M. A. (Michael A.), 1935– II. Series.
TP248.3.C47 1990
660'.63—dc20 90–2812

Printed in Northern Ireland by The Universities Press (Belfast) Ltd.

Chemical Engineering Problems in Biotechnology

Preface

Now is a very exciting time in biotechnology. The rapid advances in the high-value product processes, with their enormous investments and expected rewards, have provided the impetus for re-examination of the problems of process design, optimisation and control, and a serious consideration of the fundamental science underlying reactor design and the recovery, separation and purification of biotechnological products. In other words, the public relations bubble has burst and we are now looking at reality, like Patience on a monument, smiling at grief. Scientists and engineers, and now the general public, have been treated to starry-eyed accounts of the current biotechnological revolution, and it is worthwhile giving some time to consider a few of the problems involved. We have of course experienced several biotechnological revolutions in the past twenty years. The enzyme revolution has unblocked our drains and given us reliable medical assay procedures, even though enzymes may be going out of fashion in our laundry. The fuel alcohol revolution has faded, as a result of economic pressures and mismanagement, and I must confess to a feeling of relief that the scheme to feed the world on soya beans and bacteria has stabilised with rather more modest objectives. Biotechnology will now be expected to clear up the mess left by intensive agriculture, and fortunately has already made considerable technological progress: only financial resources are lacking.

The contributors to this volume are all engineers:

My colleague, Professor Raymond Spier, is Director of the Wolfson Cytotechnology Laboratory at the University of Surrey, as well as head of the university Microbiology Department. In addition to editing *Enzyme & Microbial Technology* and *Vaccine,* he is on the editorial board of *Cytotechnology* and is co-editor of the *Animal Cell Biotechnology* series, with its fourth volume currently in preparation; he is also Chairman of the European Society for Animal Cell Technology. To the current volume, Professor Spier has contributed an overview of problems of animal cell culture which neatly encapsulates those of biotechnology as a whole.

Dr Colin Thomas has recently been appointed a Senior Lecturer in

the University of Birmingham Department of Chemical Engineering, and has moved from the SERC Centre for Biochemical Engineering at University College London, where his research speciality involved the relation between microbial morphology and viscosity and the effects of shear on biotechnological systems.

Dr Jaap van Brakel of Delft University of Technology has contributed a personal, not to say astringent, overview of downstream processing in biotechnology which concentrates on his research speciality of selective adsorption. Since compiling his chapter, Dr van Brakel has moved to the rarefied atmosphere of the Faculty of Philosophy at the University of Utrecht.

Dr David Williams is Head of Research in the School of Information Science and Technology at Liverpool Polytechnic, and is well known for his work on adaptive control of the baker's yeast fermentation. I made Dr Williams' acquaintance when we were both lecturing at an Institution of Electrical Engineers' summer school at Cambridge (now published as *Modelling and Control of Fermentation Processes,* edited by Professor J. R. Leigh), where his exposition of 'On-line estimation of variables and adaptive control' on a hot, drowsy summer afternoon was nothing short of masterly. Dr Williams has, fortunately, found time to contribute his chapter on the problems of measurement and control in fermentation processes in spite of the demands of the privatisation of the polytechnics.

In my own chapter, I have taken advantage of my editorial privileges to include a few pages on basic theoretical aspects, such as the property transfer equations and brief notes on the significance of some key dimensionless groups. Both of these tend to be taken rather glibly (and how many of us could define the Weber number without notice?), without appreciating the pitfalls resulting from their misuse. Apart from giving some attention to my own research specialities of optimisation and variability analysis of fermentations, I have devoted a large proportion of the chapter to the problems of heat- and mass-transfer in non-Newtonian fluids.

MICHAEL WINKLER

List of contributors

Jaap van Brakel* Faculty of Philosophy, University of Utrecht, P.O. Box 80.103, 3508 TC Utrecht, The Netherlands

Herman H. Kleizen Laboratory of Chemical Technology, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands

Raymond E. Spier Department of Microbiology, University of Surrey, Guildford, Surrey GU2 5XH, UK

Colin R. Thomas School of Chemical Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, UK

David Williams School of Information Science and Technology, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, UK

Michael A. Winkler Department of Chemical and Process Engineering, University of Surrey, Guildford, Surrey GU2 5XH, UK

* Formerly of Delft University of Technology, The Netherlands.

Contents

Preface v

List of contributors viii

1 **Problems in animal cell biotechnology** 1
R. E. Spier

2 **Problems of shear in biotechnology** 23
C. R. Thomas

3 **Problems in downstream processing** 95
J. van Brakel and H. H. Kleizen

4 **Problems of measurement and control in biotechnological processes** 167
D. Williams

5 **Problems in fermenter design and operation** 215
M. A. Winkler

Index 351

1 Problems in animal cell biotechnology

R. E. Spier
University of Surrey, Guildford, Surrey, UK

1.1 Introduction 1

1.2 Upstream of the bioreactor 3
1.2.1 Water 5
1.2.2 Serum 6
1.2.3 Other selected components of the medium 8

1.3 Bioreactor conundra 10
1.3.1 The problem of choice 11
1.3.2 Shear and bubbles 12
1.3.3 To perfuse or not to perfuse? 15
1.3.4 Some options 16

1.4 The downstream arena 18

1.5 Conclusion 20

1.6 Further reading 21

1.1 Introduction

The classical definition of a 'problem' is that which lies between expectation and reality. In this sense problems can disappear by altering either one or other of the two delineated variables to eliminate the difference. However, the biochemical engineer would take the view that any process is capable of improvement, and his definition of a problem would be more concerned with his expectation that reality can be changed with increases in the cost-effectiveness of the operation or, indeed, in achieving the final product by a novel way which is not directly derivable from the existing system by modifications in degree to either process hardware, firmware or software.

In this sense he would survey the areas of the process under his

control and seek to determine those aspects of the process whose sensitivities indicate that they would be worthy of the application of his endeavours, in that the returns from a successful operation would be a significant increase in the operational efficiencies in product manufacture. He would thus recognise that the 'process' was a 'given' and that the way in which the final product was achieved could only be varied within the constraints of the definition of that product and the availability of the raw materials from which it can be made. Thus, in a process involved in the manufacture of a product from animal cells in culture, the animal cell itself and the medium in which it was to be grown would not be variables for the biochemical engineer. Rather, he would focus his attention on the equipment and operating procedures which enable a given formulation of medium to be made at a scale, with a freedom from contamination and with retention of biological activity as required by the process. Secondly, he would be concerned with the design, implementation and operation of the bioreactor in which the animal cells perform those activities which lead to the generation of product. A further requirement of the biochemical engineer is that he should be involved in a similar capacity with those unit operations downstream from the bioreactor which transform the crude product stream to the final concentrated, purified and formulated material. Generally, his duties do not extend to the filling of bottles, their labelling, storage and despatch. It is the purpose of this chapter to describe and discuss the areas of bioprocesses which involve animal cells, and to focus on those aspects in which either there is a consensus that improvement is needed to either effect scale-up or to achieve the required process economics, or in other areas where the author intuits that improvements, which are not absolutely necessary for the operation of the process, can, and probably will be, made shortly.

A second sort of problem, which is quite different from the one described above, can also be defined. This consists of the 'choice' of equipment and or procedures which can be used to achieve a given end. To some extent this represents the choice between alternatives when presented with several design solutions for the equipment for a unit operation. On the other hand, it may represent the actual situation presented to many animal cell biotechnologists when they come to decide upon which, of the several bioreactors available from the manufacturers, they should choose either for a particular process, or for a general-purpose pilot plant which can be used to investigate several of the many types of process afforded by the use of animal cells

in culture. While it could be that the engineer has been told the way in which the product can be concentrated and purified, the scale-up of such a bench process may be fraught with difficulties. It could therefore fall to the biochemical engineer to redefine the concentration and purification operations *de novo,* which would necessarily involve him in making choices about the unit operations involved, the method of their application and the order in which they are brought to bear.

1.2 Upstream of the bioreactor

The two major elements upstream of the bioreactor are the preparation of the cell inoculum and the medium in which that cell is either to grow or produce. With regard to the former, there is little involvement of the biochemical engineer in the selection, characterisation or engineering of the animal cell used in the process. Indeed, his work begins when a laboratory worker provides him with a bottle containing the cells, along with a sheaf of protocols describing how they can be grown, exploited and stored for the long term. At this time he learns that the cells either do, or do not, require a solid substratum for their anchorage-dependent growth. He also learns whether the product is one which is produced in a system which needs the cells to be in a growing state for product generation, or whether the cells can be held in a 'maintenance' condition for long periods of time with the product secreted. In addition, he is told the composition of the medium, and he takes particular notice of the quantity and quality of the serum to be used in the medium. This latter parameter has a pronounced influence on the efficacy of the downstream processing operations, as well as controlling the economics of the overall raw material costs. It should be noted that the presence of large amounts of extraneous serum in the crude product discharge from the bioreactor not only affects the unit economics of the downstream operations, but also affects the yield of the process. It is in this latter capacity that the cost of the final product can be influenced significantly, as the same cost could well be incurred for either all of the material produced in the bioreactor or the one-tenth of the material that was recovered in the final product. There are additional implications which ensue from the inclusion of serum in the growth medium, in that procedures have to be defined for the sterilisation of the serum and/or its sterile addition to the other components of the medium.

There are few choices, and therefore problems, associated with the preparation of medium fit for the growth and use of animal cells in

culture. Clearly, the medium has to be formulated and composed, and when such has been achieved there is a requirement for the medium to be tested for both its sterility and its ability to support the growth of the animal cells for which it has been designed. A further need is often required if the process is one which has been accepted for the production of a licensed biological. This additional requirement often takes the form of the acquisition of data which can be used to certify that the materials which went into the composition of the medium were actually those which are called for in the formulation documentation. Such a validation, complete with the documents or certificates derived from the performed tests, of the materials from which the medium is made can be obtained from the manufacturers of preformulated medium, for which a monetary premium over the normal cost is charged. Otherwise, medium is made up in-house according to a recipe or, if a formulation is prepurchased, then, depending on whether it has been bought as a dry powder or a liquid formulation (which could be at normal strength or ten times normal strength), the operations which have to be effected are those which result in the filling of the growth vessel with the formulated, sterilised and tested medium. As animal cell media contain materials which are either heat sensitive in themselves (glutamine) or which in combination with other materials form unscheduled compounds, materials which form the browning reaction with glucose, so the procedures used for the sterilisation of such solutions are generally those dependent upon filtration operations.

Two kinds of filtration are available. One method is based on depth filtration through a fibrous pad (once based on asbestos fibres but now transformed to cellulose and its derivatives), and the other is based on filtration through membranes. In the latter case the traditional membrane could be relied upon to exclude virtually all materials with a diameter greater than 0·2 μm. More recent membranes have halved that dimension, to 0·1 μm. In all such filtrations the prospects of losing important medium components on the surface of either the fibres or the membranes has to be considered and, indeed, most prudent operators do not use the liquids which issue from the filtration system until they are convinced that the bulked fluids which emerge are identical with the ones which have been applied. Also, further precautions are taken by:

(a) pretesting the filters by establishing that they can hold a defined pressure drop across the membrane;

(b) testing samples of filtrate taken from the beginning, middle and end of the filtration run for both sterility and growth promoting potency.

Where it is possible to presterilise and store the sterilised medium prior to use it is possible to effect the necessary checks before the medium is committed to the production operation. By such stratagems more reliable production systems can be engineered.

1.2.1 Water

It is a salutary lesson to appreciate that the quality of the water used for an animal cell biotechnology process can be the prime determinant of the success or otherwise of that process. A number of examples may be cited which demonstrate that on the changing of the source of the water for the medium formulation the consequences for cell growth have been precipitously disastrous. Much ingenuity has been expended on the preparation systems for water and such have included:

- water pyrolysed at above 200°C
- melted ice from deep within a Greenland glacier
- water made from the combination of hydrogen and oxygen gases
- water with almost zero conductivity
- water purified by reverse osmosis or ultrafiltration, passage through ion exchange resins, or passage through filters containing activated carbon
- water purified by double or treble distillation off glass or stainless steel

Once the water has been prepared it is necessary to maintain it free from pyrogens, which can be prokaryotic cell-associated glycolipid endotoxins or secreted exotoxins. These latter are often polypeptide molecules with molecular weights up to 50 000 daltons, and are made by contaminating prokaryotic micro-organisms. The ultrafiltration of the water immediately prior to use will normally eliminate such contaminants: alternatively, it is possible to hold the prepared water at temperatures above 80°C, under which conditions the pyrogen producers fail to thrive. This has implications for systems which seek to distribute water to the various rooms within a laboratory or manufacturing facility. For such a situation it is important to keep the whole system at the elevated temperature and to avoid static zones within the

pipework. The pipes should be made from welded stainless steel and the interior of the pipes should be free from pits and crevices. Attention should also be given to the valves used to control the discharges from the system, as dead legs tend to form at such positions. It is also important to use valves which can be sterilised in place and can be relied upon to obviate the possibility of the instigation of zones wherein bacteria can flourish. An alternative to the distributed water system may be obtained by the use of a central reservoir of water held at the elevated temperature, with facilities for the discharge of such water through a sterilisable heat exchanger which would be used to cool the water down to the use temperature immediately upon its discharge. It should be understood that it is often a requirement of a regulatory agency that the final product should be demonstrably free from pyrogens: were the aqueous raw materials so characterised then the stress on the tests on the quality of the final product would be lessened.

1.2.2 Serum

To the biochemical engineer, serum presents a set of problems of definition and processing requirements which can be bewildering in their complexity and intangibility. Two-dimensional electrophoretograms demonstrate the many hundreds of differentiable molecules in this compounded soup. They consist of proteins, glycoproteins, lipoproteins and glycolipoproteins, and can range in molecular weights between a few hundred to a few million daltons. From the more specific point of view of the animal cell biotechnologist, there are in this mixture both growth-promoting and growth-inhibiting materials, as well as molecules which are alleged to protect the cells against damage by bubbles or shear forces (see below, section 1.3.2). The serum may provide nutrients, and can be an important source of attachment factors for those cells which need to adhere to a surface to express their biotechnological potential. The antitrypsin in the serum serves to neutralise this enzyme when cells are released from the surface to which they have become attached, and the serum can contain immunoglobulin species which can prevent or lessen the growth of particular viruses when such are needed either for the formation of a vaccine or as a diagnostic material.

On the negative side, serum is often contaminated with viruses

derived from the site of origin of the serum. When bovine (foetal, newborn or adult) serum is prepared it can be contaminated with mycoplasmas and/or bovine viral diarrhoea virus, as well as other organisms which we do not yet know about. Sera from other sources suffer from corresponding contaminations. The elimination of such contaminants can be achieved either by the careful testing of each batch of serum or by elimination of the contaminating species. This latter can be achieved, for the viral contaminants, by gamma-ray irradiation within precise exposure limits of $2{\cdot}5\text{–}3 \times 10^6$ Rad. Mycoplasmas may also be eliminated by such irradiation; yet it is also possible to eliminate them by the use of membrane filters with a pore size of $0{\cdot}1\ \mu$m.

It is not possible to sterilise serum by the use of heat alone; rather, methods based on filtration are used. As the serum contains many complex molecules it is necessary to effect a number of presterilisation filtrations to obtain material which can pass through a sterilising filter without incurring a blockage after a low throughput of materials. Additional treatments such as UV sterilisation or heating to 50–60°C for 15–30 minutes are sometimes used to inactivate complex molecules or to alter the balance between the growth-promoting and growth-inhibiting factors in the serum. In all such events it is virtually obligatory to test each and every batch of serum for those properties which have been deemed necessary for the production process. To achieve such test systems it is advisable to present to the serum cells the quality of which can be defined (at least in their responses to other batches of serum). Aliquoted frozen cells may serve as such a referant. The concomitant use of two such batches of frozen cells is preferable to a dependence on a single batch. Also, one can test the cells of the day as an additional safeguard. The triple testing of the serum is also advised, particularly when the tests are effected on different days.

Thus stated, the problems for the biochemical engineer focus on the ability to control the quality of the raw materials. The tests described above constitute a simple though useful approach to the control problem. Other methods can be used and these can include the control of all the parameters which are involved in the production of the serum, where such would include the raising of the serum-producing animals on a controlled farm, with the production of the serum materials by a process which seeks to eliminate all extraneous contamination at the site of ingression rather than by the process of the elimination of the contaminant from the serum. Alternatively, the

engineer can question those who have designed the bioprocess to determine whether serum is as necessary as has been recommended. With modern cell-handling procedures and medium formulations, it is possible to wean the cells from their dependency on raw serum in many, but unfortunately, not all cases. The biochemical engineer should be satisfied in his own mind that the advice he has received from his colleagues is irrevocable when it comes to the necessity for the inclusion of serum in the medium formulation.

1.2.3 Other selected components of the medium

There could be some 30–70 components of an animal cell culture medium. Those that have the most variable levels are generally limited to the concentrations of glucose, glutamine, buffer material and (in serum-free media) particular growth factors such as insulin, transferrin and albumin. (There are many other such factors which can be specific for the cell type under consideration. They will not be dealt with here.)

Working on the principle that those animal cells are most likely to grow and express product when they are least stressed by their environment (a principle that could be more honoured in the breach when particular products are required), then the maintenance of a physiologically acceptable pH in the culture medium is an obvious objective. The perceptive would also recognise that as the glucose is metabolised there is an increase in the level of lactic acid produced by the cells. This acid is responsible for the decrease in the pH of the culture fluid. The problem then falls to either decreasing the amount of lactic acid derivable from a defined amount of glucose or changing the amount or the nature of the carbohydrate so that excessive quantities of lactic acid do not form, or devising a simple, effective and inexpensive means of eliminating the by-product. The controlled addition of glucose can lead to a decrease in the level of lactate produced; yet the effect on the overall productivity and growth of the cell is such that this strategy cannot be considered to be of great utility. Indeed, in those cases where the pH is controlled by the addition of alkali (generally sodium carbonate or bicarbonate), the presence of lactate does not materially affect the progress of the culture. It is also possible that the addition of glutamine decreases the amount of glucose which the culture requires. However, this causes problems of a different nature.