FUNDAMENTALS OF FERMENTER DESIGN

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ABSTRACT

The influences of environmental (macro) conditions and those on the cell level are treated from the point of view of heat- and mass-transfer in microbial systems. The effects considered are (i) consumption of substrates, (ii) output of products, (iii) production of heat and (iv) aggregation, as far as concerns direct influence on the microenvironment. The other properties which may have indirect effects on the microenvironment considered are (a) changes in the rheological behaviour of the culture, and (b) changes in interfacial tension. The problem of environmental control is treated for the case of a well-stirred fermenter, and the control factors considered are system geometry, aeration rate, intensity of agitation, temperature, pressure, nutrient supply, pH and other parameters involving specific ions, dilution rate in continuous systems, and foaming.

INTRODUCTION

The design of fermentation equipment has evolved in a largely empirical manner. The earliest fermentations required only rudimentary standards of hygiene, either because of the nature of the substrate, or because the vigour of the desired organism exceeded that of potential competitors, or because the products of fermentation were inhibitory, or because the expected shelf-life of the product was short. This applied to the fermentation processes involved in the manufacture of wine, beer, cheese, yoghurt, vinegar, sauer-kraut and so on. Even with these products the transition from domestic art to commercial practice required improved standards to increase shelf-life and to maintain acceptably-consistent standards of product quality, but equipment and process control remained essentially simple. Some sophistication occurred with the development of pure-culture techniques in beer-making, but the first really fastidious fermentation, the manufacture of acetone and butanol, was initiated on a large scale less than sixty years ago. In this system the maintenance of a strictly anaerobic environment was essential. This provided protection against a wide range of contaminants requiring atmospheric oxygen, and the principal hazard was contamination by bacteriophage.

The next major development was the adaptation of submerged-culture techniques for the production of penicillin, rapidly followed by processes for other antibiotics, vitamins, and amino acids, and the conversion of steroids. These processes have been developed over the past thirty years or so, and

have attracted the combined attention of microbiologists, biochemists and engineers to resolve a variety of problems in order to improve both yields and process efficiency. More recently, a good deal of attention has been devoted to problems associated with the replacement of batch processes by continuous processes, and the utilization of gaseous and liquid hydrocarbons as substrates, instead of the traditional carbohydrates. The latter processes, in particular, have stimulated investigations into the use of fermenter configurations other than the agitated cylindrical vessel which has been almost universally adopted for aerobic mirobial processes and for some anaerobic processes. Among the new configurations, two in particular – the air-lift and the tower—have been exploited commercially and have attracted the attention of research workers.

FERMENTER DESIGN AND THE REQUIREMENTS OF THE MICROBIAL SYSTEM

The function of the fermenter is to provide an environment suitable for the controlled growth of a pure culture or of a defined mixture of organisms. The materials of construction must be such that they will not adversely affect, nor be adversely affected by, the desired microbial activity, either by interaction with the fermentation medium or by harbouring unwanted organisms. They must be resistant to corrosion by the nutrient medium and products, and to the effects of sterilization temperatures. The actual construction of the equipment from suitable materials must also take account of these factors and of the stresses imposed by pressurization and the weight of the vessel contents. There must be provision for the regulation of temperature and of the supply of air, for charging and discharging the vessel contents, for inoculation, and generally also, for sampling and for the control of pH and foaming. Frequently, even in batchwise systems, it will be necessary to provide for controlled addition of nutrients or other materials during the course of the fermentation.

In continuous-culture systems additional facilities must be provided to control culture volume and medium flow-rate. Depending on the method of control, some system will be required to measure and control the cell concentration or the concentration of some rate-limiting substrate. These controls relate to the macroenvironment (the total culture) and only measure or control the conditions of the microorganisms in an indirect, empirical sense. Some control systems, such as those based on the rate of oxygen uptake, evolution of carbon dioxide or concentration of $NADH^+$, relate more directly to cell activity, but none gives a direct measure of the condition in the microenvironment, that is the environment in the immediate vicinity of the cell.

It is an assumption implicit in all control systems that biochemically-similar macroenvironments produce biochemically-similar microenvironments. Anomalies and difficulties which arise in scaling-up or in conducting a given fermentation in different types of equipment indicate that this assumption may be false. One possible source of anomalies is differences in the extent of cell aggregation, which, in biochemically-similar environments, may be strongly affected by hydrodynamic factors. Another likely source of

error lies in systems for assessing conditions in the macroenvironment. These will generally indicate a time-average value at a particular sampling or sensing point and may conceal considerable variations from one element of culture to another. Thus attempts to control conditions in the culture as a whole may have somewhat attenuated effects on the microenvironment.

At the same time, the activity of the microorganism will itself have effects on the microenvironment, both directly and through effecting changes in the macroenvironment. The direct effects on the microenvironment will result from: (a) consumption of substrates, (b) output of products, (c) production of heat, and (d) aggregation.

Factors (a) and (b) will result in deficiency of substrate and accumulation of product, with the possibility of resultant inhibition, unless there is adequate interchange between the region immediately adjacent to the cell and the bulk of the fluid. A similar interchange is also necessary to prevent significant and possibly damaging increase in temperature. Aggregation of cells will adversely affect both heat and mass transfer, partly by reducing the area available for transfer, partly by increasing the length of the transfer paths and partly by reducing the transfer coefficients.

The effects of the organism on the macroenvironment, which may be reflected in concomitant changes in the microenvironment, arise mainly from (a) changes in the rheological character of the culture and (b) changes in interfacial tension.

Changes in interfacial tension arise as a result of changes in the composition of the medium owing to the metabolic activities of the organism, and may affect both effective mass-transfer coefficients and the state of dispersion of the microorganisms and gas bubbles.

Rheological changes may result from the breakdown of macromolecular substrates, the formation of extracellular macromolecular products or simply from interference between cell aggregates. This latter effect is particularly pronounced for filamentous organisms and frequently results in non-Newtonian behaviour. These changes may have marked effects on rates of mass transfer and on the quality of mixing.

Although, ideally, it would be desirable to relate fermenter design and control systems to the microenvironment, in practice only the macroenvironment can be influenced directly. The factors within the control of the designer and operator are: (a) system geometry, (b) aeration rate, (c) intensity of agitation, (d) temperature, (e) pressure, (f) nutrient supply, (g) pH and other parameters involving specific ions, (h) dilution rate in continuous flow systems, and (i) foaming.

When considering the design of vertical stirred vessels, the main variables in geometry are the height-to-diameter ratio, the number, type, dimensions and positions of impellers, the number and breadth of baffles, and the design and location of coils for heating and cooling. In relation to power input, the geometrical specification for the impeller and the degree of baffling cannot be divorced from the speed of rotation. Some account must also be taken of the rate of aeration and degree of gas hold-up in the system, since this will affect the density of the culture which is the most significant property governing overall power input under conditions of fully-developed turbulence.

Control of the other factors mentioned above will have only minor effects on vessel design arising from the introduction of facilities for sensing, sampling, addition and withdrawal.

A major advantage of the stirred vessel over other designs, and one which may largely account for its popularity, is the degree of operational flexibility which it provides even when installed. This arises largely because mixing and mass transfer are influenced both by the action of the impeller(s) and by the rate of aeration, which can, within fairly wide limits, be varied independently, albeit at the expense of changing the impeller speed or geometry. By contrast, in the air-lift mixing and mass transfer are both dependent, in a given piece of equipment, on the rate of aeration, and cannot readily be varied independently. In the tower fermenter, unless provision is made for re-cycling, mixing and mass transfer are strongly affected by the dilution rate, which must be determined on biological grounds, whereas the dilution rate can be fixed independently of considerations of aeration and mass transfer in stirred vessels and air-lift fermenters.

CHEMICAL ENGINEERING ASPECTS OF FERMENTER DESIGN

The objective of fermenter design and operation is to ensure that the desired activity of the microorganisms concerned shall not be restricted by the characteristics of the equipment. It is, therefore, useful to consider the problem in terms of the physical processes which might limit microbial activity.

Mass transfer

The transfer of mass within the system is fundamental to the whole operation. There are two aspects to this process—the more-or-less uniform distribution of substrate and product molecules in the bulk of the fluid, and transfer between the bulk of the fluid and the microbial cells. The first aspect is largely governed by the forced convection and turbulence produced by agitation and the flow of gas, whereas transfer between the bulk and cells is determined by diffusional forces. It is not necessary that there should be a uniform concentration of any given component in the bulk of the fluid; only that it should not at any point fall below (in the case of substrate) or rise above (in the case of product) the level at which the desired level of microbial activity will be reduced. With materials whose solubility is many times greater than the rate-limiting concentration this generally presents no problem, although this must not be assumed to be the case in continuous flow chemostats where control is exercised by deliberately maintaining the concentration of a selected substrate at a rate-limiting level. For materials whose solubility is relatively close to the limiting concentration uniformity of distribution is more important. This could apply, for instance, to mineral salts of low solubility and, perhaps more importantly, to substrates such as steroids and hydrocarbons. Most importantly it applies to oxygen. When supplied in a stream of air, as is usually the case, the equilibrium concentration in fermentation media is only of the order of five to ten times the limiting concentration, even in the absence of microbial activity. In an actively respiring culture this margin is further reduced. Moreover, the amount in

'suspension' (in the form of bubbles) is limited by the gas hold-up in the system, a limitation which does not apply to the same extent to liquid substrates and hardly at all to solid substrates.

The diffusional mass transfer of a component between two phases may be expressed as the product (mass transfer coefficient × interfacial area × concentration driving force). In dispersed systems it is frequently difficult to determine separately the interfacial area and the mass-transfer coefficient related to unit area, and these are combined to give a modified expression (volumetric mass-transfer coefficient × concentration driving force). The volumetric mass-transfer coefficient is the product of the specific mass-transfer coefficient and the interfacial area per unit volume of dispersion. It is easier to determine this than to determine its separate components, and it is a useful parameter to employ, both in scaling-up and in comparing different forms of contacting devices. This form of expression can be used to describe the transfer of substrate to microorganisms or of product from the micro-organisms to the bulk of the fluid. It also applies to the supply of oxygen or of materials of low solubility from suspended drops or particles to the bulk of the liquid, and to the transfer of volatile fermentation products from the bulk liquid to the air-stream by which they are removed from the system.

Various models have been proposed to describe the process of mass transfer at an interface. One feature common to all models is the postulate that the mass-transfer coefficient increases as relative motion between the two phases or the degree of turbulence increases. Thus, it might be anticipated that the mass-transfer coefficients in submerged microbial systems could be influenced by the intensity of agitation.

For particles of a given size and density in a specified fluid the available evidence suggests that the specific mass-transfer coefficient is unaffected by the intensity of agitation provided that all particles are freely suspended. Calderbank and Jones[1] have shown that, for systems in which the density difference between the continuous and dispersed phases is small, the specific mass-transfer coefficient is inversely proportional to the diameter of the suspended particles. This will apply to a suspension of microorganisms in an aqueous medium, and suggests that there will be a beneficial effect from levels of agitation which prevent aggregation of the microorganisms. There will be a further advantage in that increasing the degree of dispersion will increase the interfacial area available for transfer between a given mass of organisms and the suspending medium.

If the density difference between the dispersed and continuous phases is large, the specific mass-transfer coefficient is generally independent of particle diameter. In the case of gas bubbles, however, two regimes can be distinguished[2]. Small bubbles (diameter < 0.25 cm.) behave as rigid spheres, whereas large bubbles can be distorted by hydrodynamic forces. In a given gas–liquid system the specific mass-transfer coefficient for large bubbles is greater than that for small bubbles, but in each region the coefficient is unaffected by the actual size and intensity of agitation. This suggests that any effect of agitation on the volumetric mass-transfer coefficient must arise from increases in surface area, as a result either of reducing bubble diameter within the regime concerned or of increasing gas hold-up by recirculation and back-mixing. It is generally assumed that stirred, sparged fermentation

systems operate in the small-bubble regime, but recent results obtained in our laboratories[3] suggest that some systems may operate in the transition region. If this is the case, both absolute and volumetric mass-transfer rates will be very unpredictable, and this may lead to difficulties both in scaling-up and in comparing different types of equipment.

For medium constituents which are present in the bulk fluid initially, in sufficient amounts to meet the requirements of the fermentation, only the rate of mass transfer from the fluid to the cells will be significant. When a component is added continuously during the course of the process, its availability to the organism may be determined either by the rate at which it is supplied to the nutrient fluid or by the rate at which it is transferred from the nutrient fluid to the cells. Clearly, if the overall rate of supply is below the demand of the organism microbial activity will decline. On the other hand, simply adding a component at an overall rate sufficient to match microbial demand will not be adequate unless the rate of transfer to and through the bulk fluid is sufficiently high. This is clearly seen for aeration, since, in many cases, high microbial demand rates cannot be met by sparging unassisted by mechanical agitation. A similar situation applies to the use of liquid or gaseous hydrocarbons as substrates. Although it has been suggested that hydrocarbons may be taken up directly by microorganisms, recent evidence[4] strongly suggests that for these substrates, like others, the bulk of the transfer is through the aqueous phase.

In an actively metabolizing culture, the concentration in the bulk fluid of any substrate present as suspended particles, bubbles or drops will be below the saturation or equilibrium concentration in the sterile medium under similar conditions. A steady-state or pseudo-steady-state concentration will prevail and will be determined by the balance between the rates of supply and demand. Thus we may write for the rate of supply

$$\left(\frac{dC}{dt}\right)_s = k_s a_s \Delta C_s \tag{1}$$

where $\left(\frac{dC}{dt}\right)_s$ = rate at which the substrate is being supplied to unit volume of culture.

k_s = supply-side mass-transfer coefficient

ΔC_s = concentration driving force

a_s = interfacial area per unit volume.

For the demand, we may write

$$\left(\frac{dC}{dt}\right)_d = U_0 C_0 \tag{2}$$

where $\left(\frac{dC}{dt}\right)_d$ = rate of consumption of substrate per unit volume

U_0 = specific uptake rate, i.e. rate of consumption per unit mass of organisms

C_0 = concentration of organisms.

Now $\Delta C_s = C_b^* - C_b$

where C_b^* = concentration of substrate at equilibrium in the absence of demand

C_b = concentration at any time t

Thus $$\left(\frac{dC}{dt}\right)_{nett} = \left(\frac{dC}{dt}\right)_s - \left(\frac{dC}{dt}\right)_d = k_s a_s(C_b^* - C_b) - U_0 C_0 \quad (3)$$

At steady state

$$\left(\frac{dC}{dt}\right)_{nett} = 0$$

i.e. $k_s a_s(C_b^* - C_b) = U_0 C_0$

$$C_b = C_b^* - \frac{U_0 C_0}{k_s a_s} \quad (4)$$

It will be seen that the steady-state concentration falls below the equilibrium concentration in the absence of demand, by an amount determined by the ratio of demand to the volumetric supply mass-transfer coefficient. This will result in limitation of biological activity if the bulk concentration falls below the value at which activity becomes concentration-dependent. Clearly, this can happen most readily for substrates in which k_s and the ratio C_b^* to C_b are small, conditions which apply to substrates of low solubility. Equation 4 also shows that maintenance of a relatively high bulk concentration is assisted by producing a high interfacial area between the substrate and bulk liquid.

Heat transfer

Heat is generated in submerged microbial systems, partly by the metabolic activity of the organisms and partly as a result of the mechanical work performed by the agitator and by the gas-bubbles as they expand in passing from the sparger to the head-space above the culture. In mechanically-agitated systems the work performed by gas expansion is usually a small proportion of the total.

Some heat is lost as a result of increased humidification of the gas stream in its passage through the culture and some by radiation from the outer surfaces of the fermenter. In small vessels these losses may be so large as to make it necessary to supply additional heat to maintain the desired temperature. As the scale of operation increases, the situation with regard to heat dissipation changes. Frequently, the relative volumetric gas-flow rate will be reduced, thereby reducing relative loss of heat by evaporation. More importantly, for geometrically similar vessels the volume (and, therefore, the total heat generation) increases as the third power of the linear dimensions, whereas the surface area increases only as the second power. As a result, a scale is reached at which the loss of heat by evaporation and radiation is insufficient to prevent the temperature rising above the desired level. It is then necessary to provide additional cooling, either by passing a coolant over or around the wall of the vessel or by fitting cooling coils inside the vessel. This then serves to remove heat by conduction.

The transfer of heat between the microorganisms and the surface of the vessel is somewhat analogous to that of mass transfer, involving interchange between the cells and bulk fluid and between the bulk fluid and the cooling surfaces. As in the case of mass transfer, the temperature in the bulk fluid is determined by a dynamic balance between the processes of heat evolution by the cells and agitator and of heat removal through the vessel walls. The direct determination of cell-to-fluid heat-transfer coefficients is much more difficult than the determination of mass-transfer coefficients, but it can be anticipated that the accumulation of heat within cell aggregates at a given level of metabolic activity will increase as the size of the cell aggregates increases. Thus, for a given temperature in the bulk fluid the average temperature of a cell will be expected to increase with increasing aggregation.

In one important respect the process of heat transfer differs from that of mass transfer. Whereas mass transfer takes place throughout the whole of the dispersion, the interchange of heat with the surroundings takes place at well-defined and fixed boundaries. The effects of hydrodynamic factors are more definable in this situation. In particular, changes in the velocity of the fluid over the heat-transfer surfaces may have marked effects on the rate of heat transfer, and such changes may occur as a result of changes in the rheological character of the culture. Another effect of biological activity is the fouling of heat-transfer surfaces, either by attack by materials in solution or by deposition of cells. Such fouling reduces the overall heat-transfer coefficient. Apart from the choice of constructional materials, only the size and positioning of baffles will substantially affect the situation at the vessel walls. The design, location and supporting of coils, however, should be such as to minimize the risk of deposition by ensuring free flow of the suspension over the surface of the coils.

Mixing effects

Mixing serves to minimize local variations in concentration and temperature. It arises from the random redistribution of elements of the culture suspension in the impeller zone, and from the random interaction, with the bulk of the suspension, of streams leaving the impeller. In a given system the rate of mixing is a direct, though not necessarily linear, function of the mass flow-rate from the impeller. This may be expressed as a mean circulation rate, i.e. the number of times that a volume of suspension equal to the total volume passes through the impeller zone in unit time. Alternatively, one may consider the mean circulation time, i.e. the time that is required for a volume equal to the total volume of culture to pass through the impeller zone. This will represent also the average time which will elapse between two successive passages through the impeller zone of a given element of suspension. Because of the random nature of the processes, the actual circulation times for a series of elements will be distributed about this mean.

For batch systems in which all particles are freely suspended, the distribution of circulation times is of little importance, since there will be adequate mass transfer throughout the suspension. The situation may be quite different, however, if the extent and nature of microbial growth is such as to modify the rheological properties substantially. For instance, physical interference among cell aggregates frequently results in pseudoplastic

behaviour. In the impeller region the individual aggregates are freely suspended in the nutrient medium, whilst retaining their integrity. As they pass into the bulk of the culture, they assemble into clusters as a result of experiencing less intense hydrodynamic forces. Mass transfer between these clusters and the bulk of the fluid may be much less than that characteristic of the individual aggregates. Consequently, nutrients available in limited amounts may become exhausted, or there may be excessive local accumulation of metabolic products, if such an assembly experiences a longer-than-average circulation time. This may result in reversible or irreversible loss of biological activity. Such effects may be particularly important in the case of oxygen supply, since subjection of actively respiring organisms to conditions of oxygen deficiency may result in reduction of respiratory activity, or even a transition to anaerobic metabolism for facultative organisms.

If growth of the organism results in a marked increase in viscosity and, more particularly, if the system becomes pseudoplastic, these effects are accentuated by two factors— the establishment close to the impeller of a zone of low effective viscosity, through which much of the air will be channelled, and the establishment near the walls of the vessel of stagnant zones. Such zones may become permanently depleted of oxygen, resulting, at best, in reduced productivity, or more seriously, in lysis, anaerobiosis or other undesirable metabolic activities. Channelling of air around the impeller, and around the impeller shaft in multi-impeller or top-entry configurations, may cause flooding of the impeller and marked reductions in mass transfer and mixing.

Each impeller produces two mixing zones, one above and one below the impeller level. Each of these zones can be considered to be well-mixed, but the extent to which the system as a whole can be considered to be well-mixed depends on the rate of interchange of material between the two zones[5]. This effect and the formation of stagnant zones, mentioned earlier, have important implications for the location of sensors and sampling points, and arrangements for the addition and withdrawal of material during the course of a fermentation[6]. These considerations are of particular importance in relation to continuous culture, especially in systems operating as chemostats, in which deviations from perfect mixing may lead to unpredictable performance and difficulty of control.

SCALING-UP

No matter what criteria are used for scaling-up it is not possible to reproduce exactly similar conditions on all scales of operation.

One set of criteria which has been extensively employed maintains similarity of three parameters—geometry, power input per unit volume and superficial air velocity. The effect of the latter is to reduce the relative volumetric air-flow rate as scale increases. This in itself is unimportant provided that the overall rate of supply is sufficient to meet the oxygen supply and purging requirements of the system, and that the necessary rates of mass transfer can be maintained—a criterion which depends perhaps more on the impeller than on the rate of air-flow within quite wide limits. In fact, the efficiency of oxygen utilization may improve in deeper vessels

because of the increase in contact time between the bubbles and the nutrient medium.

The changes in hydrodynamic factors which result from maintenance of geometrical similarity and equivalence of power input are much more complex[7]. The specific volumetric power input varies as the cube of the impeller speed and as the square of the diameter. This necessitates a reduction of impeller speed as the scale of operation increases. The overall effect is to reduce the average shear-rate and relative volumetric flow of the suspension through the impeller, whilst increasing turbulence and maximum shear-rate. The increases in turbulence and maximum shear-rate increase the risk of mechanical damage to the organisms, whilst the reduction of flow results in a deterioration of mixing quality. This may be a substantial disadvantage in highly viscous and non-Newtonian conditions in particular. It has already been indicated that the satisfaction of oxygen requirements throughout the culture in such conditions may depend on the frequency with which elements of the culture pass through the impeller region. In large vessels the circulation paths will obviously be longer than in geometrically similar small vessels and the distribution of circulation times will be different, as will the proportion of the culture which will be stagnant.

All these considerations indicate that, in general, the criteria quoted do not provide a satisfactory basis for scaling-up. If volumetric power input is not to increase, changes in geometry are necessary to make the best use of the power employed. In easily-dispersed, low-viscosity batch systems limitations are more likely to arise in relation to overall mass transfer than to mixing, whilst the opposite is likely to be true in pseudoplastic systems.

In continuous-culture systems mixing may be a key factor in control even in well-dispersed, low-viscosity systems. Sinclair and Brown[5] have shown that deviations from ideal behaviour, attributed to apparatus effects by Herbert *et al.*[8] can be explained by regarding a vessel with a single impeller as having two well-mixed regions, between which interchange of material is slow compared with the rate of mixing in each. This can be of particular importance when addition of rate-limiting substrate and withdrawal of product takes place in the same zone, usually above the impeller, and could be accentuated in multiple-impeller systems. Such systems are frequently employed in large vessels, and have been introduced as an empirical departure from the principle of geometrical similarity. Given proper spacing of the impellers[9] such a system may give better performance than might be expected from a single-impeller system absorbing a similar amount of power. In the absence of published data on mixing and mass-transfer rates in such systems, elucidation of this effect is not possible. It may be due to a reduction in the volume of stagnant zones, to redistribution of gas which would otherwise channel around the impeller shaft or to reduction in the length of circulation paths.

CONCLUSIONS

Biological activity depends basically on the microenvironment surrounding each cell. In practice, control of the microenvironment must be exercised rather indirectly through control of the overall conditions in the apparatus in which the process is conducted. It is clearly necessary to

ensure that the overall supply of nutrients is adequate and that there should be provision for the removal of excess heat and of volatile products of metabolism, but this is insufficient to ensure optimal activity unless local variations in conditions can be kept within acceptable limits. Mechanical agitation is frequently employed to meet this latter need, but its effects are imperfectly understood and inadequately characterized. More needs to be known for example about the effects of mechanical forces on microorganisms, the extent to which aggregation is determined by biological and mechanical factors and about the distribution of circulation times on various scales of operation, especially in non-Newtonian systems. At the same time, more information is required about the response of biological systems to transient variations in conditions which may be experienced while a particular element of culture suspension is circulated around the vessel under the action of the impeller. It may then be possible to assess quantitatively the extent, if any, to which desired biological activity is limited by variations in the physical conditions in a particular situation, and the benefits which might result from modification of those conditions.

This may be regarded as a distant and ideal objective, but benefits may accrue along the way. It is now possible to determine continuously many parameters in any biological system, and, with the aid of computers, to maintain the measured values within closer limits than has been possible by earlier methods. It does not follow that this has led to a correspondingly closer control of the microenvironment, nor that all the data which is processed is necessary to high-quality control. Fuller knowledge of physical conditions in microbial cultures and of their effects on biological activity will help to resolve these issues and to improve both equipment and process design. There seems no reason to believe that the sparged, agitated, baffled vessel will be displaced from its position of pre-eminence, but better characterization may lead to better exploitation, and to the identification on rational grounds of situations in which radically different types of contacting equipment may be justified.

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