Microbial physiology and metabolism

These are lecture notes from a series of lectures given in the Virology department of Sri Venkateshwara University in Tirupati, Andhra Pradesh, South India.

I was the guest of Professor Sai Gopal
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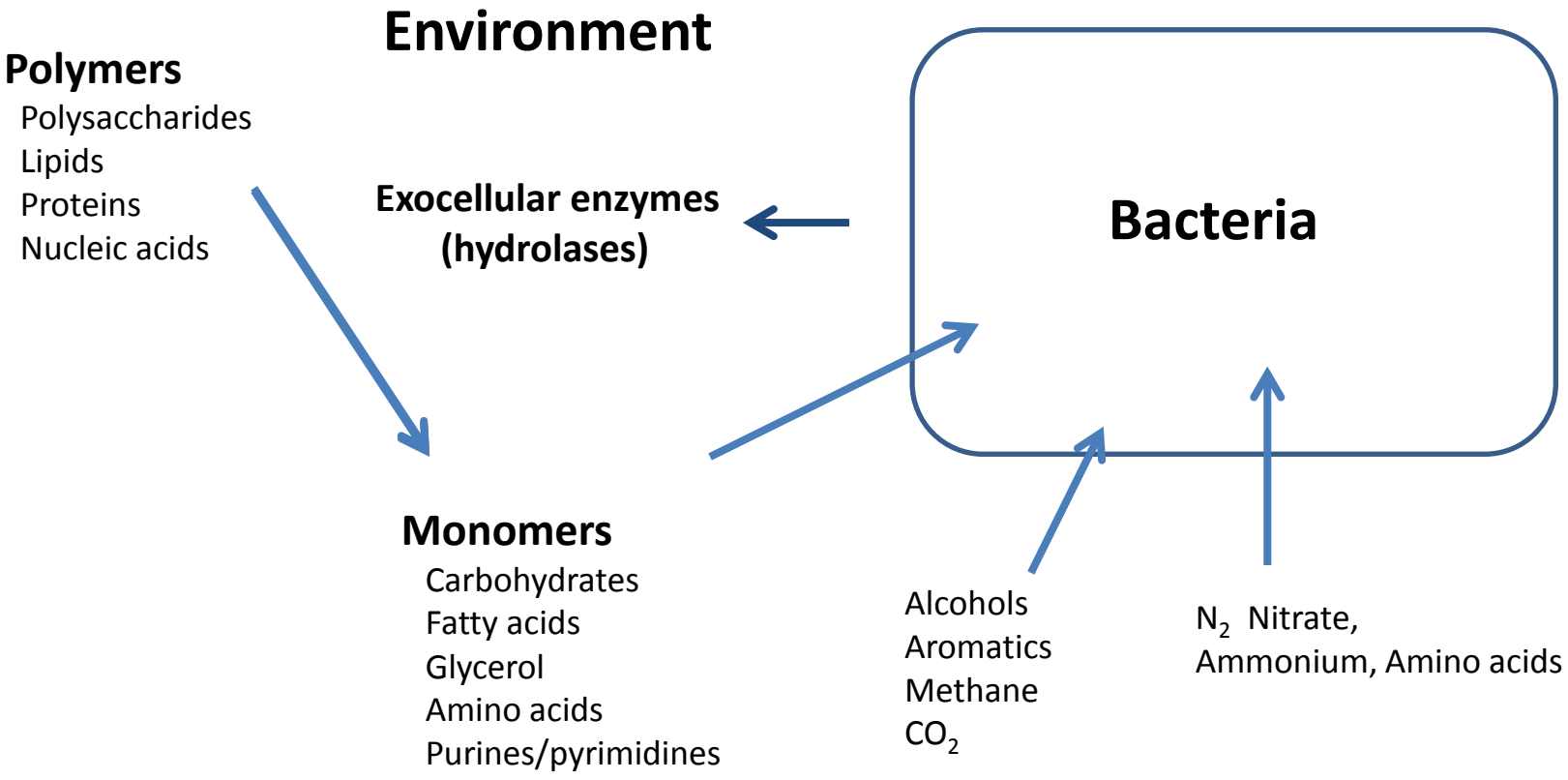
Microbial physiology and metabolism

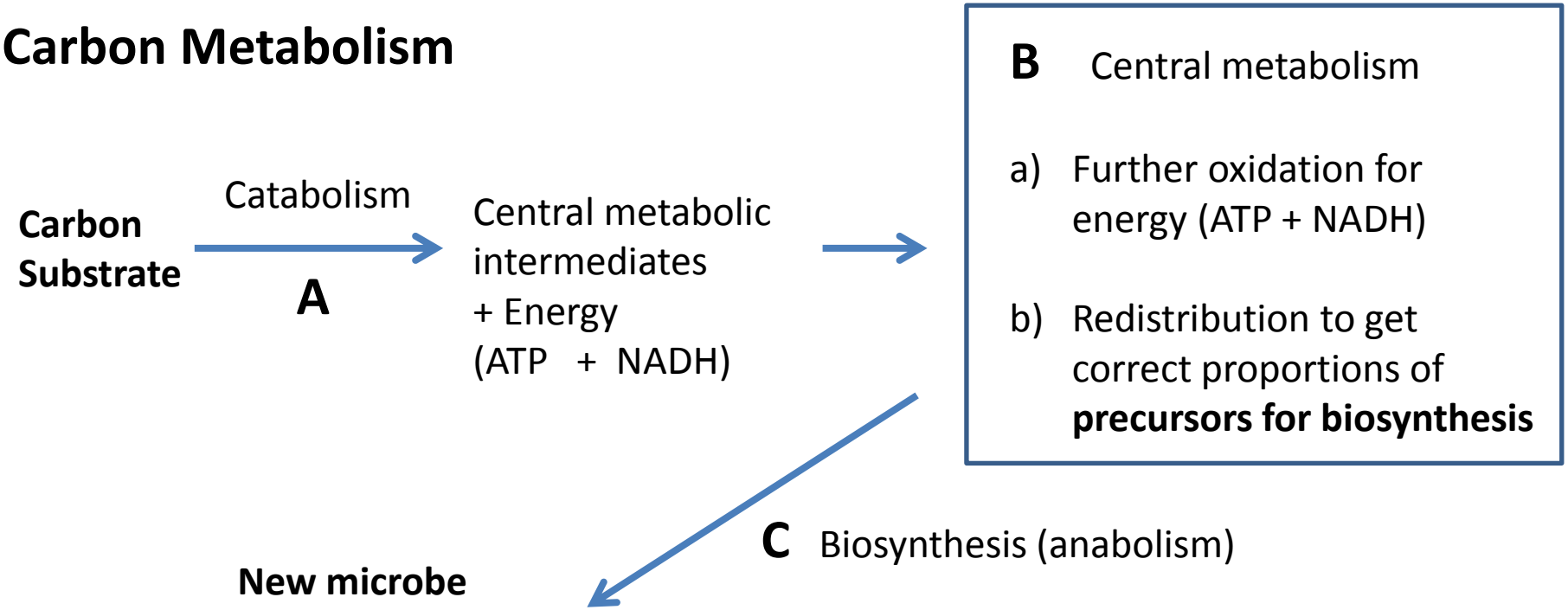
Nitrogen: N_2 Nitrate, Ammonium, Amino acids

Carbon: Almost all carbon compounds are used by bacteria, yeasts or fungi. Some microbes use only one or 2 carbon sources while others use a wide range. We will be dealing mainly with bacterial metabolism.

Methylotrophs: Grow on compounds with only one carbon atom or with no C-C bonds; they differ from most other microbes in having special pathways for Carbon assimilation and for energy production.



Carbon Metabolism



Polysaccharides [membranes and storage]
Lipids [membranes and storage]
Proteins [structural and enzymes]
Nucleic acids [genes and gene expression]

Microbes are all the same for **C** (Biosynthesis)
They are *distinguished* by their Catabolic routes
And by their energy metabolism

NOTE:
This is Primary metabolism; it is essential for life

Secondary metabolism; it is 'extra' like production of antibiotics, spores etc

Bacterial Energy Metabolism

Four main headings:

1. Carbon source: Organic or Inorganic [CO_2]
2. ATP source: Chemical oxidation or Light
3. NADH source: Organic or Inorganic
4. Energy production: Aerobic or Anaerobic

1. Carbon source

a) **Inorganic carbon** [CO_2] Autotrophs

CO_2 —biosynthesis→ **CELL**

By Calvin cycle

Light or Oxidation of inorganic mols: hydrogen, nitrite, Ferrous ion → [ATP + NADH]

This process involves electron tpt & oxid. phosphorylation

b) **Organic carbon source** Heterotrophs (including Methylotrophs)

Organic carbon → CO_2 (Aerobes; **ATP by oxid. Phosph**.)

OR

Fermentation products (Anaerobes **ATP by Substrate level phosphorylation SLP)**

Organic carbon → [ATP + NADH] → **biosynthesis** → **CELL**

‘Classification’ of microbes according to their nutrition (Carbon, ATP, NADH)

1. Carbon source	Inorganic (carbon dioxide)	Organic
2. ATP source	a) Chemical oxidations	Chemotrophs
	b) Light	Phototrophs
3. NADH source	a) Organic	Organotrophs
	b) Inorganic	Lithotrophs

	ATP	NADH	Carbon	Other name
ChemoOrganotroph	Oxid. Organic	Oxid. Organic	Organic	ChemoHeterotroph
ChemoLithotroph	Oxid. Inorganic	Oxid. Inorganic	Inorganic	ChemoAutotroph
PhotoOrganotroph	Light	Organic carbon	Organic	PhotoHeterotroph
PhotoLithotroph	Light	Inorganic	Inorganic	PhotoAutotroph

ChemoOrganotrophs: many of the common bacteria; Pseudomonas, Bacillus, Lactobacillus, Ecoli. Aerobes and anaerobes. Animals

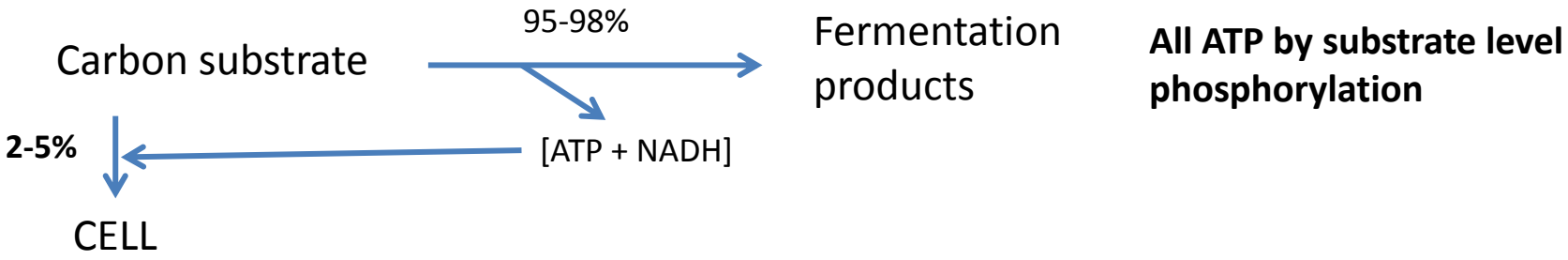
ChemoLithotrophs: Oxidise hydrogen (hydrogen bacteria), nitrite (nitrobacter), ammonia (Nitrosomonas), ferrous ions (Iron bacteria). Strict aerobes. Very important in soil and oceans

PhotoOrganotrophs; Anaerobic photosynthetic bacteria that use ethanol or acetate. Anoxygenic photosynthesis. They can also grow aerobically on ethanol or acetate as typical chemorganotrophs. Eg Rhodopseudomonas Rhodobacter

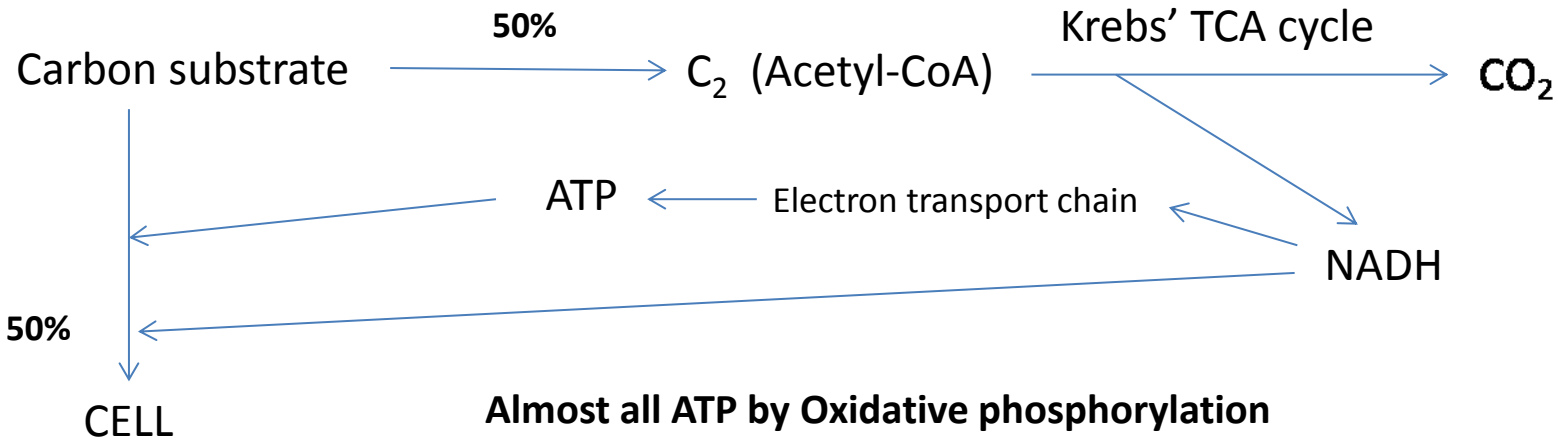
Photo Lithotrophs; plants and blue green algae (Cyanobacteria) Aerobic (oxygenic); AND some use hydrogen sulphide instead of water (anaerobic)

Oxidation of organic carbon substrates

a) Anaerobic oxidation (Fermentation)



b) Aerobic oxidation



Note: methylotrophs are special as the substrates are oxidised directly to CO_2

Some special cases of aerobic / anaerobic oxidation of organic carbon substrates

1. Acetic acid bacteria

Strictly aerobic but do not have a TCA cycle. All ATP is by oxidative phosphorylation
They accumulate reduced end products but this is not by fermentation
Acetobacter: oxidise ethanol to acetic acid
Gluconobacter: oxidise glucose to gluconic acid
Similar to methylotrophs in having special systems for oxidation of growth substrates (quinoproteins)

2. Anaerobic respiration

Exactly same as typical aerobes, using TCA cycle etc but in the absence of oxygen they can use alternatives final electron acceptors: Nitrate (reduced to nitrite, nitrogen); sulphate (reduced to sulphide)
All ATP is by oxidative phosphorylation. Typical example is *Pseudomonas*
Hyphomicrobium is a methylotroph that can grow anaerobically on methanol or methylamine with nitrate

3. Methylotrophs (Methylotrophs that use methane are *Methanotrophs*)

Grow on one-carbon compounds – methane, methanol methylamine. They cannot be Oxidised to acetyl-CoA. They are oxidised by special enzymes to carbon dioxide. ATP is by oxidative phosphorylation. Some can use nitrate or sulphate instead of oxygen [anaerobic respiration

Bioenergetics; thermodynamic principles in biology

1st Law: Total energy of a system plus surroundings is constant
Energy changes involve heat absorbed/released and work done
2nd Law: A process only occurs spontaneously (no energy input is required) only if ENTROPY (S) of a system and its surroundings INCREASES. S is a measure of Disorder For reaction A → B then ΔS Must be +ve

Problem: Cannot measure ΔS and anyway we are interested in ENERGY changes during reactions. So we use Gibbs Free Energy Change ΔG. This is the energy available to do work

$\Delta G = \Delta E - T\Delta S$ So ΔG includes change in energy and change in entropy

Relationship between ΔG, Equilibrium constant (Keq), direction of reaction and energy change

Reaction equilibrium A ⟷ B At equilibrium [B]/[A] is Keq, the equilibrium constant

A + B ⟷ C + D Keq = [c] x [d] / [a] x [b] Keq tells Direction Not Energy change

$\Delta G = \Delta G^o + RT\ln [c] \times [d] / [a] \times [b]$ At equilibrium $\Delta G = 0$ **So: $\Delta G^o = -RT\ln Keq$**

ΔG^o is Standard free energy change when reactants and products are 1.0 M

Example: If Keq = 1.0 then $\Delta G^o = 0$

If Keq = 10^5 then ΔG^o = about – 30kj/mole

If Keq = 10^{-5} then ΔG^o = about + 30kj/mole

For a reaction to occur ΔG must be –ve (an exergonic reaction); If +ve it is endergonic

If for reaction A → B $\Delta G^o = +10$ then energy must be put in to enable the reaction to occur

a) Increase concentration of A

$\Delta G = \Delta G^o + RT\ln B/A$ If [A] is increased then ln of B/A becomes more –ve until eventually ΔG is-ve

b) Put energy into the system by coupling to an exergonic reaction

e.g. couple to a 2nd reaction B → C where $\Delta G^o = -20$ Now A → B → C $\Delta G^o = -10$

NOTE:

1. The rate of reaction is not related to the ΔG value; this could be very large and negative but a slow reaction.
2. The free energy change ΔG for a reaction is the same regardless of the route taken; the oxidation of glucose to carbon dioxide is same if glucose is metabolised by the TCA cycle or if it is burnt.

The importance of ATP and other energy rich compounds

Energy-rich compounds have a high free energy change on hydrolysis; about -30 kj/mol

ATP is an energy *currency;* it is not a *store.*

Ad-rib-P-P-P	→	AdRib-P-P + Pi	→	AdRib –P + Pi	→	AdRib = Pi
ATP		ADP		AMP		
ΔG^o - 30		-30		-10		

If more energy is needed then ATP may be hydrolysed to AMP plus pyrophosphate which is hydrolysed rapidly to phosphate ($\Delta g^o = -30$); this hydrolysis drives the reaction.

As in Amino acid + tRNA → AminoacyltRNA ATP → AMP + PPi → 2 Pi

Recycling of AMP Myokinase: AMP + ATP → 2 ADP

Phosphorylation of ADP to ATP

Substrate level phosphorylation, oxidative phosphorylation or photophosphorylation

Free energy changes in redox reactions

When reduced compounds are oxidised energy is released; this can be harnessed as energy-rich compounds such as ATP or phosphoglycerate [in SLP]

Oxidation: removal of electrons $Fe^{2+} \rightarrow Fe^{3+} + e$ Reduction: addition of electrons (reverse)

In oxidation of organic mols 2 electrons are removed at a time together with 2 protons
$CH_3OH \rightarrow HCHO + [2H]$ (= 2e + 2 H^+)

A **Redox couple** is a mixture of the oxidised and reduced forms of a compound eg HCHO / CH_3OH

Redox potential (E) tells how good an oxidising or reducing agent a redox couple is
A good oxidising agent has a high + potential and a good reducing agent has a high –ve redox pot.

E^o is the standard E value when the concn. of oxidised and reduced forms are equal
The actual of E depends on the concns of ox and red $E = E^o + RT/nF \times \ln OX/RED$
If [OX] form is increased then E is more +ve That is, the couple is a better oxidiser.
If [RED] form is increased then E is more -ve That is, the couple is a better reducer.

Note: electrons flow from –ve to +ve. The difference in E (ΔE) tells how much energy is available

Ascorbate (red) + Fe (ox) → ascorbate (ox) + Fe (red)
E^o = +260V +440mV So e flow from ascorbate to iron $\Delta E = OX - RED = +180mV$
For reaction to occur ΔE must be +ve (different from ΔG^o)

How much energy is available from a redox reaction? Convert ΔE to ΔG: **$\Delta G = -nF\Delta E$**

$NADH_2 + O \rightarrow NAD + H_2O$
-320mV +880mV

$\Delta E = 880 - (-320) = +1200$ mV
ΔG = - 180 kj/mol enough for 6 ATP