Kinetika pertumbuhan mikroba

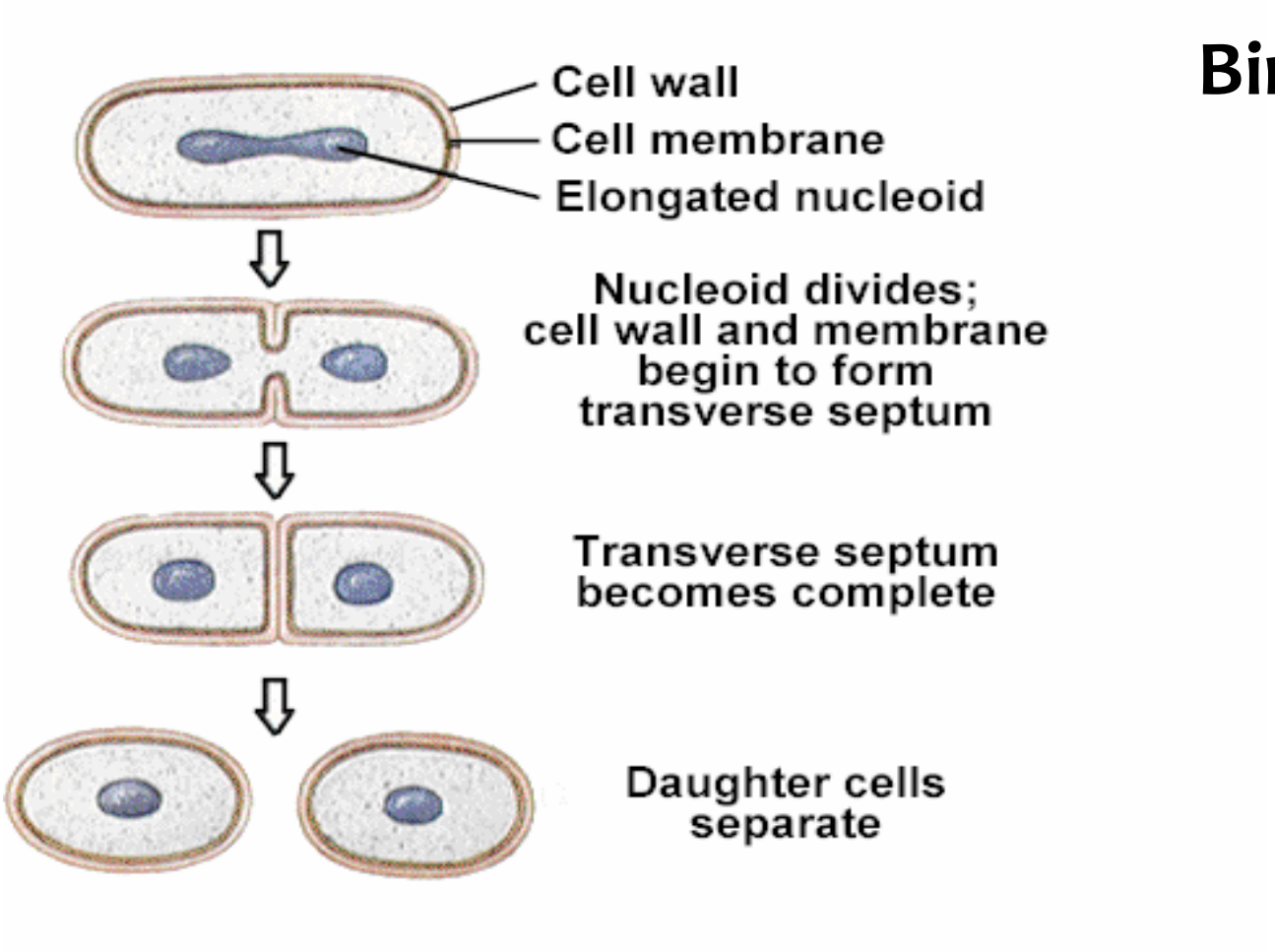
Karakteristik pertumbuhan mikroba

- ✓ Pertumbuhan mikroba merupakan pertambahan jumlah sel mikroba
- ✓ Pertumbuhan mikroba berlangsung selama nutrisi masih cukup tersedia
- ✓ Pertumbuhan mikroba dapat diukur, dengan melihat kenaikan biomassa atau jumlah sel
- ✓ Selama pertumbuhan, mikroba menghasilkan metabolit primer/sekunder berupa produk

Pertumbuhan mikroba

- ✓ Pertumbuhan bakteri : proses kompleks yg melibatkan reaksi anabolic dan katabolic
- ✓ Pd media kultur yg homogen, di bwh kondisi ideal, sebuah sel dpt membelah dalam 10 menit
- ✓ Tapi, ada juga sel yg membelah sgt lambat, hingga 100 th, pd beberapa bakteri subsurface terrestrial
- ✓ Pertumbuhan mikroorganisme dikontrol di lab, diperoleh menggunakan kultur murni

Binary Division



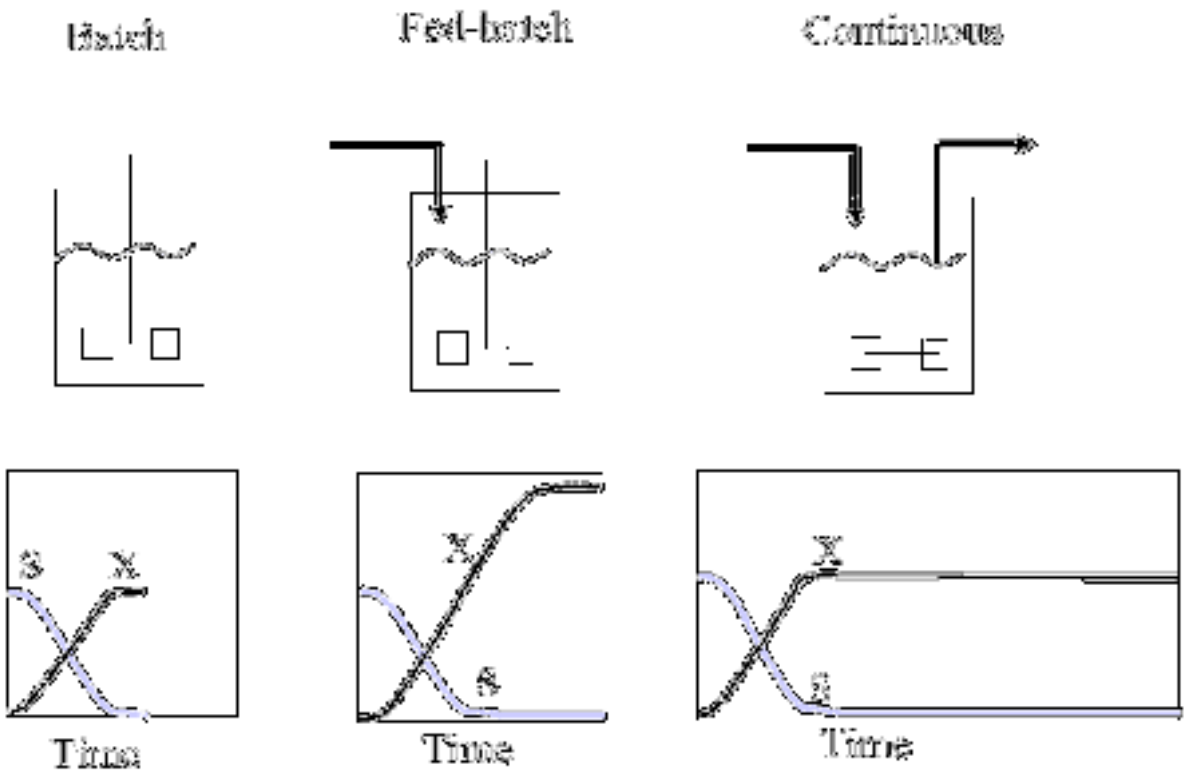
Kinetika pertumbuhan mikroba

Fermentasi dapat dilakukan secara :

1. Batch
2. Continuous
3. Fed-batch processes

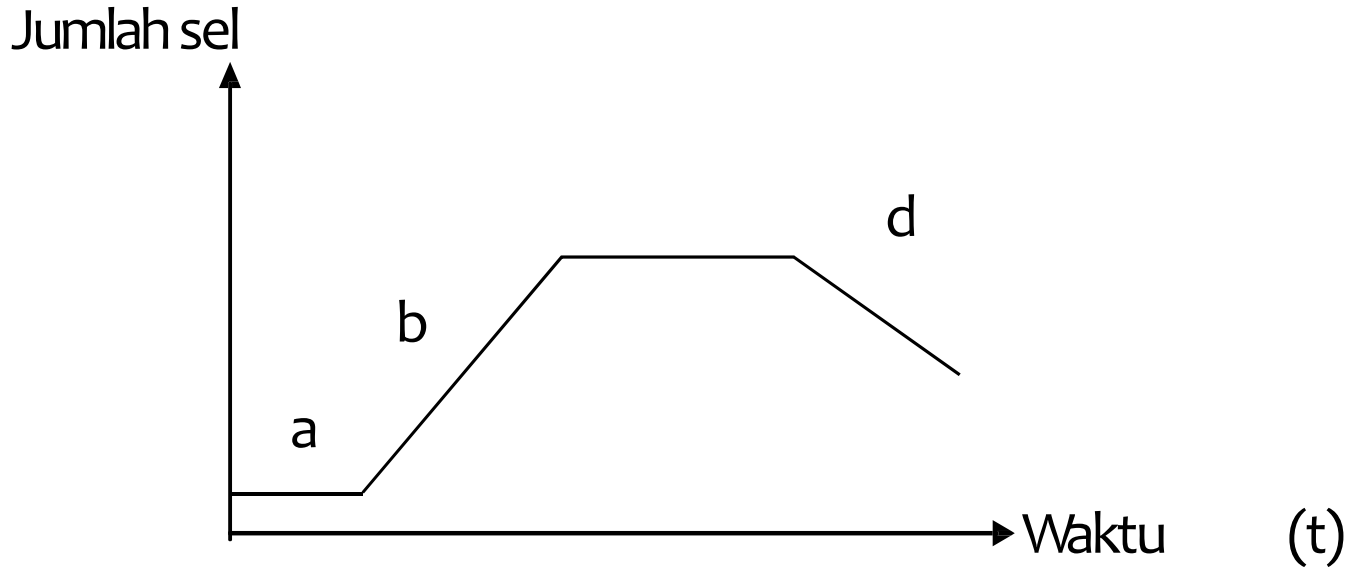
Pengoperasiannya tergantung produk yang diinginkan

Biotechnological processes of growing microorganisms in a bioreactor



Kurva Pertumbuhan mikroba

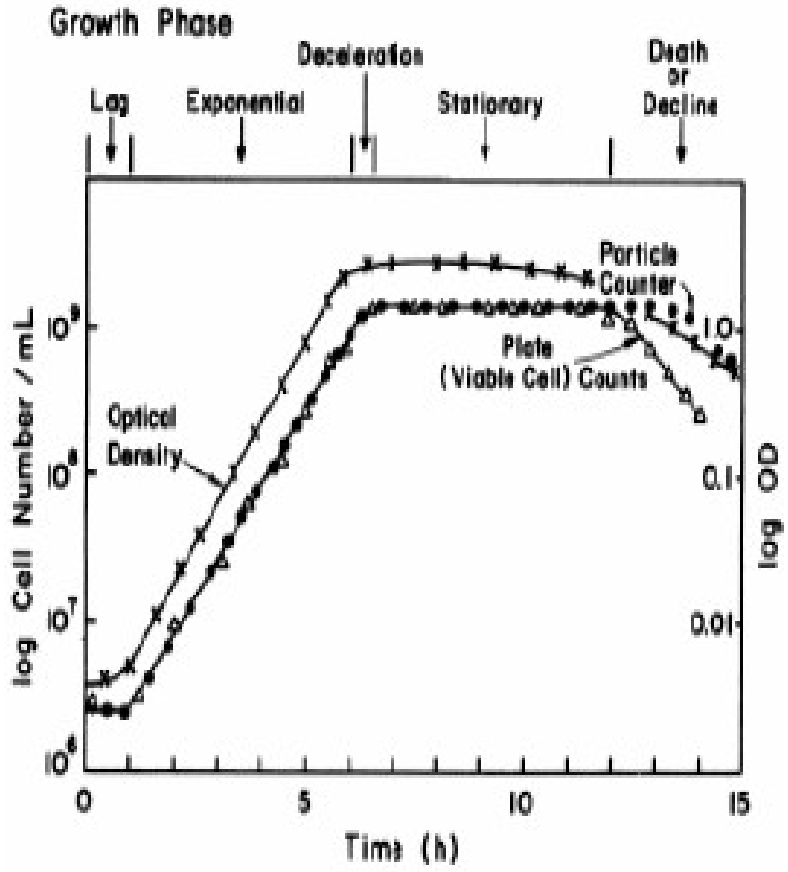
Pertumbuhan sel mikroba biasanya mengikuti suatu pola pertumbuhan tertentu berupa kurva pertumbuhan sigmoid (model Monod)



Microbial Growth Kinetics

Microbial Growth Kinetics describe how the microbe grows in the fermenter. This information is important to determine optimal batch times. The growth of microbes in a fermenter can be broken down into four stages:

- Lag Phase
- Exponential Phase
- Stationary Phase
- Death Phase



a. **FASE LAG (Fase Adaptasi)**

Fase lag merupakan suatu periode penyesuaian terhadap medium------- tidak terjadi perbanyakan jumlah sel

b. **FASE LOG (Fase Eksponensial)**

Pada fase eksponensial atau logaritmik, sel membelah dengan kecepatan konstan dan terjadi pertambahan jumlah sel menjadi 2 kali lipat (generation time)

c. **FASE STASIONER.**

Selama fase ini, jumlah sel yang hidup tetap konstan tetapi akhirnya menuju periode penurunan populasi.
Dihasilkan metabolit sekunder untuk pertahanan diri bakteri

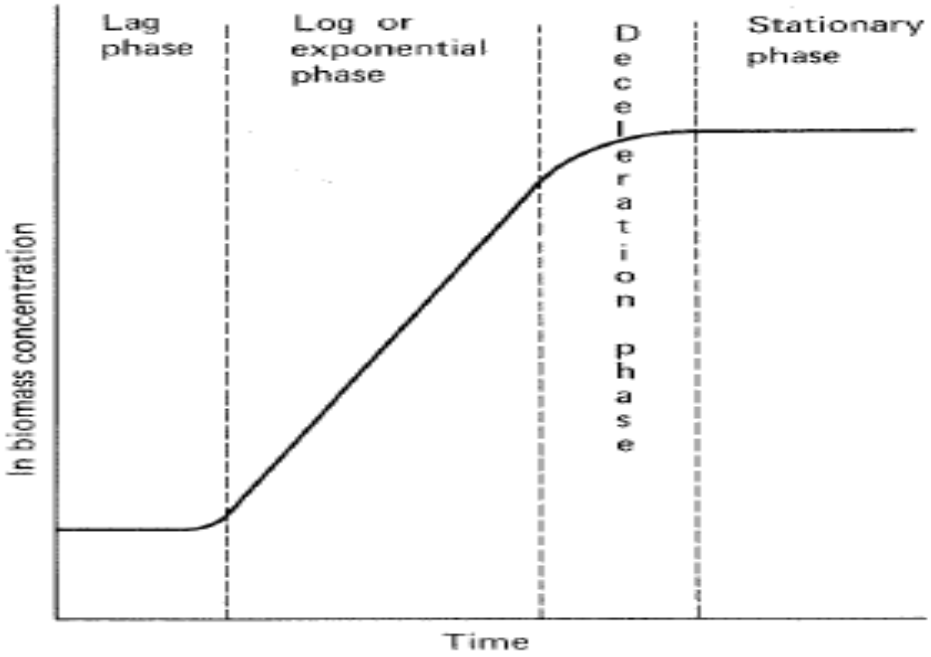
d. FASE PENURUNAN POPULASI ATAU FASE KEMATIAN

Pada saat medium kehabisan nutrien maka populasi bakteri akan menurun jumlahnya,

Pada saat ini jumlah sel yang mati lebih banyak daripada sel yang hidup.

Batch culture

- Batch culture : sistem kultur tertutup yang mengandung jumlah nutrient yg terbatas. Kultur inokulat akan melewati beberapa fase :



- ✓ Setelah proses inokulasi, tidak ada lagi pertumbuhan → lag phase, dan membutuhkan waktu utk adaptasi.
- ✓ Pada proses komersial, lama waktu lag phase sebaiknya dikurangi yaitu dg memilih inoculum yg sesuai.
- ✓ Seiring berjalannya waktu laju pertumbuhan mikroba meningkat, sel tumbuh pd kondisi konstan atau maksimum → log atau exponensial phase.

Perubahan konsentrasi microbial **pd exponential phase** dituliskan dg pers :

$$\frac{dx}{dt} = \mu x$$

Di mana x : konsentrasi microbial biomass

t : waktu (jam)

μ : laju pertumbuhan spesifik (jam^{-1})

Apabila pers di atas diintegralkan maka :

$$x_t = x_0 e^{\mu t}$$

Xo : konsentrasi biomass mula-mula

Xt : konsentrasi biomassa setelah waktu t

Pers tsb bisa diubah mjd :

$$\ln x_t = \ln x_o + \mu t$$

Apabila dibuat grafik ln x_t vs t, akan diperoleh slope = μ

Pada exponential phase, nutrient berlebih dan organisme akan tumbuh dengan laju maksimum μ_{max}.

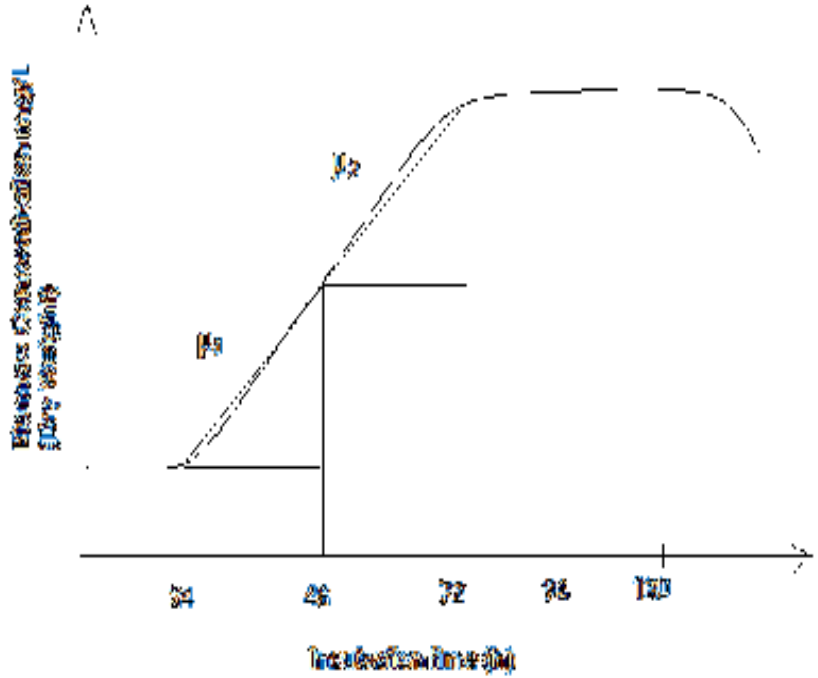
TABLE 2.1. *Some representative values of* μ_{max} *(obtained under the conditions specified in the original reference) for a range of organisms*

Organism	μ_{max} (h^{-1})	Reference
Vibrio natriegens	4.24	Eagon (1961)
Methylomonas methanolytica	0.53	Dostalek *et al.* (1972)
Aspergillus nidulans	0.36	Trinci (1969)
Penicillium chrysogenum	0.12	Trinci (1969)
Fusarium graminearum Schwabe	0.28	Trinci (1992)
Plant cells in suspension culture	0.01–0.046	Petersen and Alfermann (1993)
Animal cells	0.01–0.05	Lavery (1990)

- ✓During batch cultivation, specific growth rate changes continuosly from zero to the max value μ_{max}.
- ✓μ_{max} depends on microorganisms, physical, chemical conditions.
- ✓Typical values of μ_{max}:

Microorganisms	Cultivation Temperature	μ_{max} (h^{-1})
Bacteria	37°C	0.6-1.2
Yeast	30°C	0.3-0.5
Actinomycetes	28°C	0.1-0.3
Fungal	28°C	0.1-0.3

- ✓By plotting the growth curve of the microorganisms, then determine the instavenous μ value at each sampling time by ascertaining the tangent at the point of contact on the growth curve.
- ✓The highest value obtained (from 24-72h) is the μ_{max}.



Konsentrasi biomassa dipengaruhi oleh initial substrate, dmn konsentrasi initial substrate menaikkan produksi biomassa, sesuai pers :

$$x = Y(S_R - s)$$

x : konsentrasi biomassa yg dihasilkan

Y : yield factor (g biomas yg dihasilkan/g substat yg dikonsumsi)

S_R : konsentrasi initial substrate

s : konsentrasi residual substate

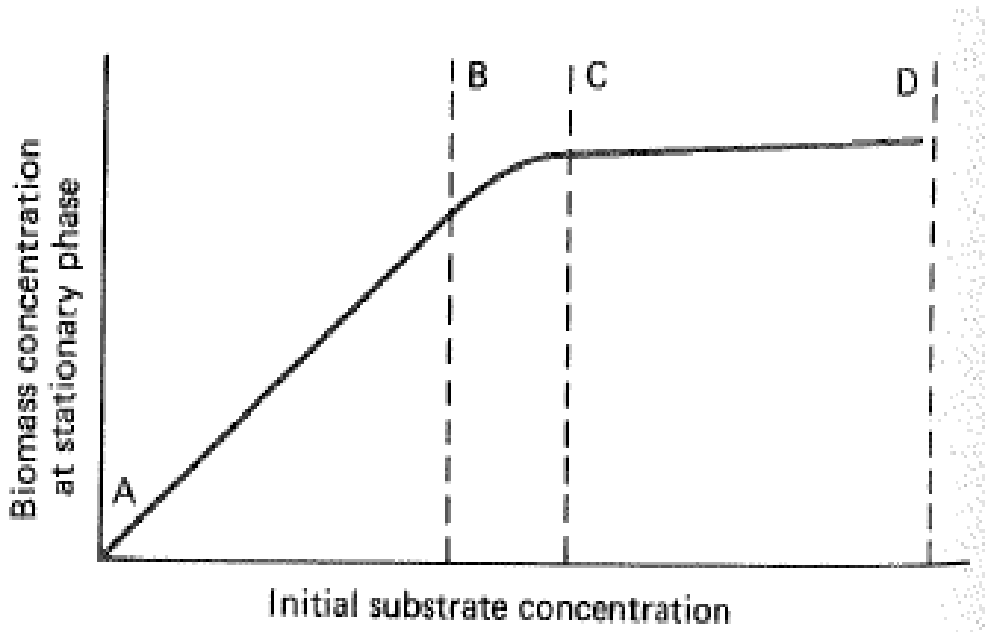


FIG. 2.2. The effect of initial substrate concentration on the biomass concentration at the onset of stationary phase, in batch culture.

- ✓Pada zona A dan B, s sama dg 0, shg pers di atas dpt digunakan utk memprediksi biomass yg mgkn terbentuk dr jml substrat
- ✓Pd zona C dan D, kenaikan konsentrasi initial substrate tdk mempengaruhi kenaikan biomassa.
- ✓Nilai Y : pengukuran efisiensi konversi substrat mjd biomassa dan digunakan utk memprediksi konsentrasi substrat yg dibutuhkan utk memprediksi biomass
- ✓Nilai Y dipengaruhi oleh laaju pertumbuhan, pH, suhu, keterbatasan substrat dan konsentrasi substrat

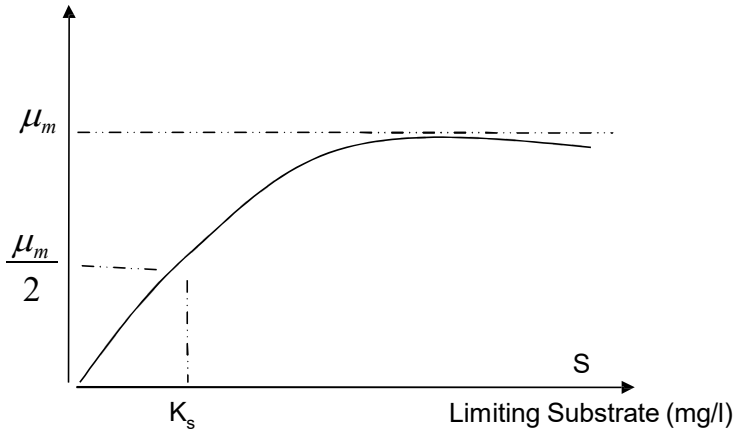
Penurunan laju pertumbuhan dan terminasi pertumbuhan dapat digambarkan dg hubungan μ dan substrat sisa:

$$\mu = \frac{\mu_{max} s}{(K_s + s)}$$

Monod equation, which developed by Jacques Monod in the 1940s

s : konsentrasi substrat sisa

Ks : konstanta kebutuhan substrat pd saat half rate



There are two constants in this equation, μ_{max} maximum specific growth rate and K_s, the half saturation constant.

Both reflect intrinsic physiological properties of particular type of microorganisms.

They also depend on substrate being utilized and temperature of growth.

Monod equation can be expressed in terms of cell number or cell mass (x) as the following:

$$\frac{dx}{dt} = \mu x, \dim ana \mu = \mu_m \frac{S}{K_s + S}$$

$$sehingga : \frac{dx}{dt} = \frac{\mu_m S x}{K_s + S}$$

The Monod equation has two limiting cases:

1. **High substrate concentration:** S >> Ks $\longrightarrow \frac{dx}{dt} = \mu_m x$

- Under these condition, growth will occur at the maximum growth rate

2. **Low substrate concentration:** S << Ks $\longrightarrow \frac{dx}{dt} = \frac{\mu_m}{K_s} Sx$

- **This type of growth is typically found in batch flask systems at the end of the growth curve as the substrate is nearly all consumed.**

- **It is also the typical growth that happened in the natural environment where substrate and nutrients are limiting.**

TABLE 2.2. *Some representative values of* K_s *for a range of micro-organisms and substrates*

Organism	Substrate	K_s (mg dm^{-3})	References
Escherichia coli	Glucose	6.8×10^{-2}	Shehata and Marr (1971)
Saccharomyces cerevisiae	Glucose	25.0	Pirt and Kurowski (1970)
Pseudomonas sp.	Methanol	0.7	Harrison (1973)

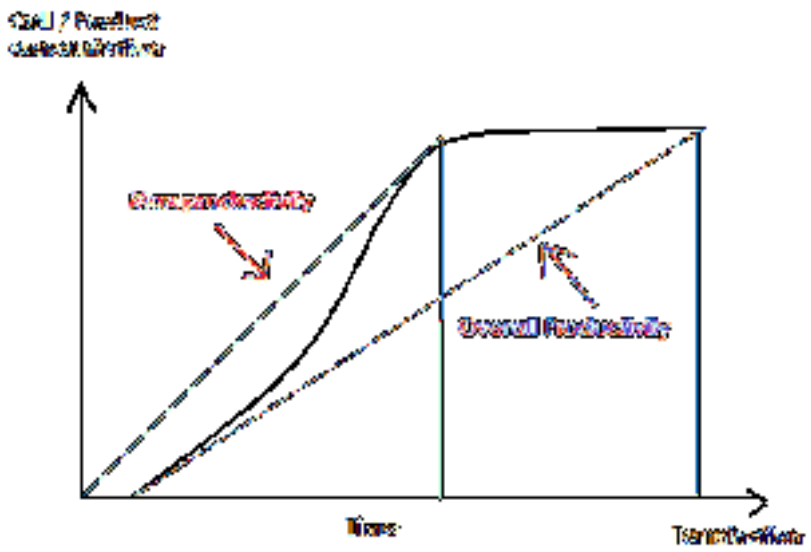
Stasionary phase

- ✓Stasionary phase pada batch culture adl titik dmn laju pertumbuhan turun ke 0.
- ✓Pd kondisi ini, mikroba mungkin akan memproduksi metabolit sekunder yg tdk diproduksi pd exponential phase
- ✓Misalnya : terjadinya biosintesis asam giberelik oleh *Gibberella fujikurio* melalui 2 fasa :
  - Balanced phase : sm dg kondisi awal hingga tengah pd exponential phase
  - Storage phase : sm dg kondisi akhir exponential phase, dmn biomassa naik krn akumulasi lemak dan karbohidrat
  - Maintenance phase : sm dg stationary phase

Batch culture

- Pd batch culture, produktivitas paling tinggi dicapai pada μmax dan akan meningkat dg naiknya μ dan konsentrasi biomassa
- Batch culture/fermentation dapat digunakan utk memproduksi biomassa : metabolit primer dan metabolit sekunder
- Productivitas – pengukuranproduk/biomassa yg dihasilkan per unit waktu (g/L/h).
- Produktivitas pd batch culture akan mencapai maksimum ketika laju pertumbuhan maksimum (μmax).

$$\text{Productivity } (R_{batch}) = \frac{X_{max} - X_o}{T_{final} - T_{initial}}$$



Where;

X_{max} *= maximum cell concentration at stationary phase*

X_o *= initial cell during inoculation*

T_{final} *= time during which organism growing at* μ_{max}

$T_{initial}$ *= time which organism not growing at* μ_{max}*, including lag phase, deceleration phase period of batching, sterilizing and so on.*

The Yield Coefficient (Y)

- *A measure of the overall efficiency of the conversion of substrate to cell mass or specific product:*

Parameter	Equation
Cell (Y x/s)	$\Delta X / \Delta S$
Product (Y p/s)	$\Delta P / \Delta S$
Product (Y p/x)	$\Delta P / \Delta X$

- *Y is not constant, will vary depending on organism, pH, temperature and substrate*

Metode mengukur pertumbuhan mikroba

- Metode langsung:
  - Penetapan konsentrasi sel: penghitungan jumlah sel dibawah mikroskop
  - Penetapan bahan kering sel----ditimbang

- Metode tak langsung
- Metode turbidity (kekeruhan)---optical density
- Penetapan penyusun sel
- Analisis persenyawaan (reaksi) biakan

Kinetika Pertumbuhan mikroba

- Merupakan suatu rangkaian reaksi kimia yang mengendalikan sintesis penyusunan biomassa yang diperoleh pada akhir biakan secara menyeluruh yang mengikuti prinsip kekekalan massa

Reaksi kimia pertumbuhan mikroba dalam suatu medium biakan

Substrat ⟶ mikroba + produk

Sumber: karbon
nitrogen
oksigen
fosfor
belerang
mineral

metabolit
CO_2
H_2O
enzim

Kesetimbangan kimia pada pertumbuhan aerobik

$$a(\text{substrat}) + bO_2 + cNH_4^+ \longrightarrow \text{Biomassa} + dCO_2 + eH_2O$$

Komposisi Substrat berkarbon: $CuOvHwNt$

Biomassa : $CxHyOzNe$

Maka:

$$aCuOvHwNt + bO2 + cNH_4^+ \longrightarrow CxHyOzNe + dCO_2 + eH_2O$$

Menghitung rendemen (yields)

$$Y_{x/s} = \frac{\text{g/L biomassa}}{\text{g/L substrat karbon yg digunakan}}$$

Menghitung Economic yield, Yp/x

$$Y_{P/X} = \frac{\text{g/l produk yg dihasilkan}}{\text{g/L biomassa yg terbentuk}} = g\ /\ g$$

Tabel rendemen biomassa dan keb.oksigen

Substrat	**Mikroba**	**Yx/s**	**Kebutuhan O2** (gO2/g biomassa kering)
Glukosa	*E.coli*	*0,53*	*0,4*
	C.utilis	*0,54*	*0,6*
Methanol	*Pseudomonas*	*0,54*	*1,2*
Ethanol	*S.cerevisiae*	*0,63*	*2,0*
Metana	biakan bakteri campuran	0,62-0,99	2,6-4,8

Latihan soal

Suatu penelitian mengenai produksi etanol oleh bakteri *Zymomonas mobilis* pada biakan curah diperoleh hasil sebagai berikut:

Waktu (jam)	Biomassa (g/l)	Glukosa (g/l)	Etanol (g/l)
5	0,05	247	1.5
9	0,15	240	5
14	0,45	225	12
18	1,20	195	22
22	2,80	130	47
24	3,40	100	63
26	3,80	75	74
30	4,15	40	90
35	4,20	25	100

Tentukanlah !

a. Laju pertumbuhan spesifik
b. Rendemen biomassa
c. Rendemen hasil (etanol yang dihasilkan)
d. Economic yield

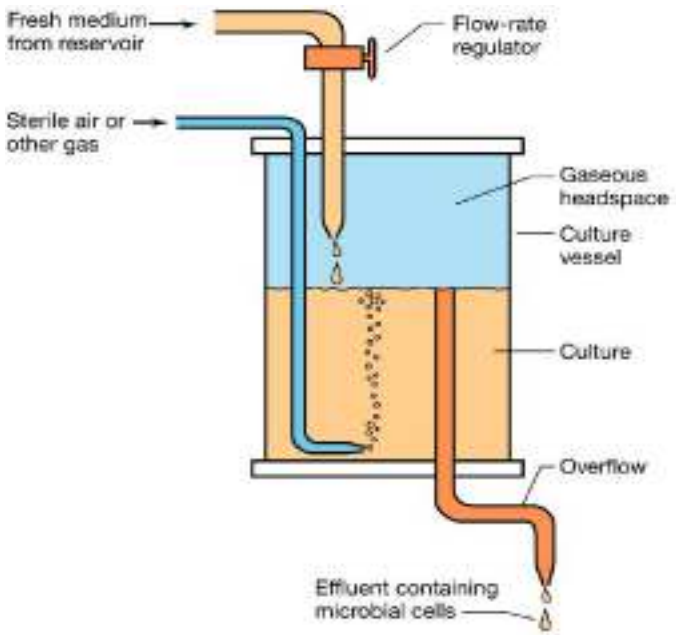
CONTINUOUS CULTURE

- Fresh fermentation media is continuosly added to the reactor while fermenter broth containing biomass, products and unused nutrient are continuosly removed.
- Exponential growth in batch culture may be prolonged by the addition of fresh medium to the vessel.
- Growth can be maintained for long duration
- Continuous feeding to a culture at a suitable rate $\longrightarrow$ formation of new biomass by the culture is balanced by the loss of cell from the vessel $\longrightarrow$ STEADY STATE.

Cells Growth in Continuous Culture

Continuous culture: fresh nutrient medium is continually supplied to a well-stirred culture and products and cells are simultaneously withdrawn.

At steady state, concentrations of cells, products and substrates are constant.



Cells Growth in Continuous Culture

The vessel that is used as a growth container in continuous culture is called a **bioreactor or a chemostat**.

Chemostat can produce microbial product more efficiently than batch fermentation.

As the chemostat can hold a culture in the exponential phase of growth.

The combination of growth and dilution within the chemostat will ultimately determine growth. Thus, the change in biomass with time is

$$\frac{dx}{dt} = \mu x - Dx$$

Where, x is the cell mass, μ is the specific growth rate and D is the dilution rate

A steady state will be reached when μ = D

$$\frac{dx}{dt} = \mu x - Dx$$

If μ > D, the utilization of substrate will exceed the supply of substrate, causing the growth rate to slow until it is equal to the dilution rate.

If μ < D, the amount of substrate added will exceed the amount utilized. Therefore, the growth rate will increase until it is equal to the dilution rate.

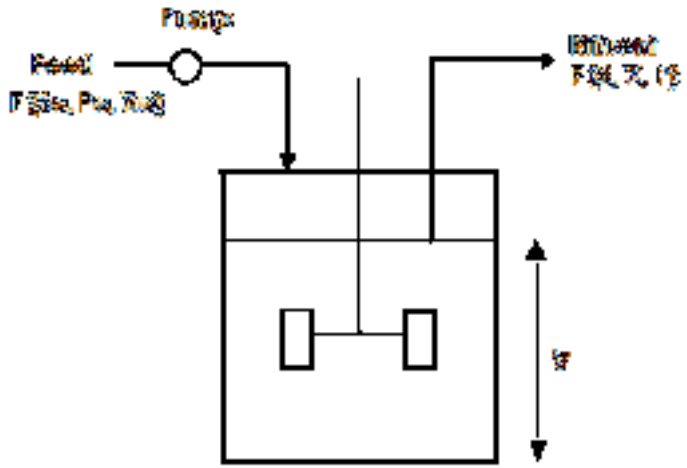
Steady state at μ = D, Such a steady state can be achieved and maintained as long as the dilution rate D does not exceed a critical rate, D_c

The critical dilution rate can be determined by substituting the value of μ in the following equation:

$$\mu = \mu_m \frac{S}{K_s + S}, \quad D_c = \mu \longrightarrow D_c = \mu_m \left(\frac{S}{K_S + S}\right)$$

Application of Continuous Culture

- Biomass production
- Growth associated product or primary metabolite – e.g: ethanol, citric acid
- Not suitable for non-growth associated or secondary metabolite – e.g: antibiotic



BATCH CULTURE	CONTINUOUS CULTURE
Nutrients added only at start	Nutrients added continuously
Product removed when fermentation stops.	Product continuously removed .
Growth rates and product formation are slower because limiting factors ex: substrate levels/ build up of toxins.	Organism held in exponential growth phase giving higher productivity so can be on a smaller scale.
Slower growth rates = Larger vessels are used.	
Easy to set up and maintain.	Can be very difficult to maintain conditions so that exponential phase is maintained. Foaming, clumping and blocked inlet pose problems.
If contamination occurs only one batch is wasted.	Contamination can afferct a huge volume of product/ organism.
Less efficient / more time wasted shutting down removing product and starting up again.	Continuous, therefore more efficient use of time.
Product quality can vary between batches.	Product quality more consistent.

FED BATCH CULTURE

- ✓Extending the batch culture by feeding continuously or periodically with medium with no removal of culture from the vessel.
- ✓Somewhere between batch and continuous culture.
- ✓A volume of medium is inoculated with the organism and allowed to grow for a batch period of time.
- ✓Subsequently, a feed is initiated into the fermenter when a "quasi steady state" is obtained.
- ✓Quasi steady state: when the growth limiting substrate has depleted.