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MATHEMATICAL MONITORING TOOLS TO WATCH MICROBIAL GROWTH FOR RECOMBINANT PROTEIN PRODUCTION IN FERMENTERS

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Abstract

The aim of this paper is to present a mathematical monitoring tool to watch microbial growth for recombinant protein production in fermenters. The method is based on a combination of engineering knowledge--microbial phenomena that includes stoichiometry, thermodynamics of cellular growth, kinetics, and physical processes (transport phenomena) like mixing, energy use, mass, and heat transfer. The development of fermentation models is assisted by the insights gained from measurements taken throughout process operations. The models have been developed using the knowledge gained from a variety of experiments. The effect on the growth rate and productivity of the microorganism(s) in the culture. Factors to consider in the design and operation of a bioreactor, as it can affect the mixing and oxygen transfer in the fermenter.

Keywords: stoichiometry, thermodynamics of cellular growth, transport phenomena.

Introduction

Chemically structured models provide data about impact of chemical species on fermentation kinetics by taking cellular functionality, parameters governing fermentation rates and transport phenomena. Insights gathered from measurements collected during process operations facilitate the development of fermentation models. Mathematical models are developed by combining engineering knowledge with microbial phenomena such as stoichiometry, thermodynamics of cellular development, microbial kinetics, and physical processes (transport phenomena) such as mixing, energy utilization, mass and heat transfer(1).

Kinetic models have evolved from simple exponential growth to sophisticated mathematical expressions for the prediction of heterogeneity within individual cells, the description of multi-reactions, the explanation of intrinsic control mechanisms, and even the prediction of genetic variation in bacterial populations. To describe the kinetics of reactions, simpler techniques and organised and unstructured models are used. Structured models are superior to unstructured models owing to their predictive abilities and capacity to show growth processes under a range of operational situations(2).

There are several models that may be used to characterise the kinetics of product production in continuous culture, including the Monod, Contois, and Andrews models. The Monod model is founded on the idea of limiting nutrients, which are the nutrients that are scarcest compared to the microbes' demands. According to the Monod model, the rate of product creation is directly proportional to the culture's limiting nutrient concentration. The Contois model is an extension of the Monod model that takes the influence of pH on product production into consideration. According to the Contois model, the rate of product production is dependent on both the concentration of the limiting nutrient and the culture's pH. The Andrews model is an extension of the Monod model that takes substrate inhibition into consideration. The Andrews model states that the rate of product production is dependent on both the concentration of the limiting nutrient and the concentration of the inhibitory substrate in the culture. By using these models and other methods, it is feasible to get a deeper knowledge of the elements that impact product

creation in continuous culture and to optimise the circumstances for maximum yield(3).

Models of Microbial Growth

A. Growth Kinetics in Fermenters

B. Rheology Study of Reactor

A. Models of Microbial Growth

Monod Equation

Monitoring the kinetics of microbial growth is necessary for determining the progression of a process. Compared to previous suggested rate equations for cell development, the empirically based monod growth rate equation has gained widespread acceptance. Coupled with the dynamic balancing equation for the substrate concentration, the Monod equation may describe or simulate the exponential growth phase followed by the decelerating growth phase in the cell concentration during batch growth dynamics(4).

$$\mu = \frac{\mu\max + Cs}{ks + Cs}$$

Webb Function

The Webb rate equation has been extensively used to characterise the kinetic behaviour of substrate inhibition and enzyme activation. As the integrated Webb equation cannot be written as an explicit closed-form reformulation of the time-dependent solution, its use is restricted to the rate versus substrate concentration study(5).

$$\mu_w(s) = \mu^* \frac{s(1 + \beta s/K_i)}{K + s + s^2/K_i}$$

Andrews function

The Andrews equation, based on specific growth rate, is one of the most often used models for understanding the growth inhibition kinetics of microorganisms, owing to its mathematical simplicity and widespread acceptance(6).

$$\mu_a(s) = \mu^* \frac{s}{K + s + s^2/K_i}$$

Han and Levenspiel

The following equation may be used to evaluate a generalised nonlinear model that describes the growth kinetics of a culture even at inhibitory levels of the substrate and throughout a broad variety of product inhibition circumstances(7).

$$r_s = r_{smax} \times S \times \left(1 - (S/S_m)\right)^n / \left(S + K_s \times \left(1 - (S/S_m)\right)^m\right)$$

Eadie-Hofstee Equation

The Eadie-Hofstee model allows for the linearization-based mathematical determination of the Monod equation. The slope is represented by a negative Ks value of the maximum y-intercept. Using the following equation, we may determine the Ks of a substrate (affinity) by producing a graph(8).

$$\frac{\mu}{S} = \frac{\mu_{max}}{K_S} - \frac{1}{K_S}\mu$$

B. Growth Kinetics in Fermenters

Growth Rate in Lag Phase

The first lag phase is a time in which there is little apparent development, yet chemical examination reveals a substantial increase. The cells' metabolic activity indicates that they are adjusting to their new environment and that growth will occur in due course(9). The kinetics of lag-phase cell development might be calculated by

$$\mu = \frac{\mu_{max}\, S}{K_S + S}\left[1 - \exp\left(-\frac{t}{t_L}\right)\right]$$

Exponential Phase Growth Rate.

Using the exponential growth phase data, specific growth rate values may be obtained for growth kinetic investigations using the following formula(10)

$$\ln\left(\frac{X}{X_0}\right) = \mu_x \times t$$

Feeding Rate

At the given time, F is the rate of pumping (1 h), S0 is the concentration of the input, and V is the volume of the reaction. The feeding method can be either constant, resulting in linear growth, or adjusted to increase exponentially, resulting in exponential growth; using either of the two equations below, we can determine the volume of feed that must be sent in fed-batch

$$F = \frac{\mu_{\text{set}} V_0 X_0}{S^*} \exp(\mu_{\text{set}}\, \Delta t)$$

F(t) is the feeding rate at time t (in L/h), F0 is the beginning feeding rate (in L/h), set is the specified growth rate (per hour), and t is the time (in h). It is known that the coefficient F0 is dependent on X0 (the initial biomass concentration in the bioreactor), V0 (the initial medium volume in the bioreactor), the specific growth rate, and biomass yield(11).

$$F(t) = F_0 * Exp(\mu_{\text{set}} * t) = L/h$$

Rate of Substrate Uptake

The rate of substrate uptake in the system may be calculated using the following equation from a basic carbon balance across the system: The inhibition of substrate absorption by acetate is based on experimental results and is expressed as a noncompetitive mechanism of inhibition(12).

$$qS = \left(\frac{qS_{max}}{1 + \frac{A_c}{K_{is}}}\right)\left(\frac{S}{S + K_s}\right)$$

Oxygen used for Glucose Oxidation

Aerobic respiration involves the oxidation of glucose in the presence of oxygen to produce energy (ATP). It contains glycolysis, the Kreb cycle, and the electron transport chain, via which the reducing chemicals NADH and FADH2 are produced. Subtracting the oxidative energy flux from the anabolic energy flux yields the oxidative energy flow, qSox,en.The oxidative energy flow (qSox, en) is the substrate utilised for aerobic energy metabolism. It is oxidised by respiration, and the stoichiometry of respiration yields the oxygen required for glucose oxidation, qOs(13).

$$qO_S = qS_{ox,en} Y_{O/S}$$

Growth Rate with acetate as energy sources

Typically, acetate is reutilized when the maintenance requirements for cell survival are exceeded (i.e., when substrate concentrations are near 0 g/L). By including the

biomass yield coefficient on acetate (YX/A) into the formula, the specific growth rate may be computed(14).

$$\mu = \left(q_{S_{ox}} - q_m\right)Y_{X/S,ox} + q_{S_{of}}Y_{X/S,of} + q_{A_c}Y_{X/A}$$

Batch Culture Logistic Model

This model is commonly used to characterise microbial proliferation in order to anticipate its inhibition and inactivation. By using this model, it is possible to more precisely characterise growth and estimate the coefficients that have biological significance(15).

$$\frac{\left(K_S Y_{\frac{X}{S}} + S_o Y_{\frac{X}{S}} + X_o\right)}{\left(S_0 Y_{\frac{X}{S}} + X_o\right)} \ln\left(\frac{X}{X_o}\right) - \frac{K_S Y_{\frac{X}{S}}}{\left(S_o Y_{\frac{X}{S}} + X_o\right)} \ln\left\{\left(S_0 Y_{\frac{X}{S}} + X_o - X\right)S_0 Y_{\frac{X}{S}}\right\} = \mu_m t$$

End Product Inhibition Model in Batch Culture Mode

To characterise the growth rate, the Taylor and Hinselwood end-product inhibition model is used. The rate of biomass generation is defined as follows(16)

$$\mu = \left(\frac{(\mu_{max} * S) * (K_P + P)}{(K_S + S) * (P)}\right)$$

Rate of biomass formation including Product Inhibition in Batch Culture Mode

With the maintenance coefficient (-kdX) included in the model, the following describes the rate of biomass production during the stationary phase(17)

$$\frac{dx}{dt} = \left(\frac{(\mu_{max} * S) * (K_P + P)}{(K_S + S) * (P)} - k_d X\right)$$

Rate of Substrate Consumption including Product Inhibition in Batch Culture Mode

The rate of substrate consumption, including product inhibition during the stationary phase, may be derived from the following equation, with the maintenance coefficient (-kdX) added(18).

$$\frac{ds}{dt} = \frac{C_Y}{\frac{Yx}{s}} * \left[\left(\frac{(\mu_{max} * S) * (K_P + P)}{(K_s + S) * (P)} - k_d X\right)\right]$$

Intracellular Enzyme Concentration rate

The initial lag and accelerating growth phases, which are not reproduced by the Monod growth rate equation, may be readily replicated by modifying the supplied Monod equation slightly. This alteration activates a growth-process rate-limiting enzyme that may not be present in adequate quantities in the inoculum or beginning culture. Despite the fact that cell development is a complicated process governed by hundreds of enzymes, it may be enough to predict a single enzyme that may be rate-limiting during the early lag growth phase in order to simulate the lag. ER may be expressed as E R = e/emax, where e is the intracellular content of the main enzyme, with units of g enzyme/g cell mass, and emax is its greatest value during the balanced exponential growth phase. The equilibrium equation for intracellular enzyme content may be expressed in terms of eCc, which has units of g enzyme per culture volume, where and are the enzyme synthesis and degradation rate constants, respectively(19).

$$\frac{de}{dt} = \frac{\alpha C_s}{K_s + C_S} - \beta e - \frac{1}{C_C}\frac{dC_C}{dt}e$$

Growth Rate in Continuous Culture

Determining the growth rate in a continuous culture by monitoring the increase in cell density over time This is stated as the "specific growth rate," which is the rate at which the cell population rises per unit of time and per unit of biomass(20).

$$X = \frac{Y(S_0 - DKs)}{(\mu_{max} - D)}$$

Dilution Rate in Continuous Culture

The volumetric flow rate of nutrients given to the reactor divided by the volume of the culture defines the dilution rate. It is helpful to remember, while employing a chemostat, that the specific growth rate of bacteria matches the dilution rate at steady state. At this steady state, the temperature, pH, flow rate, and concentration of the feed substrate will all be constant. If the dilution rate surpasses the prescribed growth rate, there may be negative repercussions. dCC/dt becomes negative when the dilution rate exceeds the specified growth rate (D > μ)(21).

$$dCC/dt = (\mu - D)CC$$

Substrate availability Rate in Continuous Culture

Substrate existing initially in the system (S_{ins}) which is accumulated during growth is given by the following equation(22)

$$S_{\text{ins}} = So + \frac{-1}{Yx/s} * \left(\frac{(\mu_{max} * S) * (K_P + P)}{(K_S + S) * (P)}\right) + k_d X - S_{\text{out}} + S_{\text{in}}$$

Product Formation Rate in Continuous Culture

The rate of product creation in CSTR can be determined experimentally by calculating the coefficients of product formation (Kp), the substrate utilisation constant (Ks), the concentration of dissolved substrate, and the final product production(23).

$$\frac{dP}{dt} = -Yp/s * \left(\frac{-1}{Yx/s} * \left(\frac{(\mu_{\max} * S) * (K_P + P)}{(K_S * S) * (P)}\right)\right)$$

Particle Suspension and Gas Dispersion

In order to maintain proper suspension of cells in the fermenter without setting at the bottom of it minimum stirrer speed required , which can be calculated by below mentioned equation(24).

$$N_i^* = C v_L^{0.1} D_p^{0.2} \left(\frac{g\left(\rho_p - \rho_L\right)}{\rho_L}\right)^{0.45} D_i^{-0.85} x^{0.13}$$

Heat transfer Coefficient

In the stirred tank reactor, heat transport is dependent on the agitation level and fluid characteristics. Typically, in small-scale fermenters, jacketed vessels are used. We can get the heat transfer coefficient by using the following equation:

$$Nu = 0.36 Re_i^{0.67} \Pr^{0.33} \left(\frac{\mu_b}{\mu_w}\right)^{0.14}$$

Where as in large scale fermenters heating is carried out by sending hot water through helical coil. By using below mentioned equation we can determine the heat transfer coefficient(25).

$$Nu = 0.87\,\mathrm{Re_i^{0.62}}\,\mathrm{Pr}\,0.33\left(\frac{\mu_b}{\mu_w}\right)^{0.14}$$

Oxygen Mass transfer coefficient

Oxygen transfer in the stirred tank is accomplished by the transfer of oxygen from the bubble to the liquid, which may be computed as the oxygen mass transfer coefficient using the formula below

$$k_L a(C^*_{AL} - C_{AL}) = q_O x$$

Empirical correlation for oxygen mass transfer in fermentation systems is kLa, where a relates directly to gas velocity and power input to the stirrer; the power term represents all the impacts of flow and turbulence on bubble dispersion and the mass-transfer boundary layer. The expression for stirred fermenters containing non-coalescing, non-viscous medium may be calculated using the equation shown below(26).

$$k_L a = 2.0 \times 10^{-3}\left(\frac{P}{V}\right)^{0.7} u_G^{0.2}$$

Presence of solutes such as salts, acids and sugars has a significant effect on oxygen solubility in water oxygen solubility is decreased by the ions and sugars normally added to fermentation media. The effect on oxygen solubility of ionic and non-ionic solutes such as molasses, corn-steep liquor, protein and antifoam agents, below mentioned empirical correlation to correct values of oxygen solubility in water for the effects of cations, anions and sugars(27).

$$\log_{10}\left(\frac{C^*_{AL0}}{C^*_{AL}}\right) = 0.5\sum_i H_i z_i^2 C_{iL} + \sum_j K_j C_{jL}$$

In general, the oxygen transfer rate (OTR) is considered to be regulated by the diffusion of oxygen over the liquid boundary layer and is thus given by a straightforward mass transfer equation. The OTR is proportional to the volumetric oxygen transfer coefficient (kLa) multiplied by the difference between the oxygen saturation concentration and the oxygen concentration in solution.

$$\mathrm{OTR} = \mathrm{kLa(CG - CL)}$$

The oxygen uptake rate (OUR) quantifies the rate at which a bacterium or collection of microorganisms consumes oxygen. Typically, it is reported in milligrammes of oxygen per litre of culture per hour (mg/L/h). Using the OUR, the growth rate of the microorganism(s) in the culture may be determined. In general, the OUR is proportional to the rate of growth (s) of the microorganism.As bacteria grow and reproduce, they use more oxygen, leading to a rise in the oxygen utilisation rate (OUR). In contrast, the OUR will diminish if the growth rate of the microorganism(s) decreases or ceases(28).

$\mathrm{OUR} = q_{o2}X = \mu_g X / Y_{X/02}$

In a gassed oxygen fermentor, power (P) is the rate at which oxygen is delivered to the culture and is commonly represented in volume per unit of time, such as litres per minute (L/min). As it may impact the development rate and productivity of the microorganism(s) in the culture, the fermentor's power is an essential issue to consider while operating it. The oxygen mass transfer coefficient (kla) quantifies the oxygen transfer efficiency from the gas phase to the liquid phase in the fermentor. Typically, it is represented in terms of mass per unit of time per unit of area per concentration gradient (mass/time/area/concentration). The kla may be modified by a number of variables, including the kind of microorganism(s) used, the characteristics of the culture medium, the mixing and aeration conditions in the fermentor, and the design of the oxygen transfer system. By regulating the power and kla, it is able to maximise the development and productivity of the microorganism by controlling the oxygen delivery to the culture (s). Increasing the power or the kla, for instance, may increase the oxygen supply to the culture and encourage quicker development, while reducing the power or the kla can reduce the oxygen supply and impede growth. It is critical to balance the oxygen supply and demand in the culture to avoid over- or under-aeration, which can stifle growth and output.

$$k_L a = C\left(P_g/V_L\right)^a V_s^b \mu^c$$

C. <u>Rheology Study in Fermenter</u>

Impeller Tip Speed

The impeller, often known as the agitator, is a crucial bioreactor component. It is responsible for mixing, aeration, heat transmission, and mass transfer inside the vessel(29).

$$V_{tip} = 2\pi N D_i$$

Mixing time

A bioreactor's mixing time is a crucial component to consider. It specifies how long and at what speed a liquid must be churned in order to achieve optimum homogeneity. The colouring method is frequently used to calculate length:A starch solution is combined with a 25 °C iodine-potassium-iodine solution. Sodium thiosulfate is then added to this solution combination, and the reaction time is monitored until the solution turns transparent. This influences how long it takes for a solution to become homogeneous. The shorter the duration, the greater the impeller's mixing qualities and the equilibrium of shear force inside the bioreactor. Cell culture procedures require longer mixing periods than microbial fermentations. This is a result of the slower speeds and the use of diverse impeller types, which reduce turbulence(30).

$$T_m = \frac{1.54V}{(ND_i^3)}$$

Power input

The torque that acts on the impeller shaft during rotation may be used to calculate the input power. As a result of its link with shear pressures, input power is an essential aspect during scale-up operations. Since the impeller may also impact product quality and cell development, it is advantageous to evaluate the input power. There are other methodologies, such as electrical power draw and

calorimetric methods. The formula for determining power input is shown below.

$$P = FR2\pi N$$

The power input to a fermenter is the rate at which energy is delivered to the fermenter in order to sustain the development and metabolism of the microorganisms in the culture. The input power is commonly stated in watts (W), and it is an essential aspect to consider while designing and operating a fermenter. Several variables, including the stirring and aeration rates, the temperature of the culture, and the heating and cooling needs, might affect the power input to a fermenter. To determine the power input to a fermenter, it is important to measure or estimate the energy needs of each of these elements and add them together. For instance, the power input to a fermenter due to stirring may be determined by multiplying the stirring rate (expressed in revolutions per minute or RPM) by the torque necessary to spin the impeller (expressed in Newton metres or Nm) by a conversion factor that converts torque to power (usually around 0.01 to 0.03). Multiplying the air flow rate (expressed in litres per minute or L/min) by the pressure necessary to transport the air (expressed in Pascals or Pa) by a conversion factor that converts pressure to power yields the power input due to aeration (usually around 0.01 to 0.03). Multiplying the heat transfer rate (expressed in watts or W) by the temperature differential between the culture and the heating or cooling system yields the power input related to heating and cooling(31).

$$P_0 = \frac{P_L}{\rho_L \cdot N^3 \cdot d^5} = \frac{2 \cdot \pi \cdot T_{eff}}{\rho_L \cdot N^2 \cdot d^5}$$

$$P_g = m\left(P_0^2 N D_i^3 Q^{-0.56}\right)^{0.45}$$

Flow Number

The flow number in a bioreactor is a measurement of the culture's flow rate compared to the bioreactor's size. The Reynolds number (Re) is commonly used to characterise the degree of mixing and oxygen transfer in a bioreactor.The flow number is determined by dividing the culture's flow rate (expressed in litres per minute or L/min) by the bioreactor's volume (expressed in litres or L) and the culture's kinematic viscosity (expressed in square metres per second or m2/s). The resultant value is a dimensionless quantity known as the flow number. During the design and operation of a bioreactor, it is essential to consider the flow rate since it might influence mixing and oxygen transfer. A high flow number suggests a high flow rate and effective mixing, while a low flow number indicates a low flow rate and ineffective mixing. For efficient mixing and oxygen transmission in a bioreactor, a flow number of 100 or higher is often deemed necessary(32).

$$Fl = \frac{Qg}{Nd\text{^}3i}$$

Reynold number

It is more difficult to estimate the power needs for non-Newtonian fluids. With very viscous fluids, it is generally difficult to create completely developed turbulence; under these circumstances, NP is always dependent on Rei, and the constant value cannot be used in power calculations. Moreover, as the viscosity of non-Newtonian liquids changes with shear conditions, the impeller Reynolds number used to correlate power needs must be revised. Some power correlations have been developed using an impeller Reynolds number based on the apparent viscosity μa(33).

$$Re_{\mathrm{i}} = \frac{N_{\mathrm{i}} D_{\mathrm{i}}^2 \rho}{\mu_{\mathrm{a}}}$$

Nusselt number

It is a number without dimensions that is closely connected to the Peclet number. The ratio between the thermal energy convecting into the fluid and the thermal energy transmitted inside the fluid is represented by both values. Nusselt's number is equal to the dimensionless temperature gradient at the surface, and it quantifies the convective heat transfer happening at the surface(34).

Nu= hL/K

Prandtl number

The Prandtl number is the ratio between momentum diffusivity and heat diffusivity. Small values of the Prandtl number, **Pr << 1**, suggest that thermal diffusivity dominates. In contrast, when **Pr >> 1**, momentum diffusivity dominates the behavior(35).

Pr = μ.Cp/k

Vessel Geometry

Unlike a vessels dimensions, manufacturing process parameters should not be scaled linearly. Linear scaling of process parameters would produce undesired results and can greatly affect the biomass generation. Usually 2:1 , 3:1 ratio is maintained while scaling up(36).

HL1/Dt1 = HL2/Dt2

Conclusion

All the listed equations in this paper are notably used to study growth kinetics of microbes, were by bioprocess developer can take close look of progress of bioreactions, metabolic state and tune the process as per the requirement by changing physical or chemical parameters that can influence fate of the fermentation process. Critical physical parameters such as effect of heat , osmolarity, pH, pressure, viscosity of liquid, mass transfer of oxygen are continuous monitored used advanced process analytical tools during the progress of bioreaction. Above listed equations are generally modified in accordance to microbial growth profile or metabolic state.

Nomenclature

μ	Specific growth rate
μ_{max}	Maximum specific growth rate
μ_{set}	Set specific growth rate
T_d	Doubling time
dp/dt	Product formation rate
ds/dt	Substrate accumulation rate

dx/dt	Biomass formation rate
S	Dissolved substrate concentration
X	Cell concentration
P	End product concentration
kS	Constant of substrate consumption dependent on pH maintenance
kP	Constant of end product production
Xdk	Biomass growth maintenance coefficient
$Y_{x/s}$	Yield of biomass from substrate
$Y_{p/s}$	Yield coefficient of lactic acid production from substrate
X_0	Biomass inoculated initially in the system
F_0	Initial flow in the system
S_0	Initial substrate concentration within the nutrients
F	Flux of nutrient per hour
t	Time step
D	Dilution rate in the system during time
V	Total volume of substrate in the system
C	Carbon concentration in moles per gram of substrate
K_s	Saturation constant
K_i	Inhibition constant
q_A	Specific acetate uptake rate
q_O	Specific oxygen uptake rate
q_S	Specific glucose uptake rate
Y	Yield coefficient
X	Biomass concentration
σ	Specific rate of substrate disappearance
α	Yield coefficient of collagen accumulation rate associated with cell growth rate
β	Variable macrokinetic parameter, yield coefficient of product accumulation rate associated with cell density
H	Vessel fill height
N	Impeller rotational speed
μ_a	Apparent Viscosity
ρ	Density
V_{tip}	Impeller tip speed
p	Power input
K	Thermal conductivity
L	Characteristic length
h	Connective heat transfer coefficient
Fl	Flow number
Re	Reynold number
Nu	Nusselt number
Pr	Prandtl number

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