THEORETICAL ASPECTS IN CONTINUOUS CULTURE

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BUZATO, J.B. Theoretical aspects in continuous culture. Semina: Ci. Exatas/Tecnol. Londrina, v. 13, n.4, p. 262-264, Dez. 1992.

ABSTRACT: Theoretical background and industrial application of continuous fermentation process are presented. Changes in concentration of organisms and substrate are discussed. Pratical difficulties are pointed out.

KEY-WORDS: Continuous fermentation, chemostat.

The continuous culture of microorganisms is a technique of well-known importance in both microbiology studies and more recently as large scale production process (ALLAIS et al., 1987; LIEVENSE & LIM, 1982; SCHIMIDT & SCHURGEL, 1987; STEVNSBORG & LAWFORD, 1986; STEWART et al., 1982). The essentia! feature of this technique is that microbial growth occurs under steady-state, which means, growth happens at a constant rate in a constant environment {PIRT, 1975). Factors such as pH values, concentration of nutrients, metabolic products, which inevitably change when a batch culture is used, are all maintained constant or independently controlled by the experimenter {HERBERT etal., 1956).

A continuous fermentation (chemostat) consists of a mixed suspension of cells into which fresch medium is continuously added at a constant rate and the culture is harvested at the same rate so that the culture volume is kept constant.

The biomass growth is limited by the concentration of a single limiting substrate, while all the other nutrients are in excess (STANBURY & WHITAKER, 1984).

A schematic representation of a chemostat is shown in the figure 1.

The flow of medium into the vessel is related to its volume by the term dilution rate (D) as:

$$D = F/V$$

That is the number of culture volumes of medium passing through the growth vessel per unit time, the dimension being reciproca of time unit.

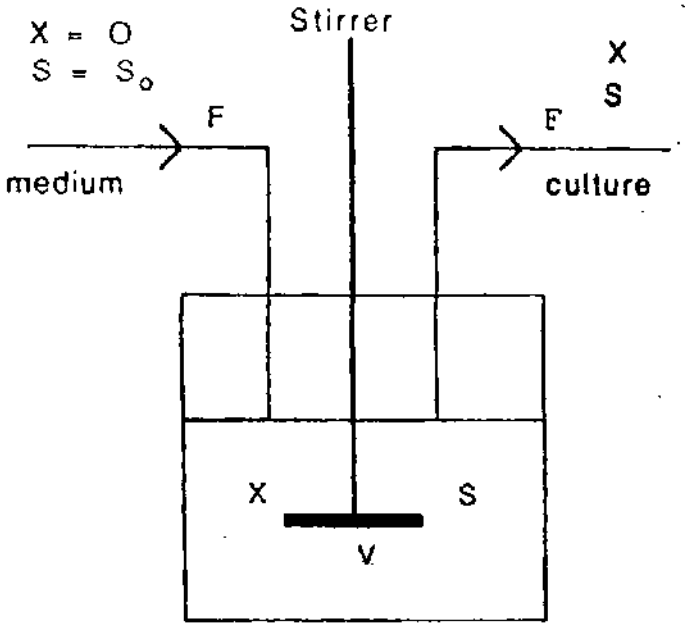

FIGURE 1 - The chemostat (diagramatic). The biomass and growth-limiting substrate concentrations are represented by X and S respectively. S_o is the limiting substrate in the feed medium; F = Flow Rate; V = Culture volume. Fonte: PIRT (1975)

Wash-out rate. Assume for the moment that the micrcorganisms in the vessel are not growing or dividing. With complete mixing, every organism in the vessel has an equal probability of leaving within a given time. The wash-out rate, i.e., the rate at which organisms

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present in the vessel would be washed out if growth ceased but flow continued is given by:

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

where x is the concentration of organisms in the vessel.

Bacterial growth kinetics. Suppose that, at first, growth is allowed to proceed batchwise without addition of medium. Following a period during which the growth rate of the cells gradually increases, the cells grow at a constant, maximum rate and this is as the log, or exponential phase. The exponential phase may be described by the equation:

$$\mu = 1/x\ dx/dt \quad (2)$$

where **x** is the concentration of organisms at time **t**, μ is the specific growth rate. In this equation μ is usually assumed to be constant. However this assumptation is correct only when all substrates necessary for growth are present in excess (STANBURY & WHITAKER, 1984).
Monod (MONOD, 1949) first showed that there is a simple relationship between the specific growth rate and the concentration of an essential growth substrate, μ being proportional to the substrate concentration when this is low but reaching a limiting saturation value at high substrate concentrations according to the equation:

$$\mu = \mu_{max}\ S/(K_s + S) \quad (3)$$

where S is the limiting substrate concentration, μ_{max} is the growth rate constant (i.e. the maximum value of μ at saturation levels of substrate) and K_s is a saturation constant numerically equal to the substrate concentration at which $\mu = 1/2\ \mu_{max}$.

Monod (MONOD, 1949) also showed experimentally that there was a constant relationship between the growth rate of a culture and substrate utilization rate.

$$dx/dt = -Y(ds/dt) \quad (4)$$

where Y is termed the yield factor. Over any finite period of time during the exponential growth phase, the yield (Y) is equal to weight of bacteria/weight of substrate consumed.

Changes in concentration of organisms. In the chemostat the organisms are growing at a rate described by equation (2) and simultaneously being washed away at a rate determined by the equation (1).

The net change in concentration of biomass with time will be determined by the relation:

$$\begin{aligned} \text{increase} &= \text{growth} - \text{outflow} \\ dx/dt &= \mu x - Dx \\ dx/dt &= x(\mu - D) \end{aligned} \quad (5)$$

It follows that if $\mu > D$, dx/dt will be positive and the concentration of organisms in the culture will increase with time. If, however, $\mu < D$, then dx/dt will have a negative value, and the cell concentration will diminish with time: the culture is washed out from the growth vessel. Only when $\mu = D$ will $dx/dt = 0$ and the concentration of organisms in the culture remains constant with the time. This condition is called steady state. Under such steady state conditions, the specific growth rate, μ, of the organisms in the culture vessel is exactly equal to the dilution rate D. Provided the dilution rate is maintained constant, the system is self-balancing.

This relationship between the growth rate of the organism and the dilution rate makes it possible to adjust the growth rate of the organism, within certain limits, to any value desired. When the dilution rate is increased above the specific growth rate, $D > \mu$, then dx/dt becomes negative, the biomass decreases, and the substrate is not utilized and increases. With $\mu < \mu_{max}$, the increased substrate concentration positively influences the specific growth rate up to its limit, where the specific growth rate equals the maximum growth rate ($\mu = \mu_{max}$). However, the specific growth rate, can not be made to exceed μ_{max} and therefore steady state conditions can not be obtained at dilution rates above a critical value (Dc), which is nearly equal to μ_{max}. If the dilution rate is set to a value greater than Dc, the bacteria will be washed out of the culture vessel faster than they can grow. Therefore the critical value of the dilution rate is of great practical importance.

Changes in Substrate Concentration. The substrate enters the growth vessel at concentration S_o, is consumed by the organisms, and emerges in the overflow at a concentration S. Therefore, the net change in substrate concentration is:

$$\begin{aligned} \text{increase of substrate} &= \text{input} - \text{output} - \text{consumption} \\ ds/dt &= DS_o - DS - (\text{growth/yield}) \end{aligned}$$

from equation (4)

$$ds/dt = D(S_o - S) - \mu x/Y \quad (6)$$

Rearranging the equation (6) and substituting μ by equation (3)

$$ds/dt = D(S_o - S) - [\mu_{max}\, X/Y][S/(K_s + S)] \quad (7)$$

Similarly μ can be substituted for equation (5)

$$dx/dt = x[[\mu_{max} S/(K_s + S)] - D] \quad (8)$$

The equations (7) and (8) define the behaviour of the culture in the chemostat. They also show that the continuous culture, in spite of the initial state of the culture, should finally establish a steady state.

Because equations (7) and (8) equal zero in a steady state, the values of x and S, which will be designated $\bar{x}$

and $\bar{S}$ can be calculated for the conditions of the steady state.

For the conditions of $\bar{x}$, equation (7) is used

$$D(S_o - \bar{S}) = [\mu_{max} X/Y][S/K_S + S)]$$

Since in the steady state $D = \mu_{max}[S/(K_S + S)$

$$\bar{x} = Y(S_o - \bar{S}) \qquad (9)$$

The concentration of the $\bar{S}$ is then determined from equation (8)

$$\mu_{max} S/(K_S + S) = D$$

$$\bar{S} = DK_S/(\mu_{max} - D) \qquad (10)$$

Substituting S from equation (10) in equation (9), it follows that

$$\bar{x} = Y[S_o - K_S\,[D/(\mu_{max} - D)]]$$

As a production process, the total output from a continuous culture unit, is equal to the product of dilution rate and concentration of microrganisms. There is a value of D for which the product $D\bar{x}$ is a maximum; in other words, for any system there is a particular dilution rate, Dm, which gives the maximum output of microrganisms without excessive substrate being left in the effluent.

The figure 2 shows the relationship between bacterial concentration ($\bar{x}$), substrate concentration ($\bar{S}$), doubling time (dt), and bacterial output ($D\bar{x}$), at different dilution rates in a chemostat.

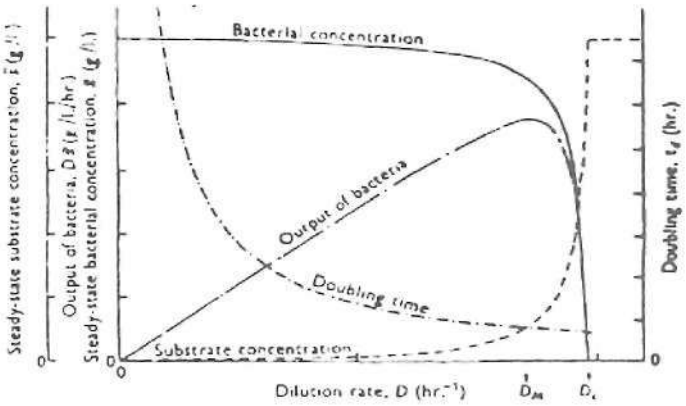

FIGURE 2 - Relationship between bacterial concentration, substrate concentration, doubling time, and bacteria! yield in the steady state, at different dilution rates (D) in a chemostat.
Fonte: HERBERT et al. (1956)

Eventhough continuous culture has advantages when compared with batch system, its use in large scale presents practical difficulties. One of the primary fears is contamination, since it is necessary to maintain sterile conditions for prolonged periods. In many cases, however, it is possible to design a fermentation to minimize the probability of contamination. The second greatest problem is mutation. Nevertheless, the event of mutation can be damaging only when it leads to an alteration of a desirable cell property and when the mutant organism is able to outgrow or successfully compete with the present organism.

BUZATO, J.B. Aspectos teóricos em cultura contínua. **Semina:** Ci. Exatas/Tecnol. Londrina, v. 13, n.4, p. 262-264, dez. 1992.

RESUMO: *São apresentados fundamentos teóricos e aplicações industriais do processo de fermentação contínua. São discutidas equações de mudanças na concentração de organismos e concentração de substrato. São apontadas algumas dificuldades práticas.*

PALA VHAS-CHAVE: *Fermentação contínua, quimostato.*

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Recebido para publicação em 21/10/92