

Avoid the Pitfalls of Bioprocess Development

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Use these tips to successfully handle the challenges that may arise during the design and scaleup of various bioprocess operations.

Many correlations and relationships have been developed for the scaleup of chemical processes, but these equations are not always applicable to their biochemical or bioprocessing counterparts. The differences between these engineering disciplines lie not in the principles of unit operations and unit processes, but in the nature of living systems. The development, scaleup and optimization of the complicated biochemical activities of microorganisms, including yeasts, bacteria, algae, and molds, and of isolated animal and plant cell systems, presents engineering challenges that are sophisticated and difficult. This article addresses a variety of issues that must be carefully considered during a myriad of bioprocess operations and discusses ways to handle them successfully. Since it is beyond the scope of this article to cover every system comprehensively, the author cites practical examples from the literature that demonstrate successful techniques for a given scenario. Additional references may be obtained by contacting the author directly.

1. Identify the operating regime

Processes that use isolated cells have a lot in common with those based on microorganisms. But unlike chemicals, the commercial use of microbes, cells or enzymes derived from cells is restricted to the conditions at which these systems can function. The vast majority of microorganisms operate in mild conditions, but some thrive at temperatures above the boiling point of water or at pH values far from neutrality. The performance of a bioprocess can suffer from

changes in the many biochemical steps functioning in concert — *e.g.*, microorganisms or enzymes may lose their activity, and biomass quantity may decrease if the operating conditions drift outside of the optimum range.

One factor that must be monitored continuously in the production of biomass is pH. Good mixing is essential to prevent the medium from being affected by pH perturbations that may result from intermittent pH-control actions. In large-scale fermenters, the lack of a completely well-mixed environment may lead to pockets with varying pH values. For example, during the use of *Bacillus thuringiensis* for thuringiensin production, pH must be maintained between 7 and 8.5. Any variation from a pH of 7.0 could decrease the biomass and product concentration. (However, short periods of pH fluctuation have a minor effect on thuringiensin production and biomass concentration.)

Taking a closer look, the organism usually produces biomass and a metabolic acidic waste during the log growth phase. The acids are used as a carbon source after glucose has been consumed. Alkali is added to maintain the pH of the broth. Once the organism enters the stationary phase, biomass production slows down considerably. At the proper operating conditions, the biomass yield is approximately 92%, with a final pH of around 8.5 (1).

In general, an organism's activity is depicted graphically as a bell-shaped curve, where the maximum point reflects a change in pH. Although the organism's rate of activity increases with increasing system temperature (per

Nomenclature

a	= ratio of the specific growth rate of the variant to that of the parent organism
C_L	= concentration of CO ₂ in the liquid phase
C_F	= fraction of CO ₂ entering the reactor feed gas
D	= dilution rate, 1/s
F	= liquid flowrate to the reactor, L/s
f	= fraction of recycle to reactor
H	= Henry's law constant
K_s	= equilibrium constant in Monod equation, concentration units
k_1, k_2	= forward and reverse rate constants for cell movement from suspended to adhered state, 1/s
m_s	= maintenance coefficient, g glucose per g dry cell mass per s
N^g	= number of generations
P^g	= reactor pressure, Pa
$[P]$	= product concentration, g/L
Q	= air flowrate, L/s
R	= universal gas constant, 8.314 J/mol-K
$[S]$	= substrate concentration, g/L
s	= substrate concentration inside the reactor or in exit stream, g/L
s_o	= substrate concentration in the feed, g/L
T	= reaction temperature, °C
t	= time, s
V	= reactor volume, L
X	= final number of cells, on a dry basis, g
X_o	= number of cells in the original inoculum
x	= amount of suspended biomass, g
x_1	= amount of adhered biomass, g
x_m	= concentration of the variant species, g/L
x_n	= concentration of the parent species, g/L
Y_p	= grams of product formed per gram of cells formed
Y, Y_s	= cell yield coefficient or yield factor = g cells produced per g substrate consumed
Y_{sp}	= yield coefficient for the production of lactic acid, g lactic acid per g glucose

Greek Letters

α	= ratio of specific growth rates, dimensionless
λ	= rate of appearance of variants, 1/s
η	= viscosity of the solution, g/cm-s
μ	= Monod exponential growth rate, 1/s
μ_m	= maximum growth rate, 1/s

the the Arrhenius equation), an increase in temperature above 70°C may result in a loss of activity.

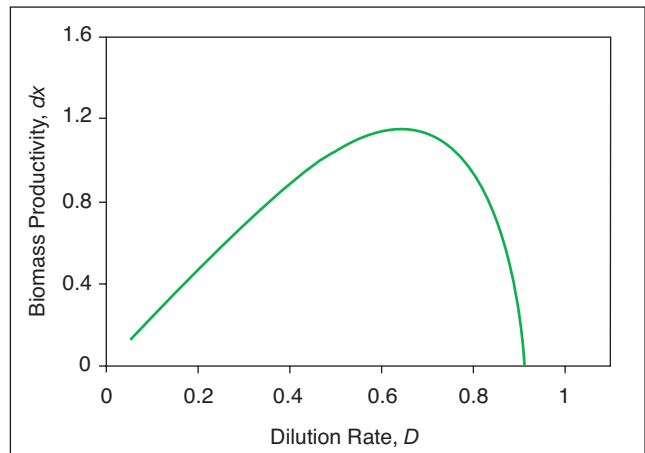
A model for a continuously stirred tank reactor in which a microorganism produces biomass (the concentration of which is x , measured in g/L) by consuming substrate (concentration noted as s ; g/L) is written as:

$$dx/dt = -Dx + \mu x \quad (1)$$

$$ds/dt = D(s_o - s) - Y\mu x \quad (2)$$

$$\mu = \mu_m s / (K_s + s) \quad (3)$$

where D is the dilution rate (flowrate/volume; 1/s); μ is the Monod exponential growth rate (1/s); μ_m is the maximum growth rate; K_s is the equilibrium constant in the Monod



■ Figure 1. Relationship between dilution rate and biomass productivity in a Monod chemostat system.

equation (dimensionless); Y is the yield factor (g of cells produced per g of substrate consumed); and s_o is the substrate concentration in the feed.

Generally, the concentration of biomass entering the reactor is assumed to be zero. This leads to a new relationship for the steady-state cell productivity:

$$Dx = DY(s_o - DK_s/(\mu_m - D)) \quad (4)$$

As D is increased, the cell productivity also increases, reaching a maximum of μ_m . At a certain value of D , the substrate is not being utilized, and both the cell growth rate and cell productivity drop to zero — the latter taking a sharper drop (Figure 1). This condition is called *wash out*. Knowledge of the wash-out flowrate is necessary in order to ensure that the reactor is operated below this value and that it can still maintain high cell productivity.

2. Effect of shear on cells

In general, whole cell systems that are used to produce biomass have higher yields in an environment where the shear forces are more uniform, such as in an airlift bioreactor. In a stirred-tank bioreactor, shear forces will be considerably higher near the impeller than in other parts of the reactor. High-molecular-weight substances such as dextran, which increase the viscosity of the medium, may be used to protect the cells against damage by absorbing kinetic energy.

Let us consider the effect of shear on various types of cell cultures. Single plant and animal cells are larger than microbial cells and are easily damaged by shear due to intense agitation; hence, stirred tank bioreactor designs tend not to work well with animal- and plant-cell systems. Recommended instead are airlift reactors. Bacterial cells are better able than filamentous fungi to survive in the shearing environment of a sparged, stirred-tank reactor

because the cell walls of filamentous fungi are composed of chitin, which is not as strong as the peptidoglycan bag that protects bacterial cells.

Animal cells do not have cell walls and are particularly vulnerable to shear forces. However, these cells are also very small and can slip between eddies, which shield them from shear forces. Nevertheless, an axial flow pattern is preferred for animal cell systems. The impeller-to-tank diameter ratio should be higher than typical values if microcarrier cultures are used.

Gas bubbles may damage cells with membranes in two ways: When bubbles collapse at the surface of the liquid, cells trapped in the wake of the bubble are subjected to relatively high stresses, which can rupture the cell membrane. Also, when bubbles or foams surrounding a cell move in different directions or at different speeds, the trapped cells are dragged along, and the membrane may be torn or sheared apart. Minimizing the formation of foam layers, for instance, by increasing the diameter of the disengagement zone, or by using slow aeration rates, will keep bubble damage in check. Also, the addition of surface-active agents protects the cells from breakage by either making them slippery enough to escape from high forces, or by increasing the viscosity of the medium, thereby facilitating its absorption of the kinetic energy of the cells.

In a similar manner, the microbial cells' mycelial pellets and hyphae may be damaged by the agitation caused by sparging and mixing. During the submerged cultivation of *Aspergillus awamori*, for instance, the change in pellet size and pellet porosity were found to be inversely proportional to the specific energy dissipation rate. In fact, a total breakup of pellets may occur in the case of hollow, centrally autolyzed aggregates (2).

Mycelial cells also produce an extracellular matrix of polysaccharides, which causes cells to clump under conditions of shear stress. This increases the overall metabolite production. However, given the strong tendency of the cells to attach to something, the fragments of hyphae from the outer pellet surface may serve as new centers for biomass growth.

3. Cell adhesion to walls

In some fermentation systems, such as those using filamentous fungi, a small part of the culture grows on the inner surface of the reactor. Hydrophobicity of the bacterial cell surface is one of the most important factors that govern the mechanism of this bacterial adhesion (3). If the concentrations of suspended and adhering biomass are x and x_1 respectively, and if there is a first-order exchange of material between suspended and adhering biomass with forward

and reverse rate constants of k_1 and k_2 respectively, then:

$$dx/dt = -Dx - k_1x + k_2x + \mu x \quad (5)$$

$$dx_1/dt = k_1x - k_2x + \mu x_1 \quad (6)$$

At steady state:

$$D = \mu(1 + x_1/x) \quad (7)$$

Equation 7 suggests that as the ratio of the concentration of adhered biomass to suspended biomass increases, the wash out flowrate increases by a factor of $(1 + x_1/x)$.

In a cylindrical vessel, the surface area of the cylindrical section is proportional to the volume expressed as $1/V^{1/3}$, indicating higher amounts of cell adhesion are likely at smaller rather than at larger-scale volumes. Figure 2 depicts the fraction effect of cell adhesion to the walls as a function of V (assuming $\mu = 1$).

Thus, the effect of cell adhesion on D will be more predominant in lab-scale vessels. Conversely, if lab-scale vessels are used for the estimation of operating conditions or for the design of a large-scale vessel, one should overestimate the dilution rate and wash out flowrate, and underestimate the reactor size for a desired cell productivity (4).

4. Fluid viscosity (non-Newtonian behavior)

Dispersed filamentous broths demonstrate non-Newtonian behavior — *i.e.*, a decrease in viscosity with increasing shear rate. Also, the apparent viscosity is normally quite high. The power law is used to describe the rheological properties of such fermentation broths.

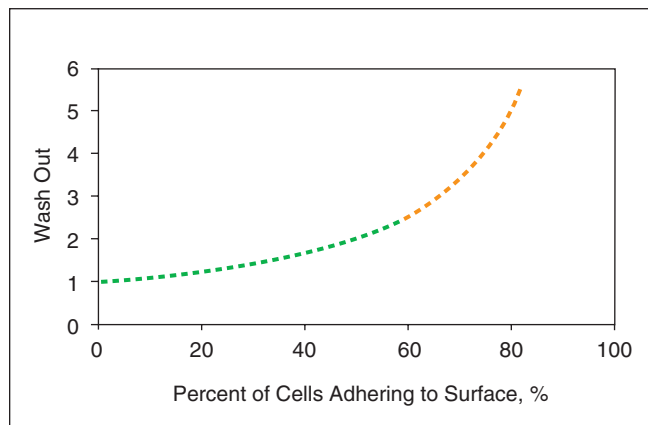
When the fermentation medium contains a polymeric substrate such as starch, the apparent viscosity will decrease during batch fermentation due to its enzymatic degradation and consumption, and a change from non-Newtonian to Newtonian behavior can occur. On the other hand, when a polymer is produced during batch fermentation, the viscosity of the broth will increase with an increase in broth concentration.

A power law correlation has been proposed for the viscosity of mold-containing solutions as a function of the biomass dry weight (X), where a ranges from 1.1 to 2.65:

$$\eta \propto X^a \quad (8)$$

Another power law equation correlates yield stress with mycelial concentration, where the exponent a ranges from 2.3 to 2.5 (5).

Oxygen transfer and CO_2 removal rates are affected by viscosity. Hence, they are important during scaleup. As vis-



■ Figure 2. Effect of cell adhesion to the walls of a vessel on the wash out ratio when $\mu = 1$.

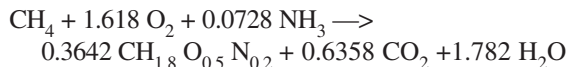
cosity increases, diffusivity decreases, which releases volumetric mass transfer. Usually, the effect of viscosity on volumetric mass transfer ranges from $a = 0.28$ to 0.60 . If flow is in the transition region, $a = 1.03$. The breakage of a bubble also depends on viscosity and liquid turbulence (2).

Biomass in the form of pellets imparts Newtonian properties to the fermentation broth. Mold suspension of *Absidia corymbifera* in filamentous and pelleted growth forms exhibits different flow behavior (6). As cited in the literature, filamentous mycelial suspensions showed non-Newtonian behavior and were correlated by a pseudoplastic model. Newtonian behavior was observed with pelleted mycelial suspensions, but at higher pellet concentrations, the rheological behavior of the broths became pseudoplastic. Non-Newtonian behavior was also observed during antibiotics production using filamentous fungi.

5. Heat removal and activity/growth rates

In the case of exothermic growth, biomass production rates are tightly coupled with heat-removal rates, since uncontrolled temperature increases could lead to loss of activity of the microorganism. If the reactor is assumed to be cylindrical and heat is removed from the surface of the cylindrical portion, one could derive from the heat balance that cell productivity is proportional to $1/V^{1/3}$. As shown in Figure 3, scaling up by a factor of 10,000 will lead to a decrease in the cell productivity by a factor of 21.5 (assuming the proportionality constant equals 1). Hence, large-scale vessels will require extra heat-transfer area to make up for large decreases in cell productivity.

Aerobic production of single cell protein from natural gas using *methylococcus capsultus* is represented by the following stoichiometry and has a heat-generation rate is 79.6 MJ per kg protein produced (4).



If the heat generation rate is balanced with the heat removal rate, cell productivity will decrease from a value of $6 \text{ g/m}^3\text{h}$ to $0.04 \text{ kg/m}^3\text{h}$ if the reaction is scaled from a 1 L to 50 m^3 cylindrical vessel, assuming heat removal is from the sides of the vessel. Hydrocarbons produce more heat than partially oxygenated species. Hence, fermentations of the former demand greater heat transfer rates than microbial consumption of carbohydrate at the same mass rate (for example, methane generates 57 times more heat than malate, acetate or glucose equivalents). Extended heating times for sterilization, and long cooling times due to insufficient heat-transfer area, can also lead to destruction of thermo-labile compounds.

6. Concentration of substrate added, and inhibition

In fed-batch or extended fed-batch fermentation processes, substrate or substrates are added to the contents of the reactor at a predetermined rate. Generally the concentration of the substrate in the feed is kept very high, while its concentration in the reactor volume is very low to avoid unnecessarily increasing the volume of the reactor contents with dilute feed.

Substrate inhibition at high concentrations of sugar is observed in many biochemical systems. The sugar concentration in the feed, typically $100\text{--}300 \text{ g/L}$, is maintained below 20 mg/L to avoid the formation of undesired byproducts. Mixing such a concentrated sugar solution into a medium with a 10,000-times-lower sugar concentration, and avoiding contact with cells containing pockets of solution greater than $50\text{--}100 \text{ mg/L}$, is a challenge.

Generally, mixing time in a vessel is inversely proportional to the agitator speed, and, for equal mass-transfer rates at all scales, mixing time will be proportional to vessel size as $V^{2/9}$. Scaleup by a factor of 10,000 will lead to about an 8 times increase in mixing time. Also, a high-viscosity medium slows down the mixing times. Hence, the microorganism should be able to survive in a highly concentrated environment for longer time durations with increasing scale of operation. So as the scale of operation increases, the problem becomes more acute. Optimal construction of feed ports and ideal mechanical agitator designs to improve mixing have to be attempted in large-scale operations. Similar issues are faced during the addition of concentrated acids or alkalis for pH maintenance.

If in aerobic yeast fermentation, oxygen tension in some areas of the reactor is too low, or the sugar concentration is higher than about 50 mg/L , undesired byproduct

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ethanol is formed. During fermentation of *Lactococcus lactis* at high sugar concentrations, lactic acid is the only product formed. At low sugar concentrations, a mixture of several acids and ethanol is formed.

Mixing time that is shorter than substrate or oxygen depletion time is crucial for substrates and oxygen that are added continuously or intermittently. If at any point the cells have depleted their critical substrates or are starved of oxygen, an irreversible apoptosis may be triggered. The mixing time (which depends on the agitator and reactor dimensions and the rpm of the agitator) has to be shorter than the time the cells take to deplete oxygen to the critical value (which depends on the rate of consumption of oxygen by the cells).

7. Product separation — an integrated approach

An integrated product-removal strategy could offer several advantages, including better economics. Continuous product removal, as in the case of alcohol fermentation, prevents deactivation of microorganisms. Such strategy is also ideal for organisms that exhibit product inhibition. Cell separation and recirculation of the liquid leads to a higher wash out flowrate, as given below:

$$D = \mu(1+f) \quad (9)$$

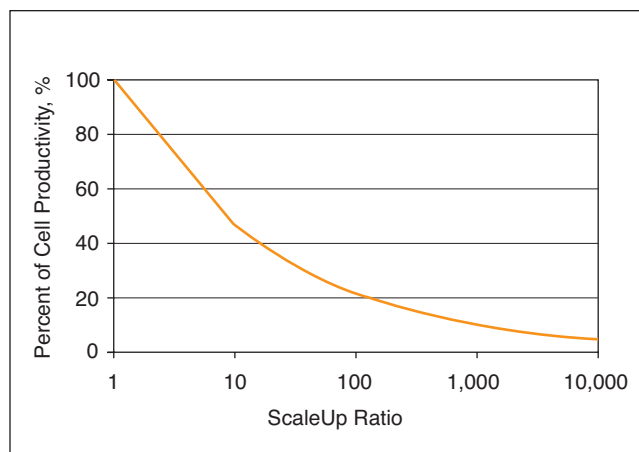
where f is the fraction recycled. Hence, cell removal followed by recycling may be useful for processing larger quantities of material.

Membrane bioreactors retain the biomass and allow only the liquid products to pass through the separation medium. Submerged membrane bioreactors, in which the membrane is submerged inside the reactant medium, can selectively retain liquid products containing the biomass. Such reactors have good application possibilities in effluent treatment.

8. Reactor selection

Generally, a stirred-tank design, which features uniformity of concentrations and temperatures within the reactor, is preferred as a laboratory chemostat. But at an industrial scale, reactor selection is based on a plethora of operating conditions.

A mechanically stirred tank is preferred for larger-scale operations, and together with the semi-batch (fed-batch) reactor, it has the advantage of operating at a low substrate level, which is usually necessary for expression of the desired proteins. Gas-agitated reactors, such as bubble columns and airlift reactors, are often preferred because



■ Figure 3. Effect of change in heat transfer area on volume during scaleup on percentage cell productivity.

the design is simple and there are no moving parts. Typically, they are specified when the gas-to-liquid mass-transfer rate is not limiting, or when the power requirements for mechanically agitated reactors are high due to the large reactor size required.

In special cases, if fouling and pressure drop are controllable, the organism may be immobilized or grown on structured packing or monolith, providing a packed-bed configuration. Such reactors are mainly used for biotreatment of waste gases and liquids in an environmental-treatment process.

Experiments conducted for gluconic acid production with *Aspergillus niger* proved equal product generation in airlift and stirred-tank reactors (7), while cellulase production by *A. fumigatus* was better in an airlift bioreactor. Airlifts are appropriate for moderate plant cell densities, *i.e.*, 10–15 g/L by dry weight, and are considered unsuitable for density more than 20 g/L due to problems related to mixing and oxygen transfer. A very wide variation in neomycin production was observed with a mutant strain of *Streptomyces marinensis* NUV 5 when the reaction was carried out in stirred-tank, packed-bed, fluidized-bed and airlift reactors. Productivities were 7,135, 1,353, 6,923 and 7,435 mg/L, respectively. The problem of oxygen transfer in a highly viscous broth during penicillin production with filamentous fungus *P. chrysogenum* was solved by growing it in a tower-loop reactor (rather than in a conventional stirred-tank bioreactor), which led to high specific productivities (8).

Reactor selection is also based on the reaction kinetics. A stirred-tank reactor is suitable for substrate-inhibition reactions, while a plug-flow design is suitable for Michaelis-Menten kinetics, as well as for product-inhibition reactions. A plug-flow design is preferred over agitat-

Table 1. Scaleup criteria in fermentation Industries (9).

Scaleup Criterion	Percentage of Industries Using this Criterion
Power per unit volume	30%
Gas-liquid mass-transfer coefficient	30%
Tip velocity	20%
Dissolved O ₂ concentration	20%

ed reactor designs for first- and second-order reactions. The type of organism and reaction medium also influences the reactor configuration.

9. Agitator scaleup

Several scaleup techniques are practiced by fermentation industries for the design of agitators. These include constant power per liquid volume, constant mass-transfer coefficient, constant tip velocity and constant dissolved oxygen concentration in the broth. Table 1 lists the percentage of fermentation industries using these criteria (9).

Scaleup methodology based on the energy dissipation rate or equal impeller tip speed were not valid as a measure of hyphal damage and production rate for the growth of *Penicillin chrysogenum*. However, the results were successfully correlated based on the circulation rate through the impeller zone. Justen, *et al.* (10) arrived at similar conclusions based on a study that used fermenters of different sizes with various types of impellers. Scaleup of linolenic acid production with *Mortierella ramanniana* in the pelleted morphological form was successfully carried out assuming equal impeller-tip-speed velocities at both pilot and production scales.

10. Aeration rates and CO₂ accumulation

Necessary oxygen is supplied to microorganisms by aeration. Hence, the design of a bioreactor for proper oxygen uptake is crucial. Improper aeration could lead to depletion of cells. Parameters to be considered during scaleup are reactor height-to-diameter ratio, total gas flowing upwards and the rate at which oxygen is transferred. If aeration is not proper, air pockets may develop around the impeller blades (11). Microbes will occasionally pass through zones of low oxygen concentration, and the time required to pass through low-oxygen-concentration zones increases with reactor scaleup.

Aeration is needed not only for cell sustenance, but also to drive CO₂ from the medium to keep toxicity low and to

keep the CO₂ concentration in the exit gas stream low, so as to achieve a large driving force for its desorption from the liquid medium. Excessive CO₂ concentration in the medium increases osmolality (the toxic level of CO₂ is 15–20%). Excess CO₂ also necessitates the addition of larger amount of base to the medium to maintain the pH. To strip off CO₂ sufficiently, a fast air flowrate must be used, which also keeps the CO₂ concentration in the exit air low, thereby achieving a large driving force. In a high-density culture with roughly 107 cells/mL, O₂ uptake and CO₂ removal rates can be approximately 5 mmol/L-h. The latter is defined as:

$$\text{CO}_2 \text{ removal rate} = (Q/V)(P/RT)(HC_L - C_F) \quad (10)$$

where H is Henry's law constant; C_L and C_F are the concentrations of CO₂ in the liquid phase and in the reactor feed gas; R is the gas constant; P the reactor pressure; and T is the temperature. Generally, C_F is roughly zero. The gas velocity in large-scale operations is kept low to avoid foaming of the liquid, which can lead to a decrease in the coefficient Q/V from small to large-scale systems, and hence an increase in C_L .

A high partial pressure of CO₂ during the batch growth of *A. niger* increases the mean hyphal length and mean branch length, inhibits penicillin production, and lowers the apparent viscosity. Thus, better oxygen transfer is seen in suspensions of *P. chrysogenum* due to enhanced chitin synthesis (12). An excess of chitin, a component of the cell wall, increases the plasticity of the cell wall and, consequently, the rheology of the fermentation broth.

11. Agitation vs. changes in cell morphology

During the continuous cultivation of the bacterium *Corynebacterium glutamicum* in a stirred bioreactor agitated at 410 and 1200 rpm and aerated at 1 and 3 vvm, cell growth profiles remained unaltered and no cell membrane damage was observed. At high agitation and aeration intensities, cells were smaller and irregularly shaped, due to the uneven accumulation of cell wall components, probably to protect their cells against high shear forces. Morphological differences of *A. niger* were observed in a pilot-scale stirred tank bioreactor. It was found that low shear rates resulted in the formation of looser pellets, while higher shear, coupled with larger-diameter cells, led to compact spherical pellets (12).

The length of a mycelium and the number of actively growing tips (by branching) increases exponentially under unconstricted conditions. But high shear results in fragmentation of the mycelium; pieces of hyphae from the outer pellet

surface serve as new centers for biomass growth. This phenomena was observed during a submerged cultivation. At times, hyphal damage can also lead to cytoplasm release.

The pellet size and porosity of *Aspergillus awamori* were found to be inversely proportional to the specific energy dissipation rate. Specific growth rates and the overall biomass concentrations of *Penicillium chrysogenum* have increased with agitation intensity, while the specific product formation was generally higher at lower impeller speeds.

12. Knowledge of microbiology and enzymology

In the cell growth process, the reactor size affects both the number of generations required to achieve the final biomass concentration and also the concentration of the variant species. The number of generations, N_g , required to achieve a final biomass concentration X starting from a known number of cells in original inoculum, X_o , is a function of the final fermenter volume, V , under exponential growth conditions (12):

$$N_g = 1.44 (\ln V + \ln X - \ln X_o) \quad (11)$$

The rate of appearance of variants, expressed as the number of variants produced per genome per generation and the fraction of parent organisms after N_g generations, will be:

$$x_n / (x_n + x_m) = (\alpha + \lambda - 1) / [(\alpha - 1) + \lambda - 2^{N_g} (\alpha + \lambda - 1)] \quad (12)$$

Refer to the nomenclature table at the beginning of this article for definitions of all terms.

Since the cellular system is very complex, the cells may ultimately adapt to the environment. However, they may respond very slowly as well as unpredictably. The response time will be a combination of widely different time constants of the various subsystems, the longest of which may be several hours. When cells are grown at a dilution rate close to wash out, they slowly adapt to this condition, increasing their μ_m by genetic changes. Similarly, reduction in glucose levels for a short period of time may induce a starvation reaction, which leads to loss of plasmids — a genetic response that is irreversible. Transient reactor dynamics can be modeled, but not much is known about the regulatory network of microorganisms. A wild-type *Aspergillus* strain used in the production of α -amylase is repressed by high glucose concentrations (15).

13. Reactor bottom clearance

Agitator location in the reactor bottom affects mixing time as well as gas-liquid mass transfer coefficient. If the

clearance is high, settling of the biomass is observed, whereas if the agitator is located too low, axial mixing is poor. In such cases, multiple axial stirrers may be required.

Similarly, the location of the concentric tube in an airlift reactor from the bottom is found to affect cell growth and the production of ginseng saponin and polysaccharide during fed-batch cultivation of *Panax notoginseng* cells. At small bottom clearances, gas-liquid mass transfer and oxygen transfer rates were limiting the extent of the reaction. At large clearances, cell sedimentation was observed (13).

14. Substrate consumption, biomass production and product formation

A simple model relating substrate consumption, biomass production and product formation is shown below. Some fermentation systems follow this type of behavior:

$$-d[S]/dt = (1/Y_s)d[X]/dt \quad (13)$$

$$d[P]/dt = (1/Y_p)d[X]/dt \quad (14)$$

where, $[S]$ and $[P]$ are the concentrations of substrate and product, respectively; Y_s = grams of cells produced per gram of substrate consumed; and Y_p = grams of product formed per gram of cells formed. The yield coefficients are considered to be constant during the course of the process (often called a “growth-related” process). Gluconic acid production from glucose by *Aspergillus niger* is a typical example of such behavior. However, simple behaviors are not always observed. For example, citric acid production exhibits varying values of Y_s . Product formation may exhibit a time delay, and Y_p may appear to be zero; maximum product is formed when the amount of biomass is low.

Cell growth and product formation oscillate during batch production of penicillin from sugar under the continuous addition of glucose. Oscillatory kinetic behavior of NADH2 in intact yeast cells (*Saccharomyces carlsbergensis*) under anaerobic conditions during the uptake of glucose has also been observed. NADH2 acts as a hydrogen donor in glucose metabolism, carrying hydrogen back and forth between reactions catalyzed by glyceraldehydes, 3-phosphate dehydrogenase, and alcohol dehydrogenase.

In other cases, *Lactobacillus plantarum* grown in media containing glucose and yeast extract exhibited a varying yield factor, depending upon the environment. The maximum growth yield based on yeast extract consumption was 0.5 g dry weight/g, whereas in the yeast-extract-limited culture, the growth yield increased with the dilution rate (14).

The depletion of fermentable sugar $[S]$ (g/L) and its conversion into lactic acid $[L]$ (g/L) may be modeled as:

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$$-d[S]/dt = (1/Y_s)d[X]/dt + m_s[X] \quad (15)$$

$$d[P]/dt = -(1/Y_{sp})d[S]/dt \quad (16)$$

where Y_s is the cell yield coefficient (in grams of cell dry mass per gram of glucose); m_s is the maintenance coefficient (in grams of glucose per gram of cell dry mass per hour); and Y_{sp} is the yield coefficient for the production of lactic acid (g of lactic acid per g of glucose). According to Ndon, *et al.*, for *L. sakei* CTC 494, Y_s and m_s depend on the temperature and pH. Y_{sp} is constant and equal to 1.0 g/g (15).

15. Dynamic operations

Generally, fermenters are operated under batch or fed-batch mode, where the substrate concentration decreases and the product concentration increases with time, leading to continuous variation in the reaction environment with time. This results in unpredictable behavior, unlike a steady-state operating environment.

For biological wastewater treatment applications, the sequential batch reactor (SBR) is gaining popularity as an alternative to conventional treatment techniques, due to its simplicity, flexibility of operation and cost effectiveness. This batch

reactor is operated in cycles consisting of various phases of operation, such as fill/draw, react, settle, discharge and idle. The reaction may consist of aeration and/or non-aeration phases. The discharge step consists of partial discharge.

It has been observed that during the treatment of dye and textile wastes, an SBR performs better than simple batch or continuous reactors with respect to decreasing the chemical and biological oxygen demands. Degradation of polyhalogenated compounds has been achieved by alternating between long anaerobic conditions, interposed by short low-oxygen tension, aerobic/methanotrophic mini-conditions. Continuous or intermittent mixing is performed to allow the settling of biomass (16).

CEP

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