The Filamentous Fungi

Volume 4 Fungal Technology

Edited by:
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Preface

The use of filamentous fungi in industrial processes is well recognized and gaining more importance with the current awareness and rapid expansion of biotechnology. The current volume will be complementary to Volume 1 *Industrial Mycology* which balanced the historical development with the current practices of industrial mycology. Volume 4 highlights the technological aspect of cultivation of filamentous fungi.

It is acknowledged that, compared to other micro-organisms, successful cultivation of the filamentous fungi may require a greater attention to detail. However, the wide range of cultivation conditions which can be tolerated offer considerable scope for commercial exploitation. Considerable attention has been given, therefore, to the technology of industrial mycology, whether in liquid or solid state fermenters. This ranges from preservation of the fungi, via the fermentation, to the separation of the biomass from the fermentation broth. A chapter on industrial genetics is also included; this is an area which, in spite of the lack of attention, holds considerable promise.

In industrial application of the filamentous fungi, attention has been given to 'new' areas in which these organisms play a major role. Again the emphasis has been on process technology. The economics of industrial mycology is discussed in relation to a well known process using a filamentous fungi.

As an illustration of the degree of attention required by the filamentous fungi, a chapter on fungal toxicity is included. If we do not get it right, it can go wrong, very wrong, as the chapter illustrates. Fungal toxicity has considerable commercial implications, however, in industrial mycology it should not be a problem we will encounter.

1983

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CHAPTER 1

Fermenter Design

B. KRISTIANSEN and H. E. CHAMBERLAIN

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1.1 Fermenter Design Considerations

Modern fermentations require that the fermenter provides an environment suitable for the growth of a pure culture or a defined mixed culture, which can be run free from contamination and under controlled conditions. A well-designed vessel will also ensure the culture is contained with no aerosol leaks of the vessel contents, since repeated exposure to even a non-pathogen can, in certain circumstances, be hazardous.

The design must incorporate a device for mixing the contents, an air supply for aerobic processes, probes to monitor the environment and regulators to control it. There must be provision for inoculation and sampling, as well as for charging and discharging the vessel. In continuous culture, it is necessary to monitor and control the flow rate of the medium as well as the culture volume and mass.

Incorporating all these features means that the construction has many potential sources for the entry of contaminants. Good aseptic design at this point is crucial. The following cardinal design rules will apply (Aiba, Humphrey & Millis, 1973):

1. There should be no direct connexions between sterile and non-sterile parts of a system.
2. Minimize flange connexions. These can move under vibration and heat and provide entry for contaminants.
3. Use all-welded construction if possible.
4. Avoid dead spaces and crevices, etc.
5. Various parts of the system should be independently sterilizable.

Materials of construction

The material of the vessel must be non-toxic, able to withstand steam under pressure so that it can be sterilized and must be resistant to corrosive effects of sterilization and high or low pH. Pits in the surface of the material can harbour micro-organisms, hence the surface should be as smooth as possible. In summary, the material used should not affect, or be affected by, the environment.

Most laboratory and many pilot scale vessels are made of glass

(borosilicate or Pyrex). These are a multitude of shapes and sizes ranging from squat stirred tank reactors, with an aspect ratio (height to diameter) ranging from 1 (laboratory scale) up to 4 (industrial scale) through tower fermenters with an aspect ratio greater than 6, to tubular loop fermenters with a length to diameter ratio >40.

Above 30 or 40 l capacity, fermenters are normally stainless steel although pilot plant vessels with no mechanical agitation can also be made of glass (Malfait *et al.*, 1981; Kristiansen & Bu'lock, 1980).

Stainless steel vessels must be highly polished and all metal parts should be made of the same grade stainless steel to minimize electrolytic corrosion.

Fermenter capacity

In industrial processes, the fermenter capacity ultimately depends on the desired product concentration. If the maximum concentration of cells that will be cultured in a given fermentation process can be decided, then the total volume of culture can be calculated from knowledge of the production requirements (Brown, 1979). Fermenter size is also affected by the choice between batch and continuous fermentation, normally continuous processes require smaller vessels to give the same productivity as equivalent batch processes.

There are a number of additional constraints not directly related to the fermentation process which also affect the size of new fermenters. These have been outlined by Brown (1981).

(*i*) Existing fermenter volumes might have to be matched to maintain production planning and to be compatible with separation and recovery equipment.

(*ii*) Foundation loadings may have upper limit.

(*iii*) Vessel diameter may be limited by structural arrangements in an existing factory building.

(*iv*) Area-to-volume ratio decreases as the diameter is increased. Space for necessary cooling surface, which is proportional to the vessel surface area becomes limiting at a diameter of about 4.5 m.

(*v*) Limits to the size of off-site fabrication facilities or to the width of load that can be transported on the highway.

(*vi*) An increase in the height of the vessel will probably mean that multiple impellers will be required, necessitating a central bearing and a large shaft diameter.

(*vii*) Increased height increases the static pressure, resulting in the need for a high pressure air supply.

(*viii*) High liquid height-to-vessel diameter ratio might cause severe foaming problems.

(*ix*) A large vessel needs a large gearbox and drive motor, both of which are usually mounted on the top of the vessel to simplify alignment problems and minimize vibration. The wall of the vessel must be thick enough to support the drive unit rather than contain the fluid.

(*x*) A large electric motor may require a non-standard 1100 V supply of electricity.

(*xi*) Circulation and, therefore, mixing times may be too great on a large scale, especially if the fluid system is a highly viscous non-Newtonian liquor.

The results of the application of the above constraints have resulted in conventional stirred tank fermenters being commonly about 125 m^3 with the dimensions of a 4 m diameter and 10 m height. Typical upper limits occur at approximately 210 m^3 with a diameter of 4.5 m and a height of 14 m.

In mechanically agitated fermenters, the energy input per unit volume required for adequate mixing will often decrease with increasing fermenter capacity. Friction in the agitator gland may consume a significant part of the power input in small fermenters. The following required power input ranges have been suggested by Solomons (1980) (Table 1.1)

Table 1.1 Effect of fermenter size on power input requirements.

Fermenter size	Power input ($W l^{-1}$)
Laboratory	8–10
Pilot plant	3–5
Plant	1–3

Mixing cost may contribute a significant part to the operating cost of fermentation plants (Ryu & Oldshue, 1977).

Process control

It is generally accepted that most fermentations could be improved by using fully monitored and controlled environments. A lack of reliable and sensitive on-line measuring instruments does not make this possible. It is necessary to improve existing as well as developing potential monitoring devices to obtain more information about the microbial activity in the fermentation broth.

In addition to the shortage of process probes, there are few structured mathematical models providing adequate description of fermentation processes. It is difficult, therefore, to analyse the data obtained for purposes of process control. The computer has become an essential tool in fermentation technology. At present it is mainly used for data logging and analysis. Its application has been the subject of a number of symposia, *e.g.* *Computer Applications in Fermentation Technology* (Philadelphia, U.S.A., 1978), and the Third International Conference on *Computer Applications in Fermentation Technology* (Manchester, U.K., 1981).

There are a number of excellent sensors for monitoring process parameters. A number of these, and the impact on fermenter design, is briefly outlined below. For further details, see Chapter 3.

Temperature There are a number of reasons for temperature changes during a fermentation (Atkinson, 1974): (*i*) Heats of reaction; (*ii*) energy dissipated by the agitator; (*iii*) energy dissipated by passing air through the culture; and (*iv*) heat loss to the air stream due to temperature changes and increase in gas humidity.

In small vessels (*iv*) may be so large as to make it necessary to supply heat to maintain the desired temperature. This is monitored by thermometers, thermocouples, thermistors or metal resistance thermometers immersed in the culture. Temperature control is normally effected by use of cooling/heating water either through internal coils or external jackets.

Filamentous fungi often have a preference for growth on solid surfaces and for successful submerged cultivation of these organisms it is important to reduce the surface area available for microbial attachment. This is an important, but often overlooked, aspect of design of laboratory scale fermenters, where hollow baffles or draught tubes are used for circulating the cooling water. This makes the available surface area for microbial attachment per fermenter volume exceptionally high and the resulting immobilized cells may contribute significantly to the fermentation, producing considerable scale-up problems.

Temperature control of large scale vessels suffers from the fact that as the vessel size increases the ratio of surface area available for heat transfer (= proportional to vessel surface area) to fermenter volume decreases. This is illustrated in Table 1.2 (Fuchs, Ryu & Humphrey, 1971). It is often stated that fermenters above 200 m^3 require external heat exchangers for temperature control. This makes the design more complex and will also increase the risk of contamination.

Table 1.2 Geometric scale factors of typical fermentation equipment.

Fermenter volume (l)	Tank diameter T (cm)	Impeller diameter D_1 (cm)	D_1/T	Liquid depth (cm)	$\frac{\text{Tank surface area}}{\text{Tank volume}}$ (cm)
51 000	333	137	0.41	622.7	0.014
3 000	152.4	66	0.43	243.8	0.030
550	74.9	30.5	0.41	95.1	0.064
230	59.7	25.4	0.43	72.2	0.08
30	29.8	12.7	0.43	42.9	0.16
10	21.0	12.1	0.58	29.2	0.22
3	14.3	5.24	0.37	19.6	0.33

pH Fluctuations in pH occur because of products or by-products of the fermentation, and since pH affects growth and metabolite production of micro-organisms, pH control has become an essential part of fermentation. It is monitored using steam-sterilizable pH probes described by Buhler & Ingold (1976). These are normally glass reference electrodes and although they deteriorate with repeated sterilization, are relatively stable.

In research fermenters, control is achieved by the addition of acid and alkali via peristaltic pumps. In industrial processes, glucose is added to drop the pH, and NH_3 gas passed in with the air increases pH. Often, buffers such as $CaCO_3$ are added to the medium at the start, to stabilize pH.

Dissolved O_2 Chemical methods are available for the absolute determination of dissolved oxygen, but these are time-consuming, and are interfered with by many solutes present. Therefore, considerable effort has been devoted to develop a device which will give a continuous read-out of dissolved oxygen levels.

The basic principle of most of these detectors is the electrochemical reduction of the uncombined oxygen at a constant potential, using the current flowing as a measure of the amount of oxygen involved per unit time.

Oxygen electrodes are either polarographic or galvanic, the former requiring an external power source and incorporating electrodes of noble