18. SECONDARY TREATMENT

Secondary treatment of the wastewater could be achieved by chemical unit processes such as chemical oxidation, coagulation-flocculation and sedimentation, chemical precipitation, etc. or by employing biological processes (aerobic or anaerobic) where bacteria are used as a catalyst for removal of pollutant. For removal of organic matter from the wastewater, biological treatment processes are commonly used all over the world. Hence, for the treatment of wastewater like sewage and many of the agro-based industries and food processing industrial wastewaters the secondary treatment will invariably consist of a biological reactor either in single stage or in multi stage as per the requirements to meet the discharge norms.

18.1 Biological Treatment

The objective of the biological treatment of wastewater is to remove organic matter from the wastewater which is present in soluble and colloidal form or to remove nutrients such as nitrogen and phosphorous from the wastewater. The microorganisms (principally bacteria) are used to convert the colloidal and dissolved carbonaceous organic matter into various gases and into cell tissue. Cell tissue having high specific gravity than water can be removed in settling tank. Hence, complete treatment of the wastewater will not be achieved unless the cell tissues are removed. Biological removal of degradable organics involves a sequence of steps including mass transfer, adsorption, absorption and biochemical enzymatic reactions. Stabilization of organic substances by microorganisms in a natural aquatic environment or in a controlled environment of biological treatment systems is accomplished by two distinct metabolic processes: respiration and synthesis, also called as catabolism and anabolism, respectively.

Respiration: A portion of the available organic or inorganic substrate is oxidized by the bio-chemical reactions, being catalyzed by large protein molecules known as enzymes produced by microorganism to liberate energy. The oxidation or dehydrogenation can takes place both in aerobic and anaerobic conditions. Under aerobic conditions, the oxygen acts as the final electron acceptor for the oxidation. Under anaerobic conditions sulphates, nitrates, nitrites, carbon dioxide and organic compounds acts as an electron acceptor. Metabolic end products of the respiration are true inorganics like CO_2, water, ammonia, and H_2S.

The energy derived from the respiration is utilized by the microorganisms to synthesize new protoplasm through another set of enzyme catalyzed reactions, from the remaining portion of the substrate. The heterotrophic microorganisms derive the energy required for cell synthesis exclusively through oxidation of organic matter and autotrophic microorganisms derive the energy for synthesis either from the inorganic substances or from photosynthesis.

The energy is also required by the microorganisms for maintenance of their life activities. In the absence of any suitable external substrate, the microorganisms derive this energy through the oxidation of their own protoplasm. Such a process is known as **endogenous respiration** (or decay)**.** The metabolic end products of the endogenous respiration are same as that in primary respiration.

The metabolic processes in both aerobic and anaerobic processes are almost similar, the yield of energy in an aerobic process, using oxygen as electron acceptor, is much higher than in anaerobic condition. This is the reason why the aerobic systems liberates more energy and thus produce more new cells than the anaerobic systems.

Catabolism and Anabolism: The most important mechanism for the removal of organic material in biological wastewater treatment system is by bacterial metabolism. Metabolism refers to the utilization of the organic material, either as a source of energy or as a source for the synthesis of cellular matter. When organic material is used as an energy source, it is transferred into stable end products, a process known as *catabolism*. In the process of *anabolism* the organic material is transformed and incorporated into cell mass. Anabolism is an energy consuming process and it is only possible if catabolism occurs at the same time to supply the energy needed for the synthesis of the cellular matter. Thus, the processes of catabolism and anabolism are interdependent and occur simultaneously.

Importance of enzymes:

- Enzymes are organic catalysts produced by the living cell.
- Enzymes are proteins, or proteins combined either with an inorganic molecule or with low-molecular –weight organic molecules.
- They have capacity to increase speed of chemical reaction without altering themselves.

Extracellular enzymes: when substrate or nutrient required by the cell is unable to enter the cell wall, the extracellular enzymes converts the substrate or nutrient to a form that can be transported in to the cell.

Intracellular enzymes: they are involved in the catabolic and anabolic reactions within the cell.

Enzymes are known for their high degree of efficiency in converting substrate to end products. One molecule can change many molecules of substrate per minute to end products.

Enzymes are also substrate specific. Hence, cell must produce different enzymes for every substrate it uses.

Generalised enzyme reaction:

(E)	+	**(S)**	$\longrightarrow$	**(E)(S)**	$\longrightarrow$	**(P)**	+	**(E)**
Enzyme		Substrate		Enzyme Substrate		Product		Enzyme

i.e, enzyme functions as a catalyst by forming a complex with the substrate, which is then converted to product and the original enzyme. These products may be acted upon by another enzyme.

- Thus series of complex product may be formed before final end product is produced
- Activity of enzyme is substantially affected by pH and temperature.
- Temp and pH of key enzymes is reflected by overall temperature & pH.

Term	**Definition-metabolic function**
Aerobic (oxic) processes	Biological treatment processes that occur in the presence of oxygen
Anaerobic processes	Biological treatment processes that occur in the absence of oxygen
Anoxic process	The process by which nitrate nitrogen is converted biologically to nitrogen gas in the absence of oxygen. This process is also known as anoxic denitrification.
Facultative processes	Biological treatment processes in which the organisms can function in the presence or absence of molecular oxygen

Hybrid (combined) processes	Various combinations of aerobic, anoxic and anaerobic processes grouped together to achieve a specific treatment objective
	Type of treatment processes
Attached-growth processes (upflow/downflow -fixed film Fluized bed reactor , moving bed bio reactor)	Biological treatment processes in which the microorganisms responsible for the conversion of the organic matter or other constituents in the wastewater to gases and cell tissue are attached to some inert medium, such as rocks, slags, or specially designed ceramics or plastic materials. Attached-growth treatment processes are also known as fixed film processes.
Biological nutrient removal	The term applied to the removal of nitrogen and phosphorous in biological treatment processes.
Carbonaceous BOD removal	Biological conversion of the carbonaceous organic matter in wastewater to cell tissue and various gaseous end products. In the conversion ,it is assumed that the nitrogen present in the various compounds is converted to ammonia
Denitrification	The biological processes by which nitrate is converted to nitrogen and other gaseous end products.
Hybrid processes	Term used to describe combined processes (e.g, combined suspended and attached-growth processes)
Lagoon processes	A generic term applied to treatment processes that take place in pond or lagoons with various aspect ratios and depths.
Nitrification	The two-stage biological process by which ammonia is converted first to nitrite and then to nitrate
Stabilization	The biological process by which the organic matter in the sludges produced from the primary setting and biological treatment of wastewater from the primary settling and biological treatment of wastewater is stabilized, usually by conversion to gases and cell tissue. Depending on whether this stabilization is carried out under aerobic or anaerobic conditions, the process is known as aerobic or anaerobic digestion
Substrate	The term used to denote the organic matter or nutrients that are converted during biological treatment or that may be limiting in biological treatment.

	For example , the carbonaceous organic matter in wastewater is referred to as the substrate that is converted during biological treatment
Suspended-growth processes	Biological treatment processes in which the microorganism responsible for the conversion of the organic matter or other constituents in the wastewater to gases and cell tissue are maintained in suspension which the liquid.

MICROBIAL METABOLISM, ENERGETICS, AND GROWTH

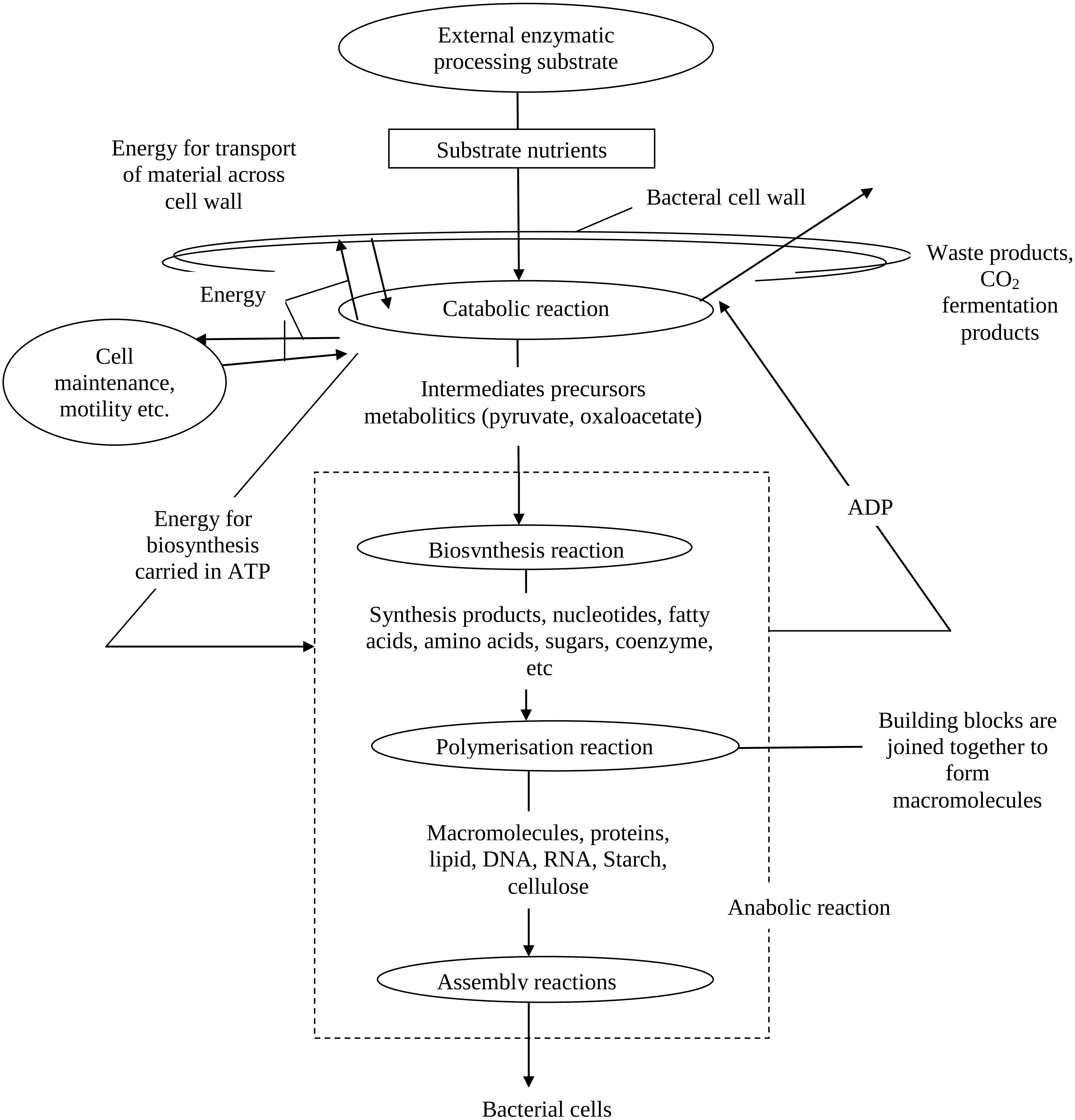

FIGURE-schematic representation of bacterial metabolism composed principally of catabolic and anabolic reactions.

18.2 Principles of Biological Wastewater Treatment

Under proper environmental conditions, the soluble organic substances of the wastewater are completely destroyed by biological oxidation; part of it is oxidized while rest is converted into biological mass, in the biological reactors. The end products of the metabolisms are either gas or liquid; and on the other hand the synthesized biological mass can flocculate easily and it can be easily separated out in clarifiers. Therefore, the biological treatment system usually consists of (1) a biological reactor, and (2) a sedimentation tank, to remove the produced biomass called as sludge.

The growth of microorganisms and the rate at which the substrate will be utilized with respect to time will depend on the type of the reactor employed and environmental conditions. This can be represented for batch process (Figure 18.1) and continuous process (Figure 18.2) differently.

18.2.1 Batch Process

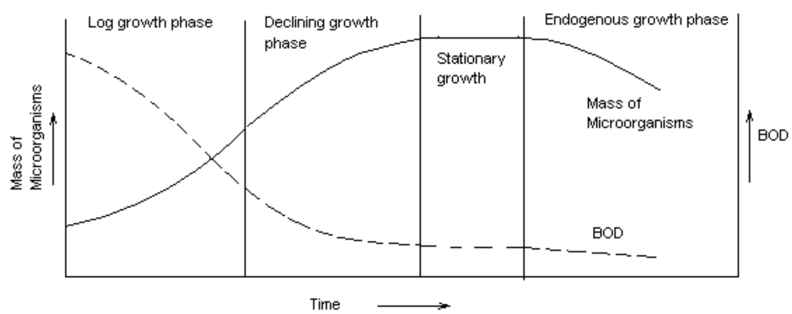

Figure 18.1 Growth of microorganisms under batch process

During fresh commissioning of the reactor if the microorganisms inoculated in the reactor are not adapted (acclimatized) to the type of wastewater being treated, there may be some ***lag phase***. During this phase there will be some lag time before the substrate is being accepted by the microbes, hence to reflect in substrate depletion and microbial growth.

Log growth phase: Substrate is adequate in this phase and rate of metabolism is only dependent on the ability of microorganism to utilize the substrate.

Declining growth phase: The rate of metabolism and hence growth rate of microorganisms decreases due to limitations of substrate supply. This is referred as substrate limited growth condition where substrate available is not enough to support maximum growth rate of microorganisms.

Stationary phase: When the bacterial growth rate and decay rate are same there will be no net increase or decrease in mass of microorganism. This phase is referred as stationary phase.

Endogenous growth phase: The microorganisms oxidize their own protoplasm for energy (endogenous respiration) and thereby decrease in number and mass.

18.2.2 Continuous System

In continuous system 'Food to Microorganism" ratio (F/M) controls the rate of metabolism. For low F/M: Food available is lower hence, it is endogenous growth of microorganisms (Figure 18.2). For high F/M: Food available is abundant; hence the growth phase is log growth phase. In between the growth rate will be declined growth phase. The biological reactors are typically operated at declining growth phase or endogenous growth phase with sufficient F/M ratio so that the microorganisms mass is at least constant, and not depleting. The sludge produced at log phase is of very poor in settling characteristics and the sludge produced in the endogenous phase has better settling properties and settles well and is more stable.

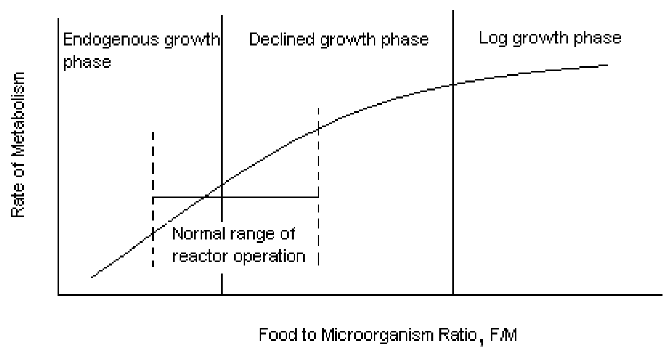

Figure 18.2 Rate of metabolism in continuous reactors for different F/M ratio

18.3 Nutritional Requirements For Microbial Growth

For reproduction and proper functioning of an organism it must have

- A source of energy
- Carbon for the synthesis of new cellular material
- Nutrients such as N, P, K, S, Fe, Ca, Mg, etc.

Energy needed for the cell synthesis may be supplied by light or by chemical oxidation reaction catalyzed by the bacteria. Accordingly the microbes can be classified as:

Phototrophs: Organisms those are able to use light as an energy source. These may be heterotrophic (certain sulphur reducing bacteria) or autotrophic (photosynthetic bacteria and algae).

Chemotrophs: Organisms that derive their energy from chemical reaction. These may be either heterotrophic, those derive energy from organic matter like protozoa, fungi, and most bacteria or may be autotrophic like nitrifying bacteria. Accordingly they are called as **Chemoheterotrophs** (those derive energy from oxidation of organic compounds) and **chemoautotrophs** (those obtain energy from oxidation of reduced inorganic compounds such as ammonia, nitrite, sulphide).

Source of Carbon*:* The source of carbon for synthesis of new cell could be organic matter (used by heterotrophs) or carbon dioxide (used by autotrophs).

Nutrient and growth factor requirement: The principal inorganic nutrients required by microorganisms are N, S, P, K, Mg, Ca, Fe, Na, Cl, etc. Some of the nutrients are required in trace amount (very small amount) such as, Zn, Mn, Mo, Se, Co, Ni, Cu, etc. In addition to inorganic nutrients, organic nutrients may also be required by some organisms and they are known as 'growth factors'. These are compounds needed by an organism as precursors or constituents of organic cell material that cannot be synthesized from other carbon sources. Requirements of these nutrients differ from organism to organism. For **aerobic pro**cesses generally minimum **COD:N:P ratio of 100:10:1-5** is maintained. In case of **anaerobic** treatment minimum **COD:N:P ratio of 350:5:1** is considered essential. The nutrient requirement is lower for anaerobic process due to lower growth rate of microorganisms as compared to aerobic process. While treating sewage external macro (N, P, K, S) and micro (trace metals) nutrients addition is not necessary; however incase of industrial effluent treatment, external addition of these may be required depending upon the characteristics of the wastewater.

18.4 Types of Microbial Metabolism

Aerobic microorganisms: When molecular oxygen is used as terminal electron acceptor in respiratory metabolism it is referred as aerobic respiration. The organisms that exist only when there is molecular oxygen supply are called as obligately aerobic.

Anoxic microorganisms: For some respiratory microorganisms oxidized inorganic compounds such as sulphate, nitrate and nitrite can function as electron acceptors in absence of molecular oxygen; these are called as anoxic microorganisms.

Obligately anaerobic: These are the microorganisms those generate energy by fermentation and can exist in absence of oxygen.

Facultative anaerobes: These microorganisms have ability to grow in absence or presence of oxygen. These can be divided in two types: (a) *True facultative anaerobes*: those can shift from fermentative to aerobic respiratory metabolism, depending on oxygen available or not; (b) *Aerotolerant anaerobes*: these follow strictly fermentative metabolism and are insensitive if oxygen is present in the system.

18.5 Types of Biological Reactors

Depending upon availability of oxygen or other terminal electron acceptor the biological reactors are classified as aerobic, anaerobic, anoxic or facultative process. Depending on how the bacteria are growing in the reactors they can be classified as (a) **suspended growth process**: where bacteria are grown in suspension in the reactor without providing any media support such as activated sludge process, and (b) **attached growth process**: where microorganism growth occurs as a biofilm formed on the media surface provided in the reactor such as trickling filters. This media could be made from rocks or synthetic plastic media offering very high surface area per unit volume. The media could be stationary in the reactor, as in trickling filter, which is called as fixed film reactor or it could be moving media as used in moving bed bioreactor (MBBR). Hybrid reactors are becoming popular these days which employ both suspended growth as well as attached growth in the reactor to improve biomass retention and substrate removal kinetics such as submerged aerobic filters (SAF).

Major biological treatment processes used for wastewater treatment		
Type	**Common name**	**Uses***
Aerobic processes		
1) Suspended-growth	Activated sludge processes	Carbonaceous BOD removal, nitrification
	Aerated lagoons	Carbonaceous BOD removal, nitrification
	Aerobic digestion	Stabilization , Carbonaceous BOD removal
2) Attached-growth	Trickling filters	Carbonaceous BOD removal, nitrification
	Rotating biological contactors	Carbonaceous BOD removal, nitrification
	Packed-bed reactors	Carbonaceous BOD removal, nitrification
3) Hybrid (combined)	Trickling filter/activated sludge	Carbonaceous BOD removal, nitrification
Suspended–and attached growth processes	Constructed wetland	Carbonaceous BOD removal, nitrification, trace metal removal
Anoxic processes:		
1) Suspended-growth	Suspended-growth denitrification	Denitrification
2) Attached-growth	Fixed film denitrification	Denitrification
Anaerobic processes:	Anaerobic contact processes	Carbonaceous BOD removal
1) Suspended growth	Anaerobic digestion	Stabilization , Carbonaceous BOD removal
2) Attached-growth	Anaerobic fixed bed	Carbonaceous BOD removal, waste stabilization (denitrification)
3) Hybrid	Upflow anaerobic sludge blanket (UASB) reactor	Carbonaceous BOD removal, especially high strength wastes
	Upflow sludge blanket /fixed-bed reactor	Carbonaceous BOD removal
Combined anaerobic, anoxic and anaerobic processes:		
1) Suspended growth	Single- or multistage processes, varous proprietary processes	Carbonaceous BOD removal, nitrification, denitrification, and phosphorous removal
2) Combined Suspended–and attached growth processes	Single- or multistage processes	Carbonaceous BOD removal, nitrification, denitrification, and phosphorous removal
Lagoon processes:		Carbonaceous BOD removal

1) Aerobic Lagoon	Aerobic Lagoon	Carbonaceous BOD removal
2) Maturation (tertiary) lagoon	Maturation (tertiary) lagoon	Carbonaceous BOD removal(nitrification)
3) Facultative lagoons	Facultative lagoons	Carbonaceous BOD removal
4) Anaerobic lagoons	Anaerobic lagoons	Carbonaceous BOD removal, waste stabilization

Biological treatment kinetics

Cell growth

In both batch and continuous culture reactors in which content of the reactor are mixed completely, the rate of growth bacterial cells can be defined by

$$\mathbf{r_g = \mu x} \qquad \text{-------------------(1)------}$$

Where, r_g = rate of bacterial growth, mass/ until vol time = dx/dt

μ = specific growth rate, $time^{-1}$

x = conc. of microorganism, mass/ unit vol.

Substrate- limited growth

In batch culture –substrate and nutrients would be depleted and growth would cease.

In continuous culture, the effect of a limiting substrate or nutrient can be defined as per Monod (1942)

$$\mu = \mu_m \frac{S}{k_s + S} \qquad \text{---------------------------- (2)}$$

Where, μ = specific growth rate, $time^{-1}$

μ_m = max. specific growth rate, $time^{-1}$

S = conc. of growth limiting substrate in solution, mg/L

Ks = half-velocity constant, substrate conc. at one-half the max growth rate, mg/L

If value of μ is substituted in eq (1)

$$r_g = \frac{\mu_m . x . S}{K_s + S} \qquad \text{---------------------------(3)}$$

Cell growth and substrate utilization

Substrate utilization and rate of growth of microbes can be correlated

$$r_g = -Y r_{su} \qquad \text{---------------------------(4)}$$

r_g = rate of bacterial growth, mass/unit vol. time

Y = max. yield coefficient, mg/mg (cell/substrate)

r_{su} = substrate utilization rate, mass/unit vol. time from equation 3 & 4

$$\boldsymbol{r_{su} = -\frac{\mu m.X.S}{Y(K_s+S)}} \quad (5)$$

$\frac{\mu_m}{y}$ = k = max rate of substrate utilization per unit mass of microorganism

$$\mathbf{r_{su}} = -\frac{\boldsymbol{K.X.S}}{\boldsymbol{K_S+S}} \quad (6)$$

Effect of Endogeneous metabolism

- Not all cells are under log growth phase due to distribution of cell ages.
- Other factors such as death and reproduction, should also be considered.

The decrease in cell mass caused by these factors is proportional to the concentration of organisms, which is identified as endogenous decay.

$$\mathbf{r'_g} = \frac{\boldsymbol{\mu_m.X.S}}{\boldsymbol{K_S+S}} - \boldsymbol{K_d.X}$$

$= - Y.\ r_{su} - K_d.X$

Effect of temperature

Effect of temperaturwe on reaction rate of a biological process is expressed as

$$\boldsymbol{r_T = r_{20}\theta^{(T-20)}}$$

r_T = reaction rate at T°C, r_{20} = reaction rate at 20°C

θ = temp. activity coefficient; 1.02 to 1.09 (1.04 typical) it is different than BOD reaction rate constant

Other rate expression:

$$r_{su} = -k$$

$$r_{su} = -k.S$$

$$r_{su} = -k.X.S$$

$$r_{su} = -k.X.\frac{S}{S_0}$$

$$r_{su} = -\frac{k.s}{k_s + S_0}$$

It is important that specific rate expression should not be generalized to cover a broad range of situations on the basis of limited data or experience.

Questions

1. Why aerobic processes produce more sludge as compared to anaerobic process?
2. With the help of figure explain how the rate of metabolism and hence the growth phase will vary with changes in food to microorganisms ratio in case of continuously feed biological reactor.
3. Explain nutritional requirements for bacterial metabolism.
4. Describe the types of microbial metabolism used in wastewater treatment.