PROCESS VALIDATION AND SAFETY IN BIOTECHNOLOGY

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ABSTRACT

A brief survey of current influences on the implementation safety in fermentation process is made. The role of validation, as intended by the Food and Drug Adminstration of USA, in improving the safety of the process is discussed, with a case study. It is concluded that the validation exercise will improve the safety of the product to the consumer and in doing so will ensure a degree of operator safety through mutual requirements. However, it is further concluded that there is still a need for devices to be invented which are capable of detecting small leakages from the fermentation, for complete operator safety. Some of the research addressing this problem is reviewed.

Keywords: BIOTECHNOLOGY VALIDATION FERMENTATION CONTAINMENT ASEPTIC BIOHAZARD

1 INTRODUCTION

The rapidly growing application of genetically engineered organisms with the subsequent ability to produce novel microbes, has raised concern of the potential risk of accidental environmental release of these organisms. However, large scale fermentations in the UK largely employ non-pathogenic microbes. On the other hand, increasing attention is being focussed on the potential for the production microbes to elicit an allergic response (1). The future use of recombinant organism (rDNA) withing fermentation practice is often thought of as being in need of more stringent containment practice and programmes in occupational safety and health due to the increasing health risk that they may pose to workers (2). The application of the general set of guidelines contained in Good Pharmaceutical Manufacturing Practice (GPMP) (3) and Good Large Scale Practice (4) should ensure that appropriate control is identified and implemented in production scale operations.

In general, control is attained by containment in a sealed system. Monitoring to ensure system integrity, and the use of personnel protective equipment should complete the control system.

"Large scale" for genetically engineered microbes, is often taken to mean fermentations in excess of 10 litres working volume, although the concentration of microbes in the fermenter is not taken into a account (5).

This paper discusses how the safety of a fermentation process is best ensured, within the limits of current technical ability. Fermentation is taken to include growth of animal and plant cells, as well as microbes.

2 OFFICIAL GUIDELINE AND REGULATORY INPUTS

Regulation of biotechnological processes covers two overlapping areas:

i) Occupational safety of the production force.
ii) Quality assurance of the product for the consumer.

The number of regulatory bodies which have become involved in biotechnological safety has grown rapidly (Table 1). However, attempts have been made to harmonise the situation in Europe via the European Federation of Biotechnology. Companies producing pharmaceuticals will often want to export to the USA. This necessitates meeting a new set of regulations (Table 1) and in particular, meeting the requirements of Process Validation set by the FDA.

Organisations Categorising Risk Of Microbes

ACDP	Advisory committee dangerous Pathogens (6)
ACSM	Advisory committee for genetic manipulation (7)
EFB	European federation of biotechnology (8)
DHSS	Department of Health and Social Security (9)
CDC	Center for Disease Control (USA) (10)

Organisations Requiring Quality and Safe Practice

GLSP	Good large scale practice (4)
GPMP	Good pharmaceutical manufacturing practice (3)
HSE	Health and safety executive (11)
CECDD	Commission of European Communities council directive (12)
NIH	National institute of health (USA) (13)
FDA	Food and drug administration (USA) (14)

Table 1. Some organisations influencing safety in biotechnology. All are UK unless indicated. (References in brackets).

A major difficulty in establishing reasonable regulations or guidelines is the fact that the infective dose of many pathogenic microbes is not known, nor is there a sensitive method for their airborn detection.

This means that the attainment of safe practice is achieved through containment of the process - no leaks to the environment. The general level of consensus by the regulatory bodies is to stipulate three levels of containment:

PRIMARY CONTAINMENT: The provision of immediate physical barriers directly on the fermenter vessel and its associated pipework.

SECONDARY CONTAINMENT: The provision of a direct back-up system to the primary containment facility, to operate in the event of primary failure.

TERTIARY CONTAINMENT: The provision of a contained environment (at plant or room level) in which the process is located.

The appropriate containment level is determined by the degree of pathogenicity posed by the organism, which in turn has been agreed by

organisations such as the EFB. Note that in nearly all large scale fermentation processes, the most common level of containment used is primary containment. This is because the microbes which are used in the fermentation are classed as being harmless to all humans (and plants and animals, where appropriate). This is good sense, since the use of primary containment is more economically sound, avoiding the provision of additional equipment and materials necessary for higher levels of containment.

Bulk processing requires the integration of both containment for protection of the personnel and the environment, and asepsis for protection of the product. Downstream processing can cause more problems than the fermentation, since more than one processing stage is often employed. Although downstream processing can often be rendered safe by killing the organism after fermentation, asepsis of the product stream will then assume the major problem. In a number of cases, some of the plant can be designated to run in a hygenic rather than sterile manner. This is achieved by ensuring continuous bulk flow of fluids (no back flow) and using sterile filters of appropriate points. This approach might be adopted for cooling water for example.

The direction of directives/guidelines for equipment design necessary to achieve the levels of containment. In summary, the EC directive (12) gives details of design specifications for a number of plant items, but at performance level, not at design level. There are three consequences of this:

i) There has to be a suitable test to show that the equipment meets the specifications.

ii) There will be a number of designs capable of meeting the performance criteria. Thus different users could incorporate different items of plant to achieve the same level of containment.

iii) There must be adequate training of personnel.

This situation is similar in the USA, where the FDA licenses drug products together with the processes and equipment in which the products are manufactured. The FDA operates on a case by case basis, inviting manufacturers to submit intentions for new biotechnology plants or operations. Further, the FDA lay much emphasis on documentary evidence on process and product validation. However, even with all this documentation that the FDA require from manufacturers, there is still no communication from the FDA to the designers and constructors of bioprocess equipment in terms of constructions standards. Contrast this with the UK BS5500 specifications for pressure vessel design (eg fermenters) which specify details such as vessel thickness and standard of welds. However, the lack of specific biotechnological specifications means, for instance, that the adoption of BS5500 for fermenter design will run into problems over provision of a pressure relief valve; through which discharge of live organisms to the atmosphere could result.

It may be argued, of course, that providing a manufacturer meets the performance criteria with his equipment, it does not matter about the designs he has used. This argument may well be sound if there were adequate methods of testing performance (see later). This subject has been extensively reviewed recently (15).

3 PRINCIPLES OF VALIDATION

The FDA definition of validation is:
"Process validation is establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes".

Process validation was first introduced by the FDA in March 1983. Process validation offers guidelines outlining general manufacturing principles for the preparation of human and animal drug products. Indeed, process validation is a requirement of the current Good Manufacturing Practice Regulations for Finished Pharmaceuticals in the USA. The operation of validation is that companies can ask the FDA for specific guidelines on what the FDA expects the companies to do in compliance for the requirements of process validation.

The FDA breaks down the validation procedure into five parts:

INSTALLATION QUALIFICATION: The verification that all portions of the installation adhere to the recommendations of the manufacturer and to local and state codes.

OPERATIONAL QUALIFICATION: The verification that equipment can operate as intended and is capable of satisfactory operations over the entire range of temperatures, pressures, time and other operational parameters (eg pressure testing and temperature mapping of process equipment).

PERFORMANCE QUALIFICATION: The performance qualification is used *in lieu* of the operational qualifications and the validation of systems or equipment that do not require challenges to prove that they are reliable and perform as specified (eg chilled or deionised water systems supplied).

VALIDATION: The performance of various challenges and completion of tests to verify that the complete process is capable of providing the required confidence level.

CERTIFICATION: The purpose of the certification document is to qualify the whole process. Through signatures on the certification document, the management expresses agreement with the data collection, methodology, background knowledge and capabilities of those involved in the writing of the protocol.

Once the total certification package has been completed, the equipment or system is considered acceptable for use under the specified conditions and functions of the protocol. Completed to be used for reference in considering changes to equipment or procedures or during maintenance.

4 CASE STUDY OF VALIDATION: A FERMENTER INSTALLATION

Before the validation exercise is carried out on the vessel, a process validation protocol must be written by the company. This incorporates all commissioning exercises to be carried out on the vessel along with validation challenge. The protocol, when installation are given in table 2.

1	The fermenter vessel has been designed to that of a standard pressure vessel (BS5500).
2	The fermenter is connected to the appropriate piping layout by flanged connections with the appropriate O-ring seals.
3	Fermentation monitoring instruments are fitted through appropriate ports.
4	The agitator is connected.
5	Air filters are connected.
6	Diaphragm valves have been fitted to the appropriate lines (eg inlet and outlet, filter, sampling, filter, some steam etc).
7	All internal finishes (including welds) that will come into contact with product or medium have been polished to an "acceptable" finishing 360 grit).

Table 2. Specifications of the fermenter system used as a case study for validation.

The first stage is COMMISSIONING OF THE VESSEL. The commissioning *exercise will include:*

i) Pressure hold testing and hydraulic pressure testing to ensure integrity of the vessel.

ii) Calibration of all instrumentation. For example pH probes against buffer solutions; temperatures probe against a heating block, pressure sensors against definitive pressure gauges.

iii) The utility lines (purified water, steam, high pressure hot water, cooling water) are checked to ensure correct flow rates and pressures.

iv) All drain lines are checked for smooth flow to drains.

v) The fermenter is then brought up to sterilisation temperature to check that this can be satisfactorily achieved. This will be done by temperature monitoring of the vessel using thermocouples. The procedure will also check that the instrumentation is not damaged by the temperature and pressure, and that none of the seals are breached.

On completion of the commissioning exercise and with satisfactory documentation of the operation*l*, PROCESS VALIDATION *can take place.*

In essence, the PROCESS VALIDATION exercise consists of a prescribed number of repeated sterility challenges on the fermentation vessel. The sterility challenge would take the form of filling the fermenter vessel with a "sterility broth" which is a rich medium allowing the growth of many different species of bacteria. This medium is subjected to the process

sterilisation procedure (eg *in situ* or aseptic filling). The sterility broth then has to pass a holding period in the vessel. Thus in more detail, for a vessel aseptically filled:

i) The fermenter is sterilised and cooled in a pre-specified manner, and the air and liquid filters are integrity tested.

ii) Upon the filters passing their integrity testing, the vessel is filled in an aseptic manner with sterility broth to the maximum normal operating level with the agitator running at its normal speed. The fermenter is operated at normal pressure.

iii) An aseptic sample is taken through be the dedicated sampling point, during the filling process. This must be achieved by a standard operating procedure.

iv) Further aseptic samples are taken through the sampling points while the vessel is being run in the normal way (with air on if appropriate). In this case the fermentation duration is 4 days, and so the validation was performed over 6 days, as a safety margin.

The results of the validation exercise should confirm with the protocol (ie sterile). If one or more of the sample fails the sterility test, a decision will have to be made as to whether the fault is post- or pre-removal from the fermenter. The validation procedure will be repeated a number of times, in any case, to show that the fermenter vessel can be operated aseptically for the production phase. The final procedure in validation will be to operate the fermentation with the production organisms and show that only the production organisms are present and meet the specified growth characteristics.

v) All samples are immediately sent for microbiological analysis.

5 THE SAFETY OF A VALIDATED PROCESS

It can be seen that Process validation is an excellent way of achieving product quality. It is also an effective way of ensuring, as far as possible, that a pharmaceutical product is manufactured by a method which minimises contamination by pathogens. However, the emphasis is undoubtedly on protecting the product. The operator safety is incidential. In spite of this, it has to be said that many of the operations necessary to protect the product will also protect the operator. Good practice and design which prevents the ingress of contaminants to a process, can often prevent the egress of the production organism to the environment.

The problem of preventing egress of microbes is obvious. At present, there is no sensitive and reliable method of detection of microbes which have leaked into the atmosphere. Leakage of microbes into the fermenter, can be readily detected by their growth - the medium and ensuing growth will amplify just a small number of contaminating microbes. On the other hand, when microbes are released from the process into the environment, no further growth can ensue. Any detection system for monitoring the air will therefore be dealing with possibly only a small number of organisms. It is possible to detect quite small amounts of biochemicals in the atmosphere. Recent work has successfully and rapidly detected microgram

quantities of protease per cubic metre of air in the factory environment (16). But even at a bacterial air concentration 1 $\mu g\ m^{-3}$, this would represent approximately 10^6 microbes, based on microbe having a dry weight of 10^{-12} g. This may be too hazardous. This might correspond to a leakage of 0.01 ml, if the fermentation had 10^8 microbes per ml. This may seem a very small leak from say a 10 m^3 fermenter. But if the fermenter is housed in a building of 10 m^3, the leak would have to be 1 ml to reach 100^6 microbes per ml. It should be added that there are a number of more sensitive specific microbial samplers being developed, which, provided they have a rapid response could improve the situation (17).

A completely different approach, and much simpler, is to check the integrity of the fermenter and its lines by pressure testing after sterilisation. If the fermenter can maintain a given pressure over a typical fermentation period, then it could be argued that there are no leaks from the vessel (note that this is subtily different from a sterility test, above, which test for ingress of microbes). However, will the pressure drop be significant to detect small leaks in a reasonable length of time? It is obviously not economical to hold a sterilised fermenter and its medium on hold for days. Also a physical test can never replace biological test of sterility (ie growth of an organism). This approach is currently being evaluated (17).

6 CONCLUSION

The general public, as consumers, cna be assured that pharmaceutical products prepared through fermentation are likely to be safe and of good quality. Quality controls through exercises like Process validation, go long way to ensuring operator safety; but there is still room for further tests to be developed for rapid monitoring of small leakages of microbes from the equipment.

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