7th Aug, 23

D.1

- Metabolic reactor coupling ATP and NAD

exergomic → energy is released.
endergomic → " consumed.

$$ATP + H_2O \rightarrow ADP + P_i$$

$$\Delta G^{0'} = -7.3 \text{ Kcal/mole.}$$

- Chemical free energy

$$RCHO + H_2O \longrightarrow 2H + RCOO^- + H^+$$

$$\Delta G^{0'} = -7000 \text{ cal/mol} \text{ --- i}$$

- Biochemical

$$RCHO + HPO_4^{2-} + ADP^{3-} \longrightarrow 2H + RCOO^- + ATP^{4-}$$

$$\Delta G_2^{0'} = 0 \text{ Kcal/mol} \text{ --- ii}$$

ii − i

$$ADP^{3-} + HPO_4^{2-} \longrightarrow ATP^{4-} + H_2O.$$

$$\Delta G_3^{0'} = +7 \text{ Kcal/mol}$$

classmate

Date

Page

2.2

Oxidation and reduction coupling NAD

$$\underset{\text{Pyruvic acid}}{CH_3-\overset{|}{C}=O-COOH} \;\underset{-2H}{\overset{+2H}{\rightleftharpoons}}\; \underset{\text{Lactic acid}}{CH_3-CHOH-COOH}$$

$+NADH \quad + NAD^+$

hydrogen will come from NADH molecules.

S.3 Carbon metabolism:-

3.1 EMP pathway

[Embden - Meyerhoff - Parnas]

6C

Glc $\xrightarrow{ATP \to ADP}$ G6P $\rightarrow$ F6P $\xrightarrow{ATP \to ADP}$ F-1,6-biphosphate $\rightarrow$ Dihydroxyacetone $\rightleftharpoons$ G3P $\rightarrow$ 1,3-DPG $\xrightarrow{ADP \to ATP}$ 2PG $\rightarrow$ PEP $\xrightarrow{ADP \to ATP}$ PYR

taken twice.

→ overall 2 mole ATP is Generated.

→ Amphibolic.

$$C_6H_{12}O_6 + 2P_i + 2ADP + 2NAD^+$$
$$\longrightarrow 2\,C_3H_4O_3 + 2ATP + 2(NADH + H^+).$$

3.2. <u>Glycolysis :-</u>

$$\underset{\text{Pyruvic acid}}{C_3H_4O_3} + NADH + H^+ \longrightarrow \underset{\text{Lactic acid}}{C_3H_6O_3} + NAD^+$$

$$\text{glucose} + 2P_i + 2ADP \rightarrow 2\,\text{lactate} + 2ATP + 2H_2O$$

$$\Delta G^{\circ\prime} = -32,400 \text{ cal/mol.}$$

in free state

$$\text{glc} \rightarrow 2 \text{ lactic acid} \quad \Delta G^{\circ\prime} = -47,000 \text{ cal/mol}$$

efficiency for free energy transfer

2c-

$$= \frac{-47000 - 32400}{-47000}$$

$$= 31\%$$

3.3 Pentose Phosphate (PP) pathway.

$$\text{Glucose} + 12\,NADP^+ + 7H_2O + ATP \longrightarrow 6CO_2 + P_i + 12(NADPH + H^+) + ADP$$

(Provide NADPH)

ribose - 5 - phosphat + erythrose - 4 - phosphat

Purine / pyrimidine.

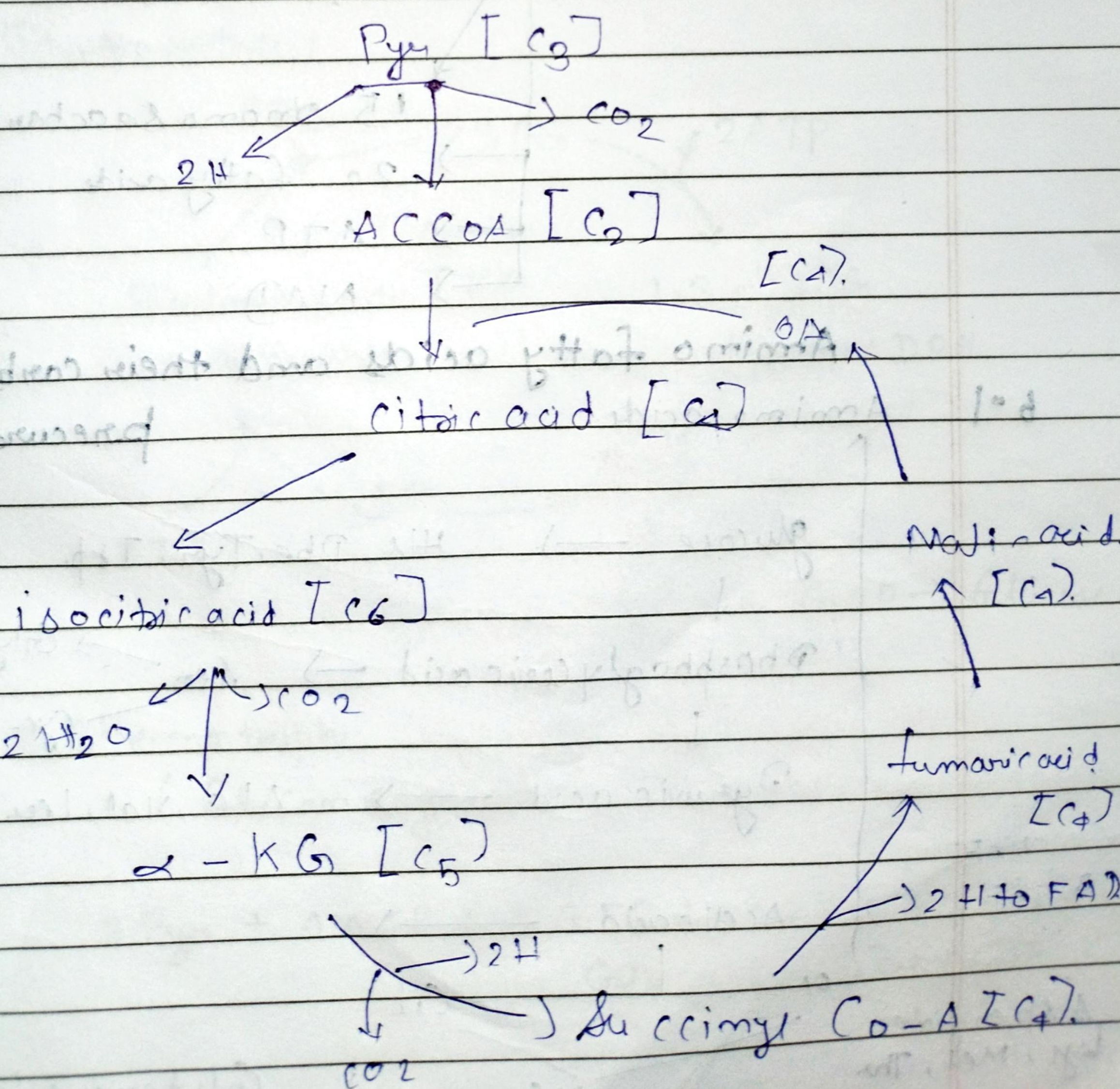
4. Respiration

Reductant	oxidant	Product
H_2	O_2	H_2O
H_2	SO_4^{2-}	$H_2O + S^{2-}$
Organic comp.	O_2	$CO_2 + H_2O$
NH_3	O_2	$NO_2^- + H_2O$
NO_2^-	O_2	$NO_3^- + H_2O$
Fe^{2+}	O_2	Fe^{3+}
orgain comp	NO_3^-	$N_2 + CO_2$

*

5.4 Respiration → organic comp oxidised to $CO_2 + H^+$
→ H^+ passed through serie of reaction H_2O.

5.4.1 TCA [citric acid cycle]



10th Aug, 2023

5.6 Biosynthesis

Small molecule biosynthesis

70 compounds → 4 Ribonucleotide
→ 4 Deoxyribonucleotide
→ 20 amino acid
→ 15 monosaccharides
→ 20 fatty acids
→ ATP
→ NAD

Amino fatty acids and their carbs precursor.

6.1 Amino acids

glucose → His, Phe, Tyr, Trp.
↓
Phosphoglyceric acid → Ser → Gly, Cys.
↓
Pyruvic acid → Ala, Val, Leu.

Acetic acid →

OA — Cit

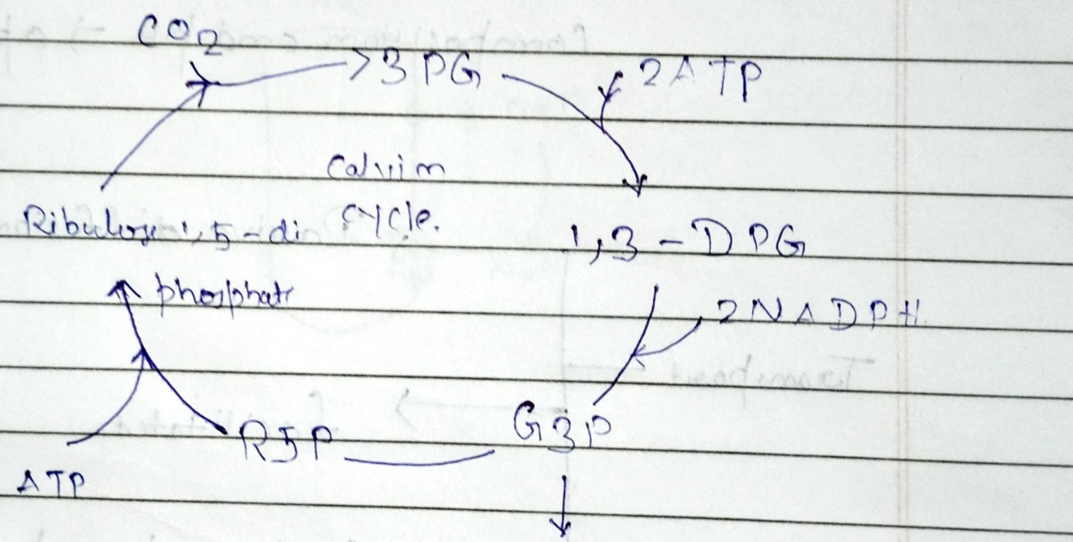
Aspargine
Lys, Met, Thr

αKG → Glutamine, Lys, Pro, Arg

$$AcCoA + ATP + CO_2 \xrightarrow{\text{Accase}} \text{Malonyl-CoA} + ADP + P_i$$

$$AcCoA + 7\ \text{Malonyl-CoA} + 14(NADPH + H^+) \longrightarrow \text{Palmitate} + 7CO_2 + 14\ NADP + 6H_2O$$

= Photosynthesis



= Chemotrophs
(Gluconeogenesis).

$$2\ Pyr + 4ATP + 2GTP + 2NADH + 2H^+ + 4H_2O \longrightarrow Glc + 2NAD^+ + 4ADP + 6P_i$$

Photosynthetic.

$$6CO_2 + 12NADPH + 18ATP + 12H_2O \rightarrow C_6H_{12}O_6 + 12NADP^+ + 18ADP + 18P_i$$

5.7 Transport Across cell membrane.

ions and molecules

↓

composition and pH → optimal activity.

Transport →
- Passive diffusion
- facilitated
- Active transport.

free energy change. diffusion rate ∝ conc. diffusion

to acompany $\Delta G^\circ = RT \ln \frac{C_1}{C_2}$

transport from $C_2 \rightarrow C_1$

in passive diffusion.

if compoment is charges.

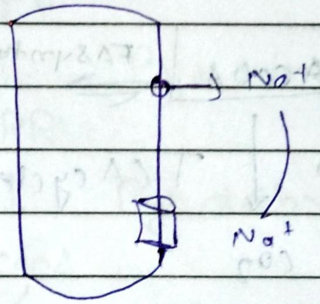
$$\Delta G^{o} = RT \ln \frac{C_1}{C_2} + Z_1 \mathcal{J} \Delta \Psi$$

Z_1 = No. of charge on transported molecule

$\mathcal{J}$ = Faraday = 23062 kcal/v/mol.

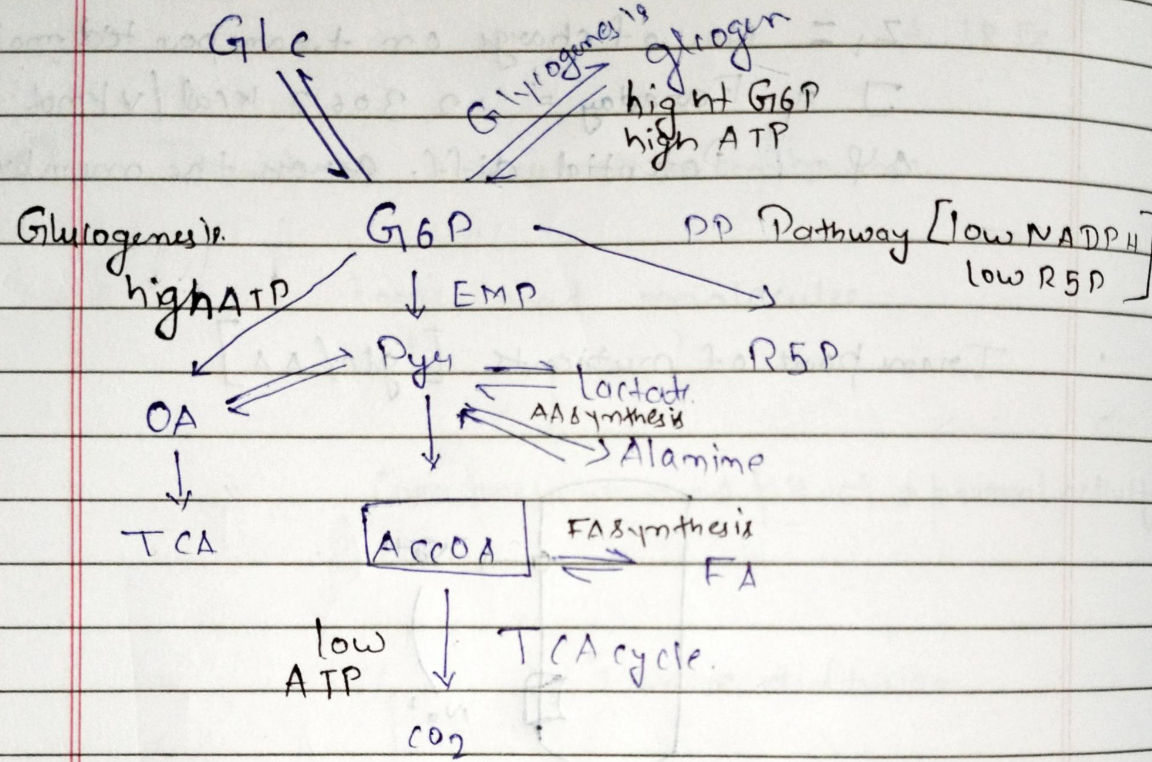
$\Delta \Psi$ = Potential diff. across the membrane

- Transport of nutrients [glc/AA]



14th Aug, 23

S·8 Metabolic Organisation and Regulation



8·2. Energy State of a cell

ADENYLATE energy charge

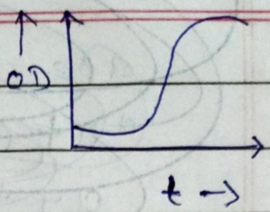
[0.87 – 0.94]

energy charge of most cells is

$$= \frac{[ATP]}{[AMP] + [ADP] + [ATP]}$$

- Regulation is at enzyme Level [Allosteric regulation]

• growth in sigmoid fashion



glc

↓

g6P

⇅

F6P

↓ Phosphofructokinase ← ATP⁻ , ← ADP⁺

PEP

↓ Pyr. kinase ← AMP⁺

PYR

↓ Pyr dehydrogenase ← ATP⁻

ACCOA

ACCOA + OA → Cit — cit synthase ← AMP⁺

ADP⁺ → Icit

Cit → Icit

TCA cycle

Icit → αKG — Icit dehydrogenase

ATP⁻ → Icit

21st Aug, 23

S-10 Detailed Stoichiometric representation of cell growth (chemoheterotropic)

1. Energy source dissimilation

a. $CH_xO_y + \text{coefficient}(H_2O) + \frac{\delta_S}{2} a NAD^+$

$\longrightarrow aCO_2 + \frac{\delta_S}{2} a(NADH + H^+)$

$\varepsilon_S a(ADP + P_i) \longrightarrow \varepsilon_S a(ATP + H_2O)$

b. Oxidative phophorylation :-

$2b(NADH + H^+) + bO_2 \longrightarrow 2bNAD^+ + 2bH_2O$

$\underbrace{2b(P/O)}_{\text{No. of ADP phosphorylation}}(ADP + P_i) \longrightarrow 2b\left(\frac{P}{O}\right)(ATP + H_2O)$

c. **Biosynthesis:-**

$$(1+\sigma)\,CH_xO_y + \frac{\delta}{N}\,H_lO_mN_n + \frac{1}{2}\left[\delta_B - \delta_S(1+\sigma) - \frac{\delta}{n}\,\delta_N\right]$$

$$+ (NADH + H^+)$$

$$\longrightarrow CH_\alpha O_\beta N_\delta + \sigma CO_2 + \text{coeff. } H_2O.$$

$$\frac{MW_B}{Y^{max}}(ATP + H_2O) \rightarrow \frac{MW_B}{Y^{max}}(ADP + P_i)$$

$$Y_{ATP} \Rightarrow \frac{\text{Mass of cell formed}}{\text{Moles of ATP consumed}} \quad Y_{ATP}$$

D. Maintenance.

$$C(ATP + H_2O) \rightarrow C(ADP + P_i)$$

Source.

Biomass — δ_B, δ_S, δ_N – Nitrogen

Degree reductance No of mole of e^- available / g-atom of C.

C + 4, H + 1, O − 2, N − 3

$CH_\gamma O_S N_v$ $\quad \delta = 4 + \gamma - 2s - 3v$

10.4 "Metabolic Energy" Stoichiometry.

(Total combustion)

$$O_2 + \text{Substrate} \xrightarrow[\Delta H_S]{\text{I}} CO_2 + H_2O$$

II — Respiration yielding cell mass.

ΔH_c — III — heat obtained by combustion of cell mass.

$$x + H_2O + CO_2$$

$$Y_\Delta \left[\frac{g \text{ cell}}{\text{kcal heat evolved}} \right] = \frac{Y_S \left(\frac{g \text{ cell}}{g \text{ of substrate}} \right)}{(\Delta H_S - Y_S \Delta H_c) \frac{kcal}{g \text{ of } S}}$$

heat of combustion of substrate.

ΔH of cell.

Q. $CH_{1.66}N_{0.2}O_{0.27} + 1.28\,O_2 \rightarrow CO_2 + 0.1N_2 + 0.83H_2O$

$$\Delta H_c = \frac{1.28 \times 104}{12 + 1.66 \times 1 + 0.2 \times 14 + 16 \times 0.27}$$

$= 6.47$ kcal/g of cell

if 30% is ash.

then,

$\Delta H_c' = 0.7 \times 6.47$ kcal/g cell.

- Presence of multiple substrate:

$$\bar{\Delta G}^{0'} = \frac{\Sigma \left[M_i \Delta G_i^{0} \right]}{\Sigma M_i}$$

New chapter

23rd Aug, 23

- Kinetics of substrat, utilization, biomass Product, formation.

i. for batch

$$r_{fi} = \frac{d}{dt}(C_f)$$

ii. for continous → volumetric flow rat L/s.

$$r_{fi} = \frac{F}{V_R}(C_i - C_{if})$$

iii. CSTR :-

$$F(\cancel{x_f}^{\,0} - x) + V_R r_x = 0$$

$$\Rightarrow r_x = \frac{F x}{V_R}$$

$$r_x = \mu x = \frac{F}{V_R} x.$$

μ - specific Growth rate.

$$\Rightarrow \mu = D$$

$D = \frac{F}{V_R}$ - dilution rate (min^{-1})

24th Aug, 23

$$S_{sterile} = \frac{D K_S}{\mu^{max} - D}$$

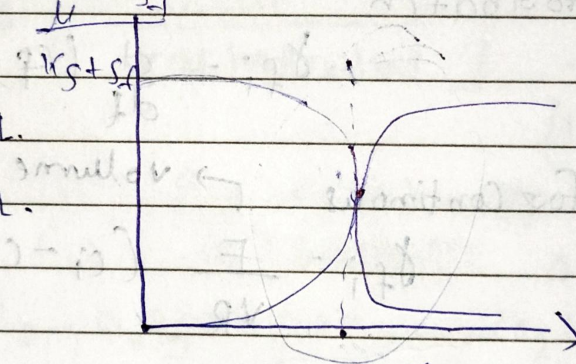
$$x_{sterile} = Y_x \left[S_f - \frac{D K_S}{\mu^{max} - D} \right]$$

Case i $D \to 0$, $S \to 0$, $x = Y_x S_f$

Case ii, D increase continously $D \to \mu^{max}$ [wash-out]

$x = 0$

$$D_{max} = \frac{\mu^{max} S_f}{K_S + S_f}$$



$D = \mu^{max}$

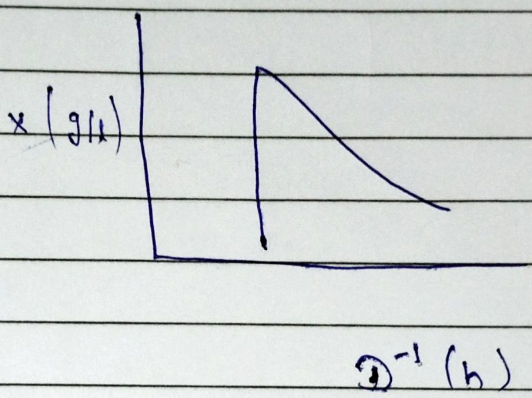
washout $D \to$

Cell productivity = Dx.

$$\frac{d}{dD}(Dx) = 0.$$

$$D_{max, output} = \mu^{max} \left(1 - \sqrt{\frac{K_S}{K_S + S_f}} \right)$$

$$r_x = \frac{\mu^{max} \cdot S x}{S + K_S} - K_e \cdot x$$ Endogenous metabolism



BT 303

Tutorial 1

Q1. Clostridium acetobatilicum carries out anaerobic fermentation and converts glucose into acetone, butanol along with small conc. of butyrate, acetate etc. In fermentation, the following are obtained from 100 moles of glucose and 11.2 moles of NH_3.

Product formed	moles
Cells	13
Butanol	56
Acetone	22
Butyric acid	0.4
Acetic acid	14
CO_2	221
H_2	135
Ethanol	0.7

a. By performing C, H, N, O balance determine elemental composition of cells?

b. Find if the fermentation is balanced with respect to its oxidation - reduction (redox) states?

Sol^n Given,

100 moles of glucose $[C_6H_{12}O_6]$

11.2 moles of NH_3.

from glucose,

600 C

1200 H

600 O

from NH_3

11.2 N

33.6 H

total C = 606

H = 1233.6

O = 600

N = 11.2.

Now,

Butanol → $C_4H_{10}O$

Acetone → C_3H_6O

Butyric acid → $C_4H_8O_2$

Acetic acid → CH_3COOH

CO_2

H_2

ethanol → C_2H_5OH.

2400

$+4 \quad +12-12$

$100\, C_6H_{12}O_6 + 11.2\, NH_3 \longrightarrow 13\, C_aH_bO_cN_d$

$(24) \qquad (16) \qquad (24)$

2400

$+ 56\, C_4H_{10}O + 22\, C_3H_6O + 0.4\, C_4H_8$

$+ 14\, C_2H_4O_2 + 221\, CO_2 + 135\, H_2$

$+ 0.7\, C_2H_6O$

$\Rightarrow$ C : $100 \times 6 = 13a + (56 \times 4) + (22 \times 3) + (0.4 \times 4)$
$+ (14 \times 2) + 221 + 0.7 \times 2$

$\Rightarrow \quad a = 4.46$

H : $b = 16.01$

O : $c = 3.88$

N : $d = 0.86$

$C_{4.46}H_{16.01}O_{3.88}N_{0.86}$

1200

3)

Product:

$13[4 \times 4.46 + 1 \times 16.01 - 2 \times (3.88) - 3(0.86)]$
$+ 56[4 \times 4 + 1 \times 10 - 2]$

C $\rightarrow$ 2400

H = 1233.6

O = −1200

N = −33.6

$e^- = 2400$

29

1. A chemostat culture growing on hexadecane was carried out a dilution rate of $0.18 h^{-1}$. The following data were reported working volume $- 0.015 m^3$

Aeration rate $- 1.8 m^3/h$

feed substrate concentration $- 10 kg/m^3$

Reactor substrate concentration $- 0.032 kg/m^3$

conc. of cell $- 10.2 kg/m^3$

% CO_2 in exit gas $- 1.0$ v/v

% O_2 in the exit gas $- 2.5$ v/v

$$\mu_{max} = 0.27 h^{-1}$$

Calculate the following parameters

$Y_{x/s}$, K_s, specific substrate uptake rate, O_2 evolution rate and RQ?

Soln:- $Y_{x/s} = 1.016 = \frac{\text{feed substrate}}{\text{Conc. of cell}} = \frac{\text{Conc. of cell}}{\text{feed substrate}}$

$$= \frac{10.2}{10} = 1.02$$

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S}$$

$$K_s = \frac{\mu_{max} \cdot S}{\mu} - S$$

at steady state $\mu = D$

$$K_s = \left[\frac{\mu_{max}}{D} - 1\right] S = \left[\frac{0.27}{} - 1\right] S$$

$$\varphi_S = \frac{\mu}{Y_{x/s}} = \frac{0.18}{1.016} = 0.18 \ kg/kg/h.$$

$$\varphi_{CO_2} = \frac{1.8 \frac{m^3}{h} \times 0.61 \times 1.98 \frac{kg}{m^3} \times \frac{1}{44}}{10.2 \frac{kg}{m^3} \times 0.015 \ m^3}$$

$$\varphi_{O_2} = \frac{1.8 \times 0.025 \times 1.43 \times \frac{1}{32}}{10.2 \times 0.015}$$

$R_Q = \frac{\varphi_{CO_2}}{\varphi_{O_2}}$ Respiratory quotient = $\frac{\text{moles of } CO_2 \text{ formed}}{\text{moles of } O_2 \text{ formed}}$

2. A $5 m^3$ fermented is operated continously with feed substrate conc. $20 \ kg \ m^{-3}$. The microorgⁿ cultivated in the reactor has the following characteristics;

$\mu_{max} = 0.45 \ h^{-1}$

$K_S = 0.8 \ kg \ m^{-1}$

$Y_{x/s} = 0.55 \ kg/kg.$

a. What feed flow rate is required to achieve 90% substrate conversion.

b. how does the biomass productivity at 90% substrate conversion compare with the maximum possible?

Soln:- a. at steady state

$$D = \frac{\mu_{max} \cdot S}{K_S + S}$$

$$= \frac{0.45 \times}{0.8 +} = 0.32 h^{-1}$$

$F = D \times V$

$= 0.32 \times 5$

$= 1.6 \ m^3/h$

b.

$$Q_x = D\cancel{X}$$

$$= D\left[S_i - \frac{D K_S}{\mu - D}\right]$$

Chapter 5

5.2. $ATP + H_2O \rightarrow ADP + P_i$; $\Delta G^{\circ'} = -7.3$ Kcal/mol

5.3 In Embdem - Mayerhoff - Parnas [EMP] overall 2 ATP produce.

5.4. Respiration potentially make available much more energy for use by the cell than glcolysis

$$Gly + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$$\Delta G^{\circ'} = -686 \text{ Kcal/mol.}$$

And
in Cell

$$C_6H_{12}O_6 + 38ADP + 38P_i \longrightarrow 6CO_2 + 38ATP + 44H_2O.$$

$$\Delta G^{\circ'} \approx +38 \times 7.3 \text{ Kcal/mol}$$
$$+ 277 \text{ Kcal/mol.}$$

energy capture efficiency $= \frac{277}{686} \approx 40\%$

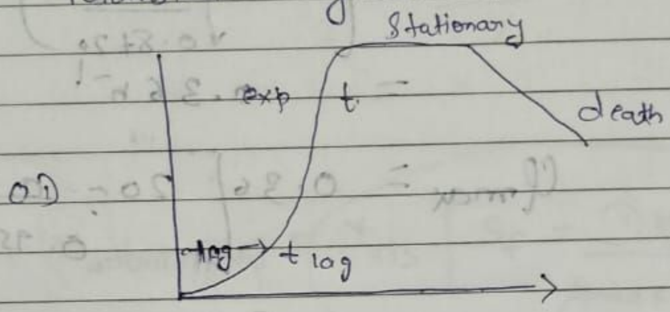
5.10. yeild factor $Y_{x/s} = \frac{\text{cellmass formed}}{\text{Mass of substrate utilized.}}$

$$Y_{x/s} = \frac{\Delta x}{\Delta S}$$

- RQ, Respirotary quotiemt $= \frac{\text{moles } CO_2 \text{ formed}}{\text{moles } O_2 \text{ consumed}}$

14th Sept, 23

"Transient" growth kinetics.



t

Log.

$$\frac{dn}{dt} = \mu n \qquad , t = t_{log}$$
$$n = n_0 \simeq 0.$$

$$\ln \frac{n}{n_0} = \mu [t - t_{log}].$$

$$\Rightarrow n = n_0 e^{\mu [t - t_{log}]}$$

$$t_d = \frac{\ln 2}{\mu} \qquad [\text{doubling time } n = 2n_0]$$

rate of nutrient consumption $\propto$ mass of conc. of cells.

$$\frac{da}{dt_a} = -K_a n.$$

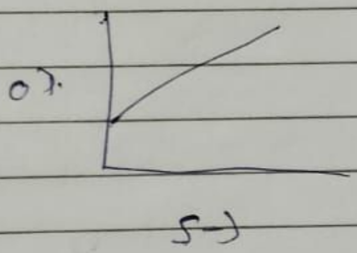
$$\Rightarrow a - a_0 = \frac{-K_a}{\mu} [n - n_0]$$

if a is fully Consume

$$a_0 = \frac{K_a}{\mu}(x_s - x_0)$$

$$\Rightarrow x_s = x_0 + \frac{\mu a_0}{K_a}$$

x_s = Complete consumption of carbon source.



toxin accumulation.

$$\frac{dx}{dt} = Kx\left[1 - f(\text{toxin conc.})\right]$$

assume toxin linearly decreases the growth

$$\frac{1}{x}\frac{dx}{dt} = K(1 - bC_t)$$

$\hookrightarrow$ toxin conc.

$$\frac{dC_t}{dt} = qx.$$

$$\Rightarrow C_t = q\int_0^t x\,dt$$

$$\mu_{eff} = \frac{1}{n}\frac{dn}{dt} = K_0\left[1 - bq\int_0^t n\,dt\right]$$

- <u>Death phase</u>

$$n = n_S\, e^{-K_d t}$$