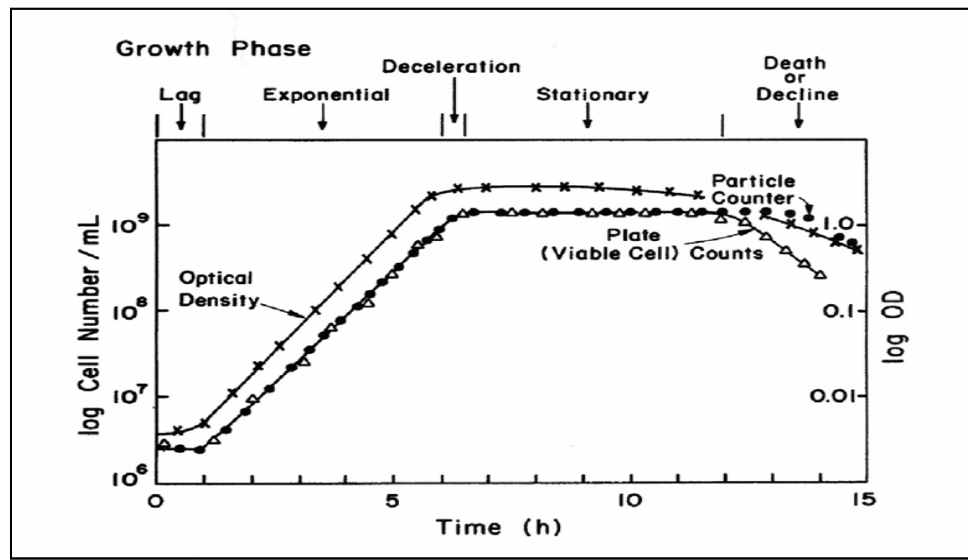


Growth Kinetics in Batch culture:

Lag phase or Stationary phase

It is a period of adaptation for the cells to their new environment. New enzymes are synthesized. A slight increase in cell mass and volume may occur, but there is no increase in cell number. Prolonged by low inoculum volume, poor inoculum condition (high % of dead cells), age of inoculum, nutrient-poor medium. Cell lysis may occur and viable cell mass may drop.



Log Phase

At the end of lag phase the cells in the medium get well adjusted to the new environment and are ready for growth. During log phase growth reaches maximum (max).The cells multiply rapidly and the cell mass or number of living cells double regularly with time during the growth phase.

Accelerated Growth Phase

This is a brief transient period during which cells start growing slowly. This phase connects the lag phase

Exponential growth phase

In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially)

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- Balanced growth

–all components of a cell grow at the same rate.

- Growth rate is independent of nutrient concentration, as nutrients are in excess.

The balance of cell mass in a batch culture gives:

$$\frac{dX}{dt} = \mu_{net} X, \quad X = X_0 \text{ at } t = 0$$

Integration of the above equation yields :

$$\ln \frac{X}{X_0} = \mu_{net} t, \text{ or } X = X_0 e^{\mu_{net} t}$$

X and X₀ are cell concentrations at time t and t = 0

$$\mu_{net} = \mu_r = \mu_m$$

μ_m is the maximum specific growth rate (1/time)

Doubling time of cell mass: the time required to double the microbial mass:

$$\tau_d = \frac{\ln X / X_0}{\mu_{net}} = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$

Stationary Phase

Some cells divide while some others die. The dead cells in the inoculums break open(lysis) releasing carbohydrates, amino acids and other components into the inoculums. The components from the lysed cells are used as nutrients by the other living cells in the inoculums causing division of the matured cells. Thus population size in an inoculums is maintained constant during the stationary phase.

Deceleration growth phase

Very short phase, during which growth decelerates due to either:

- Depletion of one or more essential nutrients
- The accumulation of toxic by-products of growth (e.g. Ethanol in yeast fermentations)

- The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis:
-

$$\frac{dX}{dt} = -k_d X$$

k_d is the rate constant for endogenous metabolism.

Kinetics of the death phase is not given importance because industrial processes are terminated before death phase begins. Death of cells like growth is assumed to follow an exponential decay and is given by

$$X_t = X_0 e^{-k_d t}$$

Where k_d is the rate constant during the death phase.

Production of Extracellular metabolites

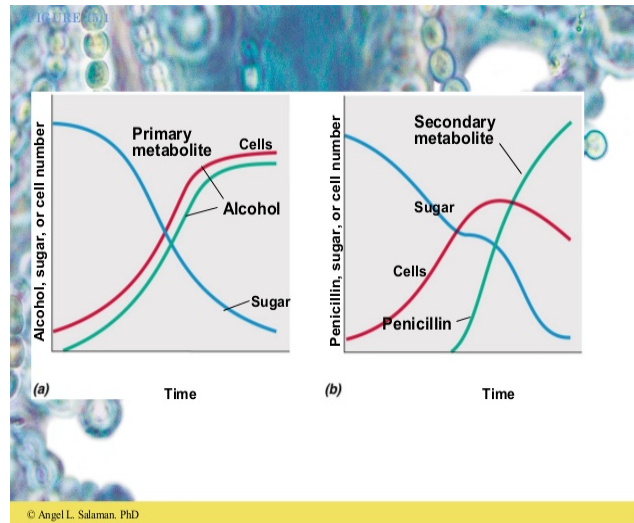
Microbial metabolites can be divided into two groups: Primary metabolites and secondary metabolites.

PRIMARY METABOLITES

Are formed in trophophase (log phase). They are compounds made during the ordinary metabolism of the organism during the growth phase. Citric acid is produced by some strain of *Aspergillus niger* as part of the citric acid cycle to acidify their environment and prevent other organism from taking over. These products are released into the environment without cell lysis.

SECONDARY METABOLITES

Are compounds made in the stationary phase. For e.g., penicillin prevents the growth of bacteria which could compete with penicillium molds for resources. These compounds are of value to human beings either as antibiotics or as antiseptics. Like primary metabolites, these products are released into the environment without cell lysis.



Medium Formulation and Yield Factors

During the growth of cells, substrates that provide energy and raw materials are needed for the synthesis of additional cell mass. Generally, in a biochemical process, the cell environment should contain elements required in order to form additional cell mass and the free energy from the substrate consumed should exceed the free energy of cells and metabolic products formed.

In order to obtain the amount of every element that took part in a growth process, the cellular content of all elements should be known. These include carbon, nitrogen, oxygen and hydrogen (C, N, O and H respectively). For the same strain that grows in different environment, composition of the elemental basis of the cell mass is consistent. However, the formulation of the medium is highly complicated due to:

- some substrate elements are released in products and not assimilated into cell material.
- Consideration of the limiting rate and limiting stoichiometry.
- Specific nutrients may be limiting/specific products may be inhibitory due to metabolic properties of cell strain.

The two limiting factors above may not occur at the same time as the other. One type of nutrient may limit the rate of cell growth, but it may also cause by the depletion of a different compound that stops the cell to grow. Such difficulties can only be observed by conducting an experimental analysis which can distinguish between the rate limiting and stoichiometric limiting.

In order to obtain a balance amount of cell formed from the amount of substrate used, a ratio between the cell and nutrient is used. It is normally termed as the yield factor, and has the unit of the cell and nutrient/substrate concentrations.

$$\text{Growth yield: } Y_{x/s} \equiv -\frac{\Delta X}{\Delta S}$$

$$\text{Product yield: } Y_{p/s} \equiv -\frac{\Delta P}{\Delta S}$$

Growth yield based on consumption of oxygen :

$$Y_{x/o_2} \equiv -\frac{\Delta X}{\Delta O_2}$$

Calculate the yield factor for the production of acetic acid using *Acetobacter aceti* bacteria. The chemical equation for the conversion of acetic acid is given below;



Acetobacter aceti is added into a vigorously aerated medium containing 10gl ethanol. After some times, ethanol and acetic acid concentrations become 2gl and 7.5gl respectively.

1. What is the overall/observed yield of acetic acid produced from the batch culture.
2. Determined the percentage of the observed yield in relation to the theoretical yield.

Answer:

1. By using a basis of 1 litre, the overall yield over the entire culture period is given as;

$$Y_{P/S} = 7.5\text{gl}/(10-2)\text{ gl}$$

$$= 0.94\text{gproduct gl substrate}$$

2. The theoretical yield is based on the mass of ethanol actually used for synthesis of acetic acid, thus, from the stoichiometric equation given above;

$$Y_{P/S} = 1\text{gmol of acetic acid}/1\text{gmol of ethanol}$$

$$= 60\text{g}/46\text{g}$$

$$= 1.30\text{gacid gl ethanol}$$

and the percentage of observed yield can be calculated by putting;

$$\text{observed yield/theoretical yield}$$

=

$$0.94/1.30 * 100 = 72.3\%$$

Material Balance of Cell Growth

The method of obtaining a balanced growth equation is very much similar to the equation describing chemical reactions. One needs to establish a chemical formula for dry cell material if the elemental composition of a particular strain growing under conditions is known, the ratios of subscripts in the empirical cell formula can be determined.

In order to get a unique bacterial cell formula and corresponding formula weight, one

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should employ a formula which contains 1 gram-atom of carbon; such that the formula is consistent with the known relative elemental weight content of the cell. Consider an aerobic growth which the products are cells, carbon dioxide and water, and the initial components such as carbon and nitrogen sources; CH_xO_y and $HlOmNn$.

Stoichiometry of Microbial Reactions

One mole of biological materials is defined as the amount containing 1 gram atom of carbon, such as $CH_\alpha O_\beta N_\delta$. Assumption: No extracellular products other than H_2O and CO_2 are produced.

Respectively, the equation can be written as follows;



from the above stoichiometric equation, 4 relationships consist of the stoichiometric coefficients a , b , c and d are;

$$C : a = 1 + e$$

$$H : aX + cL = \bar{\quad} + 2d$$

$$O : aY + 2b + cM = \circ + d + 2e$$

$$N : cN$$

The above equations is accompanied with an additional relationship obtained from experimental determination, i.e. *respiratory quotient* (RQ);

$$RQ = \frac{\text{moles of } CO_2 \text{ formed}}{\text{moles of } O_2 \text{ consumed}} = e/b$$

We have five equations and five unknowns (a , b , c , d , e). With a measured value of RQ, these equations can be solved to determine the stoichiometric coefficients.

Degree of Reduction

In the case of the formation of extracellular products, an additional stoichiometric coefficient is added, therefore, the element balance is not appropriate/applicable. The concept of degree of reduction is introduced to solve such problems. The technique is used for proton-electron balances in bioreactions. The degree of reduction for organic compounds is denoted as γ and it is defined as the number of equivalent of the available electrons per gram atom carbon.

The available electrons are the electrons that would be transformed to oxygen upon oxidation of a compound to carbon dioxide, water and ammonia.

The degree of reduction of any element in a compound is equal to the valence of this element, for instance, the valence of carbon in CO_2 is +4 and the valence of nitrogen in NH_3 is -3.

Degree of reduction for some key elements

$$C = 4$$

$$H = 1,$$

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$N = -3,$

$O = -2,$

$P = 5,$

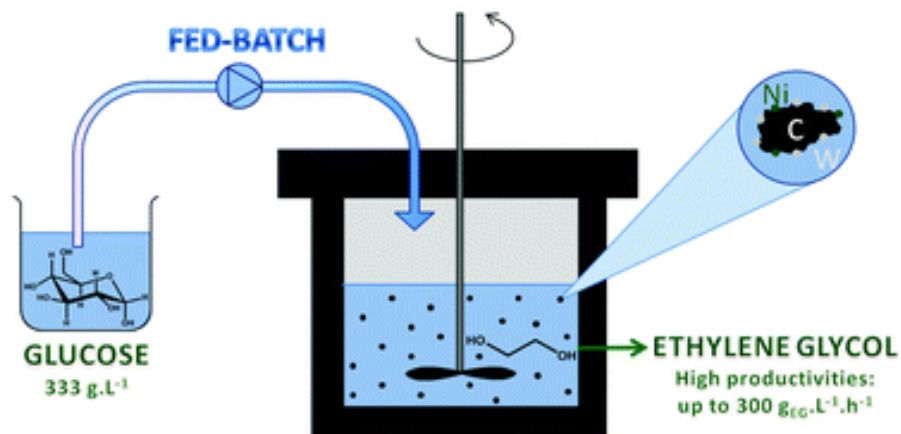
$S = 6 f$

Calculation of γ □ Methane (CH_4): $1 \times 4 + 4 \times 1 = 8 \quad \gamma = 8/1 = 8 \quad \square$

Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$): $6 \times 4 + 12 \times 1 + 6 \times (-2) = 24 \quad \gamma = 24/6 = 4 \quad \square$

Ethanol ($\text{C}_2\text{H}_5\text{OH}$): $2 \times 4 + 6 \times 1 + 1 \times (-2) = 12 \quad \gamma = 12/2 = 6$

Fed-Batch Culture



The main reason of having a fed-batch or *semi-continuous* type bioreactor is to avoid over-fed of substrate that can lead to cell inhibition and high growth rate for cell culture. The substrate feeding is continuously VR . Sterile air or oxygen. Substrate inlet F_i added until a maximum liquid in a fermenter is reached. When this happened or the substrate/nutrient is completely depleted, a fermenter may be allowed to continue or be partially/completely emptied depending on the process. The effluent resulted from the conversion of substrate/side-products from a particular fermentation are not removed and thus such a reactor can be used to monitor cells under LOW substrate/nutrient conditions without *washout* occurring.

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Fed-Batch Model Formulation

Mathematical model of a fed-batch bioreactor can be written as;

for the reactor volume:

$$d(xVR)/dt = F x_i$$

assuming that $\frac{1}{2} \text{feed} = \frac{1}{2} \text{liquid in fermenter}$

for biomass:

$$d(xVR)/dt = F x_i + rVR$$

$$VR \frac{dx}{dt} + x \frac{dVR}{dt} = F x_i + (\mu x) VR$$

$$VR \frac{dx}{dt} = F x_i - x \frac{dVR}{dt} + \mu x VR$$

with $F_i = F$;

$$\frac{dx}{dt} = \frac{F}{VR} x_i - \frac{F}{VR} x + (\mu_{max} s / K_s + s) x$$

$$\frac{dx}{dt} = D x_i - D x + (\mu_{max} s / K_s + s) x$$

$$\frac{dx}{dt} = (x_i - x) D + (\mu_{max} s / K_s + s) x$$

for a sterile feed,

$x_i = 0$ (free from micro organism)

hence;

$$\frac{dx}{dt} = -x D + (\mu_{max} s / K_s + s) x$$

for substrate/nutrient:

$$d(sVR) / dt = F s_i - 1/YX/S rVR$$

$$s \frac{dVR}{dt} + VR \frac{ds}{dt} = F s_i - 1/YX/S + (\mu_{max} s / K_s + s) x VR$$

$$VR \frac{ds}{dt} = F s_i - s \frac{dVR}{dt} - 1/YX/S + (\mu_{max} s / K_s + s) x$$

let $F_i = F$

$$\frac{ds}{dt} = \frac{F}{VR} (s_i - s) - 1/YX/S + (\mu_{max} s / K_s + s) x$$

$$\frac{ds}{dt} = D(s_i - s) - 1/YX/S + (\mu_{max} s / K_s + s) x$$

for product:

$$d(pVR/dt) = YP/S/YX/S rVR$$

$$p \frac{dVR}{dt} + VR \frac{dp}{dt} = YP/S/YX/S + (\mu_{max} s / K_s + s) x VR$$

$$VR \frac{dp}{dt} = YP/S/YX/S + (\mu_{max} s / K_s + s) x VR - p \frac{dVR}{dt}$$

Divide by VR

$$\frac{dp}{dt} = YP/S/YX/S + (\mu_{max} s / K_s + s) x - p \frac{dVR}{dt} / VR$$

$$\frac{dp}{dt} = YP/S/YX/S + (\mu_{max} s / K_s + s) x - Dp$$

Comparison Between Fed-Batch and Continuous Bioreactors

Major difference is the liquid effluent in a fed-batch system is not continuously removed, thus washout could not occur.

Can be set and maintain their specific growth rate and substrate concentration to an optimal level.

Advantages of Fed-Batch System

Because cells are not removed during fermentation, therefore this type of fermenter is well suited for production of compounds produced during very LOW or zero growth.

- The feed does not need to contain all the nutrients needed to sustain growth. Feed may contain only the nitrogen source or metabolic precursor.
- Contamination is highly unlikely for fed-batch system unless it has occurred during the early stage of a fermentation.
- Can operate in a number of ways; batch to fed-batch to batch in an alternate manner. The feed for substrate/nutrient can be manipulated in order to optimise the product formation. For instance, during fermentation, the feed composition or the flowrate can be adjusted to match the physiological state of cells.

Application of Fed-Batch System

Since the system can maintain the growth/reaction at LOW nutrient/substrate concentrations, therefore;

- it can be used to get the product or cells when the nutrient/substrate is inhibitory to the cells or affect the mass transfer rate. This can be maintained by controlling the substrate feeding such as during the production of citric acid, amylase enzyme and vinegar.
- can get higher biomass. cells yield, low substrate concentration is important especially in the production of mammalian cells, baker's yeast and antibiotic.
- the production is dependent on a specific nutrient composition, carbon to nitrogen ratio.
- oxygen uptake rate (OUR) must be restricted in order to maintain LOW substrate concentrations.