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STRATEGIES AND KINETICS OF INDUSTRIAL FERMENTATION FOR THE MASS PRODUCTION OF VARIOUS PRIMARY AND SECONDARY METABOLITES FROM MICROBES

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ABSTRACT

This review is mainly focused on the microbial cultivation, microbial growth kinetics, factors affecting the microbial growth and the metabolites production from various microbes. Different physical and chemical conditions play a vital role in the microbial cell growth by suppressing and as well as flourishing their growth. Several fermentation strategies are available for microbial cultivation such as batch cultivation, fed batch cultivation and continuous cultivation. For batch culture, a closed system is needed having minimum amount of important nutrients initially whereas for fed batch cultivation, new fresh medium are added continuously resulting in the increase in biomass concentration with time. Continuous cultures are the systems which are open and in which fresh medium is added continuously in fermentation chamber however the amount of medium present in the container remains constant. All above strategies are being used for production of various useful products that are of great importance to human beings as they are being directly used as food or drugs, or indirectly used as materials in food, chemical and pharmaceutical industries. The growing list of fermentation products includes primary metabolites (ethanol, citric acid and glutamic acid), secondary metabolites (antimicrobial drugs), enzymes (amylase, cellulase, xylanase, lipase and protease), vaccines (hepatitis A, B and some combination vaccines) and therapeutic proteins (interferon, human growth hormone and insulin).

KEYWORDS: Microbes, Batch cultivation, Fed-batch cultivation, Continuous cultivation, Primary metabolites, Secondary metabolites.

1. INTRODUCTION

Fermentation can be defined as the process in which a substrate is transformed into a useful product through the use of microorganisms (molds, yeasts, bacteria etc.) which produces enzymes and complex organic catalysts. Fermentation word is derived from the Latin word "*fermentare"* which means "to leaven" and a verb "*fervere*" thus meaning "to boil" which was coined for its appearance by the action of Yeast. The microorganisms in the process consume the sugar which is present in the extract. In this action of anaerobic catabolism they break down the sugars and produces carbon dioxide bubbles. Thus this appearance gave birth to the name fermentation. Eukaryotic and prokaryotic cells are a vital cause of cell division. Cells of plants and animals include culture of tissues, while bacterial cells include bacterial culturing. Development of microorganisms can be clarified as steady increment in the cell components causing increase in the cell numbers driving by the division of cell.

Fermentation process using different microbes is one of the ancient methods for synthesis of many useful products, even from prehistoric times of Egyptians and Chinese where the process was accidentally developed but scientific principles were not well known at that time. The initial fermentation process was used to make beer then followed by bread making, cheese, milk, yogurt, vinegar and wine.[1] Fermentation in modern industrial term is said as the procedure for the manufacture of any product through the culturing of microorganisms. This process is carried out through various cultures. Some of the commercially used culture techniques are Batch culture, Fed Batch culture and Continuous Culture. The reaction occurring in the bioreactor can be static or agitated, or may be in liquid form (Submerged) or solid state substrate.

2. Batch Cultivation

Cultivation using batch fermentation requires an enclosed system having a confined measure of non-contaminated media with required microbial population into a fermenter and permitted to go before the biochemical responses in light of enzymatic catalysis at streamlined natural conditions (temperature and gases accessibility and so on.) for a defined duration.[2]

Throughout the whole procedure of fermentation process no extra supplements are included aside from development control components; antifoaming chemicals, oxygen if there should arise an occurrence of aerobes and an acid or base for the control of pH. Particular development rate in the batch fermentation process isn't influenced until the point when the amount of substrate declined to a low level.[3] Where the microbial development is restricted by the depletion of that constraining element in a medium, the microbial cell concentration at the stationary stage could be communicated by the equation, (2.1);

$$x = Y.SR \quad (2.1)$$

Where x = cell concentration, Y = yield factor for the limiting substrate and SR = initial substrate concentration present in the medium. *Y* represents measure of efficiency of the cell in converting substrate into biomass. The biomass in a batch culture can be given as equation, (2.2); where S is the residual concentration at the point in time.

$$x = Y\,(SR - S) \quad (2.2)$$

Throughout the whole process of fermentation under the physiological situations medium synthesis and biomass concentration are continuously altered in consequence of microbial metabolism and supplement utilization, distinctive eight stages of batch cultivation showed up (Figure, 1).[4]

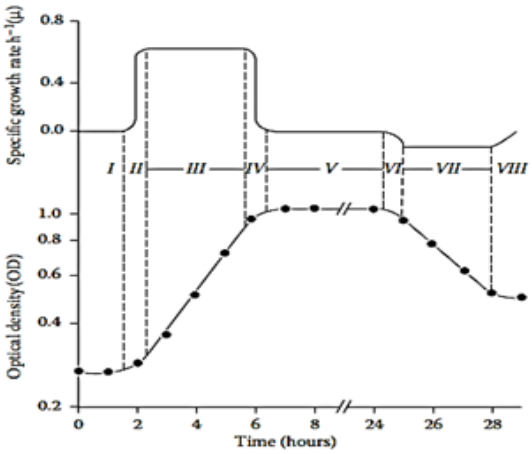

Figure 1: Typical growth curve in batch cultures: roman numerals and vertical dotted lines are showing behavior of microbial growth pattern.

2.1. Phases of Growth Curve

The microbe follows the different growth phases during its multiplication and product formation.[5,6] These phases are facilitated by the certain parameters like the nutrients, pH, temperature, antifoaming agents, growth inhibitors, aeration and absence or presence of certain gases. In 1909 it seems that at first Lane-Claypon put forward a section of "growth curve" into different stages.[7] She defined four discrete growth phases, namely, an early phase in which cell division does not takes place, a phase of maximum growth or the log phase, a phase at which microbial growth becomes constant and a decline phase. Afterwards in 1918 three phases of growth were added by Buchanan, linking these linear portions with the growth curves to provide a more practical depiction of the practical phenomenon.[8]

In 1952, Browning *et al*. used numerous common assumptions regarding the consumption of a restrictive essential elements by the microbial cells; he did a practical on profusion of organic kinetics to develop statistical growth models of the parasite.[9] One more physiological hypothesis for later growth was proposed by the investigator[10], to develop a logical expression. Starting with the initial identification of the distinctive sigmoid form of the microbial growth curve, many efforts were done to signify the characteristic of the growth curve in a logical expression. In 1949, Monod classified these perspectives into a number of categories, the growth model presented by Monod is the one most widely used formulations defining microbial growth kinetics.[11]

(i) Lag Phase

Lag stage is the modifications stage to the medium condition with no obvious development. In this stage cells begin amalgamation of biochemicals for their growth and its length relies on past history of microbial cells.[12] The definition of latent phase or the lag phase was first presented by Coplan's in conditions that may possibly be indicated quantitatively. He put forward a degree of "restraint of growth" which expresses the variation among the extent of a logarithmically increasing microbial growth and the microbial mass it would include at the similar moment.[13] The length of this phase may vary due to certain factors. This is one of those phase in which maximum time may be lost. In some cases this period may be prolonged or may get shortened.[12,14] Hence in the industrial fermentation this phase is minimized for less time wastage. Buchanan was the first person to present analytical expression of the physiological theory of the lag. He described lag as the period necessary entirely for the cells in the microbial community to "germinate," and the period of "germination" is the time at which the cell divisions takes place into two.[8] In 1943 two investigators, Lodge and Hinshelwood, worked on the consequences among the size of inoculums and the age of primary microbial culture. They developed "lag-age" association in which the duration of the lag tends to be increased apart from very immature parent cultures.[15]

(ii) Transient Acceleration Phase

Acceleration phase is the very next phase after the lag phase. It is not much prolonged phase as lag phase. In very less time it reaches to exponential phase. During this phase the microbes or inoculum starts to grow. It has all the important enzymes and nutrients for its further metabolism and product formation.[5] The certain amount

of growth is required to enter the exponential phase. The maximum growth rate is indicated by "μ_{max}".

(iii) Exponential / Log Phase

Following transient acceleration stage where cells step by step increment their development scale prompting the most extreme rate of microbial development period known as the log or exponential stage. Period of perfect development ecological conditions amid which development step by step increments to its most extreme rate without presence of development inhibitors. The products produced at this time are called as primary metabolites which are quiet essential for the microbial cells. This phase is also equivalent to Trophophase.[16] Each cycle of cell growth or cell division takes a certain time of increasing their number. This time taken between each cycle is called as generation time or doubling time. The generation time defines the average time taken by the cell to divide in the culture. In the terms of Mathematics log growth is judged in two ways; one is the cell biomass (x) and other one is cell number (N),[5] The biomass of cell development can be considered as an autocatalytic response, so the development rate is straightforwardly reliant upon the biomass concentration. This response can said to be depicted as takes after: rate of progress of biomass is;

$$\mu x = \frac{dx}{dt} \qquad (2.3)$$

Where "*x*" is concentration of biomass (g/L), "μ" is specific growth rate (per hour) and "t" is time (h). Some filamentous bacteria (e.g.; *Streptomyces*) and fungi are remarkable fermentation organisms. Maximum growth rate values of some industrially important organisms are given in table 1.

Table 1: $\mu_{max}(h^{-1})$ values of some organisms.

Organisms	μ_{max}(/h)	Reference
Vibrio natriegens	4.24/h	[17]
Methylomonasmethanolytica	0.53/h	[18]
Aspergillus nidulans	0.36/h	[19]
Penicilliumchrysogenum	0.12/h	[19]
Fusarium graminearumSchwabe	0.28/h	[20]
Animal cell	0.01-0.05/h	[21]

(iv) Deceleration phase

Deceleration stage is the most imperative period of development in industrial biotechnology, otherwise called disintegration stage, late log stage and early stationary stage.

(v) Stationary phase

Period of development bend where microbial cells can't develop and moved towards stationary stage due to inaccessibility of supplements and formation of harmful metabolites by microorganisms is known as stationary stage.[5] Some auxiliary metabolites including certain anti-toxins and a few compounds are formed in stationary stage. To check the condition of the restrictive growth component some scientists explored to check the relation of growth under limiting substrate.[14] They checked the concentration of the biomass in stationary phase in comparison to the initial concentration of substrate. This expression may be given as (eq. 2.4);

$$x = Y\,(SR - S) \qquad (2.4)$$

"x" is Biomass production concentration, "Y" is the factor for yield (g biomass production g^{-1} consumed substrate), "SR" is early concentration of substrate, "S" is concentration of the residual substrate. The ceasing of the growth in this phase is may be due the limitation of the essential nutrients (substrate limitation). Due to this it is possible that there is the start of toxic accumulation in the batch and this has other adverse effect on the product and the microorganism itself. According to a scientist Bull, this phase is still one of the most metabolically active period for microorganisms as they can produce certain metabolites which may be useful for them known as secondary metabolites.[22] This period can also be referred as the Idiophase. Products produced in this phase can be linked to the growth rate. In 1975, Pirt described this link as kinetics of product formation via microbial culture.[23] To express this link an equation was derived as (eq. 2.5);

$$dp / dt = q_p x \qquad (2.5)$$

"*p*" can be said as product concentration, and "q_p" is the specific product rate of formation. Another relation was also derived between the product formation and the biomass production by equation 2.6;

$$dp / dx = Y_{p/x} \qquad (2.6)$$

"$Y_{p/x}$" is product yield via biomass (g product g_{-1} biomass).

Some of the product or secondary metabolites formation can be related to non-growth linked products. But these products are only produced under specific conditions.

(vi) Death phase

The last period of development cycle where microbial populace is nearly stopped and its rate is more noteworthy than the development rate is known as the death stage. Viable amount of microbial populace diminishes without change in steadiness of turbidity because of the presence of the dead cells.[14]

2.2. Types of Batch Culture

Many types of batch culture are present e.g.; solid state culture, submerged culture, shaking culture, spinning culture and stirred culture. However solid state culture and submerged cultures are widely used.[24] Solid state culture is a type of batch culture, the procedure of cultivation of organisms on a compact medium even in absence or with minimum amount of water. Many products are produced using this method like fuel, nutrients enriched feed for animals, some of the enzymes important for industrial use, pharmaceutical products and food.[25] Solid state fermentation usually uses natural raw material which is comprised of carbon source essential for the microbe's growth. The moisture level on the solid medium depends upon the absorption rate of the material used. Hence enough moisture is required for the production or growth of microorganisms. Some products developed under solid state fermenter are enumerated in table, 2.[24]

Table 2: Development of products in a solid state fermenter.

Microorganism	Substrate used	Product	Type of bioreactor
Aspergillus niger	Cassava bagasse	Citric acid	Column fermenter
Bacillus subtilis	Rice bran	Iturin A	Tray
Lactobacillus species	Tamarind seed powder	Tannase	Static flask
Trichoderma reesei	Wheat bran	Cellulase	Static flask
Penicillium simplicissimum	Soybean cake	Lipase	Static flask
Lactobacillus delbrueckii	Cassava bagasse	Lactic acid	Static flask

The significance of solid state fermentation is much more than the submerged as it cost effective due to the use of simple growth media and it equals the value of nutrients. Another reason is the low quantity of water content usage. Oxygen problem is also resolved due the particular particle size which helps in aeration and respiration.[24] Submerged culture is another method for batch culturing in which nutrient enriched liquid medium is used for microbial growth. Many industries prefer using submerged method as it is space efficient and have less chances of contamination. During the process it is supplied with high amount of oxygen and aeration. The parameters including temperature, pH, and oxygen consumption and CO_2 formation are monitored constantly for controlling and optimizing it throughout the process.[26] The submerged method is significant in a way that it does not occupy much of the space and the capacity is much greater than the solid state fermentation and this method is also designed by order of controlled engineering. A number of enzymes are being produced by submerged fermentation; some of them are listed in table 3.

Table 3: Enzyme formation utilizing submerged fermentation.

Sr. No.	Microorganism	Substrate	Product	Reference
1	*Streptomyces spp., Serratiamarcesce*	Yeast extract medium	L-Asparaginase	[27]
2	*Bacillus spp.*	Starch broth	Amylase	[28]
3	*Thermotolerant bacillus spp.*	Corn cob and yeast extract peptone	Xylanase	[29]
4	*Bacillus species*	Carboxymethyl cellulose	Cellulase	[30]

3. Fed Batch Cultivation

Fed batch culture is also an enclosed system; it is the modification of batch culture system in which nutrients are fed continuously with fresh medium. The amount of the culture medium gradually increases with time. The kinetics related to fed-batch cultivation was proposed by the investigators.[31] [32] There is a great influence of feeding profile on the microbial growth rate, rate of production of a specific product[33], permeability of the outer membrane and on the composition of protein[34], so the feeding must have all the nutrients required for microbial growth even ammonia and amino acid can be added when they are needed.[35] The concentration of biomass at constant microbial phase or the stationary phase under growth limiting environment can be expressed as (equation 3.1);

$$Xmax - Y.SR \qquad (3.1)$$

The inoculum used for cultivation is supposed to be non-significant in evaluation with the final biomass. If the fresh medium is added to the closed cultivation system at dilution level which is lesser than X_{max}, then substrate present will be utilized as rapidly as it is added in the cultivation chamber (equation 3.2).

$$F.SR = \mu \left(\frac{X}{Y}\right) \qquad (3.2)$$

Where F = flow rate of the medium added, X = total amount of biomass present in the fermenting vessel. The microbial cell concentration remains the same even when the total biomass 'X' increases with the time. This can be given as (equation 3.3);

$$\frac{dx}{dt} = 0 \qquad (3.3)$$

And thus u = D, such a system than shows a 'quasi steady state'. As the time passes, microbial biomass

concentration increases and rate of dilution decreases which can be expressed as equation 3.4;

$$D = F/\ (Vo + Ft) \quad (3.4)$$

Where F is the flow rate of the cultivation medium, V_o is the initial volume and t is the time required while working with fed batch surroundings. After World War I production of yeast cell at industrial level was the first and crude method with regards to the fed-batch cultivation. Observations and experiments have effectively improved the situation for Fed-batch cultivation with a few added substances like supplements at research level and industrial level additionally for the production of a wide range of items including protein and yeasts[36,37], amino acids[38] some solvents, vitamins and many more (table, 4). The applications of fed batch culture include the development of many antimicrobial drugs, various proteins, glycerol and many vitamins etc.

Table 4: Examples of some of the products produced.

Product		Reference
Amino Acids	Glutamic acid	[39]
	Lysine	[38]
	Tyrosine	[40]
Antibiotics	Cephalosporin	[41, 42]
	Penicillin	[43, 44]
	Tetracycline	[38]

4. Continuous Culture

The microbial growth rate and its conditions can be kept constant by the process of continuous cultivation. The medium is introduced into a vessel through a reservoir at a constant rate. To keep the volume constant in the vessel, the culture media along with the accumulated waste produced is removed at the same rate at which the new medium is introduced in the vessel. The fixed restrictive concentration of the particular nutrient in the vessel is maintained which allows controlling the growth of microbes. The other components which are needed for microbial growth are added in excess in the vessel in order to fulfill growth requirements. The required concentration of growth restrictive nutrient is added as well in the vessel to achieve maximum growth rate.[11,45] Most widely used continuous cultivation systems are the chemostats.[45]

Specific Growth Rate (μ): It can be evaluated by using equation 4.1. Graphical representation of specific growth rate determination is also given in Figure 2.

$$\frac{dx}{dt} = \mu(/h) \quad (4.1)$$

Where; 'dx' is change in the biomass, 'dt' is time period and μ is the rate of specific growth.

Doubling time: Doubling time can be determined by applying:

$$T_d = \frac{ln2}{\mu} \quad (4.2)$$

Where; ln2 is equals to the natural logarithm of 2, and μ is the rate of specific growth (h^{-1}).

Dilution Rate: Rate of dilution represented by D, can be calculated by applying equation 4.3,[46]:

$$D = \frac{F}{V} \quad (4.3)$$

Where; *F* is the rate of flow (ml/min) in vessel and *V is* culture volume (ml) in fermenter. The degree of change in the concentration of biomass can be estimated via using equation 4.4:

$$\frac{dx}{dt} = \mu - D \quad (4.4)$$

The Monod Constant

It can be determined by applying equation 4.5:

$$\mu = \mu maxS/S + Ks \quad (4.5)$$

Where; μ_{max} = maximum rate of specific growth, *S* = substrate concentration and *Ks* = affinity constant or also known as half saturation constant.

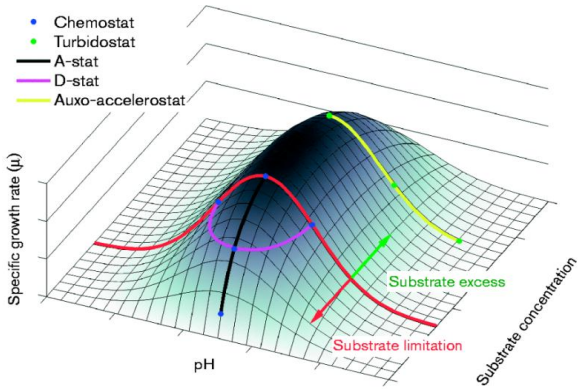

Figure 2: High-resolution steady-state Growth space analysis (GSA) using different changestats. The surface was generated based on the Monod equation[46] and near-to-real effects of pH and substrate concentration on μ. Note: residual substrate concentration starts inhibiting μ_{max} above a certain concentration due to osmotic stress. Blue and green points represent chemostat and turbidostat experiments, respectively, which can be carried out during one A-stat or auxo-accelerostat experiment, respectively.

4.2. Types of Continuous Cultures

Continuous cultivation systems are basically sorted into three kinds by modifying different components; chemostat, turbidostat and auxostat.

4.2.1. Chemostat

In a chemostat experiments about the substrate concentration is acclimated to unaltering state that controls the microbial development. Sterile cultural media storage is associated with a production chamber, where when the development is started new sterile broth is continuously provided by means of supply. New sterile

media is just permitted at constant rate to enter the development chamber that would restrict the microbial development (figure, 3). In view of this reason the rate of restricting amount of new sterile media decides the rate of microbial growth.[6]

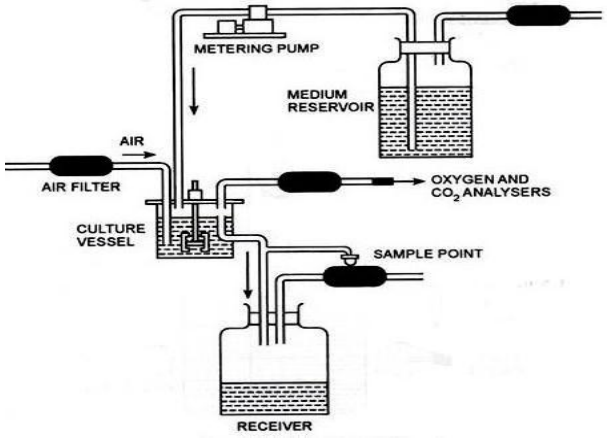

Figure 3: Chemostat setup which contains medium reservoir, culture vessel with control parameters.

4.2.2. Feedback Systems of Chemostat

In a chemostat experiments, biomass concentration can be acquired by means of two sorts of input frameworks; internal and external feedback systems (Figure, 4). In 1975, Pirt depicted an entire demonstration of the feedback system.[23]

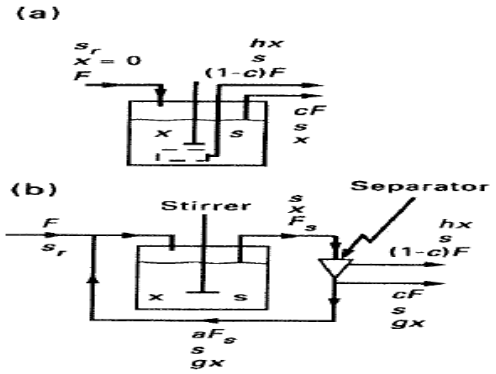

Figure 4: Illustrative demonstration of the feedback systems; (a) internal feedback system, (b) external feedback system.

a) Internal Feedback System

Biomass expulsion from profluent stream is constrained by a vessel that is partitioned into two streams from which one is unfiltered and the second is the filtered one. Level of input can be estimated by extent between the viability and outflow from the filtration channel.

b) External Feedback System

In this system the effluent is provided through an isolating gadget simply like a channel or ceaseless axis that further formed into two sorts of profluent streams like in internal feedback system. Either outer or interior feedback framework can demonstrate; development rate is not as much as dilute, in the vessel, concentration of biomass is raised, lingering concentration of substrate reduction is caused by upsurge in the concentration of biomass, biomass and product yield is raised to its extreme.

4.2.3. Auxostat

Auxostat also called nutistat and pH-stat is a kind of continuous cultivation in which constant factor is maintained by the dilution rate. During an auxostat study, fresh media addition rate is synchronized equivalent to the rate of cellular metabolism.

4.2.4. Turbidostat

In turbidostatic cultivation, the microbial growth rate is kept steady by evaluating turbidity of development to precisely record the biomass formation rate of balanced supplement feed.[47] Concentration of the cell is kept up consistent in turbidostatic experiments, while the chemical compound conditions are kept steady in chemostat cultivation systems (figure, 5).

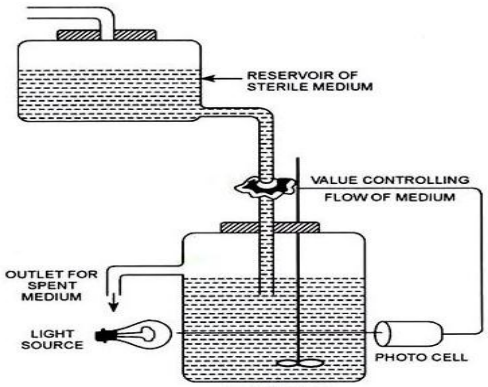

Figure 5: Turbidostat setup containing sterile medium reservoir, culture vessel, light source and photo cell for observation.

4.3. Continuous Microbial Cultivation Arrangement Classification

With a specific end goal to outline a systematic continuous development bioreactor setup, physiology of microorganisms and production by means of fermentation are vital contemplations that should be totally known. Classification of continuous cultivation system is given below (figure 6).

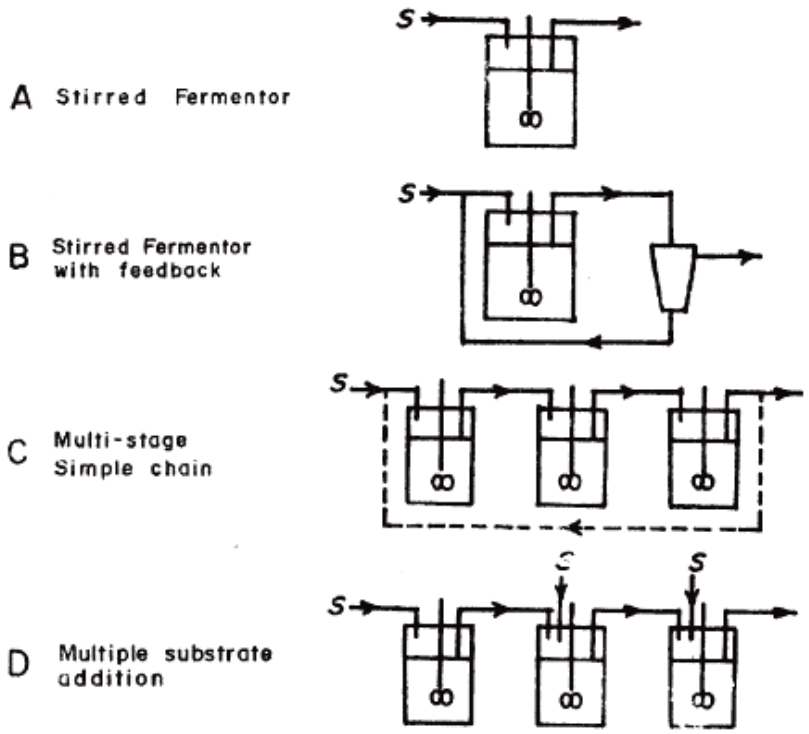

Figure 6: Continuous culture arrangement types; (A) Single Stage, (B) Multiple stages, (C) Recycled continuous cultivation, (D) Semi-continuous cultivation.

1. Single State Continuous Cultivation

The whole fermentation procedure takes place in a single vessel where the essential components are introduced in the vessel and at the same time the medium flows out of the vessel. This type of continuous system is best for the growth associated fermentation processes namely yeast or alcohol production (table, 5).[48]

Table 5: Historical outline of single-stage continuous processes for Polyhydroxyalkanoate (PHA) production.

Sr. No.	Year	Strain	PHA Produced	Work Significance	Reference
1	1972	*Azotobacter beijerinkii* NCIB 9067	PHB	PHA production	[49]
2	1986	*Hyphomicrobium* ZV620	PHB	Confirmation of carbon- nitrogen ratio to PHA mass fraction in biomass	[50]
3	1990	*Cupriavidusnecator* DSM 545	PHBHV	Continuous PHA and copolyester production	[51]
4	1990	*Haloferaxmediterranei*DSM 1411	PHBHV	PHA production using extremophiles	[52]
5	1991	*Ps. putida* GPo1	*mcl*-PHA	Continuous *mcl*-PHA production	[53], [54]
6	1995	*Cupriavidusnecator* DSM 545	PHBHV	Impact of *D* on PHA production	[55]
7	2000	*Ps. putida* GPo1	*mcl*-PHA	Dual Nutrient Limited (DNL) continuous PHA production	[56]
8	2005	*Cupriavidusnecator* DSM 545	PHBHV	Triggering of *scl*-PHA copolyester composition	[57]
9	2004	*Ps. putida* GPo1	*mcl*-PHA	Triggering of *mcl*-PHA copolyester composition and aromatic *mcl*-PHA production	[58]
10	2009	*Ps. putida* GPo1	*mcl*-PHA	Continuous production of crystalline *mcl*-PHA	[59]

2. Multiple Stages Continuous Cultivation

This type of fermentation consists of fermentation vessels connected to each other. The culture medium is introduced into the first vessel and the medium flowing out will go into the rest of the vessels. Growth of the microorganism will takes place in the first vessel and the production of the desired product will takes place in the subsequent vessels. This type of fermentation is

commonly used for the production of the metabolites. Different viruses have been cultivated in multi-stage continuous fermenters some of them are listed in table 6.

Table 6: List of viruses cultivated in continuous multi-stage bioreactors.

Sr. No.	Virus	Multi-Stage Configuration	Cell Line	Reference
1	Poliovirus 1	Two stirred tank reactor (STR) stages	Hela S-3-1	[60]
2	Adenovirus	Three STR stages	Hela-derived KB cell line	[60]
3	Baculovirus E2-strain	Two and three STR stages	Sf-AE-21	[61]
4	Recombinant baculovirus - AcMNPV	Semi-continuous repeated fed-batch (two STR stages)	Sf-9	[61, 62]
5	Recombinant baculovirus-vIBD-7	STR followed by a tubular reactor (two-stage system)	Sf-9	[63]
6	Recombinant baculovirus	Two STR stages	Se301	[64]
7	Influenza A/PR/8/34 (RKI)	Two STR stages	AGE1.CR.pIX	[65]

3. Multiple Stage or Recycled Single Continuous Cultivation

This type of fermentation consists of fermentation vessels connected to each other. The culture medium is introduced into the first vessel and the medium flowing out will go into the rest of the vessels. Growth of the microorganism will takes place in the first vessel and the production of the desired product will takes place in the subsequent vessels. This type of fermentation is commonly used for the production of the metabolites

4. Semi-continuous cultivation

This type of continuous fermentation involves intermittent addition of cultivation broth in the fermentation vessel and the removal of the outflow from the vessel instead of adding and removing nutrients continuously. Two types of this type of fermentation are known; Cyclic- semi continuous fermentation and Cell reuses semi continuous fermentation. In the former, a single vessel is frequently in use, though a series of the vessels could be used as well. The amount of the fresh medium added in the vessel must be equal to the amount of outflow. By decreasing the volume of the fresh medium in the vessel, the time required to terminate the fermentation process is also decreased. On the other hand it has been shown that the production rate of the cyclic fermentation tends to be lower than continuous fermentation.

5. Factors affecting growth

Microbes grow happily under their favorable environment. The effect of condition (chemical and physical) plays a vital role in the microbial cell growth. These conditions are acting in suppressing as well as flourishing their growth. Time and productivity are linked with certain conditions. To overcome the deleterious production effects the environment provided to microorganism is considered the most. Certain factors to be checked are as follows:

5.1. Temperature and Growth

Every organism differs from each other if mentioning their optimum temperature. The temperature fluctuation can inhibit or exhibit the growth in many ways. Microbes possess lipids, proteins, nucleic acids etc. that can experience structural change by the temperature change. These are the sensitive molecules which could denature by a slight change. If there structure is damaged there working efficiency decreases and thus the resulting product won't be beneficial for use. With the rise in temperature the chemical reactions rate might increase. If the temperature is raised 10^{o}C the chemical reaction rate doubles. According to this the cell growth rate should be faster.

On the basis of the temperature many groups are classified that are used accordingly on the basis of their need. Certain microorganisms can survive up to temperature range of 30^{o}C these microbes are termed as stenothermal. And the microbes which can grow beyond this range are called as eurythermal. If optimum temperature is referred then Mesophiles can grow in the optimum range of 20-45^{o}C, beyond or below this range the working efficiency will decrease and the microbial structure may also denature. While the Psycrophiles can grow in the range of below 15^{o}C. These can survive in low temperature and show effective working. The other group of temperature microbes is Thermophiles. These microorganisms can survive up to 50^{o}C of temperature and above. It includes fungi, algae and protozoan species that may survive up to 55^{o}C. Other than these groups there is also another group known as thePsychrotrophs, their temperature range is form 0-20^{o}C. They will work effectively at 20^{o}C but as long as they remain in their temperature range but cannot survive below the 0^{o}C. Some are Extreme thermophiles that can grow up to 100^{o}C such as *Bacillus stearothermophilus* (60–65^{o}C).

5.2. pH and Growth

There is not only single property on which a microbe resides. Besides optimum temperature microbes possess

the optimum pH range at which their working is not hindered. There are certain groups such as Acidophiles that have their optimum range from 1 to 5.5 pH. In general fungi are known as diverse microorganism that can survive a wide range of pH. But the good growth is observed from 4 to 6 pH. Apart from this group some microbes are classified in the group of Neutrophiles that can grow between the ranges of pH 5 to 9. This is most favorable range of pH in which most of the microorganisms grow. Alkalophiles are such groups which have optimum pH range from 8.5 to 11.5. *Bacillus* and *Micrococcus* range among this group.

5.3. Oxygen and Growth

Oxygen is the basic necessity of every living being on earth. Microbes have wide range of oxygen requirement. On this basis microorganisms are classified in groups such as obligate aerobes which can grow only in the presence of oxygen. They need oxygen to consume for the further chemical reaction process. Second group of classification is Facultative Anaerobes. This is such group that can live in both absence and presence of oxygen. But it will grow efficiently if oxygen is present. Third group is Microaerophilic which require oxygen in certain amount for the synthesis of particular compounds. Fourth group is of aerotolerant anaerobes. As the name indicating these can grow very well in absence of oxygen. Last but not the least group is Obligate Anaerobes which are highly non tolerant to oxygen. The presence of oxygen exhibits adverse effects on these microbes like death.

5.4. Solutes and Water Activity and Growth

Water is the essential element for the working and survival of living things. Without water life is somehow ceased and affected in harmful way. Every living thing requires a certain amount of water to survive. Many microorganisms already consist of 70 to 80 % of water and require additional solute or water to flourish in working environment. The growth of microorganisms can be suppressed or increased by adjusting water activity to certain optimum limit. If enough solute is not present in environment for the microbe there are the chances of microorganisms cell death.

The presence or requirement of water for microorganism can be expressed as Water Activity (Aw). As there are ranges of microbes on the basis of temperature and pH, same in this case they are classified accordingly. The need of water varies as some may sustain the dry environment while some flourish in extreme osmotic environment. One of the groups is Xerotolerent that can survive in low water activity. Fungi such as *Aspergillus* can survive in low osmotic level. Another group is Halophiles which are mostly known as marine microorganism groups where the concentration of salts is much higher than the water availability e.g. *Halobacteriumhalobium*. This group can survive in little amount of water activity. Bacteria usually requires 0.9 Aw for their functioning, below this their activity is hindered.

5.5. Effect of Substrate Concentration

In 1940s, Jacques Monod built up the Monod Equation by carrying out chains of experiments to portray the connection among the substrate concentration and the growth rate.

$$\mu = \mu \max S/S + Ks \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(5.1)}$$

Where μ is specific growth rate, μ_{max} is maximum μ, S is concentration of substrate, Ks is affinity constant or half saturation constant

Monod equation has two constants: μ_{max} which is the maximum specific growth rate and Ks which is the half saturation constant. These constants are affected by physiological conditions of the organisms, usage of substrate and temperature of the surroundings. By comparing the Eq. (5.1), number of cells represented by X, are used to describe the Monod equation Eq. (5.2);

$$dX/dt = \mu \max SX/Ks + S \qquad \text{(5.2)}$$

6. Monitoring Cultivation Process

Many methods are available to monitor microbial cell population and biomass concentration. Direct methods include microscopic cell counting, determination of dry weight, plate counting methods. Indirect methods include turbidimetry, spectrophotometry, and estimation of cell protein, nucleic acid or ATP.[66] In direct microscopy, the number of microbial cells is determined by using a counting chamber. In case of bacteria Petroff- Hauser chamber is the preferred one. Direct microscopic count is the fast technique but it has some limitations. It cannot distinguish between viable and non-viable cells. Dry weight estimation method measures the total cells weight including the non-viable cells in the sample. This method is usually done to separate biomass from the liquid medium. Large amount of sample is needed to get enough biomass for precise weighing of the biomass. Filtration by using filter paper with a standard pore size of 0.22nm is usually used to attain cells separation. Filtrate obtained is dried and the results are given in mg of cells per ml. Electronic cell counters are also rapid methods of microbial detection however many of these are more appropriate for large microorganisms like yeasts and protozoa rather than bacteria. Coulter Counter is an electronic cell counter which relies on measurement of the electric resistance changes which are produced by the particles present in an electrolyte. These electrical changes produced by microorganisms are transformed into a countable pulse however due to the presence of any debris or cells clumping, there are chances of errors.

Another direct technique is the plate count method which detects living cells. Spread plate and pour plate techniques are commonly used for this purpose. Serial dilutions are required for highly concentrated samples on the other hand concentration step are required in case of

small amount of microbial cells in the samples. In spread plate method, sample is spread on the surface of the solid medium whereas in pour plate method, sample is mixed with agar before it solidifies. An incubation of 1-2 is required to get the results in CFU (colony forming units). Turbidimetry and spectrophotometric methods are used to measure overall biomass concentration by using appropriate calibration curves. These methods are typically carried out at specific wavelength. The light scattered by the microbial cells is determined through spectroscopy which equals to microbial cell concentration. ATP bioluminometry is used for the quantification of ATP present in the sample which is very important in the measurement of living microorganism. A complex of enzyme and substrate that is luciferase- luciferin is utilized. For each ATP molecule, a photon of light is produced which is being detected by a bioluminometer. This technique is very sensitive and is most suitable for colorless samples. The results are expresses in femtomoles and it is estimated that around 10 femtomoles are equivalent to one yeast cell.[6]

7. Productivity and Efficiency of Cultivation Processes

Efficiency rate examination is viewed as a huge factor while choosing cultivation system to be applied [4]. Pirt clarified such sort of examination can be evaluated by applying Equation 6.1.[23]

$$Productivity\ Index = lnXm / X_0 + 0.693t_L/t_d \ldots\ldots\ldots\ldots\ldots\ldots (6.1)$$

Where X_0 = initial biomass concentration, Xm = maximum biomass concentration, t_L = total shutdown time duration of batch cultivation, t_d = doubling time.

Productivity index of whole cultivation process can be easily estimated by substituting the values in Equation, 6.1 given by Pirt in 1975.

CONCLUSION

Most of us educate and apply the microbial growth kinetics almost daily. The principles appear to have been worked out long back, and we feel little inclination to address them. As an end result, to maximum of us the field of microbial growth kinetics has turn out to be a (dull and off) requisite rather than a discipline of energetic research. This review is an attempt to critically access and talked about the foundation whereupon the presently used kinetics for depicting microbial growth. Metabolites production via microbial cultivation is a phenomenon that has been extensively discussed in literature. In literature most of the studies have been done in chemostat cultivation that is also known as contrivance of precedence for studying physiology of microbes. Although many of the new industrial fermentation processes exploit the fed batch cultivation process which can not only be utilized to restraint metabolism overflow but can also be used to gain high cell density products.

All above fermentation strategies are in use for production of several valuable products for the welfare of mankind. In future more automation and scrutiny of cultivation strategies is required while microbial culturing for the vast level production of various gainful products such as primary metabolites, secondary metabolites, vaccines, therapeutic drugs, enzymes and recombinant proteins.

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