Problems in design and operation of bioreactors for plant and animal cell cultures

Ryszard Pohorecki
Faculty of Chemical and Process Engineering
Technical University of Warsaw

1. Introduction

Plant and animal cell cultures are promising ways to produce a number of high value products. Although animal cell culture techniques are more advanced than those of plant culture, both are still in their developmental stage (Fig. 1). Moreover, they pose a number of specific design and operation problems, quite different from those characteristic of microbial cultures. At present, more than 70 different bioreactor types for animal cell cultures are available on the market (2), and the number of those suggested for plant cell cultures is also significant. However, there is no universal design suitable for all culture types, and development of such a universal design is probably impossible. It would therefore be impractical to try to discuss all the different designs available. Instead, I shall try to discuss some problems characteristic of bioreactors for plant and animal cell cultures, and to describe a number of typical designs suggested to overcome these problems.

From an engineer's point of view, the main features distinguishing plant and animal cell cultures from microbial cultures are the following:

— animal cells and plant cells are usually significantly bigger than microbial cultures;
— they grow much more slowly;
— they are usually more vulnerable to hydrodynamic stresses;
— they require less oxygen than typical aerobic microbial cultures;
— in the case of plant cells, they often require illumination;
— they often are anchorage dependent and differentiate during cultivation;
— their cultures are usually more dense than microbial cultures.

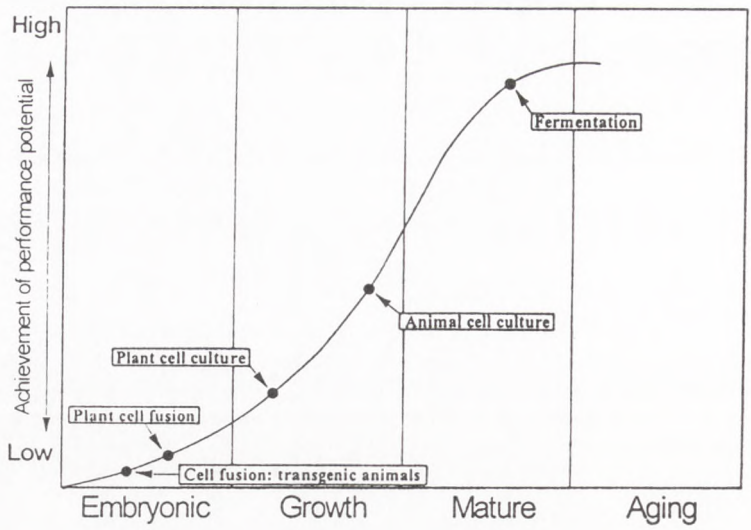

Fig. 1. Stage of development of different biotechnology branches, after (1).

2. Modes of operation and culture systems

There are three basic modes of bioreactor operation (3):

— batch mode, in which after seeding a liquid medium with an inoculum, nothing (except possibly of some gas) is added to the culture or removed from it as it grows;

— semicontinuous mode, in which some material is added to the culture or removed from it;

— continuous mode, where a culture is maintained in a steady state by removing from it a suspension of cells in the liquid medium at the same rate as the cells are produced within the culture.

Most of the animal and plant cell cultures are carried out in batch or semicontinuous manner. In the repetitive batch mode, a fraction of the culture at the end of a batch is used as the inoculum for a refilled reactor. In the fed batch mode, nutrients are added to the culture as the cultivation takes place. In the perfusion mode, medium is continually flowing through a culture, which is retained in the reactor. Continuous cultures are sometimes carried out, usually as two-stage operations.

Another classification distinguishes between three fermentation systems (4):

— suspended growth;

— attached growth;

— hybrid systems.

In the suspended growth system, the cells or cell aggregates are freely suspended in the nutrient medium and move along with the fluid in the

reactor. In the attached growth system, the cells are immobilised on a supporting surface, acting as an anchor, in the form of a layer in direct contact with nutrient medium. In the hybrid systems, the cells are attached to small particles (microcarriers), or entrapped into small capsules, which are suspended in the nutrient medium. Another possibility is to immobilise the cells between membranes or between a membrane and a wall, with nutrients supplied through the membrane.

3. Main bioreactor types

Different combinations of the above described operation modes and fermentation systems, together with different geometrical and hydrodynamical concepts, resulted in an abundance of bioreactor types. The most popular among them are:

— stirred tank reactors (STR);
— bubble columns (BC);
— gas (air) lift reactors (ALR);
— liquid jet reactors (LJR);
— packed bed reactors (PBR);
— fluidized bed reactors (FBR);
— membrane reactors (MR);
— surface reactors (SR).

Schematic sketches of these reactors are shown in Fig. 2.

In a stirred tank reactor (STR), cells, cell aggregates or microcarriers are freely suspended in a nutrient liquid agitated by a mechanical stirrer, with possible addition of air by a sparger.

In a bubble column (BC), the nutrient liquid is being agitated by a flow of sparged gas, the cells or cell aggregates being freely suspended in the liquid.

In an air lift reactor (ALR), the circulation of liquid and suspension of cells is effected by the gas supplied (sparged) into the liquid.

In a liquid jet reactor (LJR), the agitation of liquid and the entrainment of air is caused by a jet of liquid injected into the reactor.

In a packed bed reactor (PBR), a stationary bed of solid elements, acting as a support for the cells, is penetrated by the flowing liquid.

In a fluidized bed reactor (FBR), the cells, aggregates or microcarriers are suspended in a stream of upward flowing liquid, forming the so called fluidized bed.

In membrane reactors (MR), the culture of cells is immobilized between flat membranes, or contained outside the membrane capillaries in the reactor shell.

In surface reactors (SR), the cells are adhering to flat or coiled support sheets.

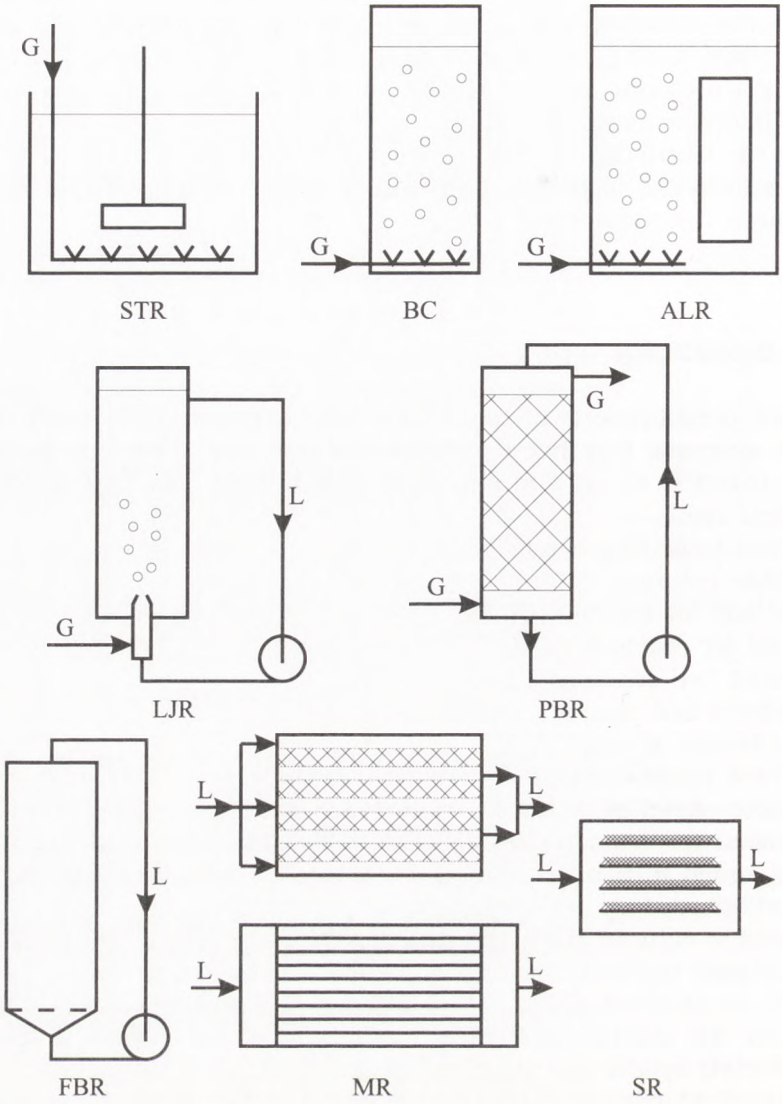

Fig. 2. Schematic sketches of main bioreactor types.

4. Hydrodynamics

Let us now examine some of the essential features of bioreactors. The hydrodynamic characteristics of a bioreactor should ensure:

— maintaining of the cells/cell carriers in a suspension;
— mixing of the bulk liquid and elimination of the dead zones;

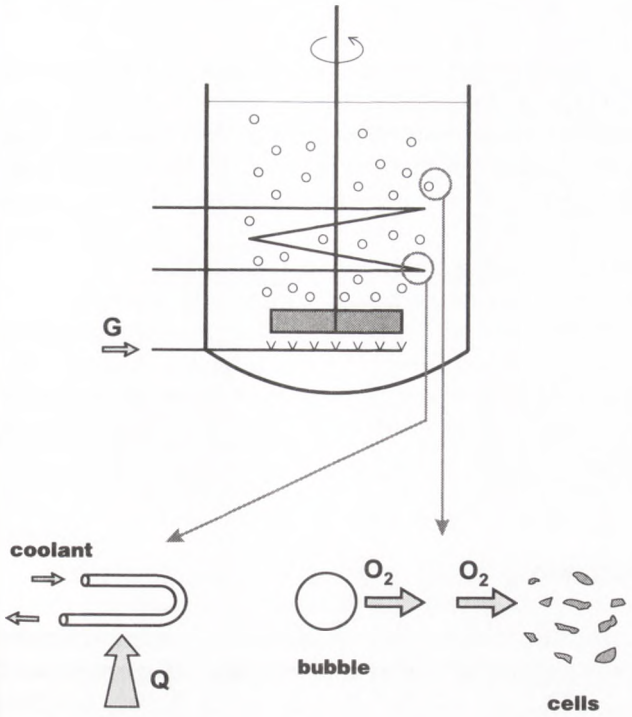

Fig. 3. Transfer processes in a bioreactor.

— good mass transfer from the gas-liquid interface into the bulk liquid and from the bulk liquid to the liquid-cell interface (Fig. 3);

— heat transfer from the liquid to a cooling coil or jacket surface (Fig. 3).

All these tasks must be performed with minimum power dissipation and without introducing too high shear stresses into the liquid, as animal and plant cells are sensitive to shear. This problem shall be discussed later in a greater detail.

5. Mixing systems

In order to agitate the culture mass, power must be introduced into the system. This may be done by:

— mechanical stirrers;

— gas flow;

— liquid flow.

A number of stirrer geometries have been developed in addition to the conventional Rushton turbine. They may provide either radial or axial flow and usually have to be individually selected for a given task (given culture requirements).

6. Heat and mass transfer

As mentioned in the previous section, one important feature of a bioreactor must be good heat and mass transfer characteristics.

Heat transfer is usually not crucial in animal and plant cell cultures, but mass transfer is. There are three mass transfer processes to be considered:

— mass transfer of nutrients to and of the products of metabolism from the cell surface;

— mass transfer of oxygen from the gas phase to the liquid;

— mass transfer of oxygen from the bulk liquid to the cell surface.

Oxygen transfer has been distinguished here, as, owing to low solubility of oxygen it is often of paramount importance. Fortunately, the oxygen demand of animal and plant cells is usually low (typically at least of an order of magnitude lower than that of microbial cultures), it is, however, often a matter of concern for the designer.

7. External and internal diffusion

In the case of cell aggregates or layers, the problem of mass transfer becomes further complicated. Apart from the mass transfer of oxygen and other nutrients from the bulk of liquid to the aggregate (or layer) surface (called external diffusion or external mass transfer), there is mass transfer inside the aggregate (layer), called internal diffusion or internal mass transfer (Fig. 4). This internal diffusion is solely governed by the size and structure of the aggregate (layer), and therefore can be influenced only in very indirect ways.

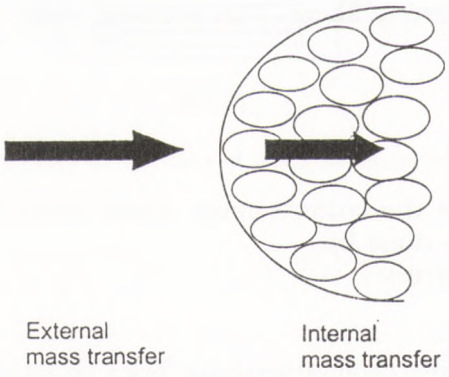

Fig. 4. External and internal mass transfer.

8. Air supply systems

Air is most often introduced by direct sparging. However, as it shall be explained in the next section, bubble generation and bubble disintegration regions are dangerous from the point of view of viability of the cells. Alternative oxygen supply systems have therefore been developed, such as introduction of oxygen or air through membranes, or presaturation of the liquid with the air.

9. Cell viability

As mentioned earlier, plant and animal cells are vulnerable to hydrodynamic stresses (the so called "problem of shear" in biotechnology). Therefore, apart from the need of ensuring proper biological conditions for their cultures, there is also a necessity of avoiding these stresses. The main regions in which cells may be damaged include:

— the vicinity of the stirrer;
— the gas bubble formation regions;
— the bubble bursting regions.

Surprisingly, the most important mechanism of cell damage seems to be that associated with bubble bursting. The cells tend to stick to the bubble surface, and very high stresses produced during the bursting process effectively damage them. There is also evidence that the cells may be damaged in the regions of bubble formation.

10. Power dissipation

In the vicinity of the stirrer, cell damage can result from:

— direct interactions between cells and/or microcarriers and turbulent eddies;
— collisions between microcarriers in turbulent flow;
— collisions of microcarriers against the impeller.

The first two of the above mentioned mechanisms are further complicated by the so called turbulence intermittency (5). The mixing energy supplied to the system is dissipated and eventually converted into heat. However, the dissipation field is not uniform not only macroscopically, but also locally (microscopically), with violent outbursts of the turbulence ocurring in a haphazard way in the fluid. This phenomenon, called local intermittency, complicates the mathematical description of the hydrodynamic situation in the vessel (Figs. 5 and 6). Another important aspect of hydrodynamic stresses action on cells is that they not only influence cells viability, but also can change their metabolism and secretion.

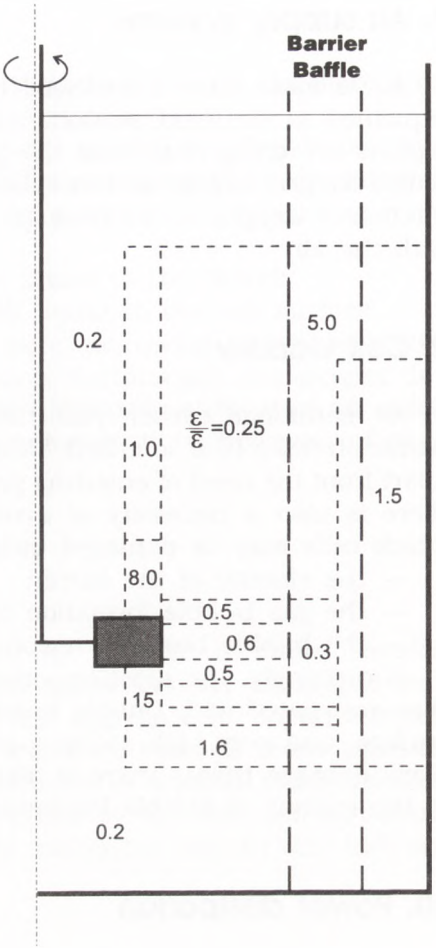

Fig. 5. Macroscopic nonuniformity of power dissipation field (20).

11. Measurement and control

Most commonly measured and controlled process variables include (6):
— temperature;
— pH;
— dissolved oxygen concentration;
— agitation speed;
— air flow rate;
— liquid flow rate;
— foam level;
— pressure.

Control of plant and animal cell cultures is particularly difficult inasmuch as:
— slow growth rates require long cultivation times, which calls for particularly sterile conditions;

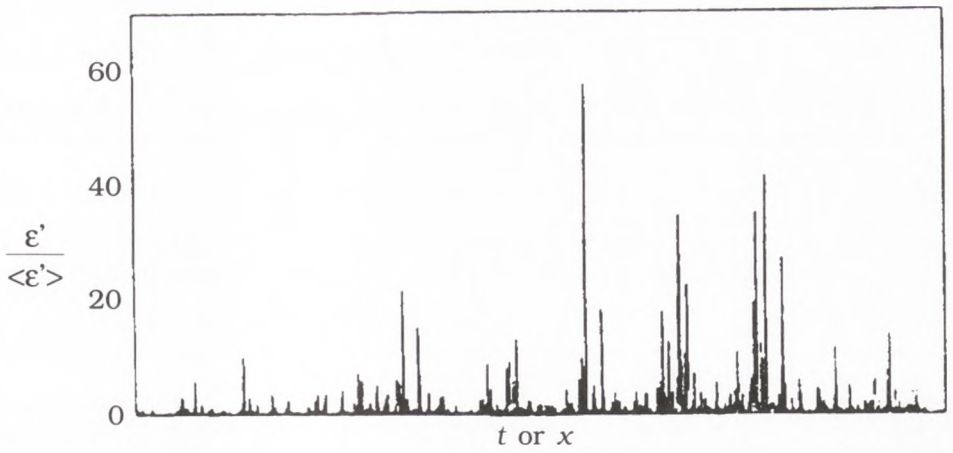

Fig. 6. Microscopic nonuniformity of power dissipation field, after (19).

— modern control techniques making use of computerized controllers require quantitative information about culture behaviour (mathematical models), which, in the case of animal and plant cell cultures, is hardly available. As a result, simple control loops have to be used, usually giving only suboptimal control.

12. New research problems

Among the new, challenging research problems at the interface of biology and engineering, the following are particularly important for the plant and animal cell cultures:

— development of reliable mathematical models of the culture growth kinetics, necessary for the development of effective, optimal control algorithms as well as for reasonable bioreactor design;

— investigation of the effects exerted on cells by hydrodynamic stresses, enabling prediction of cell viability and metabolic changes;

— investigation of the effects of the local intermittence of turbulence;

— investigation of the mass transfer in cultures, especially of the internal mass transfer in cell aggregates and layers.

This short review gives, of necessity, but a glimpse of the vast area. More detailed information can be found in the literature (7-18).

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Summary

Basic modes of bioreactor operation (batch, semicontinuous and continuous), and main culture systems (suspended growth, attached growth and growth on microcarriers) are shortly described.

Main problems in bioreactor design and operation, including: hydrodynamics, heat and mass transfer, cell viability, measurement and control, are discussed.

Some particular problems: mixing systems, air supply systems, power dissipation, external and internal diffusion are discussed in a greater detail.

Examples of specific reactor design are given, namely: stirred tank reactors, bubble columns, gas lift reactors, liquid jet reactors, packed bed reactors, fluidised bed reactors, membrane reactors. New research problems are outlined.

Key words:
bioreactor, plant cell, animal cell, cell culture.

Address for correspondence:
Ryszard Pohorecki, Faculty of Chemical and Process Engineering, Technical University of Warsaw, ul. Waryńskiego 1, 00-645 Warszawa, fax (022) 251440.