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Bioreactor Systems and Effects

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Industrial Animal Cell Reactor Systems: Aspects of Selection and Evaluation

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1 Introduction

The selection, evaluation and development of animal cell reactor systems for industrial applications are, under ideal conditions, aimed at determining an economic and quality optimum for the interactive factors for biology, chemistry, engineering and operations. In this paper we will present an overview of some of the considerations pertaining to these factors and discuss some of the topical aspects of reactor design based on the current state of technology.

The prominence of animal cell technology is largely based on the fact that it is still the only vehicle enabling the correct translation of genes into the complex structures required for functional fidelity (this is not to say that structural alterations necessarily entail functional changes).

Cultured cells vary in their response to the combined effects of physical and chemical variables, so that the optimization of a medium, for example, should therefore also take into account the reactor configuration and operating conditions, and vice versa.

Similarly, cellular activities determine the consumption and secretion of substances which in turn will affect the cellular activities, thus forming a mutually dependent feed-back loop.

2 Complexity of Cells, Reactors and Operations

The trend towards using conventional cell lines, such as Vero, BHK and CHO [1–3] reflects an uncertainty with respect to this technology. Their widespread use cannot be explained on the basis of any inherent benefits of these lines over other potentially available lines. A driving force for their selection as production lines is the availability and participation in knowledge presented in the public domain. The choice of such common lines may also reduce the potential risk and effort involved in the course of product registration. This "bandwagon effect" is similar to that observed with *E. coli*, which was stylized into the standard organism for recombinant DNA work. Ignoring the disadvantages inherent in some of these conventional lines, they do offer the very practical, but short-term benefit of a quick start into a new technology development, by following procedures already established in the literature.

On the other hand, the strategy of employing the same cell line(s) (not necessarily the conventional lines) for a variety of products has a strong economic appeal; it would not only simplify the effort required in the development of the different production processes, but also reduce the work load expended in quality control and risk assessment (i.e. regulatory requirements).

The widespread application of a few "conventional" cell lines as fusion partners for lymphocytes (e.g. SP2/0, NS-1, [4]) has led to hybridomas with similar culture characteristics. To a certain degree this is enabling the development of processes with similar technological features and requirements. However, these technical commonalities are not sufficient to support direct comparisons of the performance characteristics of different reactor systems using different hybridoma lines. The physiological, cultural and product formation characteristics still vary significantly between cell lines.

These qualitative and quantitative differences between cell lines used in the investigations of reactor systems hampers the direct comparison of reactor performance.

The process characteristics described below for individual reactors and conditions must be interpreted critically in the context of what has been discussed so far. The generalizations are intended to serve merely as a guide through the maize of reactors and operating conditions; it is by no means comprehensive nor definitive.

3 Reactor Systems, Elements and Operating Features

In principle suspension-type cells are generally propagated as single cell or aggregate suspensions, whereas anchorage-type cells require a solid attachment matrix to support propagation. This matrix may be a static surface, such as a Roux-type flask, or may also be held in liquid suspension, as is the case with microcarriers and macroporous carriers (see below). In addition to providing an environment "low" in hydrodynamic shear stress, we must satisfy the mass transfer requirements for oxygen, nutrients and products, in order to propagate and maintain the culture.

These basic requirements are then improved upon by the introduction of process control features. Both mass transfer and process control are easier to implement in reactors in which the cells are present in homogeneous suspension.

This was first achieved, on an industrial scale, with standard (microbial) Stirred Tank Reactors (STRs) (Table 3) for the production of Foot and Mouth Disease vaccine [5]. The experience gained from their extensive application in the bioindustry has compensated for complex scale-up characteristics. To-date, the largest reactors of this kind used for animal cells have working volumes of between 5000 and 10000 l.

Prompted by the simplified scale-up and operating features a number of companies have instead adopted for the Airlift Reactor configuration (ALR) (Fig. 1 and Table 1), which has now been used for more than a decade for the industrial propagation of hybridomas and other suspension cells [6, 7].

Instead of using an impeller the cells are held in suspension by sparging alone, which also serves to oxygenate the medium. The oxygenation efficiency has been shown to

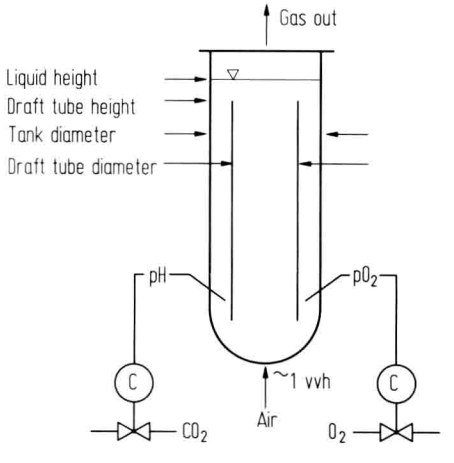

Fig. 1. Basic design of the airlift reactor configuration. Scale-up based on:
Hl/Dt > 6
(Dt^2 − Dd^2)/Dd^2 ~ 1
Hl ... Liquid Height *Dt* ... Tank Diameter
Hd ... Draft Tube Height *Dd* ... Draft Tube Diameter

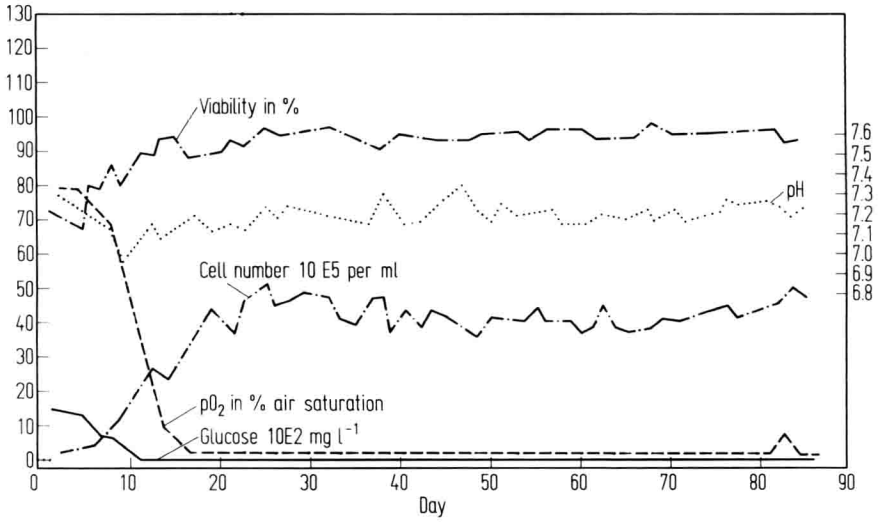

Fig. 2. Continuous propagation of Namalva cells in an 80 l airlift reactor

improve on scale-up and, when using air, is generally sufficient to support cell densities of up to 4 E6 cells per ml; increasing the oxygen concentration in the sparge gas potentially enables increases of up to E7 cells per ml, which of course also depends on the properties of the cell line [8]. Figure 2 presents data from a continuous culture of Namalva cells (used in the production of Alpha Interferon) in an 80 l airlift reactor.

Disadvantages of Airlift Reactors arise from the possible cell damage associated with direct sparging, particularly with microcarrier-based cultures. Operations may also be complicated by the coupling of the agitation and oxygenation functions, and by the probable need for the addition of an anti-foam agent (although this is not always necessary).

Bubble-associated shear damage is avoided in Fluidized Bed Reactors as the cells are propagated within macroporous carrier particles, which are held in liquid suspension. In addition, these reactors may be oxygenated bubble-free using gas permeable membranes.

The concept of the Fluidized Bed Reactor (FBR; Fig. 3) has been applied to many areas of the chemical and microbial processing industry (see [9, 10] and Table 2). In principle the fluidized bed reactor consists of a cylindrical or conical reactor vessel; medium is pumped through the vessel in an upward flow, thereby suspending solid carrier particles in which most (but not all) cells are entrapped. Particle fluidization is determined by such parameters as the particle (material) density relative to the liquid, by the size, shape and the uniformity of the particle, and the liquid velocity. Sparging will also significantly affect the performance characteristics.

The liquid velocity, which should not be less than 1 cm s^{-1}, determines the medium gradient across the length of the reactor and the mass transfer efficiency through the particle.

Solid carrier matrices have been developed so that anchorage dependent cells may be grown in homogeneous suspension. However, cells grown on the surface of such

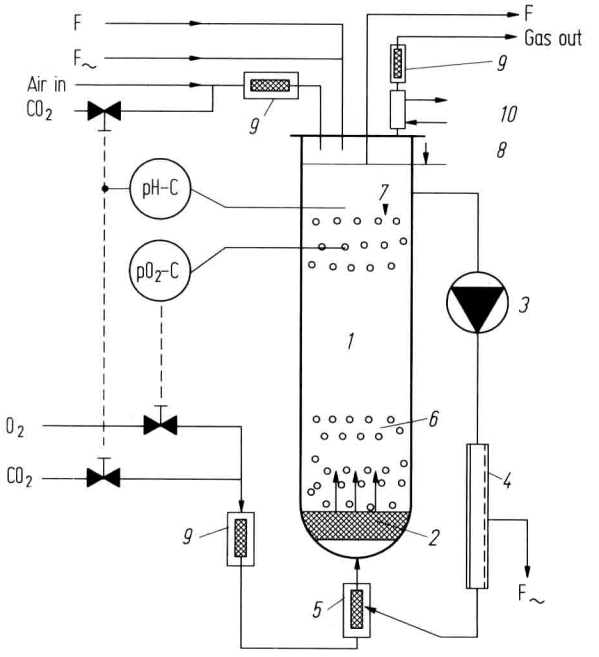

Fig. 3 Configuration features of a fluidized bed reactor.

1 Fluidized bed reactor
2 Flow distributor
3 Low shear pump
4 Cross flow ultrafiltration module
5 Gas microsparger
6 Porous carriers
7 Level of fluidized bed
8 Liquid level
9 Air filter
10 Reflux cooler

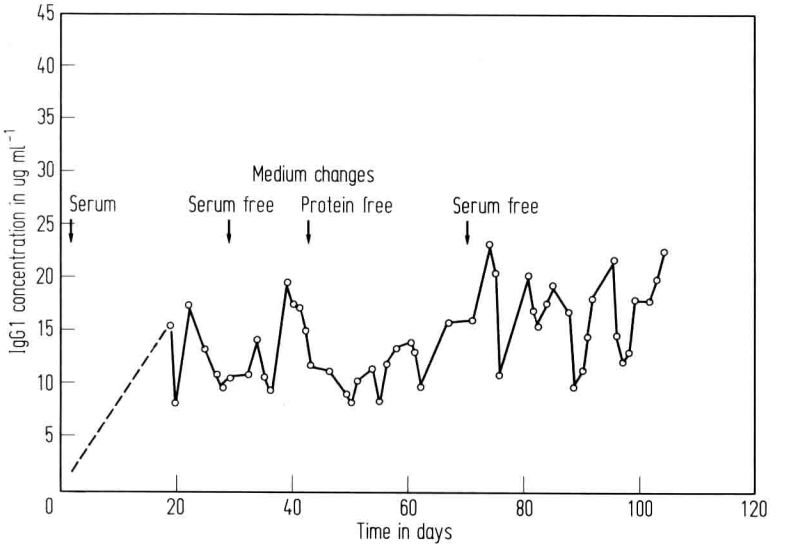

Fig. 4. Continuous propagation of murine hybridoma cell line in a scaled-down glass bead packed bed reactor.
Total bed volume: 1 liter; ~ 35% void volume between beads; medium feed rate: ~ 18 volumes per void volume per d; medium recirculation rate: 1.5 to 2 volumes per void volume per min

matrices (microcarriers) are generally sensitive to bubble associated damage, whereas cells grown within the matrices of macroporous carriers are protected from these effects (macroporous carriers are generally larger than microcarriers, $>500\ \mu$ vs. $<200\ \mu$ diameter respectively). On the other hand the cells may encounter mass transfer limitations, although experimental data on diffusion characteristics within such macrocarriers is only beginning to emerge. These carriers must of course consist of an inert material such as glass (Katinger, unpublished), alginate [11], collagen (Verax Corp., USA; Perstorp Biolytica AB, Sweden). With collagen carriers, care must be taken to ensure that the collagen is neither enzymatically degraded nor introduces contaminants into the raw product (see [12] for immobilization techniques).

The volume fraction of the suspended carriers can comprise up to 90% of the working volume of the reactor, thereby enabling cell concentrations which are much higher than those typical for stirred tank reactors.

With appropriate carriers the fluidized bed system can be scaled up as a unit process to any currently relevant capacity using standard bioprocess equipment. Although there is still room for improvement, especially with respect to the carriers, the fluidized bed concept comprises many of the features desirable for animal cell propagation, such as high cell retention and perfusion capabilities, simple scale-up parameters and bubble-free operation. The mixed particle suspension also facilitates process control and uniform medium dispersion.

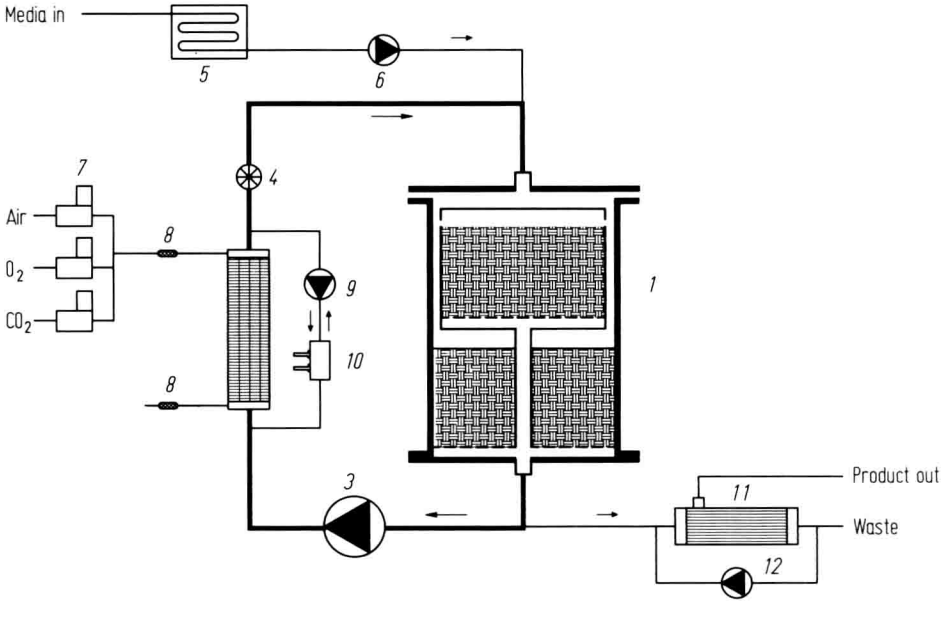

Fig. 5. Packed bed reactor with improved scale-up features

1 Packed bed reactor	*7* Gas mass flow meter-controllers
2 Silicone membrane lung	*8* Gas filters (0.2 μ)
3 Low shear pump	*9* Reversable sample pump
4 Mass flow meter	*10* pO_2 & pH sensors
5 Media pre-heater	*11* Cross flow clarification module
6 Media pump	*12* Recirculation pump

Typical problem areas concerning this reactor type, apart from the carriers, are the design and operation of the recirculation pump and of the oxygenator (fouling problems, oxygen transfer limitations etc.), if it is to be operated bubble-free.

A low shear and bubble-free alternative to the use of microcarriers and macroporous carriers for the propagation of anchorage-dependent cells lies in the application of Packed Bed Reactors (PBR). However, it has been demonstrated that this reactor type can also be employed as an efficient means of propagating suspension cells (demonstrated with hybridoma cell lines, see below).

It also appears that the high local and overall cell densities (based on the cell aggregates and low total reactor volume, resp.), a feature of this reactor, enable a significant reduction in the amount of protein required for serum-free cultures. For example, it has been possible to propagate some hybridoma lines continuously (over several weeks) using protein-free medium alone; the same lines could not be maintained without the addition of transferrin and insulin in a stirred (low shear) spinner flask or 15 liter reactor under otherwise comparable culture conditions (Bliem R, Varecka R, Oakley R, publication in preparation; Fig. 4).

This reactor type comprises a culture vessel packed with inert particles (glass, polyurethane, ceramic, etc.), usually solid or porous spheres in the range of 2 to 5 mm in diameter [13–16]. This packed vessel is incorporated within a medium recirculation

Table 1. Selection criteria for airlift reactors

Process requirements	Reactor features (current status)
Volumetric capacity	preferable: > 10 l reactor volume; largest units: 800 to 2000 l reactor volume (Bender Austria; Celltech, UK);
Typical cell count per reactor unit	1 E10 to 8 E12[a]
Typical cell density	2 to 4 E6 cells per ml
Number of parallel products	few
Mode of operation	batch or continuous (perfusion difficult);
Typical length of run	weeks to months;
Process control	homogeneous cell suspension; direct on-line monitoring and control of pH, pO_2 etc.
Cell line stability	generally required to be > 50 generations;
Product type	cell mass production; secreted or cell-associated product; virus production;
Medium	protein and anti-foam additions usually required;
Scale-up	simple design and engineering as unit process system; economic automation; good economy of scale;
Operational constraints	bubble-associated damage to microcarrier-based cultures likely;
Operating benefits	no pumps; no oxygenator; no moving parts;
Maintenance	simple maintenance; steam sterilization;

[a] E represents the computer notation for "10 to the power of"

loop, connecting the reactor with an oxygenator. Cells initially occupy the surface of the packing. As the cells multiply they begin to fill the void space between the particles, the extent of which depends on the structural features of the packing. As the cells fill the void space, and over extended culture periods (months), the reactor becomes prone to channelling and gradient formation; these effects are exacerbated on scale-up. A new reactor configuration alleviates these effects (Fig. 5).

Scale-up of this reactor configuration is simple and may be achieved on the basis of linear velocity alone, provided that the packing is uniform and has been well charac-

Table 2. Selection criteria for fluidized and packed bed reactors

	Reactor features (current status)	
Process requirements	Packed bed reactors	Fluidized bed reactors
Volumetric capacity (continuous perfusion)	up to approximately 300 l per reactor per day (~ 1000–2000 l batch equivalent; Bio-Response, USA; Verax, USA; IAM, Austria)	
Number of parallel products	several possible	
Typical cell count per reactor unit (current status)	up to 2 E12 cells	
Typical cell density (based on bulk working volume)	5 E6 to 5 E7 cells per ml (aggregate densities up to 5 E8 cells per ml)	
Mode of operation	continuous operation over extended periods most economic, although batch possible;	
Process control	indirect measure of cell number and condition only	macroporous carriers can be sampled but representative cell measurements problematical
	pH, pO_2 etc. measured on-line in recirculation loop;	
Cell stability	cell line should be stable over > 50 generations;	
Product type	preferably secreted products; virus production possible;	
Medium	use of protein-free media apparently facilitated;	theoretically high medium utilization due to high cell densities and mixed suspension;
Scale-up	economy improves on scale-up; simple scale-up design;	
Operational constraints	oxygenators and pumps can present engineering problems; complicated to use as seed stock reactors; potential diffusion limitations;	
	no direct measurements of cell mass;	cost and design of carrier matrices; representative cell measurements difficult;
	handling effort of carrier matrix	
Operating benefits	cell retention; low shear stress; reusable packing; low inoculation densities (possible (0.01 of harvest count);	homogeneous aggregate suspension (facilitates control); cell retention; low shear stress;
Maintenance	packing and carriers require handling effort; steam sterilization;	

terized, both under cell free and under culture conditions. This system therefore also offers many of the features desirable in cell culture, i.e. high cell retention and perfusion capabilities, simple scale-up and bubble-free operation, in addition to the potential medium benefits enabled by the high cell densities. The ability to obtain a direct and representative measure of the cell densities and viability remains to be developed. The recirculation pumps and the oxygenators are also critical elements of this system (and have therefore been the focus of a development program at Bio-Response).

In principle membrane reactors, such as hollow fiber (see below) or flat sheet membrane reactors [17] differ from the packed bed reactor in that the packing consists of a permeable membrane, seperating the bulk medium flow from the cell mass. The medium may be perfused (convective flow) through the cell mass and collected on the cell side, or exchanged (largely) by diffusion across the membrane. This type of reactor is very well suited to the production of moderate quantities of several different antibodies simultaneously; a typical reactor unit may contain between 1 E10 and 1 E11 (E denotes "to the power of 10") cells, although the viability may vary considerably with operating conditions [18–21]. Whereas suspension cells perform well in this reactor type, anchorage-dependent cells are prone to clog the reactor membrane and introduce significant diffusion limitations. The membrane material should also be tested for product binding properties.

Table 3. Selection criteria for hollow fiber and stirred tank reactors

	Reactor features (current status)	
Process requirements	Hollow fiber reactors	Stirred tank reactors
Volumetric capacity	4 to 8 l per unit per d (per ~ 50 ml extracapillary space and ~ 0.5 m^2 extracapillary extracapillary surface) up to 200 units per facility practical (Xoma, USA; Bio-Response, USA)	Preferable: > 5 l per reactor per d; Largest reactors: 5000 to 10000 l reactor volume (Wellcome, UK; Genentech, USA);
Typical cell count per reactor unit	2 E9 to 5 E10 (flat-membrane reactors up to 5 E11)	1 E9 to 5 E13
Typical cell density	1 E7 to 5 E8 cells per ml;	2 to 4 E6 cells per ml; up to 3 E7 cells per ml possible with cell retention;
Number of parallel products	several products (flexible production system)	few products
Mode of operation	continuous perfusion preferable	batch or continuous; (perfusion possible)
Typical length of run	months	weeks (months)
Process control	indirect measure of cell density and condition; heterogeneous cell mass;	direct monitoring and control of homogeneous cell mass;
Cell stability	cell line stability dependent on scale and duration of culture (generally > 50 generations) (both systems);	

Table 3. (Continued)

	Reactor features (current status)	
Process requirements	Hollow fiber reactors	Stirred tank reactors
Product type	secreted product;	cell mass production; secreted or cell-associated product; virus production;
Medium	high cell densities facilitate use of serum-free media;	optimum medium supply to cells in single-cell suspension;
Scale-up	linear increase in cost; good predictability if increase in capacity by multiple units; (reactor scale-up difficult); low initial capital costs	economy improves on scale-up; scale-up as unit process; economic automation; low running costs (compared for example to HFRs);
Operational constraints	control limitations; increasing number of units on scale-up; impractical as seed stock reactors; diffusion limitations;	using direct sparging: bubble-associated cell-damage; foaming; (bubble-free oxygenation now possible for industrial scales);
Operating benefits	bubble-free oxygenation; cell-free supernatant (simple cell retention); on-line concentration possible; low space requirements for small product quantities;	good in-process control possible;
Maintenance	disposable units; labor intensive routine; sterilization: irradiation, ethylene oxide (rarely steam);	sterilization: steam;

The relatively simple operating requirements of hollow fiber reactors are a distinct benefit, although they rely on special techniques and skills. The reactor units may be obtained at a cost at which their disposability is still economic. The reactors may be operated in such away as to yield cell free and product-concentrated supernatant, which translates into significantly lower down-stream processing costs.

Scale-up is currently only possible by multiplying reactor units. Although this limits their economy of scale, their small size and simple design facilitates easy handling, and the simultaneous production of several products within the same facility (Bio-Response Inc. uses Hollow fiber reactors routinely for the production of up to 100 g quantities of several monoclonal antibodies simultaneously).

Finally, in Tubular Reactors the cells are propagated whilst slowly flowing along the length of a tube or along a series of connected reactors. A reactor of this type is currently being developed for industrial application [8, 22].

4 Propagation Mode : Batch vs. Continuous

A determination of the propagation mode, batch or continuous, should be based on a careful analysis of the product formation characteristics, capital and operating expenses, and the required level of process control, raw material and product quality.

A typical batch culture lasts between 5 and 15 days, depending on the inoculation density and the culture growth characteristics. After an initial adaptation period (lag phase), the growth rate of the culture increases, until nutrient depletion and waste product formation limit continued growth and subsequently lead to culture degeneration and lysis.

Some two thirds of the duration of a batch process comprise culture growth. Therefore this form of propagation is an option for products which are formed preferentially during culture growth (growth-associated product formation), or in which the product is formed both during growth and non-growth (lag or stationary) phases (constitutive product formation). However, if the product is formed constitutively, as appears to be the case with most proteins of current industrial interest, including monoclonal antibodies, continuous production systems offer economic advantages; the underlying principles are well established for microbial and chemical processes [23–25] and equally apply to animal cell culture [8]; Bender GmbH and Bio-Response, unpublished information).

One obvious advantage of continuous culture therefore lies in the savings of repeated growth cycles. Continuous cultures are generally operated well below the maximum growth rate, thereby reducing the medium wasted on biomass production (this is of course of little value if the desired product is biomass associated, e.g. membrane antigens, or involves lytic virus production).

Operating expenses are also comparatively lower for continuous culture as a result

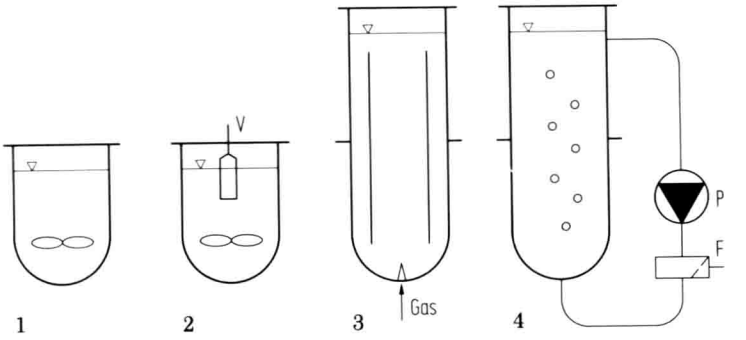

Fig. 6. Exchangeable reactor configurations (Unit Process Modules) for continuous process operations at IAM
1 STR, standard microbial reactor configuration with axial flow impeller; Hl/Dt ~ 3; *2* STR, as 1., with vibro-cage (*V*) for oxygenation and cell (microcarrier) retention; *3* Airlift, with or without vibro-cage; Hl/Dt ~ 6; *4* Airlift configuration as Fluidized Bed with on-line cross-flow filter module (*F*); (*Hl* ... Liquid Height *Dt* ... Tank Diameter
P ... Recirculation Pump)