THE DILUTION RATE (D) AND THE CONTINUOUS FERMENTATION PROCESS

CONTACT DETAILS

info@bionet.com
sales@bionet.com

www.bionet.com

When a continuous fermentation process is carried out, it is critical that the condition of steady-state is reached. The main process parameters that can be used for the chemostat technique are the dilution rate, the specific growth rate and the yield of the product on the substrate.

The dilution rate (D), usually in units per hour (h^{-1}), describes the relationship between the flow of the media into the bioreactor (F), that can be expressed in $L{\cdot}h^{-1}$, and the culture volume within the bioreactor (V) in L

$$D = F/V$$

The residence time (t) is the inverse of the dilution rate and is also related to the reactor volume and the flow rate:

$$t = V/F$$

In general, the continuous culture must go at least through four or five residence times before it can be considered to be in a steady-state (McNeil and Harvey, 2008).

The net change in cell concentration over a time period can be calculated using the biomass equation for the continuous reactor, where the term with the dilution rate is included:

$$\frac{dX}{dt} = \mu X - DX$$

The biomass concentration is expressed as X and μ is the specific growth rate in hours (h^{-1}), basically, the term μX refers to the growth and DX is the output, therefore, under steady-state conditions, the cell concentration remains constant when:

$$dX/dt = 0 \qquad \text{And} \qquad dS/dt = 0$$

Therefore, the changes in both biomass concentration (X) and in substrate concentration (S) over time (t) are zero, which means no net accumulation of biomass or substrate. Reaching a perfect steady-state in some systems is very difficult, which instead reach a pseudo-steady state. (McNeil and Harvey, 2008).

To reach a culture at steady-state, the specific growth (μ) must be equal than the dilution rate (D)

$$\mu = D$$

This equilibrium can be achieved controlling the substrate concentration (S) in the fermentation medium by the media addition of fresh media. The specific growth rate was demonstrated by Monod and is determined by the rate of flow of nutrient solution to the culture according to the next equation:

$$\mu = \mu max \frac{S}{(Ks + S)}$$

The inlet flow of fresh media and the outlet flow of product must be regulated to maintain a constant concentration of substrate (S) in the culture medium in the bioreactor, where the right equipment and a proper control SW are a key factors for this purpose (figure 1 represents an example of this system of a BIONET F0-BABY bioreactor connected to a Continuous Process Module, CPM). To reach the steady-state, the substrate concentration (S) determined by the dilution rate (D)

can be predicted by the next equation:

$$D = \mu max \frac{S}{(Ks+S)} \rightarrow S = \frac{Ks{\cdot}D}{(\mu max - D)}$$

The expression to calculate the biomass concentration (X) according to the yield coefficient (Yx/s) on the S at steady-state will be:

$$X = Yx/s\ (So - \frac{Ks \cdot D}{(\mu max - D)}$$

It is very important to establish experimentally a proper dilution rate for each process, given that different dilution rates lead to different product yields and qualities (Collet et al., 2004).

Inappropriate dilution rates (D) rate can result in unwanted situations:

- If the D is greater than the maximum specific growth rate (μ_{max}), the result is washout, dX/dt becomes negative
- The substrate concentration in the vessel can be accumulated
- The accumulation of substrate in the bioreactor can turn the relation of biomass concentration and be increased without control.

(McNeil and Harvey, 2008; Stambury et al., 2003)

The schematic representation of a simple chemostat system at lab scale is illustrated in figure 1, where a BIONET F0-BABY bench-top bioreactor can is operating in continuous mode via the Continuous Process Module (CPM), controlled by ROSITA Software. This allows for an easily scalable strategy in the development of experiments to determine the most accurate dilution rates.

The continuous fermentation processes are more difficult to carry out, compared with batch and fed-batch fermentation processes. he main reasons being that establishing a steady-state is a challenge, and that the contamination risk is higher and thus requires specific additional equipment. Nevertheless, if these drawbacks can be solved, the main advantages are a cost-effective fermentation process, which reduces the number of process stages (bioreactor cleaning, starter culture preparation, propagation, sterilization cycles, etc), saving energy and time and thereby increasing the productivity of the process, and avoids the accumulation of toxic substance.

More info of Bionet Continuous Process Module for bench-top bioreactors.

REFERENCES

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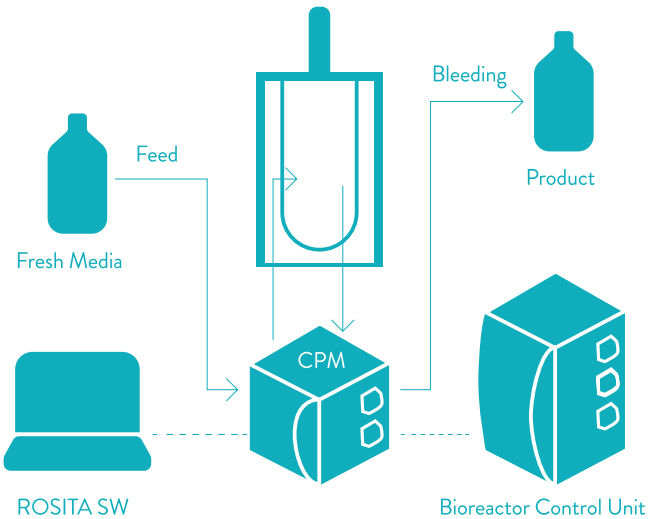

Figure 1.

Continuous fermentation process at lab scale by using a BIONET F0-BABY bioreactor with a Continuous Process Module (CPM).