

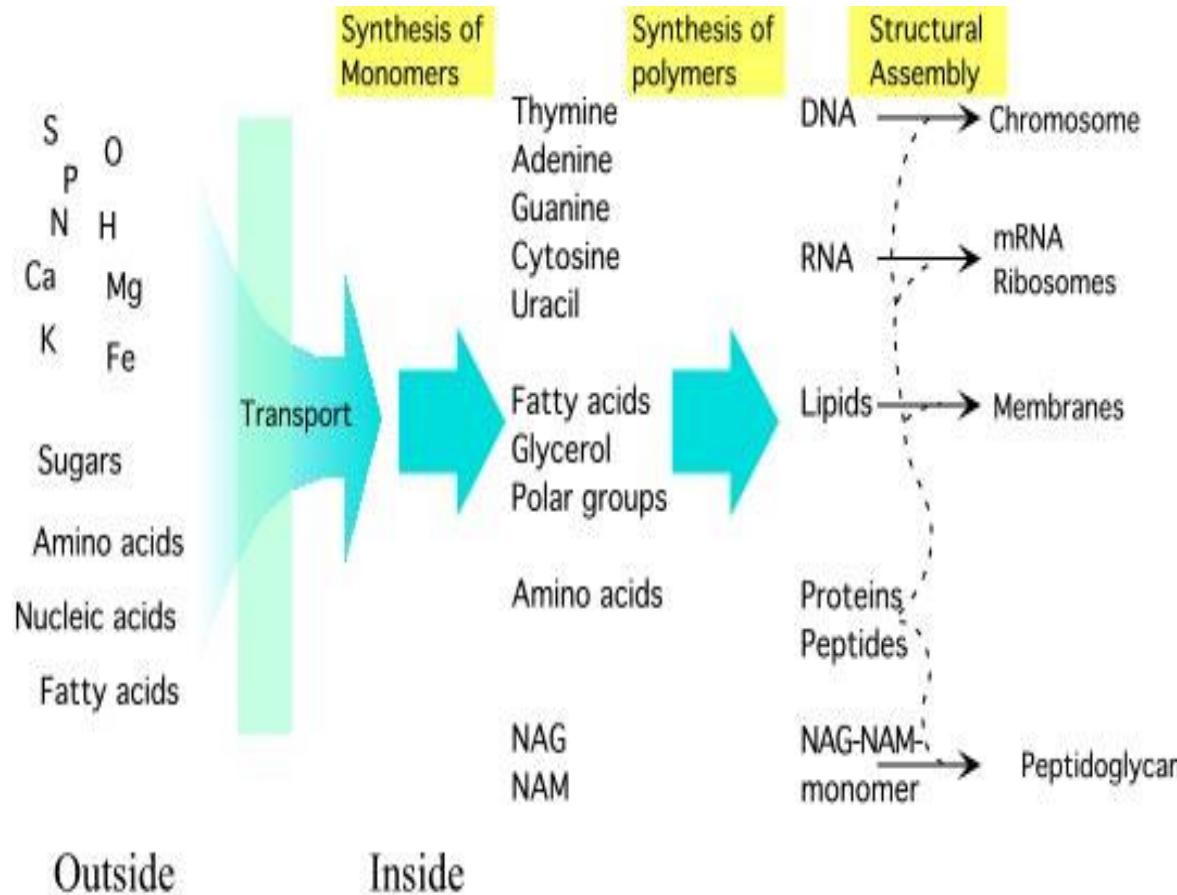
## CHAPITRE III

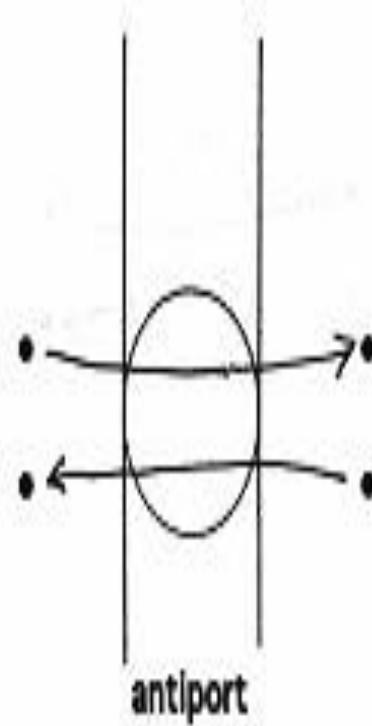
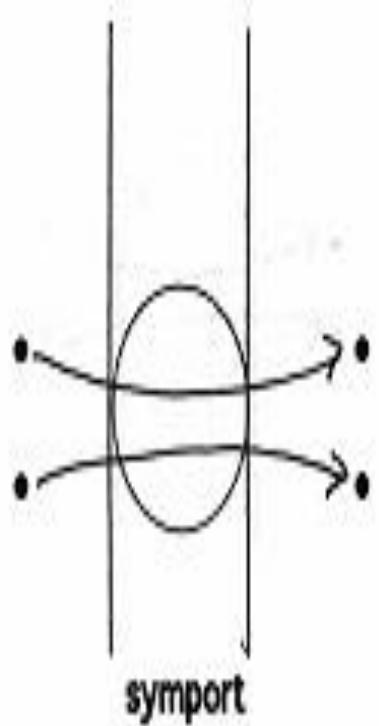
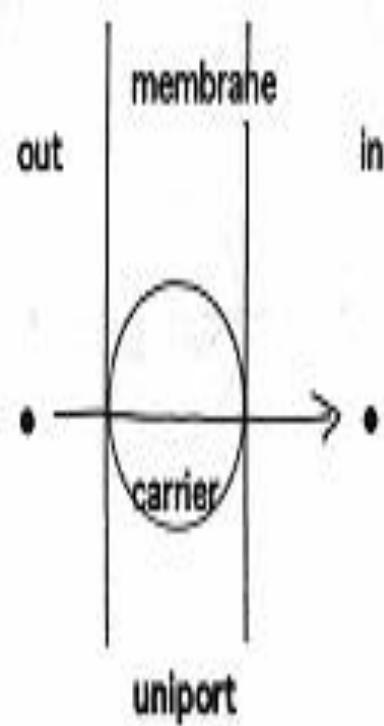
# NUTRITION CROISSANCE ET CULTURE BACTERIENNES

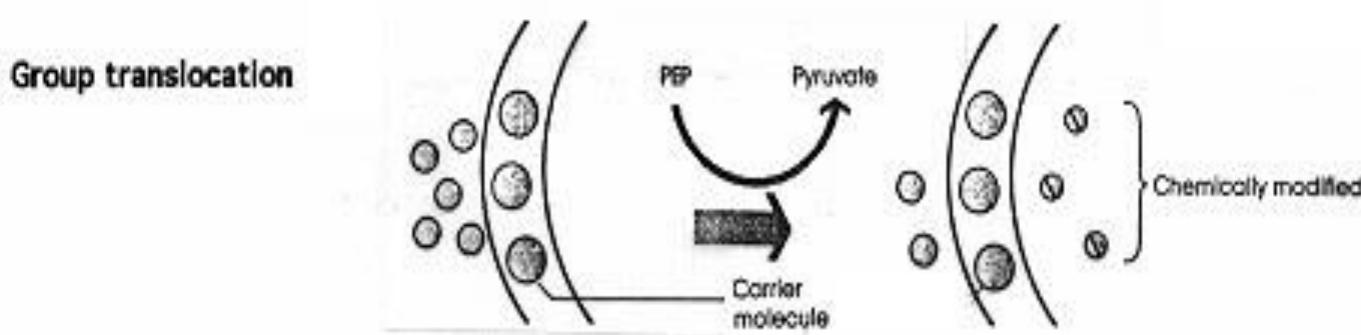
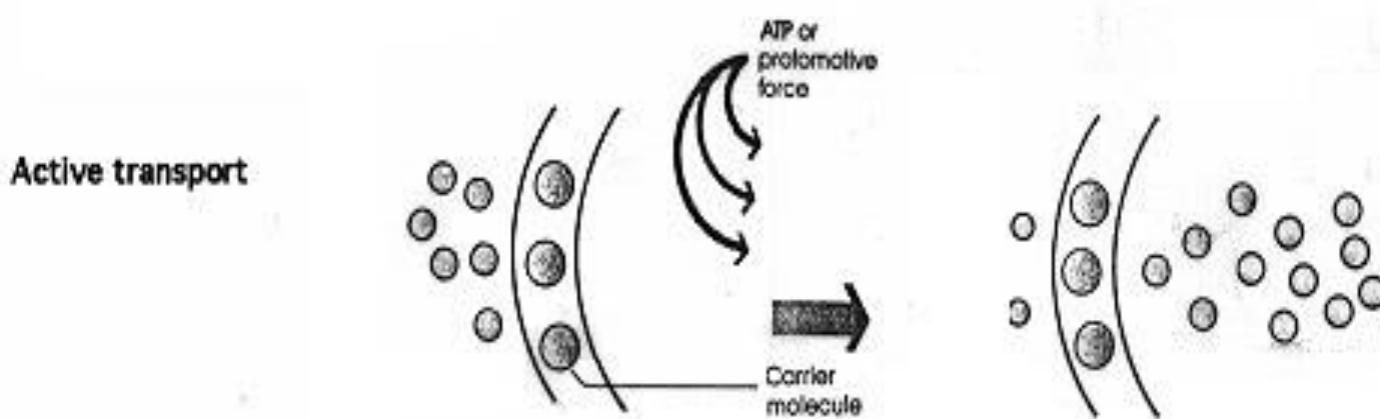
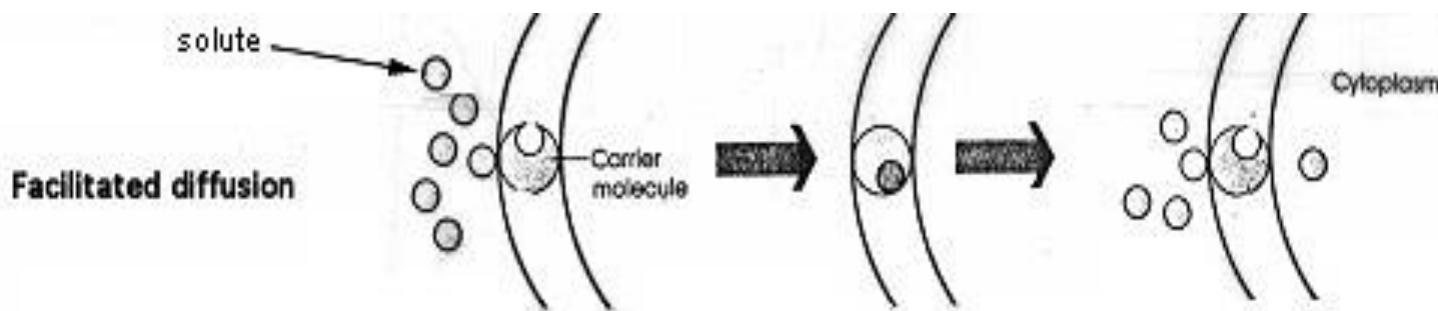
## Toutes les cellules y compris les bactéries

- Sont composées des mêmes éléments chimiques: Protéines, Acides Nucléiques, Minéraux, Sucres, Acides Gras, Vitamines
- Se reproduisent et sont le résultat de la reproduction
- Nécessitent de l'énergie extérieure
- Régulent le flux des nutriments et des métabolites qui entrent et quittent la cellule
- Sont très finement régulées en élaborant des systèmes senseurs des signaux environnementaux

# NUTRITION







Property	PD	FD	IDT	BPDT	GT
<b>carrier mediated</b>	-	+	+	+	+
<b>conc. against gradient</b>	-	-	+	+	NA
<b>specificity</b>	-	+	+	+	+
<b>energy expended</b>	-	-	pmf	ATP	PEP
<b>solute modified during transport</b>	-	-	-	-	+

**Table 7. Distinguishing characteristics of bacterial transport systems.**

**PD = passive diffusion**

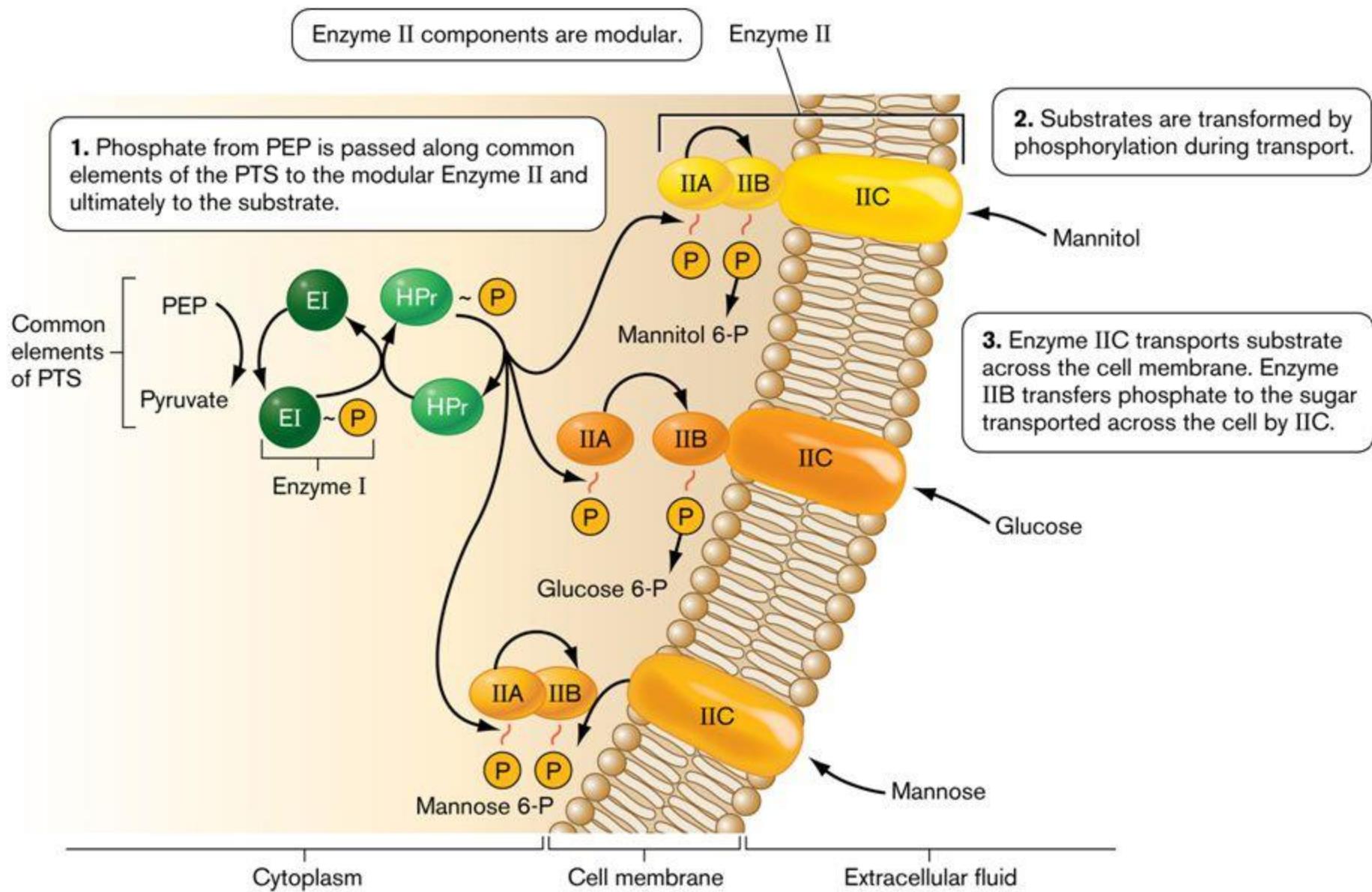
**FD = facilitated diffusion**

**IDT = ion-driven transport**

**BPDT = binding protein dependent transport**

**GT = group translocation**

Figure 4-10 Group translocation: the phosphotransferase system (PTS) of *E. coli*.



# Les milieux de culture

Utilisés pour croissance et maintien des microorganismes au laboratoire.

Composition dépend de l'espèce à cultiver:

- Nutriments essentiels carbonés et azotés
- Minéraux: P, S, Mg, Fe, ....
- facteurs de croissance: AA, Purines , pyrimidines, vitamines ...

On peut distinguer:

- **Milieu liquide, milieu solide ou semi liquide**
- **Des milieux Complexes** (ou riches): Leur composition chimique précise est inconnue, mais ils assurent la croissance d'une très large gamme de microorganismes.

Composant de base: Peptones, Tryptones, Hydrolysats de viande, de soja, de caséine de gélatine ..

Exemple: Bouillon nutritif, GNO, Bouillon de soja, ....

- **Des milieux synthétiques (minimum)**

Ils sont constitués de composés chimiques de nature et de quantités connues et précises.

Le dosages des différents nutriments minéraux et organiques sont connus et bien précis.

# Types de milieux de culture

- Milieux enrichis: Ajout de sang pour espèces exigeantes.  
Il existe des espèces non cultivables, et de espèces intracellulaires obligatoires.
- Milieux sélectifs: Favorisent sélectivement la croissance de certaines espèces.

## Exemples:

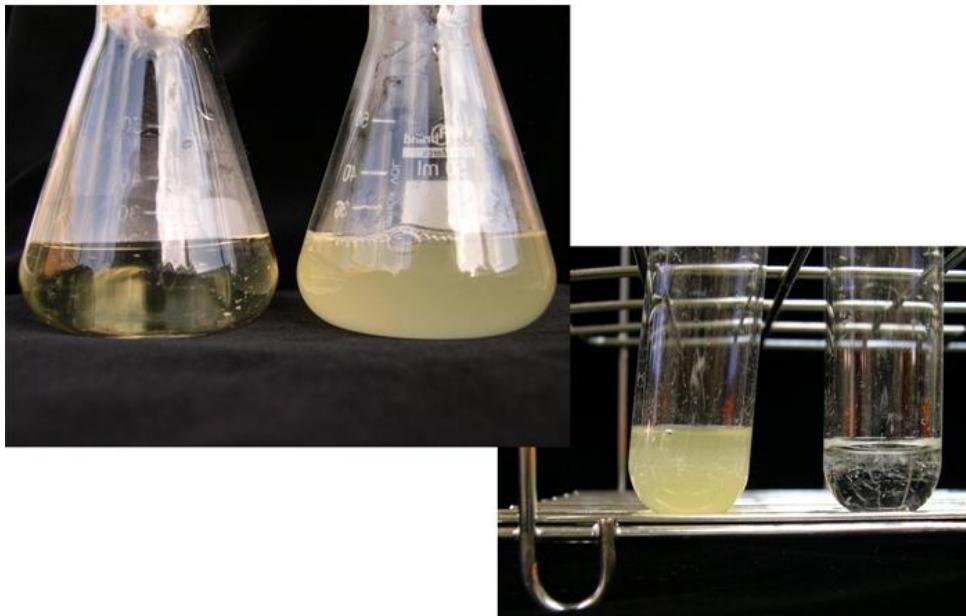
- Milieu de Drigalsky contient le cristal violet, favorise les G-
- Milieu Endo-Agar ou Eosine méthylène bleu agar inhibe les G+
- Milieu SS contient des sels biliaires sélectionne **Salmonella** et **Shigella**.
- Chapmann contient NaCl et favorise les **Staphylocoques**.
- Ajout d'antibiotiques

Remarque: On peut aussi sélectionner par la température.

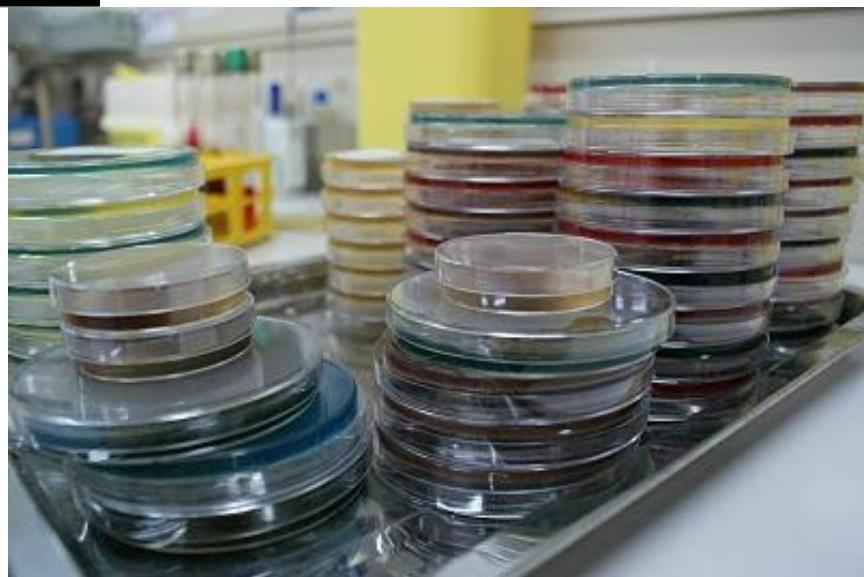
Facteurs de croissance		Fonction ou coenzyme	Organismes auxotropes
Bases puriques ou pyrimidiques	Adénine Guanine Uracile Thymine	constituants des ac. nucléiques	<i>L. plantarum</i> <i>L. casei</i>
Acides aminés	Acide glutamique Lysine Arginine Tryptophane Tyrosine	constituants des protéines	<i>L. arabinosus</i>  <i>S. typhi</i>
Vitamines	B <sub>1</sub> - Thiamine	cocarboxylase (TPP)	<i>St. aureus</i> <i>L. fermenti</i>
	B <sub>2</sub> - Ribo-flavine	FMN, FAD	<i>L. casei</i> <i>Str. hemolyticus</i> <i>Cl. tetani</i>
	B <sub>5</sub> - Acide pantothénique	coenzyme A	<i>Lactobacillus</i> <i>Pr. morganii</i> <i>Zymomonas mobilis</i>
	B <sub>6</sub> - Pyridoxal	pyridoxal-phosphate	<i>L. casei</i> <i>Str. faecalis</i>
	B <sub>12</sub> - Cobalamines		<i>L. lactis</i> <i>L. lichenimii</i> <i>Euglena gracilis</i> <i>Ochromonas sp.</i>
	PP - nicotinamide	pyridine-nucléotides	<i>Y. pestis</i>

Facteurs de croissance		Fonction ou coenzyme	Organismes auxotrophes
Vitamines	Acide nicotinique	pyridine-nucléotides	<i>Pr. vulgaris</i> <i>L. arabinosus</i>
	Acide pimélique	biotine	<i>Alphafæcaciae</i>
	Acide folique	formylations	<i>L. casei</i> <i>B. subtilis</i>
	Acide para-amino-benzoïque	acide folique	<i>Cl. acetobutylicum</i> <i>Cl. tetanomorpha</i> <i>Acetobacter suboxydans</i>
	Acide lipoïque	transporteur d'électrons	<i>L. casei</i> <i>L. delbrueckii</i> <i>Cl. tetani</i>
	Biotine	« coenzyme R » (carboxylations)	<i>L. arabinosus</i> <i>Rhizobium trifolii</i> Streptocoques <i>Sc. cerevisiae</i> et autres levures
	Choline	synthèse des phospholipides	Pneumocoque type III
	Hème (facteur « X »)	synthèse des hémoprotéines	<i>H. influenzae</i> <i>H. comis</i>
	K <sub>3</sub> - Ménadione	transporteur d'électrons	<i>M. paratuberculosis</i>

## Cultures en milieu liquide



## Culture sur milieu solide



## **MacConkey** (lactose+ rouge neutre) :utilisation      acidification

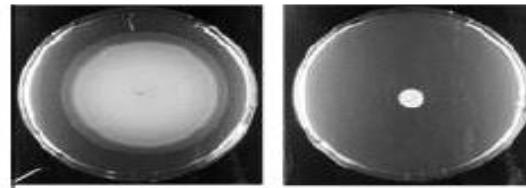


**Lac<sup>+</sup>** (colonies rouges)



**Lac<sup>-</sup>** (colonies incolores)

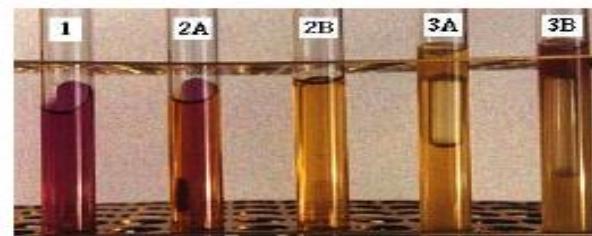
### **Mobilité** (0,5% agar)



### **Fermentation du glucose**

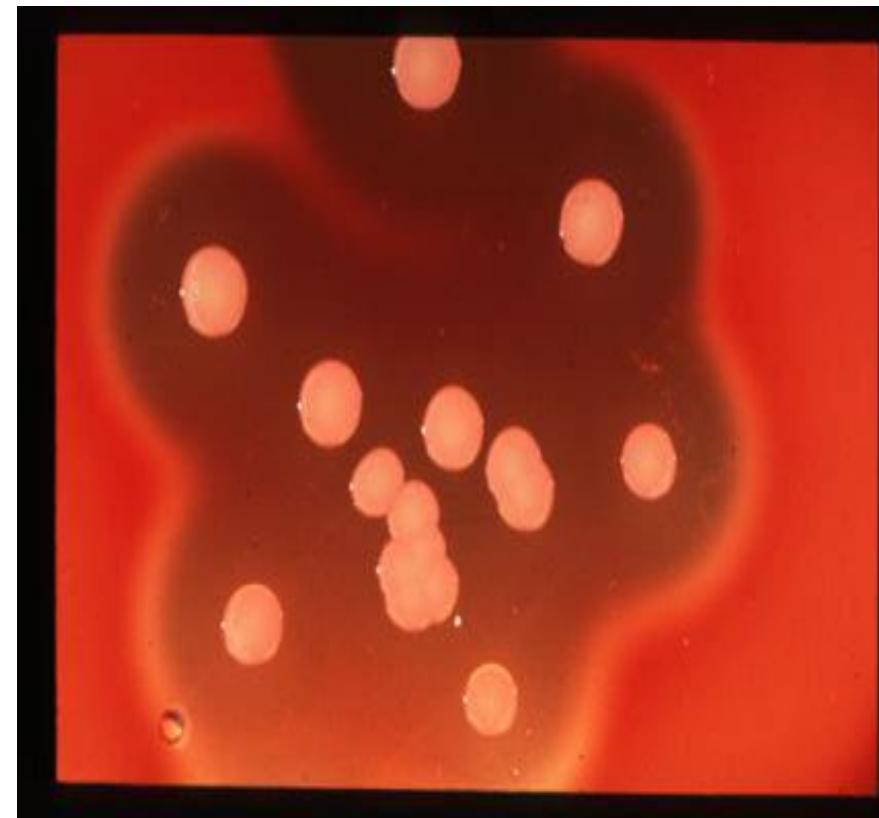
Production de déchets acides (indicateur de pH, rouge de phénol)

Production soit de CO<sub>2</sub> soit de CO<sub>2</sub> et H<sub>2</sub> (insoluble)



## Milieux sélectifs différentiels

Milieu MSA  
(mannitol + sels  
+ rouge phénol)



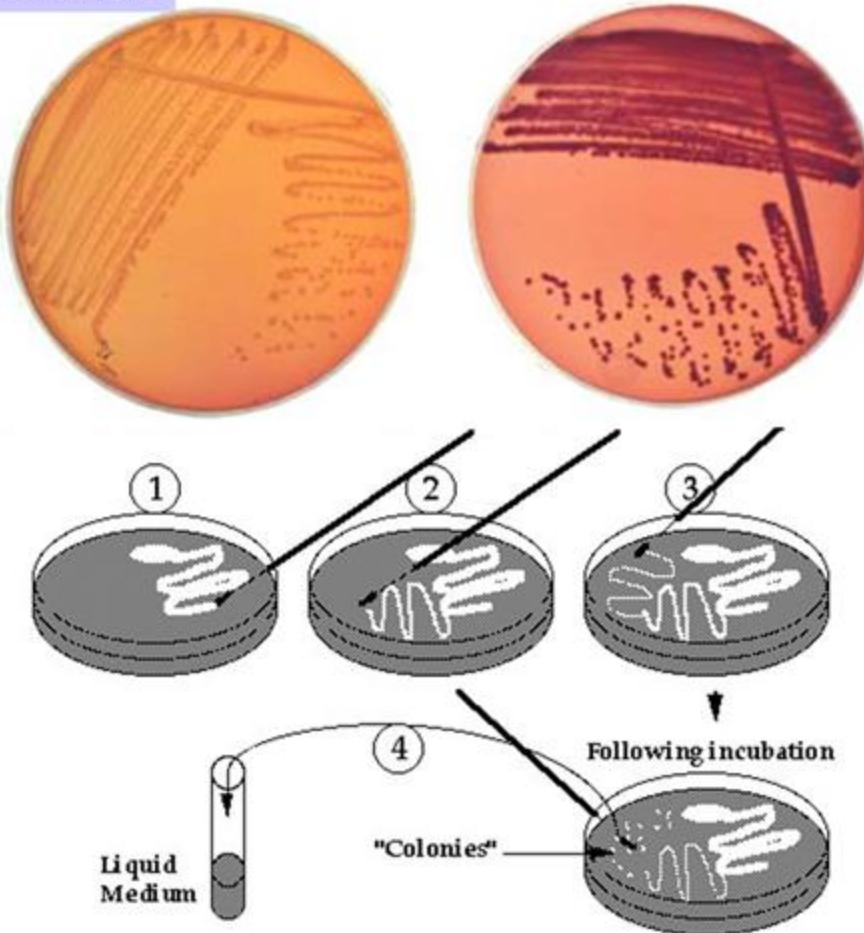
- **ISOLEMENT DES CULTURES PURES :**

Dans le milieu naturel, mélanges de plusieurs espèces. Leur étude nécessite la réalisation de cultures pures.

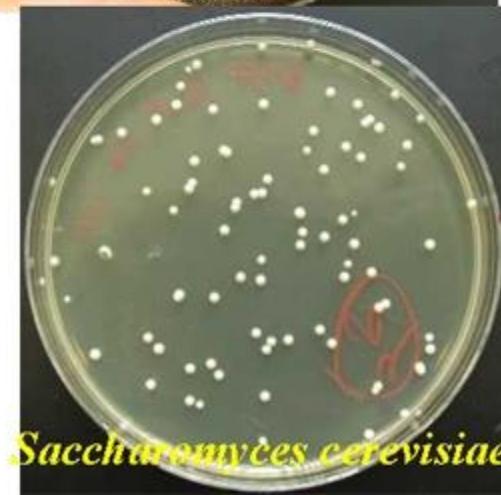
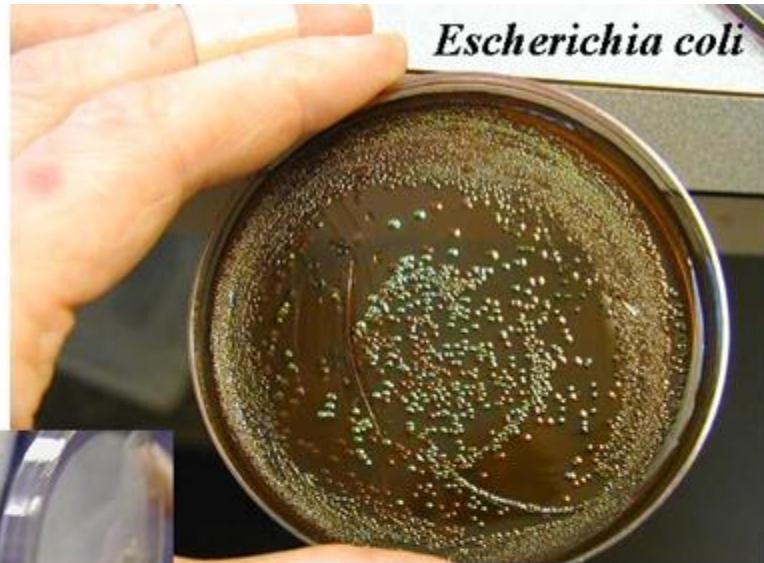
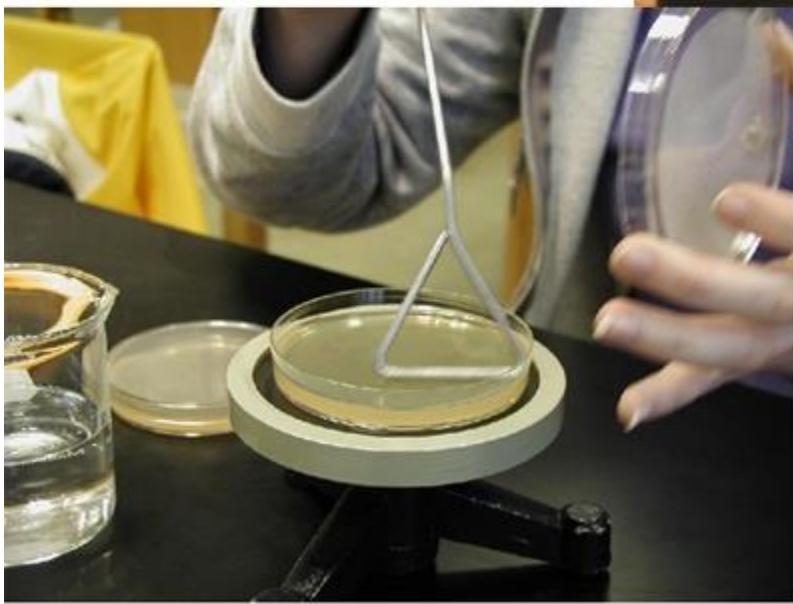
Pour cela, on fait:

- 1) Ensemencements par stries (streak plate), ou un
  - 2) Etalement à l'aide d'un râteau de verre (spread plate), ou alors un
  - 3) Ensemencement après dilutions dans la gélose en surfusion ou en sandwitch entre deux géloses.
- Ces techniques peuvent être combinées avec l'utilisation de milieux sélectifs et différentiels pour l'obtention de souches ou de cultures pures.
  - On obtient ainsi des colonies qui parfois nécessitent une ou plusieurs opérations de séparation pour aboutir à une culture pure.

## Isolement de colonies sur milieu solide



Chap. II : Techniques  
de base en microbiologie



## COLONIES :

- MORPHOLOGIE : elle peut avoir des aspects très variés, on trouve généralement les cas suivants :

Forme : Punctiforme, Circulaire, filamenteuse, Ondulée, rhizoïde, Fuseau, ...

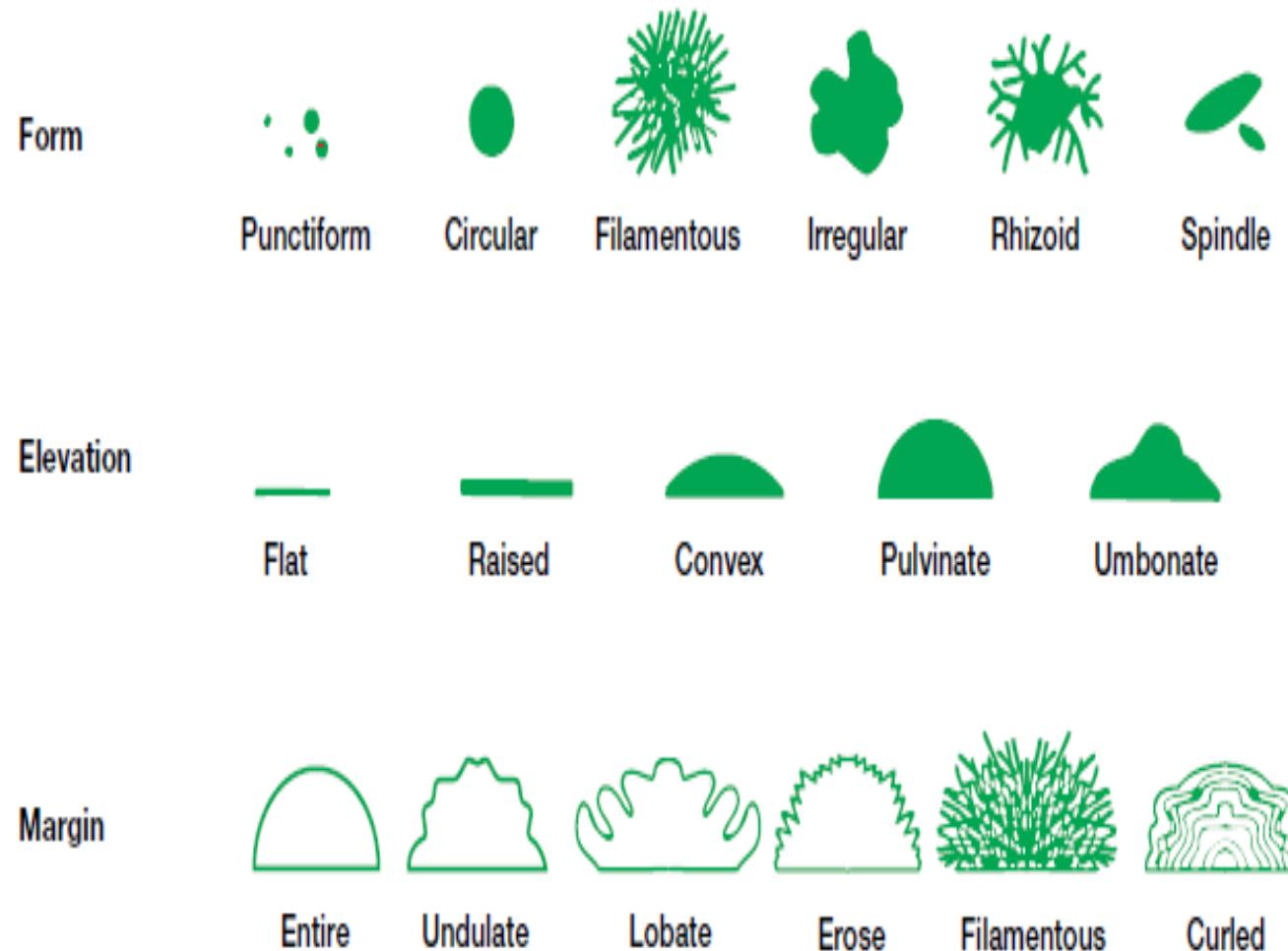
Elévation : Plate, Elevée, Convexe, Dôme, Ombiliquée, ...

Bordure : Entière, Ondulée, Lobée, Erodée, Filamenteuse ou duveteuse, Plissée, ...

- CROISSANCE DES COLONIES :

Forte croissance en bordure et faible croissance au centre de la colonie (car au centre de celle-ci il y a diminution des nutriments, accumulation des toxines et mauvaise circulation des gazes tels que O<sub>2</sub> et CO<sub>2</sub>).

**Figure 15.1** Bacterial Colony Characteristics on Agar Media as Seen with the Naked Eye. The characteristics of bacterial colonies are described using the following terms.



Appearance: Shiny or dull

Optical property: Opaque, translucent, transparent

Pigmentation: Pigmented (purple, red, yellow)

Nonpigmented (cream, tan, white)

## Isolement de colonies sur milieu solide



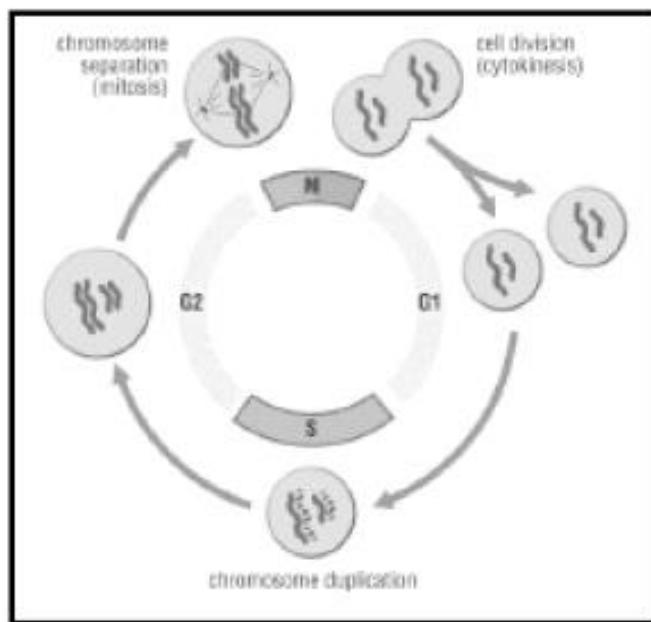
*Bacillus subtilis*



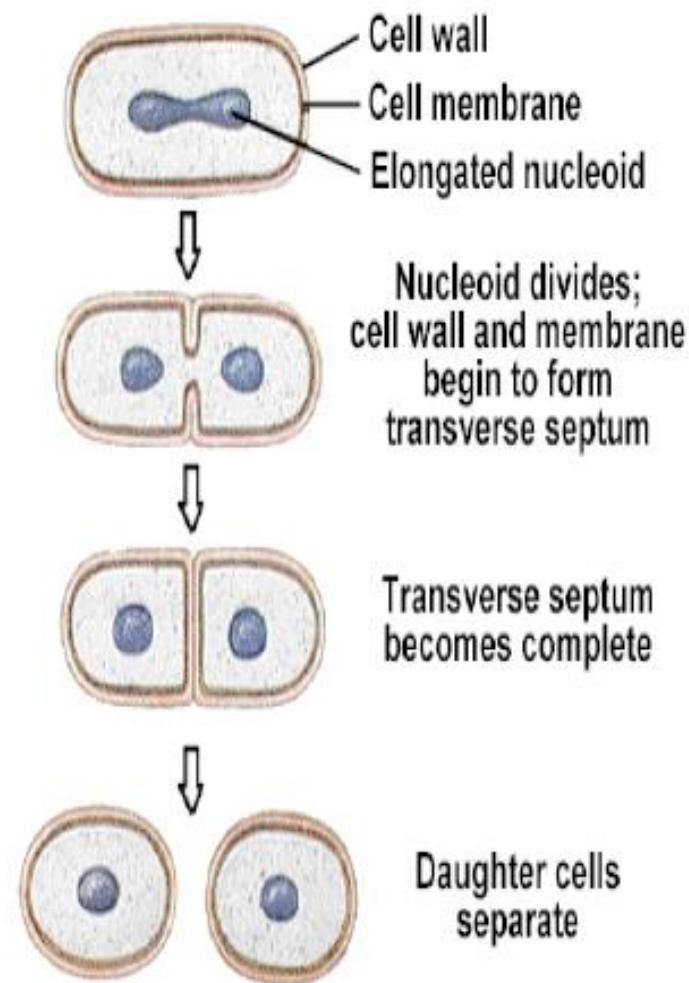
*Staphylococcus aureus*

# Division cellulaire

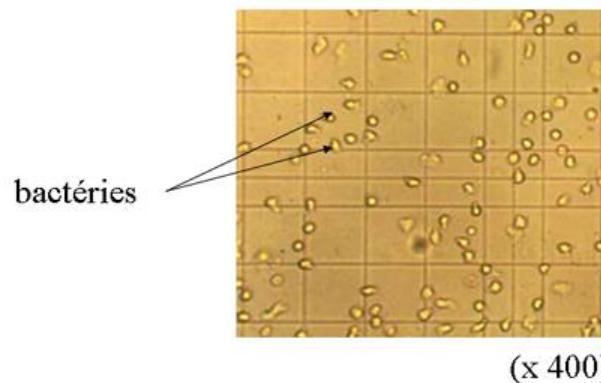
1 puis 2 puis 4 puis ...



cycle cellulaire eucaryote

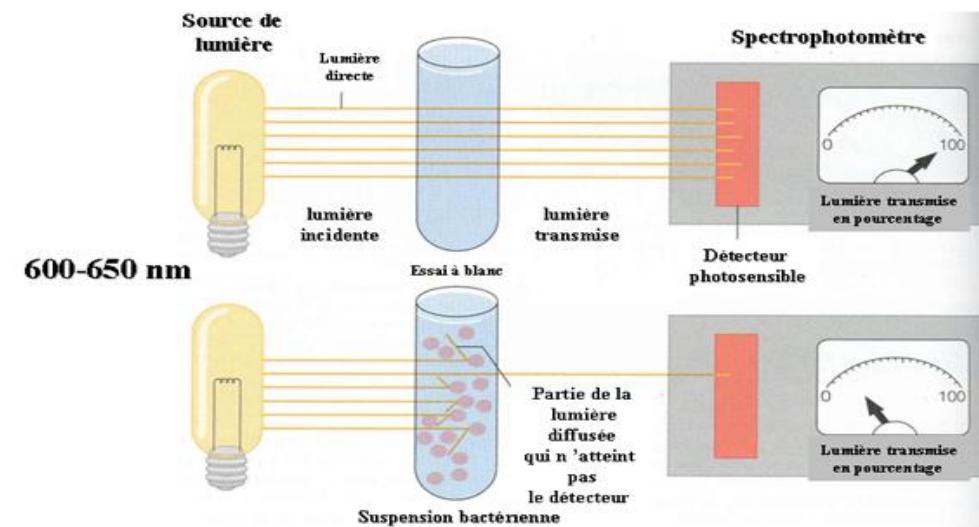


## Dénombrement direct



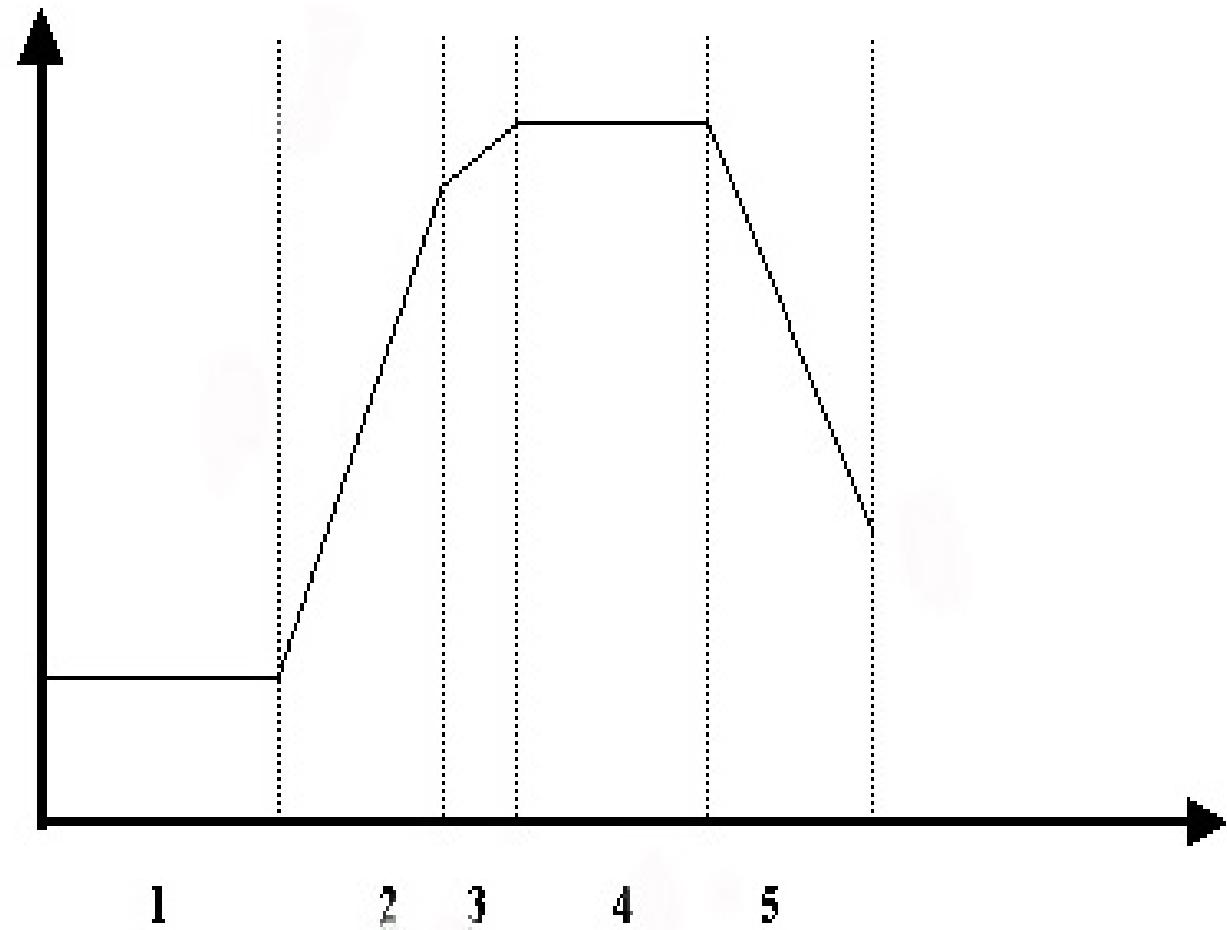
### Mesure de la turbidité

- Absorbance, (A) ou Densité Optique DO.

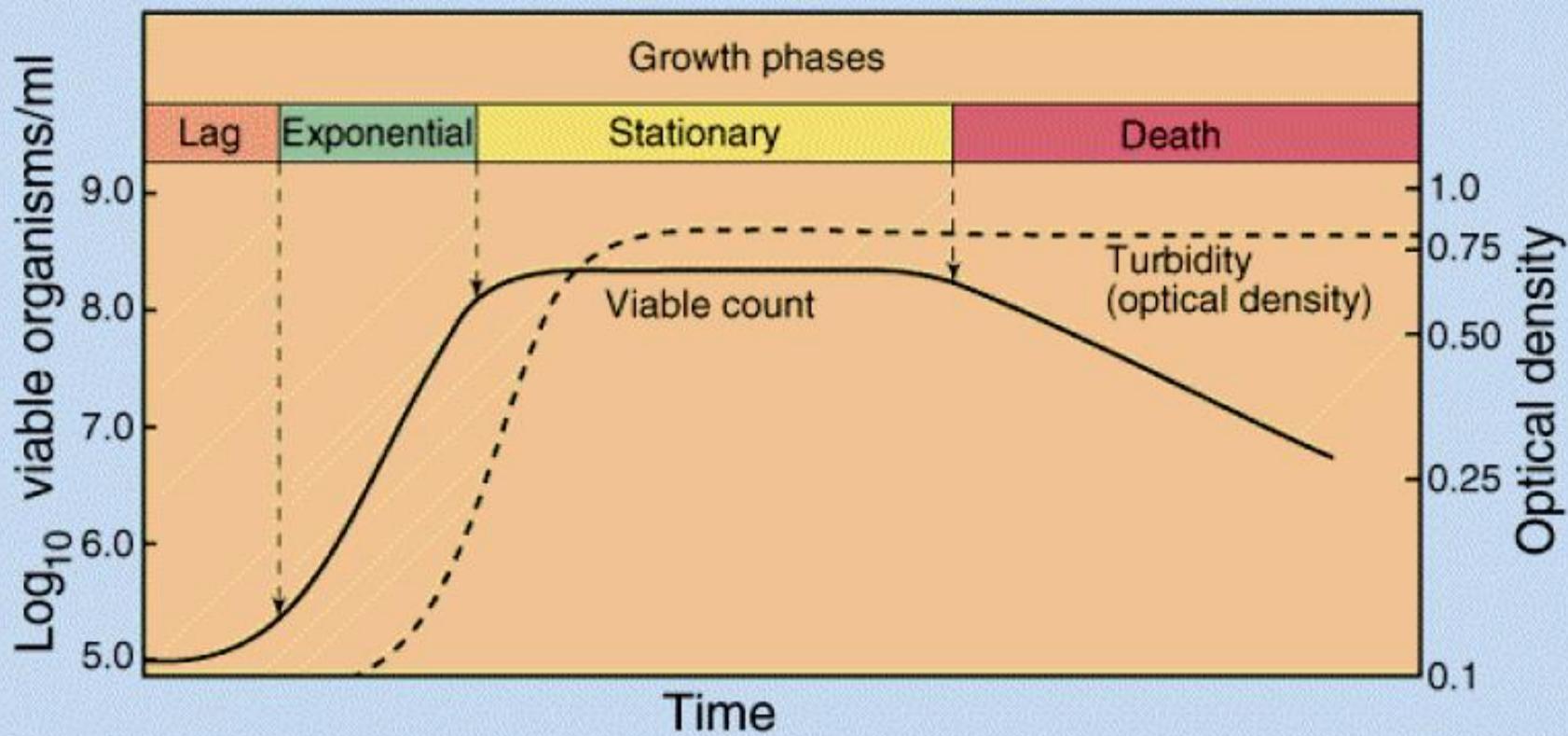


## Exemple d'une courbe de croissance (Log nombre de cellules en fonction du temps)

- 1 : phase de latence,**
- 2 : phase de croissance exponentielle,**
- 3 : phase de ralentissement,**
- 4 : phase stationnaire,**
- 5 : phase de déclin.**



# Les phases de croissance



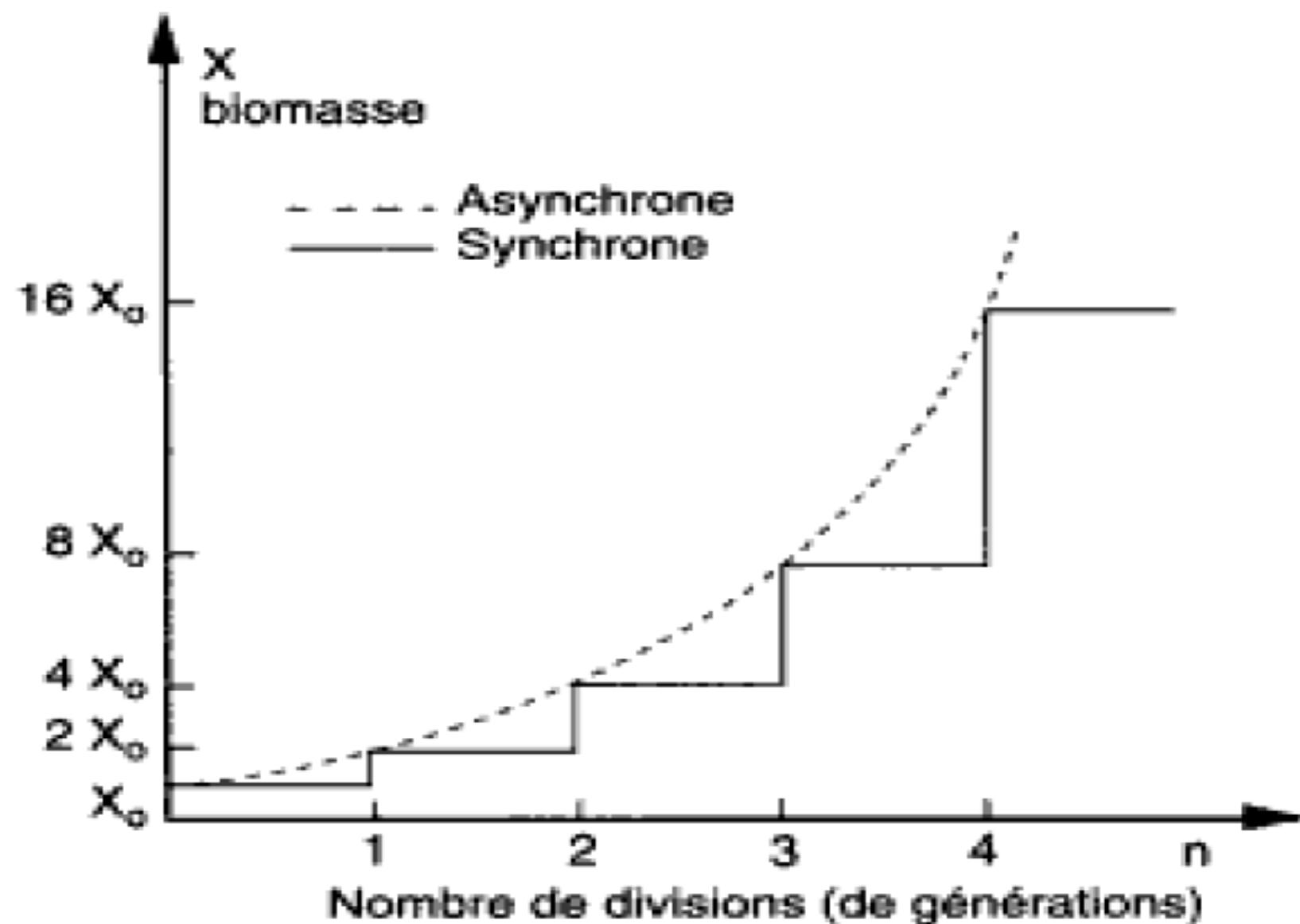
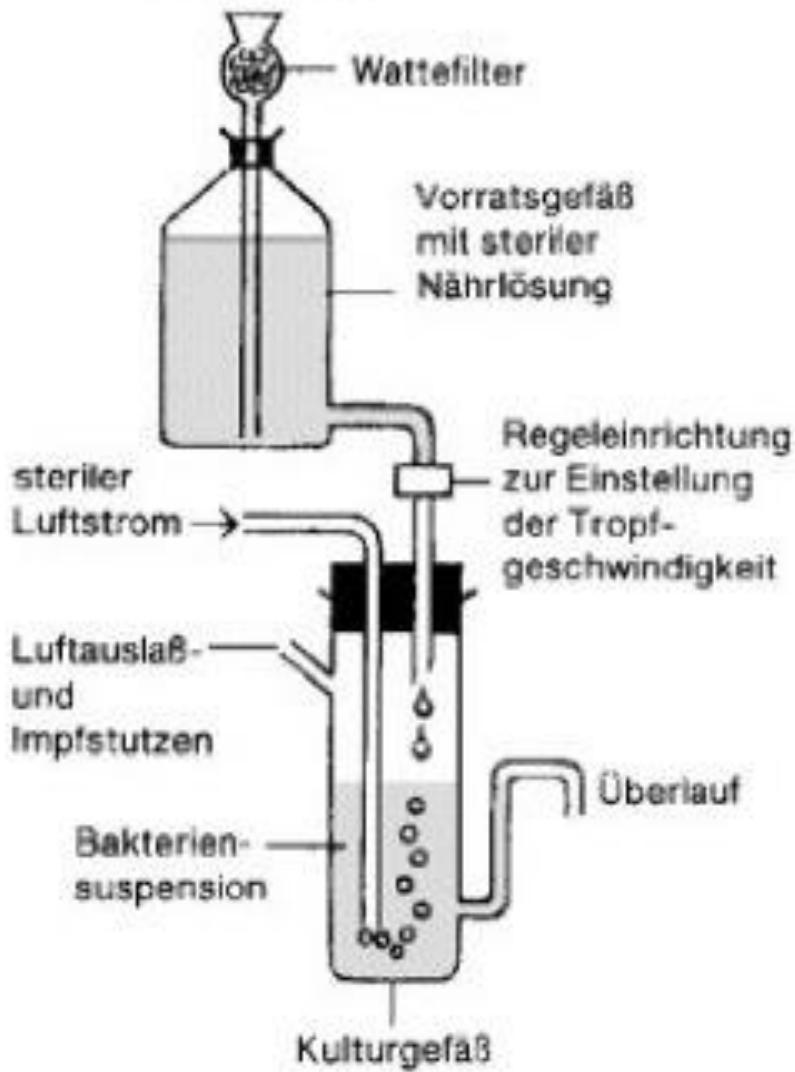


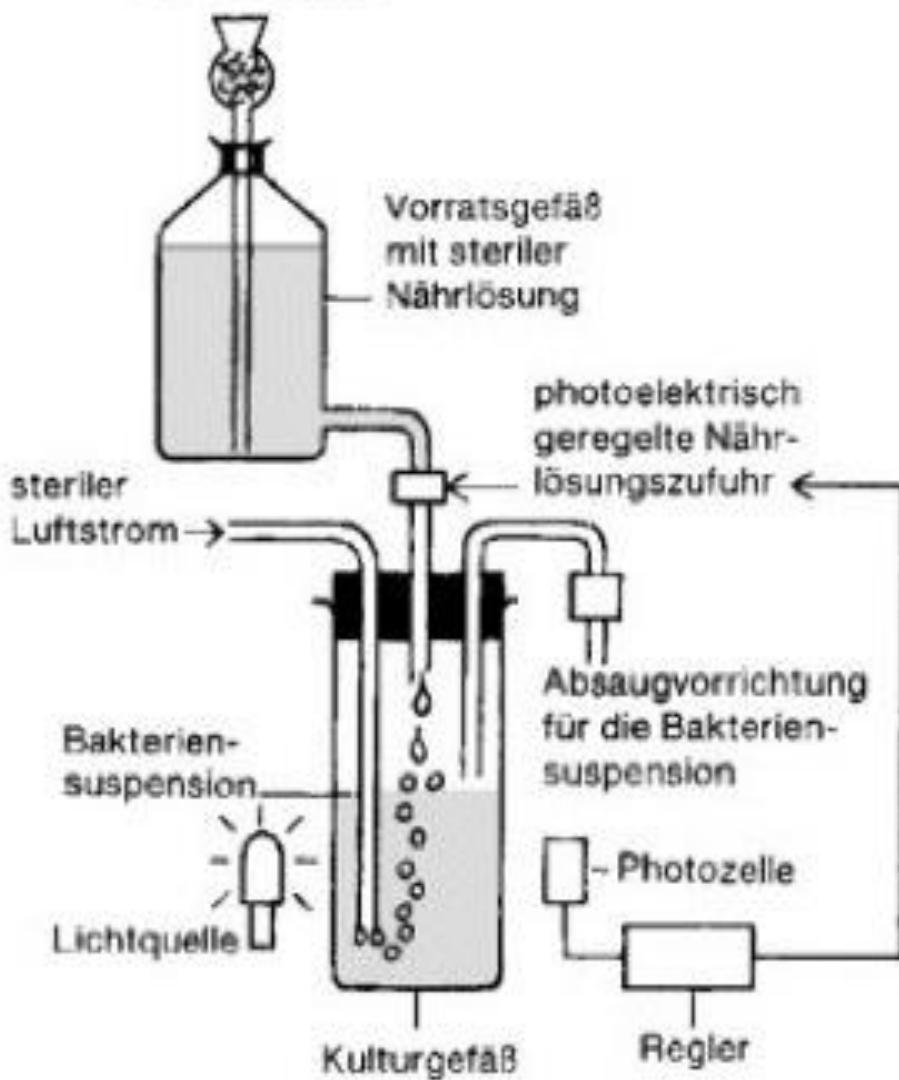
Figure III.8 – Croissance synchrone, croissance asynchrone.

## SCHEMAS DES SYSTÈMES DE CULTURE EN CONTINU

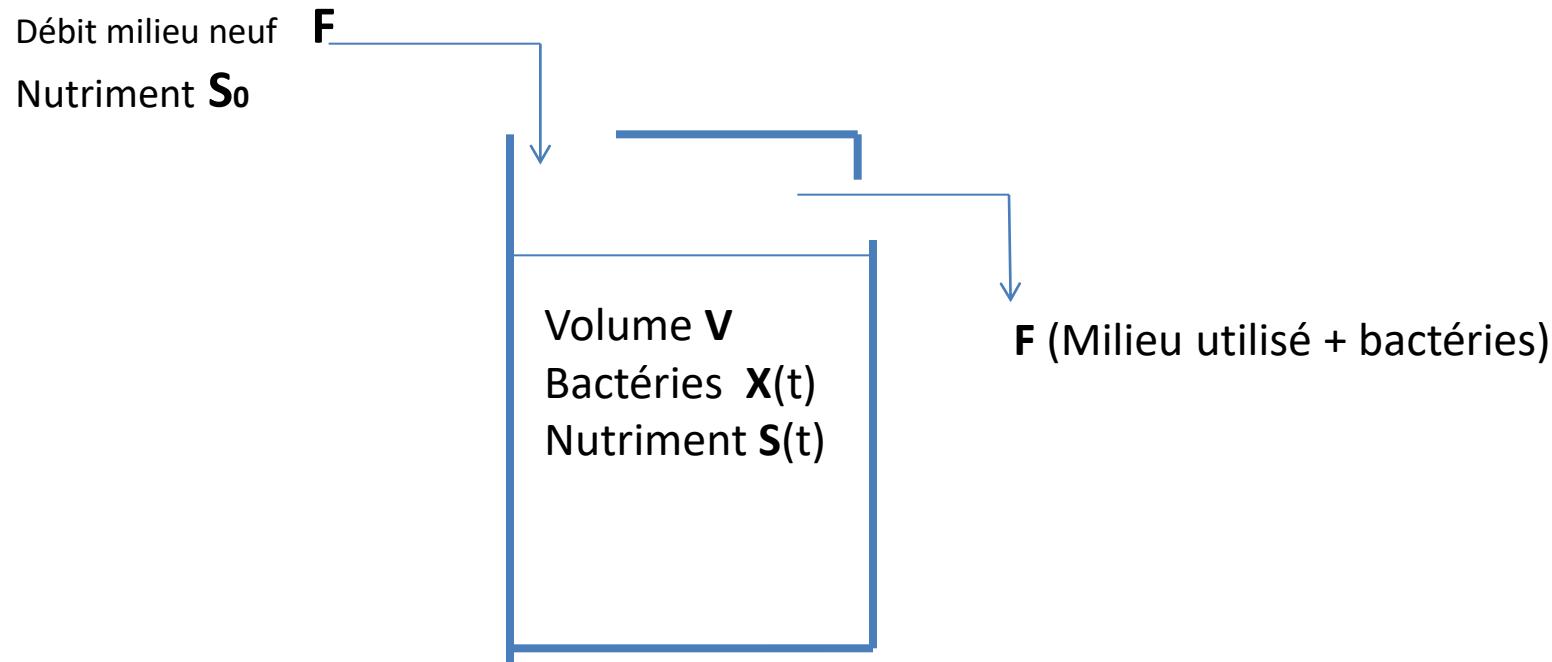
### Chemostat



### Turbidostat







$$\frac{dx}{dt} = \text{rate produced} - \text{rate out}$$

$$\frac{dS}{dt} = \text{rate in} - \text{rate out} - \text{rate consumed}$$

Let  $g(S)$  be the growth rate of the organism with units of 1/time and is an increasing function of nutrient. Let  $\gamma$  be the *yield* constant which is the mass of the organism produced per unit mass of nutrient, i.e.,

$$\gamma = \frac{\text{mass of organism produced}}{\text{mass of nutrient used}}.$$

Then the system of differential equations becomes:

$$\frac{dx}{dt} = g(S) x - \frac{F x}{V}$$

$$\frac{dS}{dt} = \frac{F S_0}{V} - \frac{F S}{V} - \frac{1}{\gamma} g(S) x.$$

### → 3 - Principe de la culture continue

#### 3.1 Modèle à un compartiment : Le chémostat

$$D = \frac{F}{V} \cdot dt$$

éq. 1),

où : - le flux  $F$  est le volume de milieu qui s'écoule dans et hors du réacteur de culture pendant l'intervalle de temps  $dt$ ,  
-  $V$  est le volume du réacteur.

Dans le réacteur de culture est placé une culture d'organismes unicellulaires dont le taux de croissance  $\mu$  est constant pour une concentration donnée en élément limitant. Le changement de biomasse  $B$  en fonction du temps est donné par la relation :

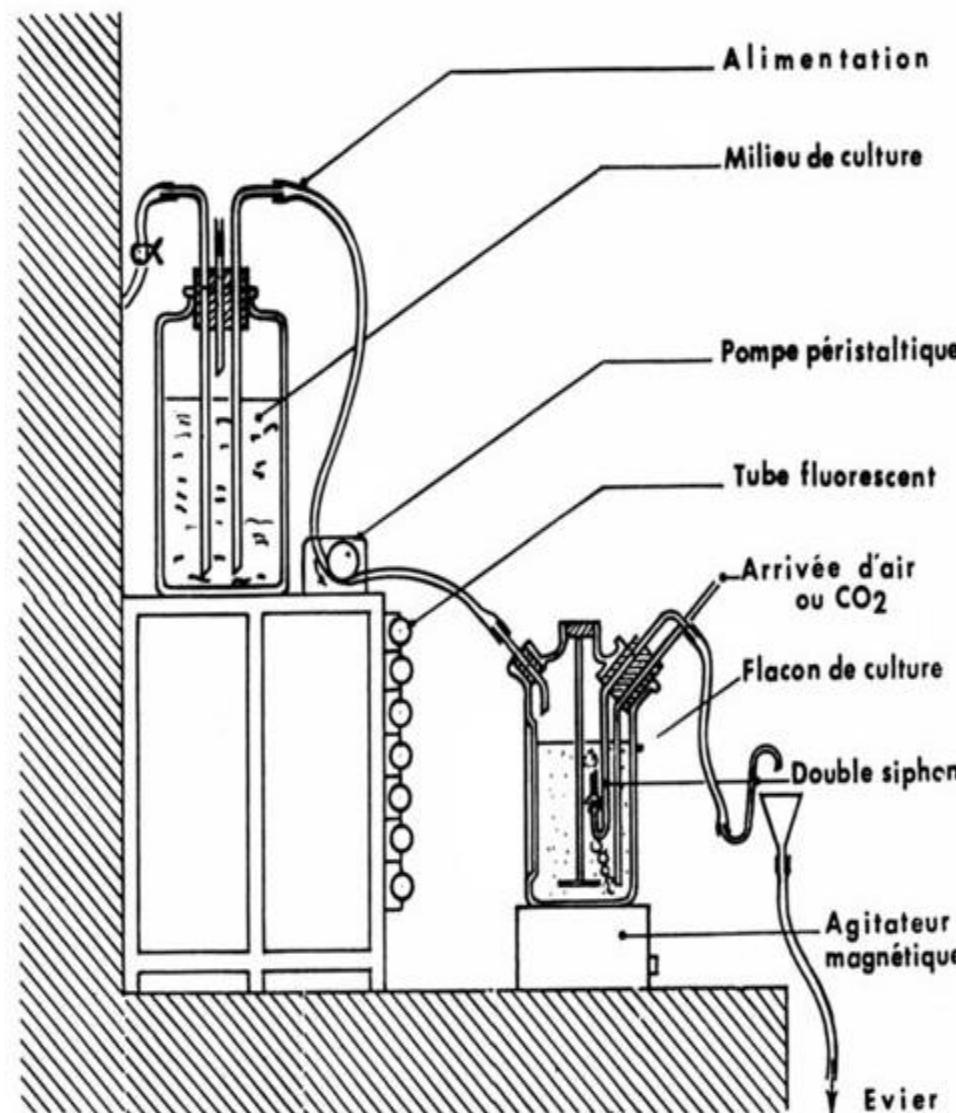
$$\frac{dB}{dt} = B \cdot (\mu - D)$$

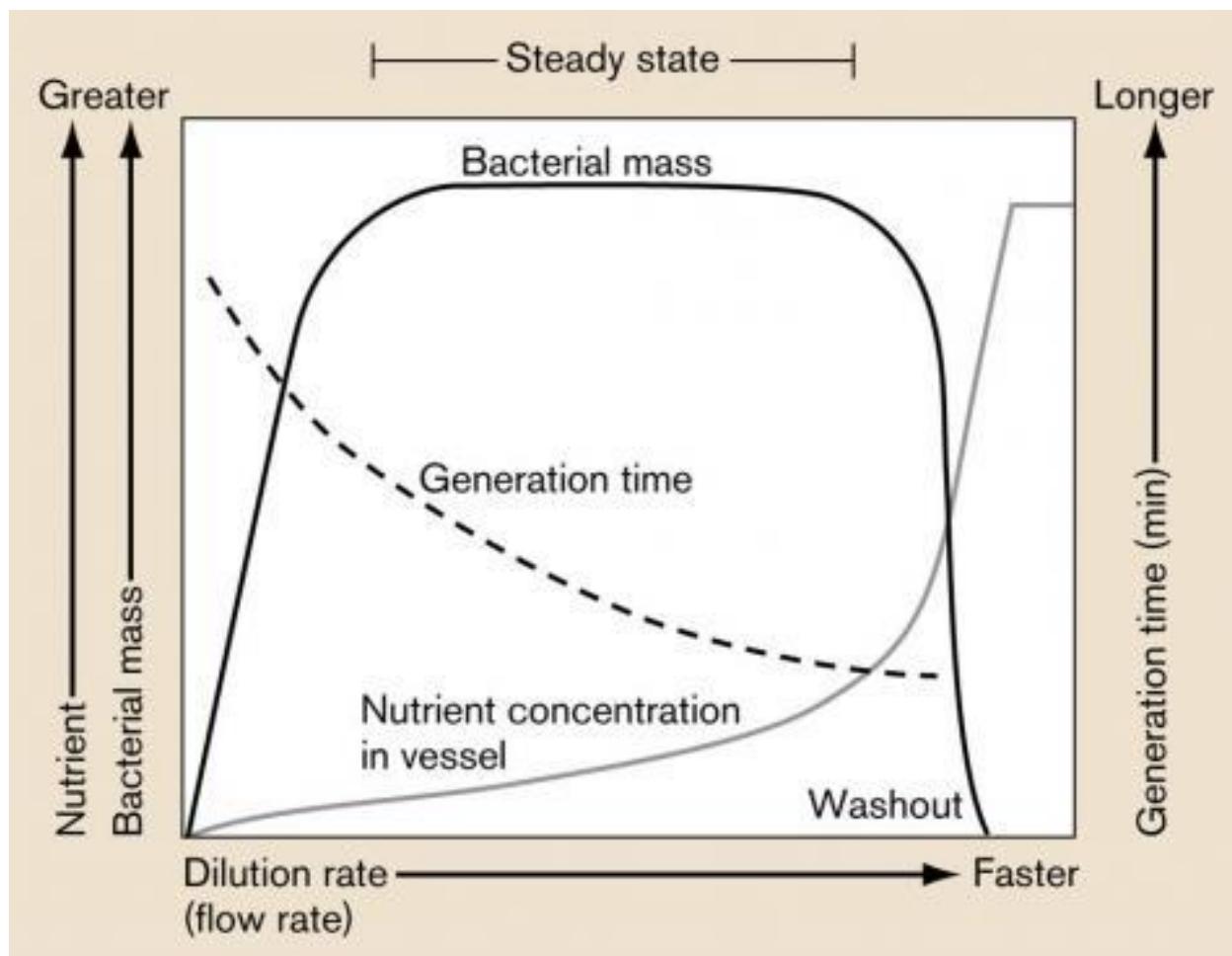
(éq. 2),

Si on se trouve dans une situation d'équilibre dans laquelle  $\mu = D$ , on obtient la solution :

$$\frac{dB}{dt} = 0$$

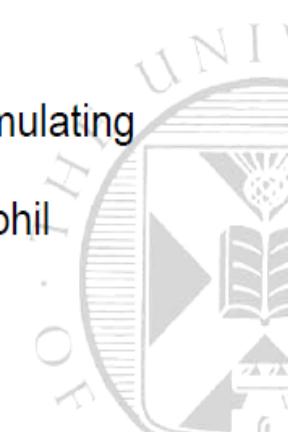
(éq. 3).





# Commercial applications of metabolic analysis

- Why do we need to improve bacterial growth? Shouldn't we try and kill all those nasty bacteria? Think again...
- Optimising *E. coli* growth is a commercial challenge.
- Recombinant *E. coli* bacteria (*through the addition of plasmids containing an inserted gene*) are grown in tanks and used for industrial production of:
  - insulin for diabetics
  - hepatitis B surface antigen (HBsAg) to vaccinate against the hepatitis B virus
  - factor VIII for males suffering from haemophilia A
  - erythropoietin (EPO) for treating anaemia (*and doping athletes...*)
  - human growth hormone (HGH)
  - granulocyte-macrophage colony-stimulating factor (GM-CSF) for stimulating the bone marrow after a bone marrow transplant
  - granulocyte colony-stimulating factor (G-CSF) for stimulating neutrophil production e.g., after chemotherapy
  - tissue plasminogen activator (TPA) for dissolving blood clots
  - and many others...





# CONDITIONS PHYSICO-CHIMIQUES DE LA CROISSANCE

## A- Effet de l'oxygène

1 - Les bactéries aérobies strictes ne se développent qu'en présence de l'oxygène. Respiration. L'oxygène moléculaire, accépteur final d'électrons, est réduit en eau

Exemples: (*Pseudomonas, Acinetobacter, Neisseria*).

2 - Les bactéries micro-aérophiles se développent mieux ou exclusivement lorsque la pression partielle d'oxygène est inférieure à celle de l'oxygène dans l'air

Exemples: (*Campylobacter, Mycobacteriaceae*).

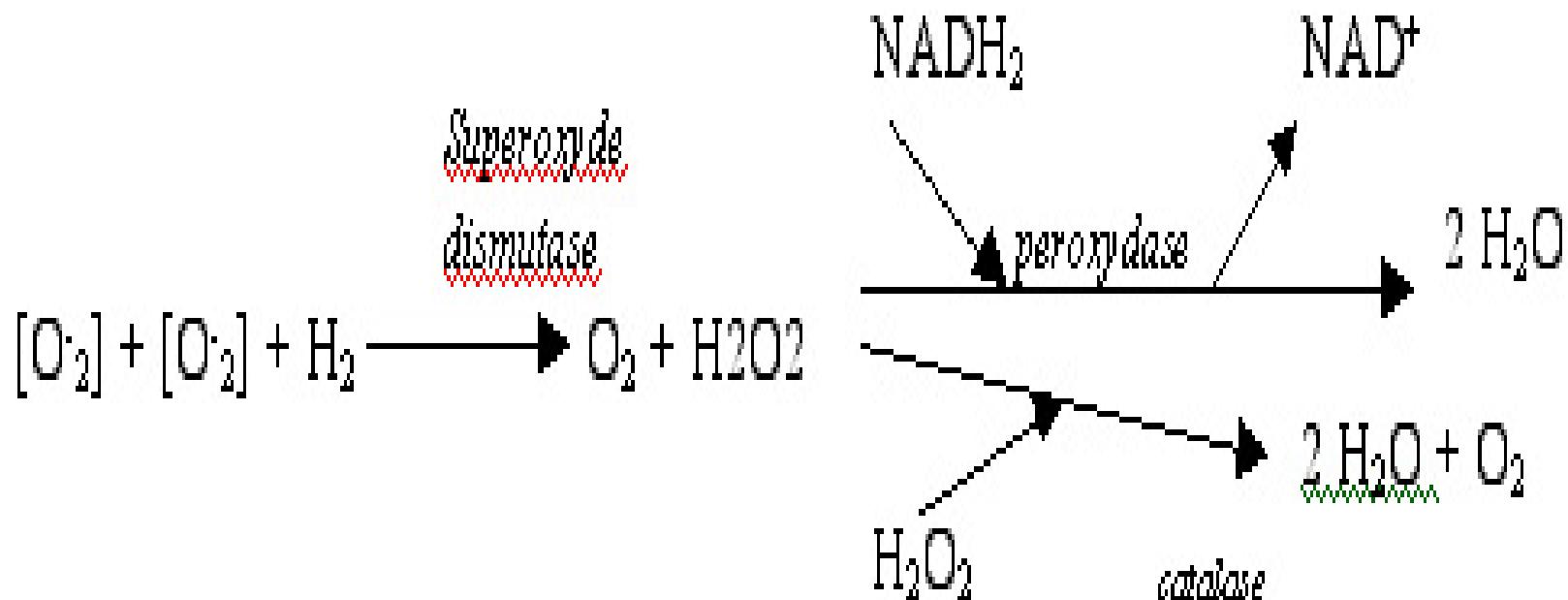
3 - Les bactéries aéro-anaérobies facultatives se développent avec ou sans air. C'est le cas de la majorité des bactéries de pathologie médicale : les entérobactéries Exemples: (*Escherichia, Salmonella*), les streptocoques, les staphylocoques. Fermenteurs.

4 - Les bactéries anaérobies strictes ne se développent qu'en absence totale ou presque d'oxygène. (culture sous atmosphère réductrice).

Exemples: bactéries intestinales (*Bacteroides, Fusobacterium, Clostridium*) et bactéries des flores normales de l'organisme.

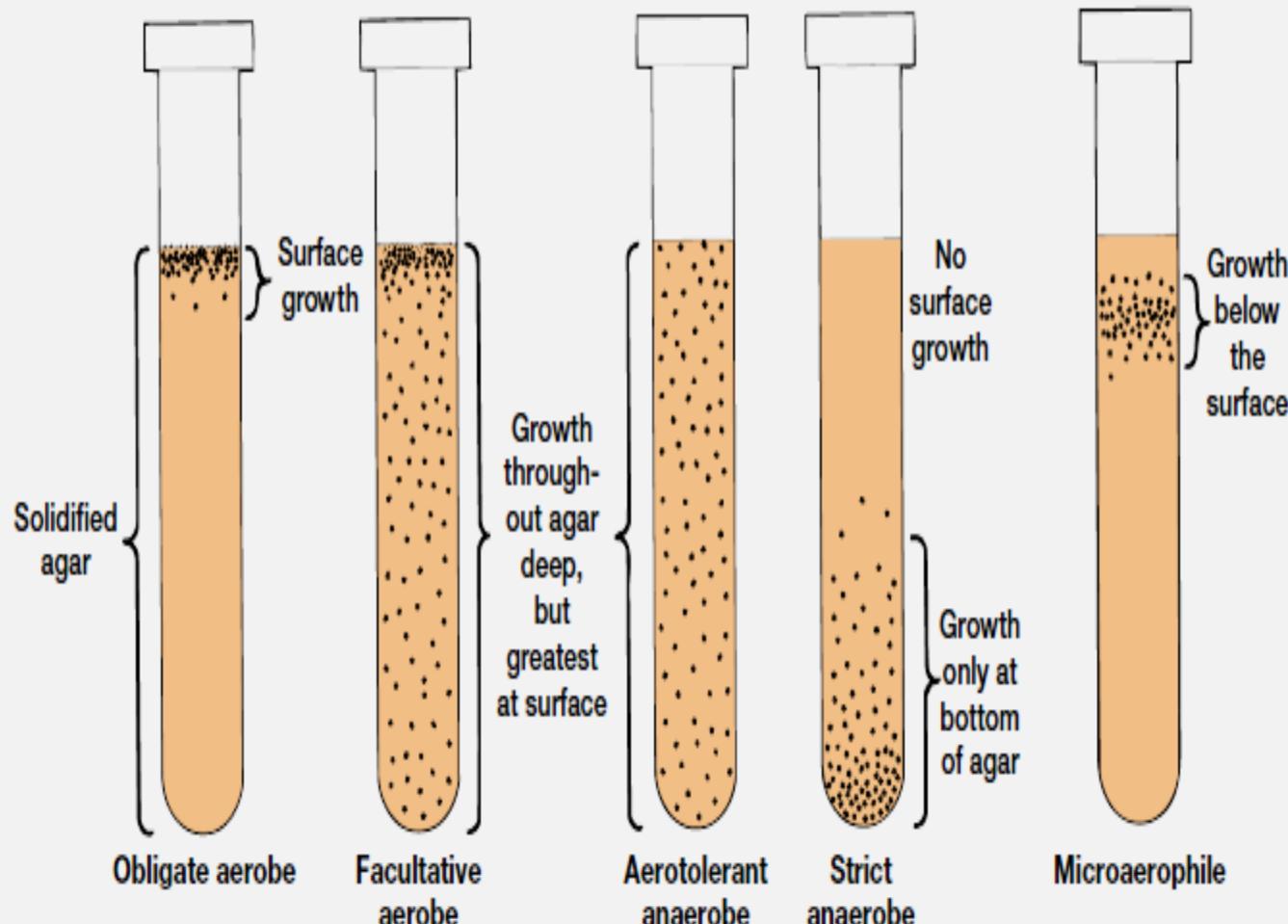
La production de radicaux superoxydes que les bactéries anaérobies ne peuvent pas éliminer  Toxicité de l'oxygène.

Chez les anérobies stricts les ions superoxyde et hydroxyle qui sont des oxydants très forts ne peuvent pas être détruits (absence de superoxyde dismutase) et/ou par l'absence d'une activité enzymatique catalase et peroxydases.

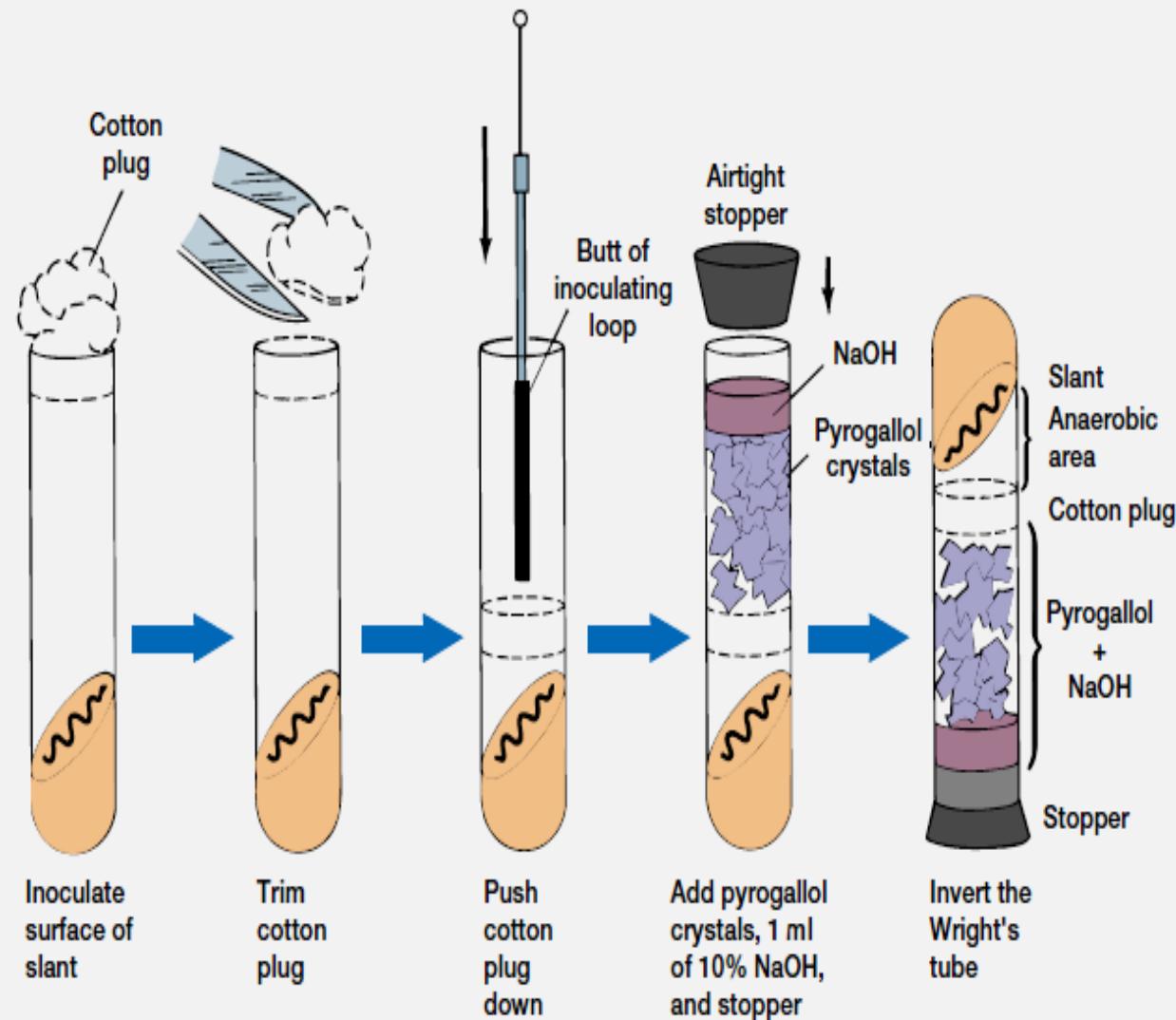


**La superoxyde dismutase (SOD), est une métalloprotéine avec une activité enzymatique: la catalyse de la dismutation du superoxyde en oxygène et peroxyde d'hydrogène. Pour cette raison, cette enzyme est une partie importante du système de défense contre les radicaux libres.**

**Figure 18.1 The Appearance of Various Agar Deep Cultures.** Each dot represents an individual bacterial colony within the agar or on its surface. The surface, which is directly exposed to atmospheric oxygen, will be aerobic. The oxygen content of the medium decreases with depth until the medium becomes anaerobic toward the bottom of the tube.



**Figure 18.2 Preparation of an Anaerobic Wright's Tube.** Pyrogallol is a reducing agent that is activated by NaOH to remove oxygen from the tube and create anaerobic conditions.



## B- Effet de la pression osmotique

Les bactéries sont assez tolérantes aux variations des concentrations ioniques. Certaines espèces sont osmotolérantes (*Staphylocoques, Vibrio cholerae*).

## C- Effet de l'eau libre (activité de l'eau)

La disponibilité de l'eau présente dans l'atmosphère ou dans une substance intervient dans la croissance bactérienne. L'activité de l'eau ( $A_w$ ) est inversement proportionnelle à la pression osmotique d'une solution ou d'un composé. Elle dépend de la présence plus ou moins importante de solutés (sels, sucres ou autres) dissous dans l'eau.

$$A_w = P_{\text{solution}} / P_{\text{eau}} \quad (P = \text{pression de vapeur})$$

**Tableau III.6 – Valeurs d'Aw pour quelques solutions de NaCl et de saccharose.**

Aw à 25 °C	NaCl en g/100 g d'eau	Saccharose en g/100 g d'eau
0,99	1,75	11
0,96	7,01	25
0,94	10,34	93
0,92	13,50	120
0,90	16,50	144
0,85	23,60	208

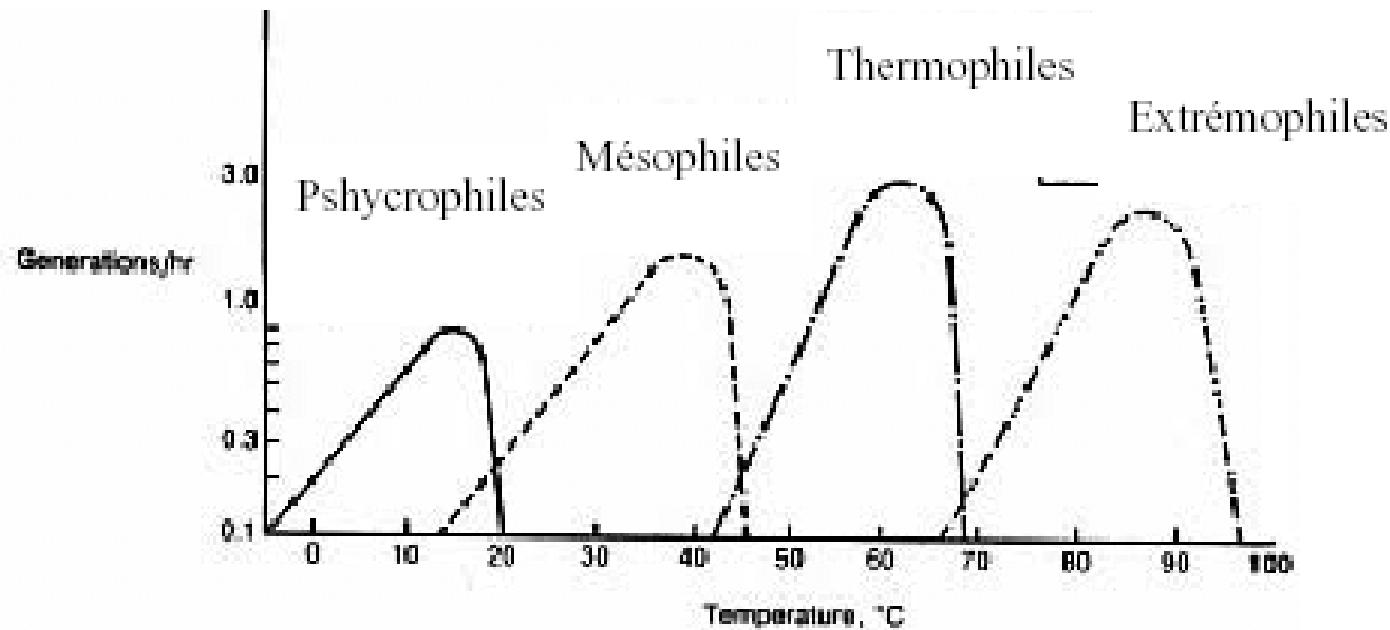
Aliments	aw	Micro-organismes pouvant s'y développer à température ambiante	Lieu de stockage et température
Viande fraîche	0,96	Bactéries	Réfrigérateur + 4°C
Poisson	0,99	Bactéries	Réfrigérateur + 4°C
Fruits et légumes	0,98 à 0,95	Bactéries	Réfrigérateur + 4°C
Confiture	0,75 à 0,8	Moisissures	Température ambiante ou réfrigérateur
Fruits secs	0,6	Aucun micro-organisme	Température ambiante dans un endroit sec
Céréales	0,7	Moisissures	Température ambiante
Biscuits secs	0,55	Aucun micro-organisme	Température ambiante, dans un endroit sec
Café soluble	0,2	Aucun micro-organisme	Température ambiante dans un endroit sec

## D- Effet de la température

### Effet de la température

1966: découverte par le Pr. Brock de bactéries vivant dans des sources d'eaux chaudes ( $>100^{\circ}\text{C}$ ) du parc national Yellowstone (USA)

On trouve aussi des bactéries poussant à très basse température : eaux glacées de l'Antartique... ou dans nos frigos!!!



**Figure 2.** Courbes de croissance des microorganismes psychrophiles, mésophiles et thermophiles selon la température

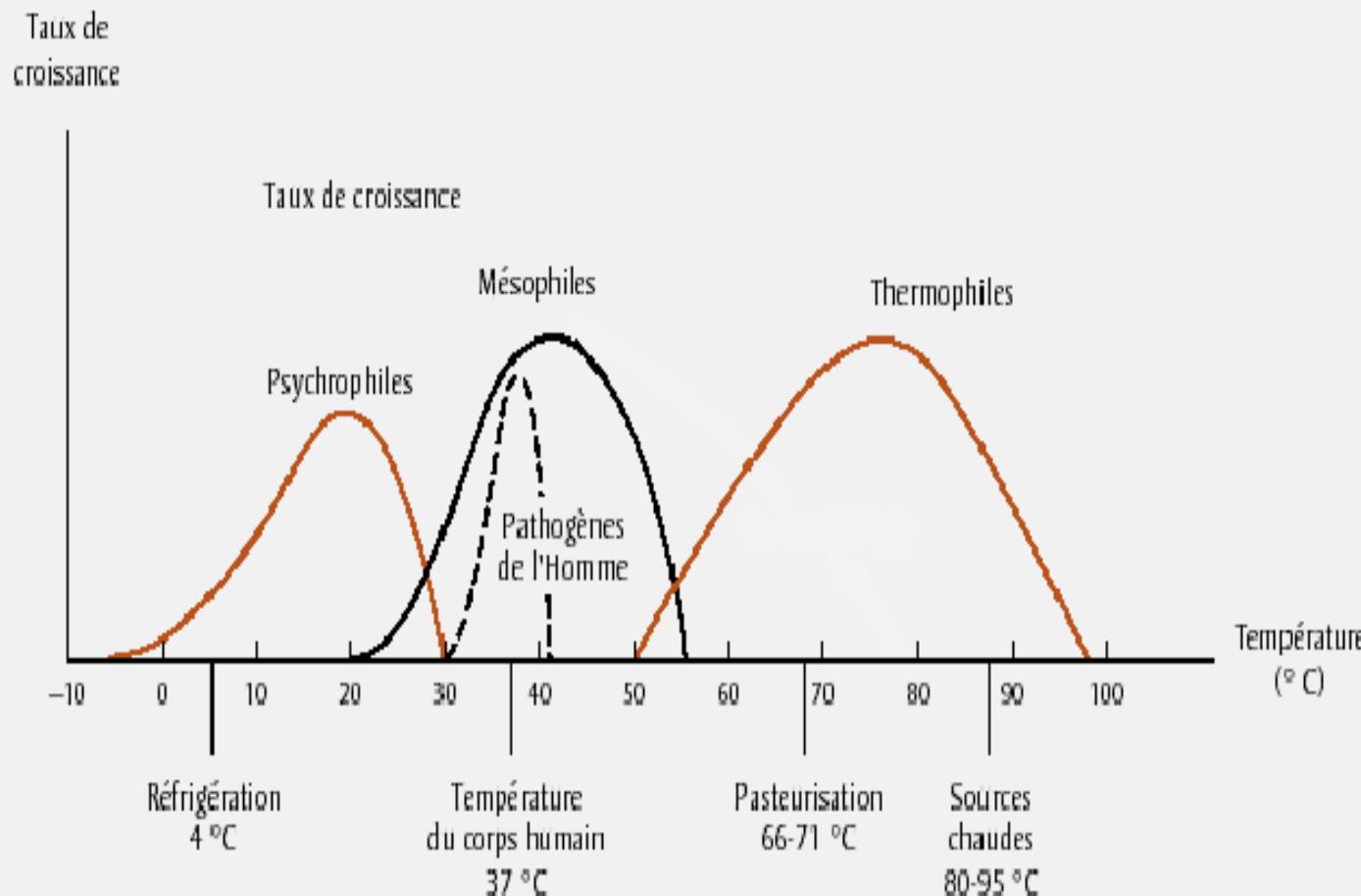


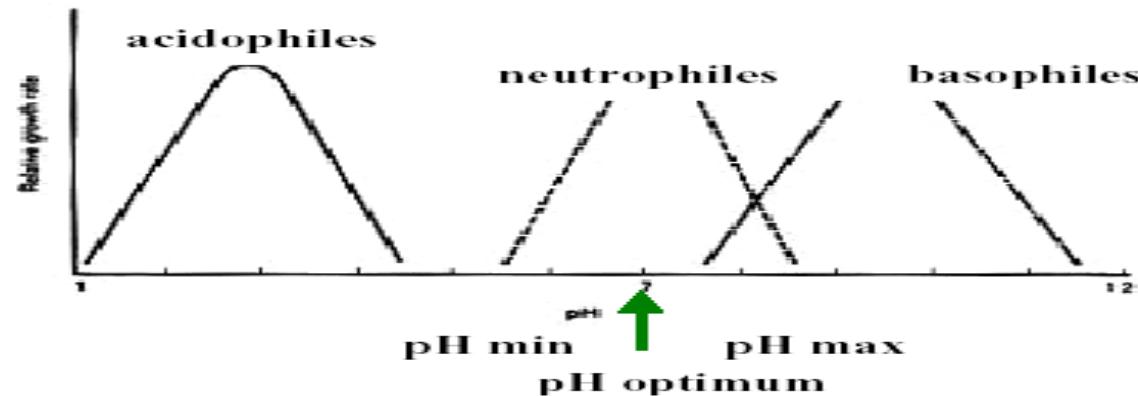
Tableau III.4 - Classification des micro-organismes en fonction de leur température optimale de croissance.

Catégorie	$\theta$ minimale	$\theta$ optimale	$\theta$ maximale
Thermophiles extrêmes	> 50 °C	> 70 °C	> 80 °C
Thermotrophes	> 25 °C	35-50 °C	> 50 °C
Thermophiles	> 25 °C	> 45 °C	> 50 °C
Mésophiles	5-10 °C	30-37 °C	40-43 °C
Psychrotrophes	- 5 °C	20-25 °C	35 °C
Psychrophiles	- 15 °C	5-10 °C	20 °C

## E- Effet du pH

### Effet du pH

pH des environnements naturels varient de 0,5 pour les sols les plus acides (volcans) à 10,5 pour les lacs les plus alcalins (lac de soude au Kenya)



pH milieu de culture doit se rapprocher du pH optimum de la bactérie étudiée  
est tamponné pour être maintenu (accumulation déchets)

La plupart des milieux pour diagnostic de pathogènes humains est vers pH 7

#### **Acidophiles**

*Lactobacillus*

*Thiobacillus* (dissolution des membranes et lyse des cellules à pH neutre)

NB extraction de minéraux

Archéobactéries (*Sulfolobus, Thermoplasma*) sont acidophiles obligatoires  
le + d'ex de bactéries extrémophiles

maintien pH interne entre 5 et 8 grâce des pompes membranaires  
efflux de  $H^+$  et entrée de  $K^+$  (cyto +)

#### **Basophiles**

pompes membranaires      efflux de  $Na^+$

*Nitrobacter*

## **F- Effets du rayonnement:**

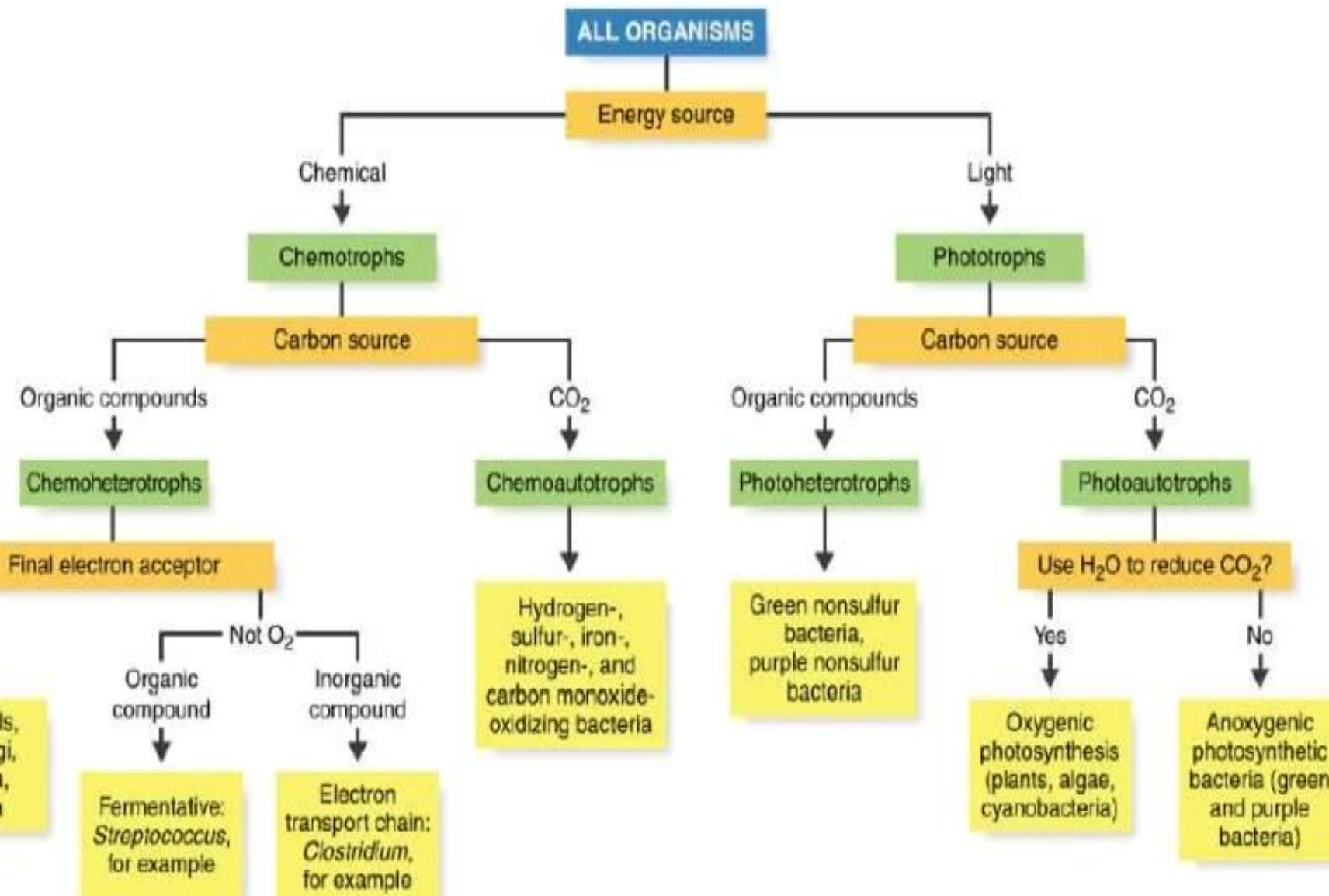
**Rayons UV, Rayons Gamma (Action sur les structures des cellules , action sur l'ADN).**

**Utilisation en stérilisation**

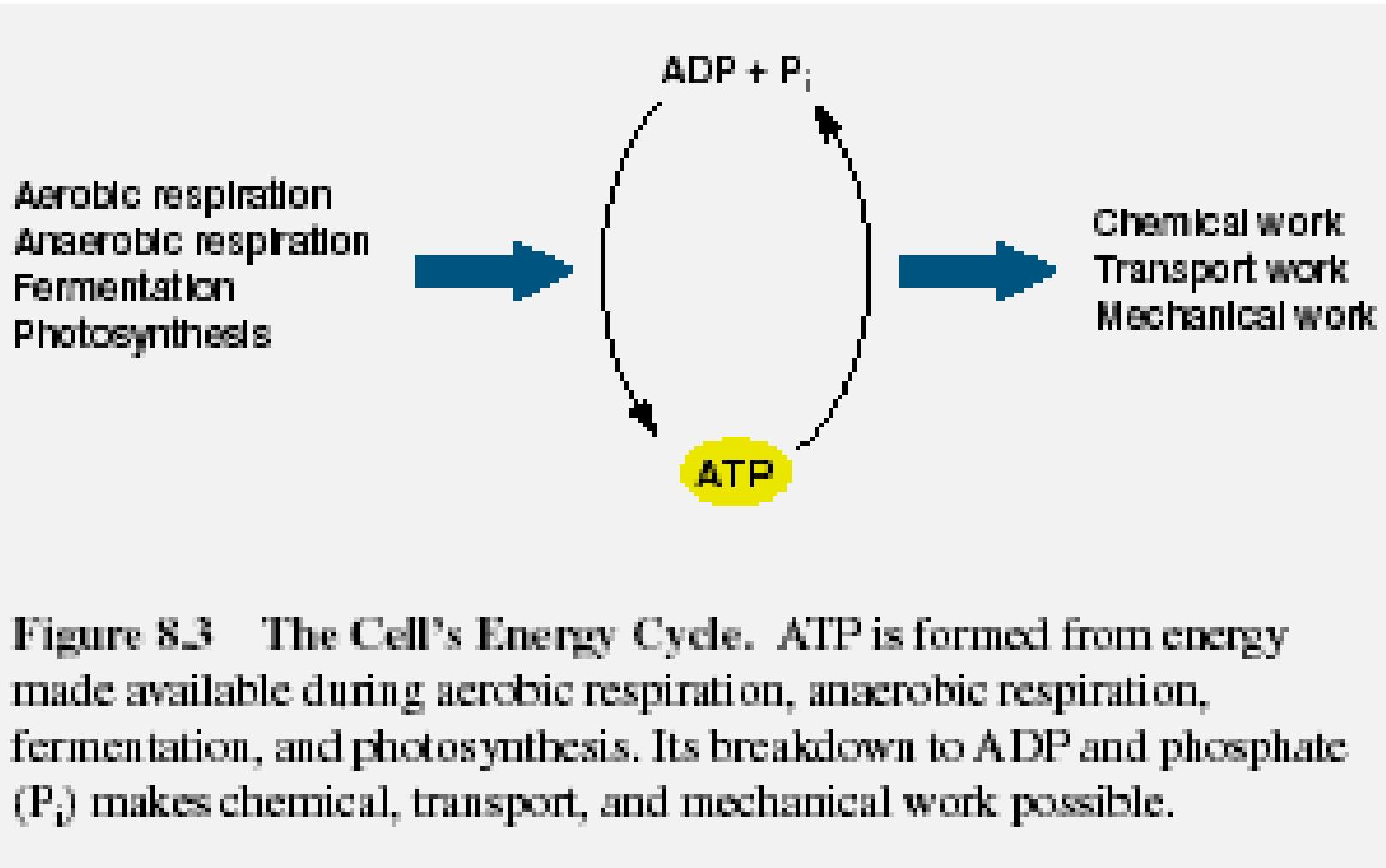
**Interaction avec les pigments protecteurs des structures cellulaires , ADN notamment.**

# **CHAPITRE IV**

# **METABOLISME BACTERIEN**



## METABOLISME



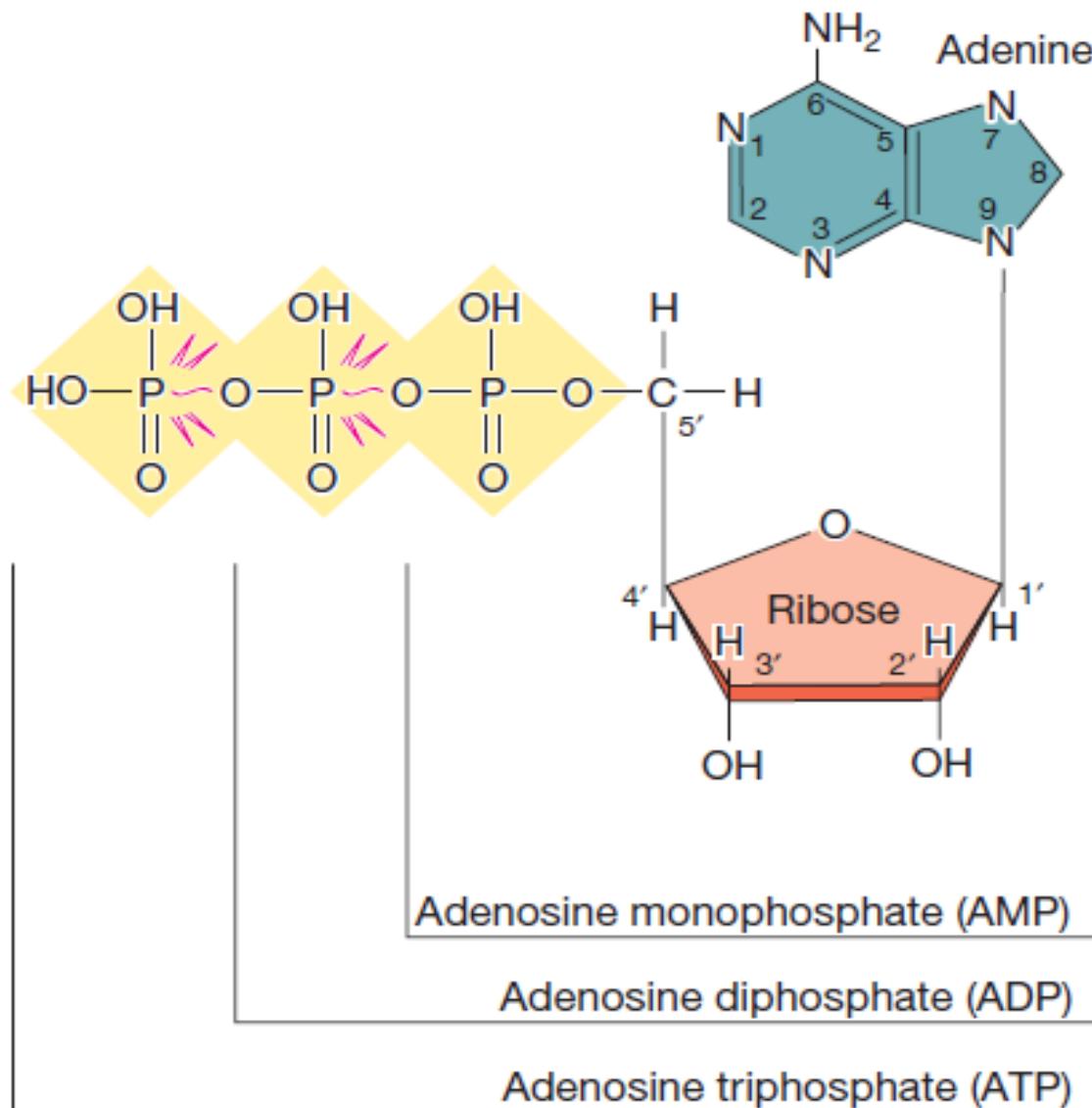
**Figure 8.3** The Cell's Energy Cycle. ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, and photosynthesis. Its breakdown to ADP and phosphate (P<sub>i</sub>) makes chemical, transport, and mechanical work possible.

# Concepts

---

1. Energy is the capacity to do work. Living organisms can perform three major types of work: chemical work, transport work, and mechanical work.
2. Most energy used by living organisms originally comes from sunlight trapped during photosynthesis by photoautotrophs. Chemoheterotrophs then consume autotrophic organic materials and use them as sources of energy and as building blocks.
3. An energy currency is needed to connect energy-yielding exergonic reactions with energy-requiring endergonic reactions. The most commonly used currency is ATP.
4. All living systems obey the laws of thermodynamics.
5. When electrons are transferred from a reductant with a more negative reduction potential to an oxidant with a more positive potential, energy is made available. A reversal of the direction of electron transfer—for example, during photosynthesis—requires energy input.
6. Enzymes are protein catalysts that make life possible by increasing the rate of reactions at ambient temperatures. Enzymes do not change chemical equilibria or violate the laws of thermodynamics but accelerate reactions by lowering their activation energy.

# ATP



(a) Bond that releases energy when broken

# METABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS

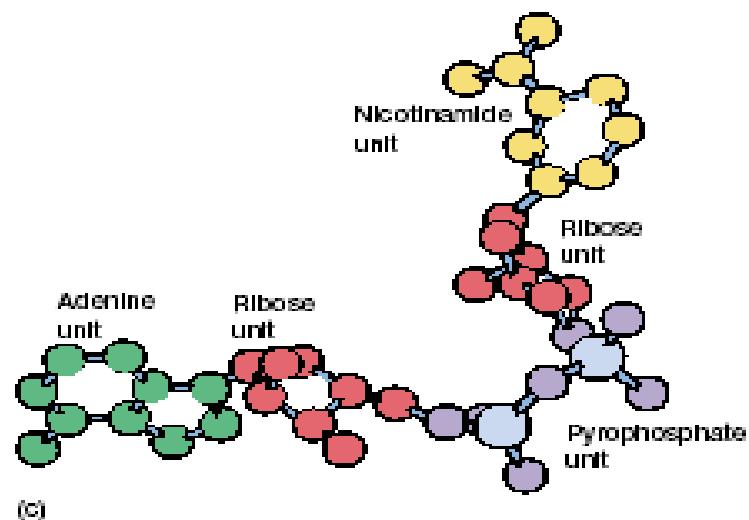
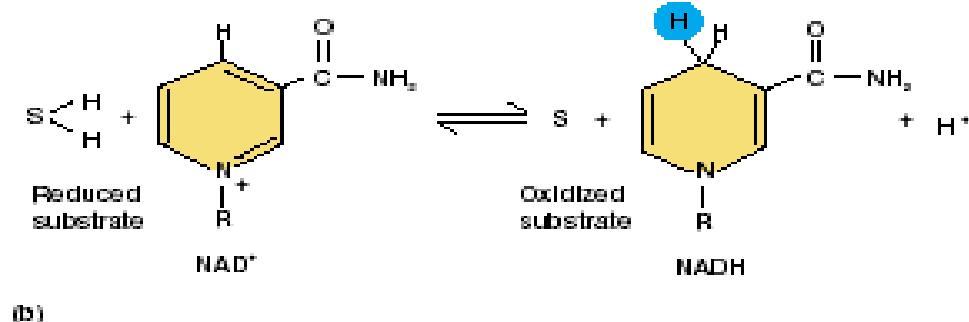
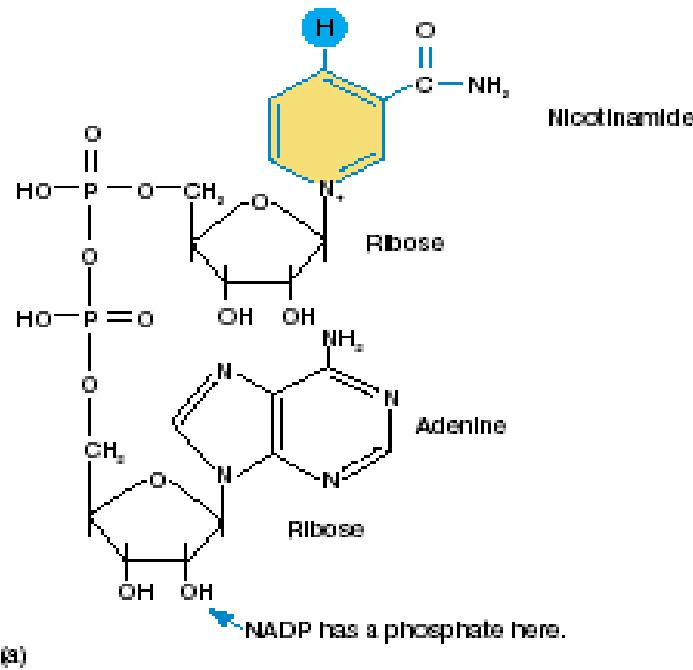
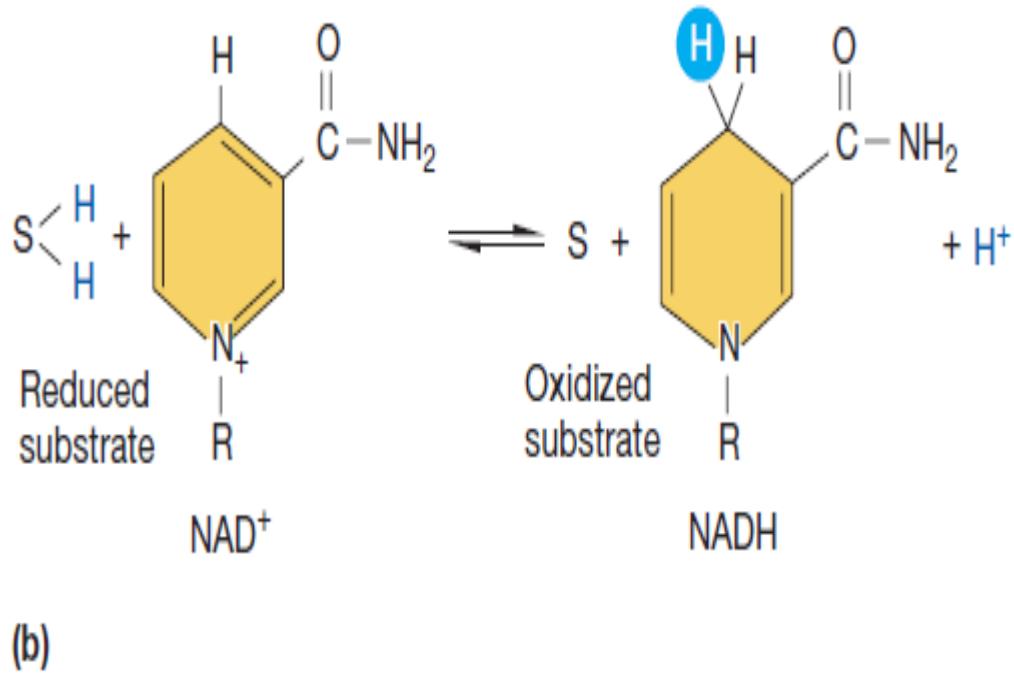
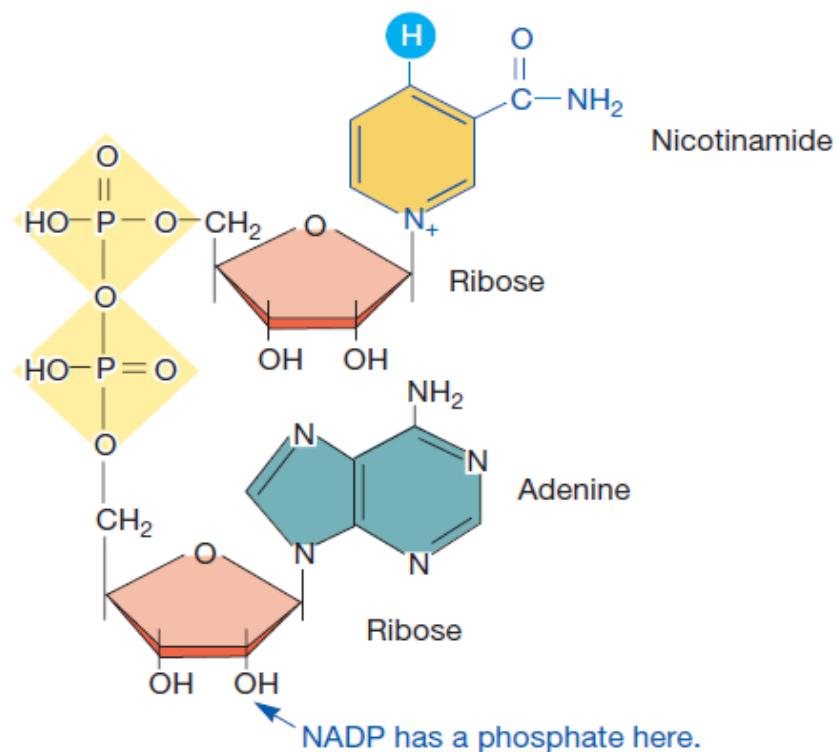


Figure 8.9 The Structure and Function of NAD. (a) The structure of NAD and NADP. NADP differs from NAD in having an extra phosphate on one of its ribose sugar units. (b) NAD can accept electrons and a hydrogen from a reduced substrate (SH<sub>2</sub>). These are carried on the nicotinamide ring. (c) Model of NAD<sup>+</sup> when bound to the enzyme lactate dehydrogenase.

## Guanosine Di et Tri Phosphate

Le coenzyme GDP/GTP (GTP 523 daltons) = Nucléotide ayant une structure très voisine de celle de l'ADP/ATP

## MÉTABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS



## METABOLISME: TRANPORTEURS D'ELECTRONS ET DE PROTONS

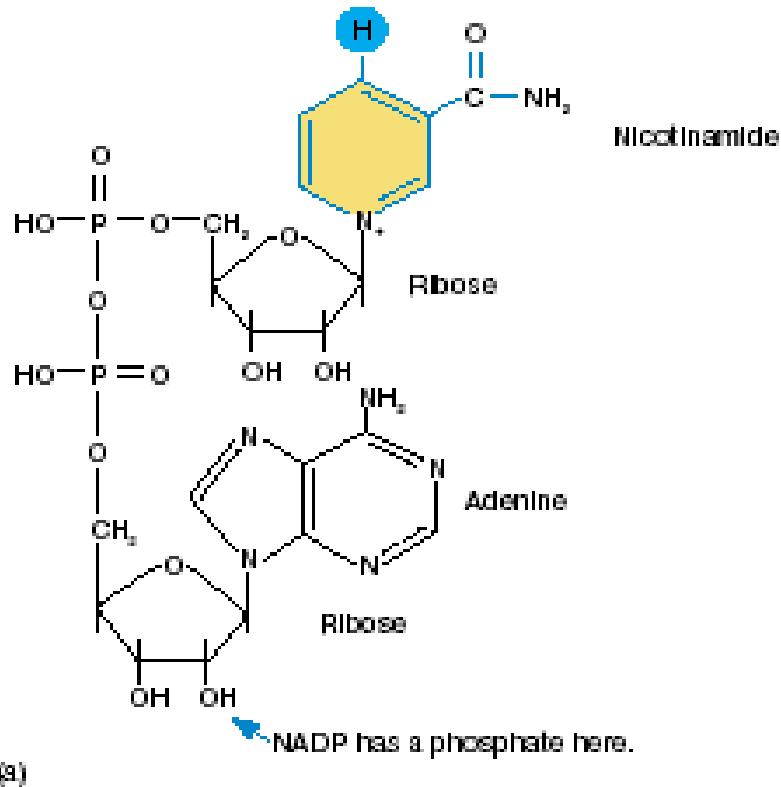
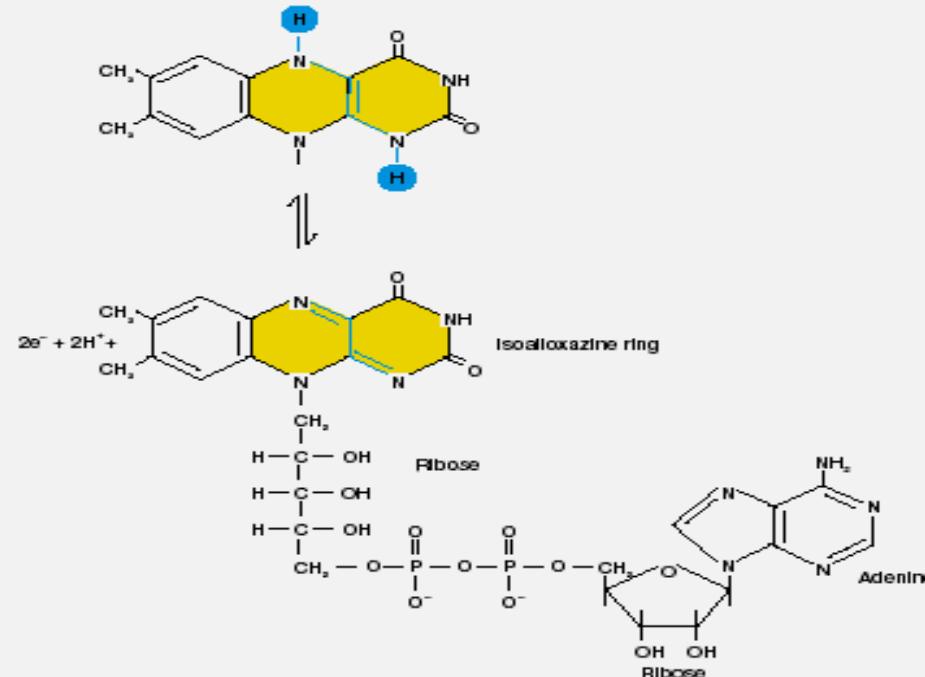


Figure 8.9 The Structure and Function of NAD. (a) The structure of NAD and NADP. NADP differs from NAD in having an extra phosphate on one of its ribose sugar units. (b) NAD can accept electrons and a hydrogen from a reduced substrate ( $SH_2$ ). These are carried on the nicotinamide ring. (c) Model of  $NAD^+$  when bound to the enzyme lactate dehydrogenase.

## METABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS

### Dérivé de la Riboflavine qui est la vitamine B 2



### Ubiquinone

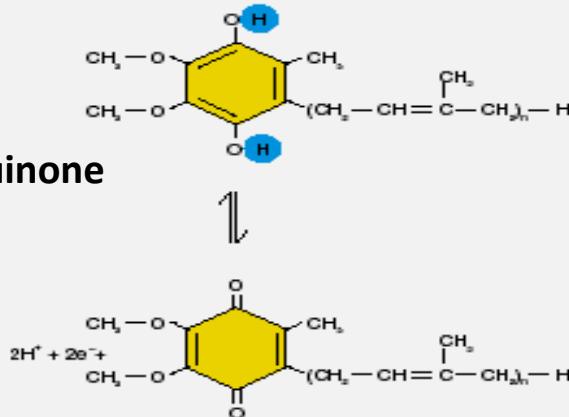


Figure 8.11 The Structure and Function of Coenzyme Q or Ubiquinone. The length of the side chain varies among organisms from  $n = 6$  to  $n = 10$ .

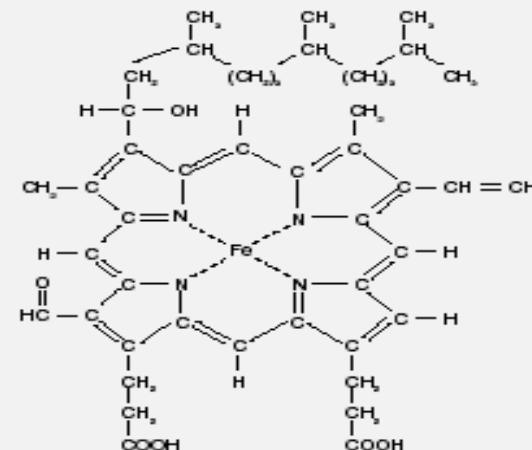
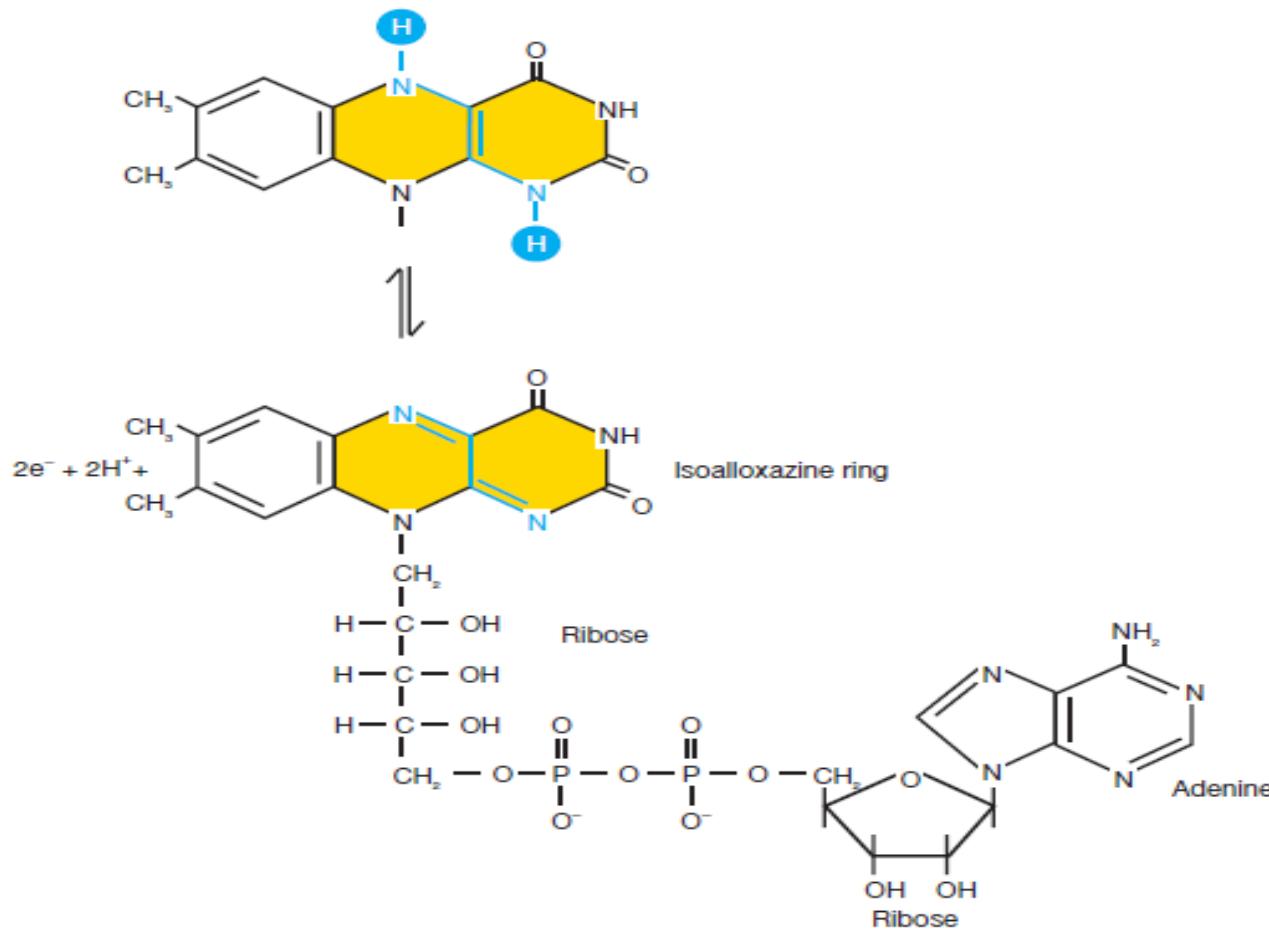


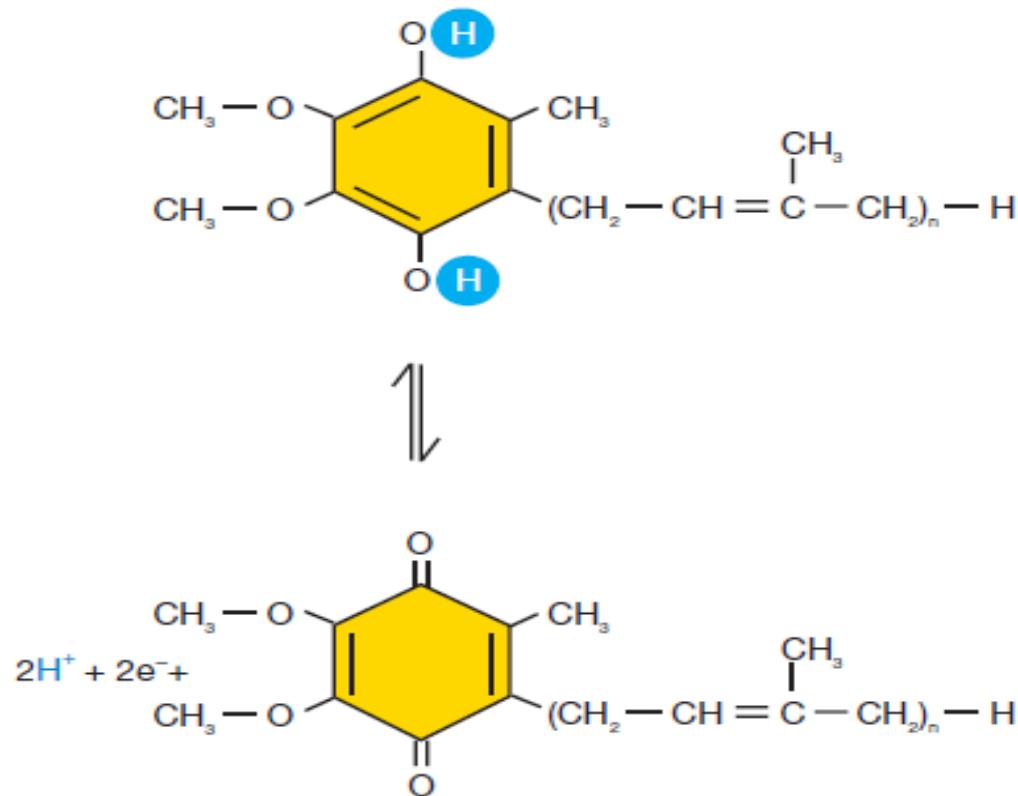
Figure 8.12 The Structure of Heme. Heme is composed of a porphyrin ring and an attached iron atom. It is the nonprotein component of many cytochromes. The iron atom alternatively accepts and releases an electron.

## METABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS



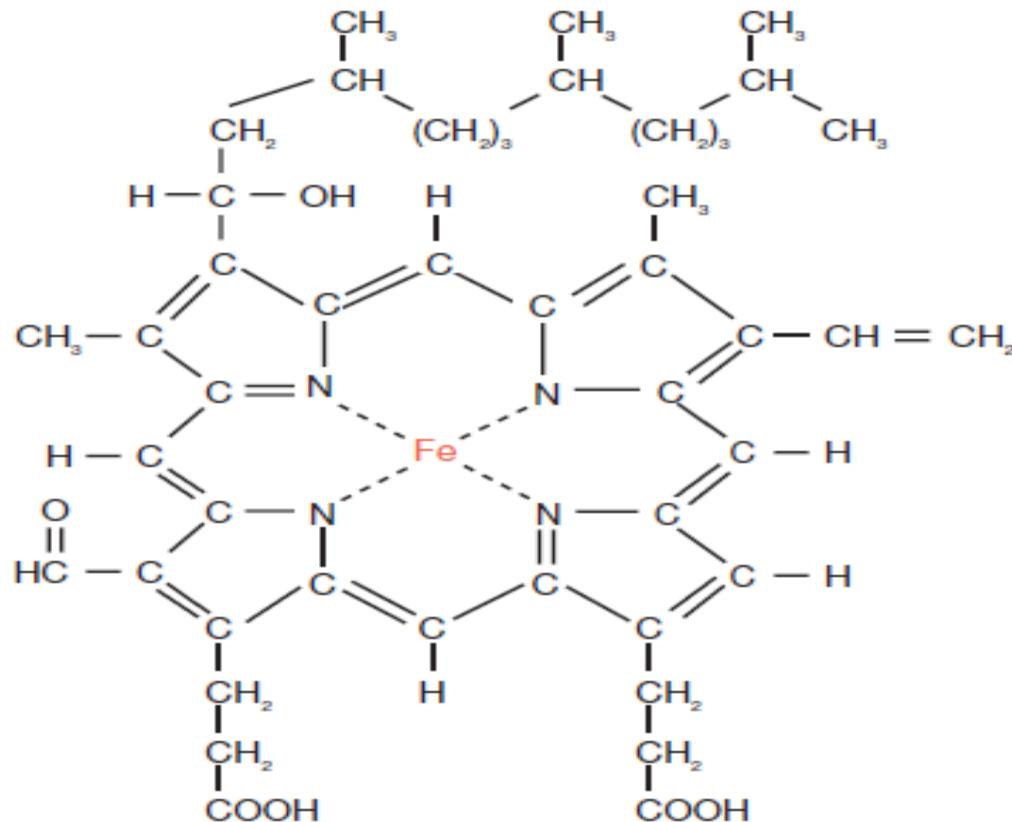
**Figure 8.11 The Structure and Function of FAD.** The vitamin riboflavin is composed of the isoalloxazine ring and its attached ribose sugar. FMN is riboflavin phosphate. The portion of the ring directly involved in oxidation-reduction reactions is in color.

## METABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS



**Figure 8.12 The Structure and Function of Coenzyme Q or Ubiquinone.** The length of the side chain varies among organisms from  $n = 6$  to  $n = 10$ .

## METABOLISME: TRANSPORTEURS D'ELECTRONS ET DE PROTONS



**Figure 8.13 The Structure of Heme.** Heme is composed of a porphyrin ring and an attached iron atom. It is the nonprotein component of many cytochromes. The iron atom alternatively accepts and releases an electron.

## METABOLISME

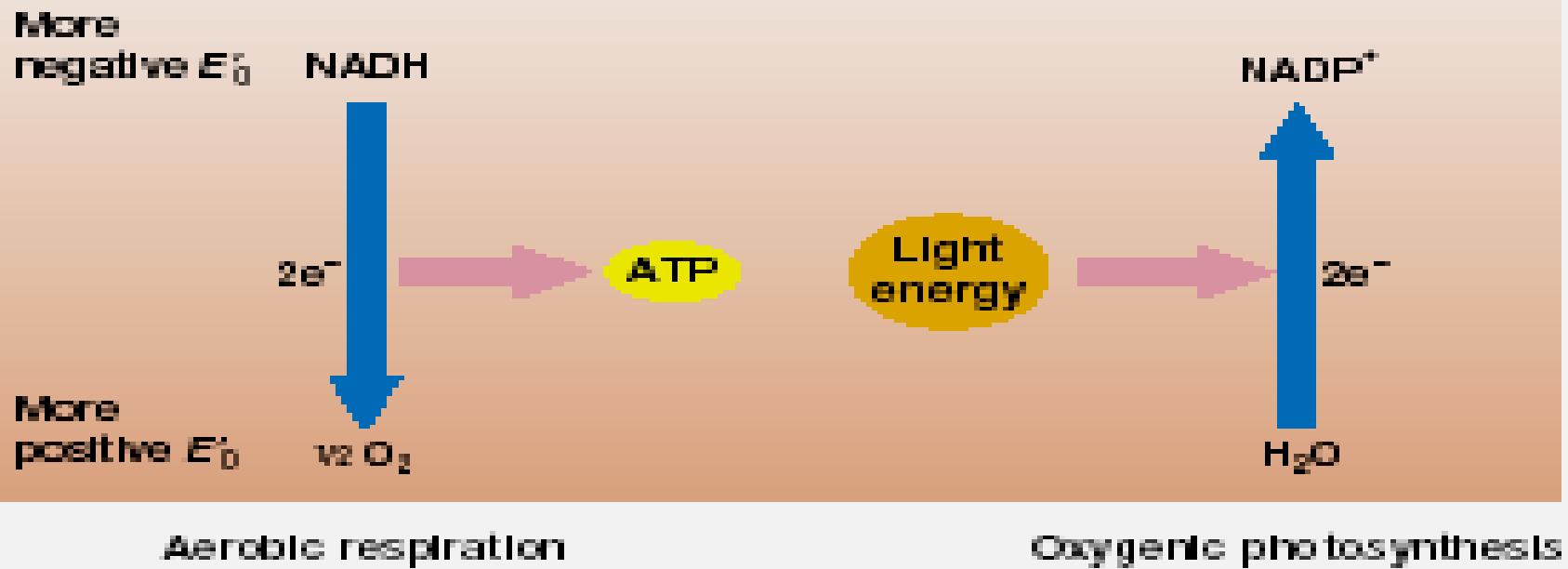
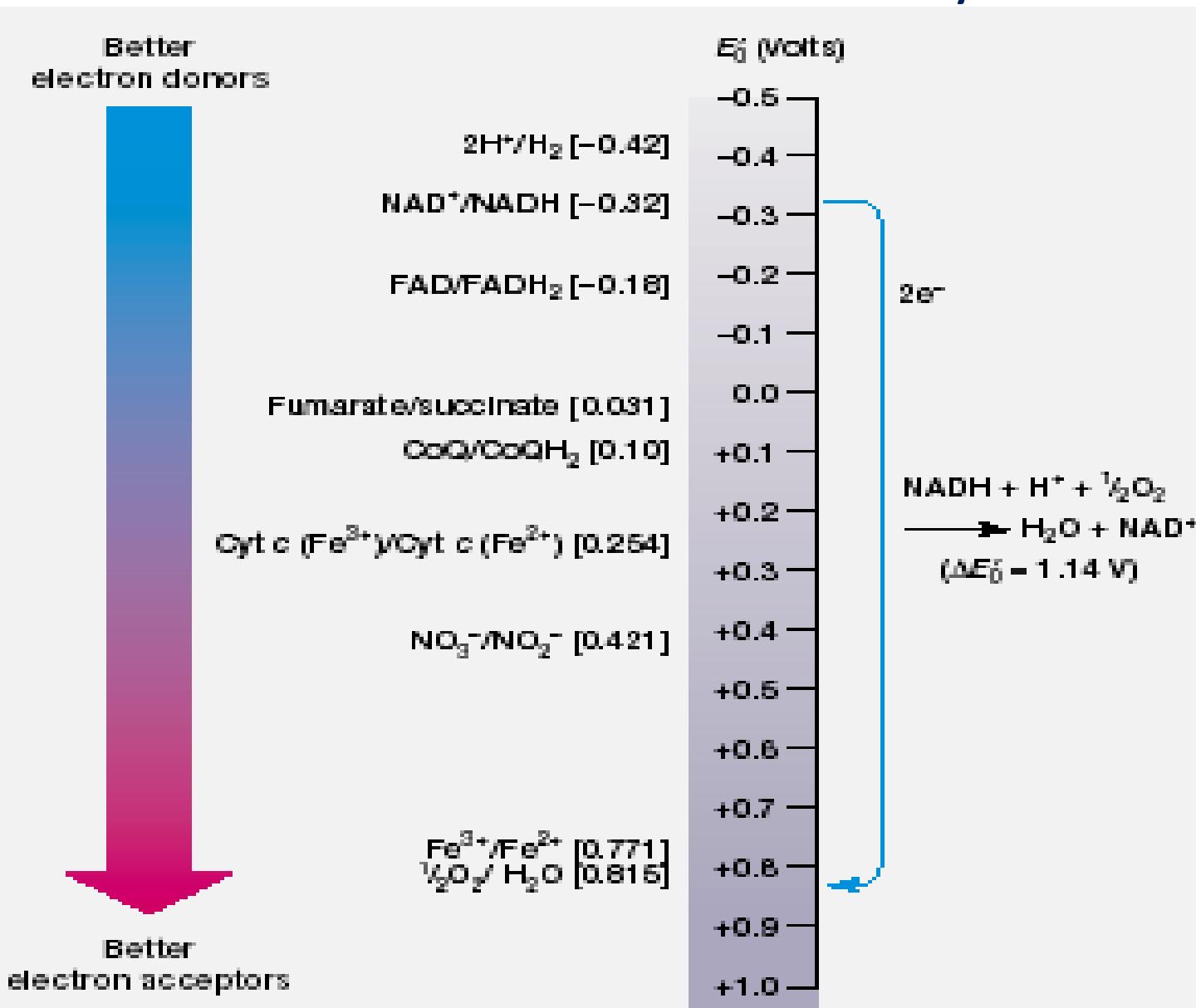
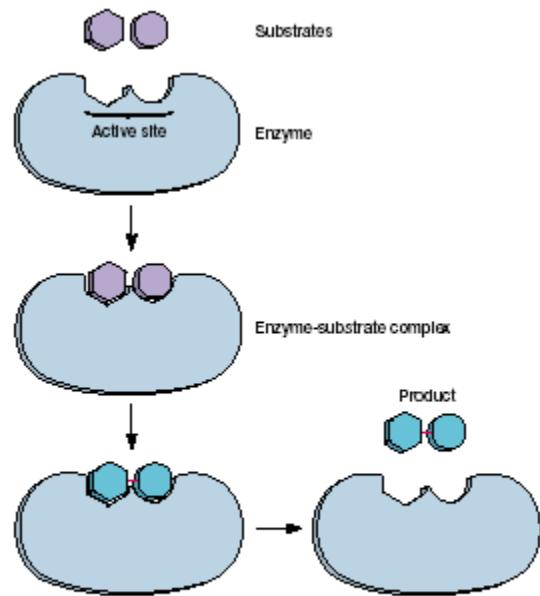


Figure 8.8 Energy Flow in Metabolism. Examples of the relationship between electron flow and energy in metabolism. Oxygen and NADP<sup>+</sup> serve as electron acceptors for NADH and water, respectively.

Le transfert d'électrons s'effectue vers l'agent oxydant avec le potentiel d'oxydo-réduction ( $E_0$  le plus élevé)





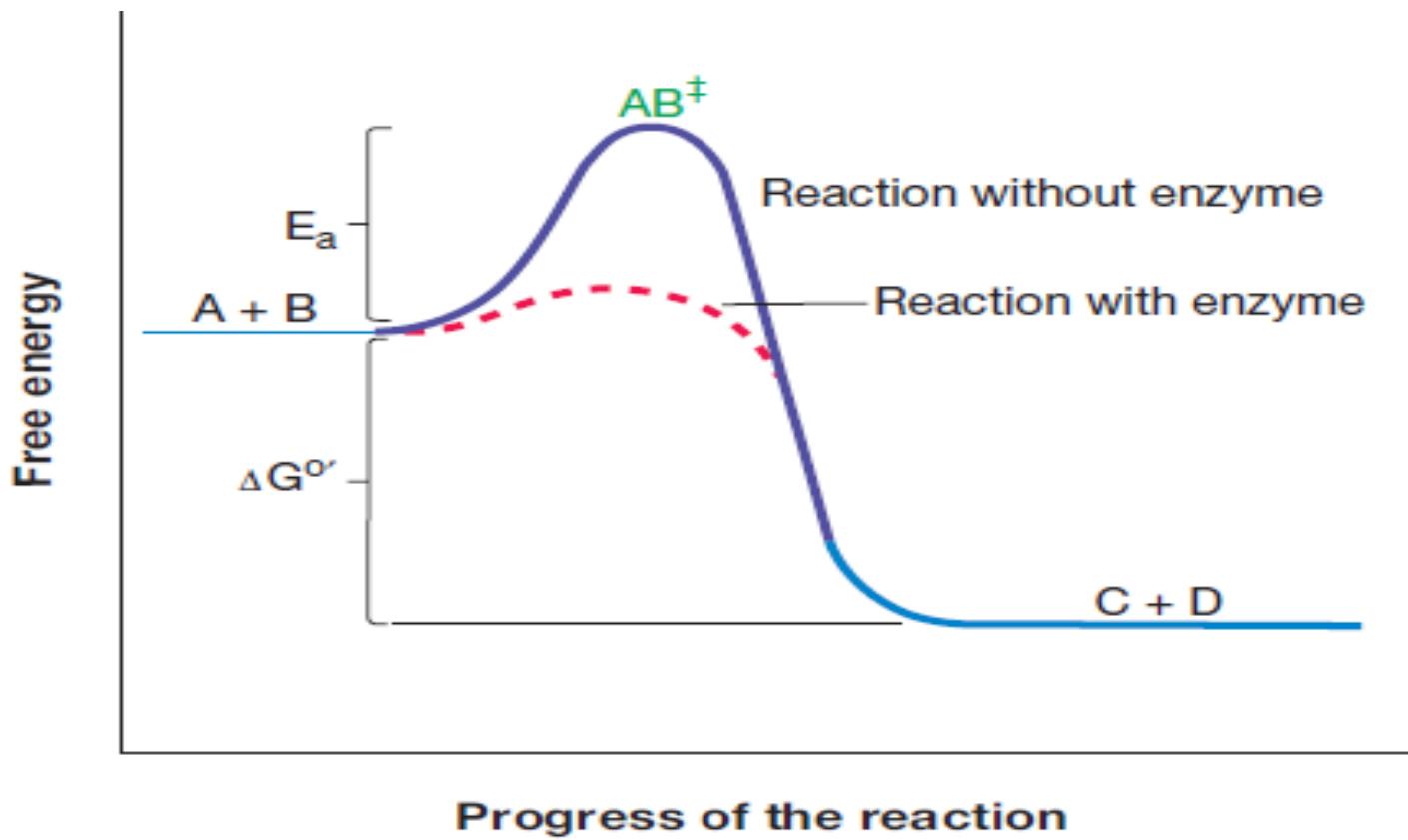
lactate dehydrogenase (LDH) removes hydrogens from lactate.

Figure 8.15 Enzyme Function. The formation of the enzyme-substrate complex and its conversion to products is shown.

Table 8.2

## Enzyme Classification

Type of Enzyme	Reaction Catalyzed by Enzyme	Example of Reaction
Oxidoreductase	Oxidation-reduction reactions	Lactate dehydrogenase: Pyruvate + NADH + H $\rightleftharpoons$ lactate + NAD <sup>+</sup>
Transferase	Reactions involving the transfer of groups between molecules	Aspartate carbamoyltransferase: Aspartate + carbamoylphosphate $\rightleftharpoons$ carbamoylaspartate + phosphate
Hydrolase	Hydrolysis of molecules	Glucose-6-phosphatase: Glucose-6-phosphate + H <sub>2</sub> O $\rightarrow$ glucose + P <sub>i</sub>
Lyase	Removal of groups to form double bonds or addition of groups to double bonds	Fumarate hydratase: L-malate $\rightleftharpoons$ fumarate + H <sub>2</sub> O
	$  \begin{array}{c}  & & x & y \\  & &   &   \\  \backslash & / & & \\  c = c + x - y & \rightleftharpoons & - c - c - \\  / & \backslash &   &   \\  \end{array}  $	
Isomerase	Reactions involving isomerizations	Alanine racemase: L-alanine $\rightleftharpoons$ D-alanine
Ligase	Joining of two molecules using ATP energy (or that of other nucleoside triphosphates)	Glutamine synthetase: Glutamate + NH <sub>3</sub> + ATP $\rightarrow$ glutamine + ADP + P <sub>i</sub>



**Figure 8.15 Enzymes Lower the Energy of Activation.**

This figure traces the course of a chemical reaction in which A and B are converted to C and D. The transition-state complex is represented by  $AB^\ddagger$ , and the activation energy required to reach it, by  $E_a$ . The red line represents the course of the reaction in the presence of an enzyme. Note that the activation energy is much lower in the enzyme-catalyzed reaction.

$$\Delta G = \Delta H - T \cdot \Delta S$$

**$\Delta G$**  Variation de l'énergie libre de réaction (qté d'énergie pour réaction à T et P ctes)

**$\Delta H$**  Variation de l'enthalpie

**T** Degrés Kelvin

**$\Delta S$**  Variation de l'entropie (énergie non exploitée perte)

$$\Delta G = \Delta H - T \cdot \Delta S$$

**$\Delta G$**  Variation de l'énergie libre de réaction (qté d'énergie pour réaction à T et P cstes)

**$\Delta H$**  Variation de l'enthalpie

**T** Degrés Kelvin

**$\Delta S$**  Variation de l'entropie (énergie non exploitée perte)

$$\Delta G^\circ = - 2,303 \text{ RT. Log } K_{\text{eq}}$$

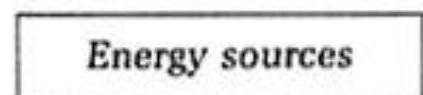
$$\Delta G^\circ = - nF \cdot \Delta E'_0$$

$$\Delta G^\circ = - nF \cdot \Delta E'_0$$

in which  $n$  is the number of electrons transferred and  $F$  is the Faraday constant (23,062 cal/mole-volt or 96,494 J/mole-volt). For every 0.1 volt change in  $\Delta E'_0$ , there is a corresponding 4.6 kcal change in  $\Delta G^\circ$  when a two-electron transfer takes place.

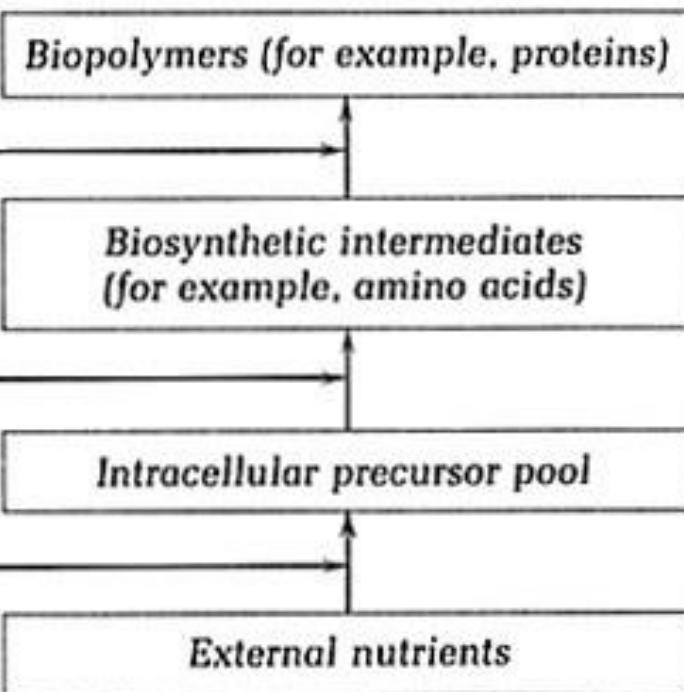
## Catabolism

Energy-yielding metabolism



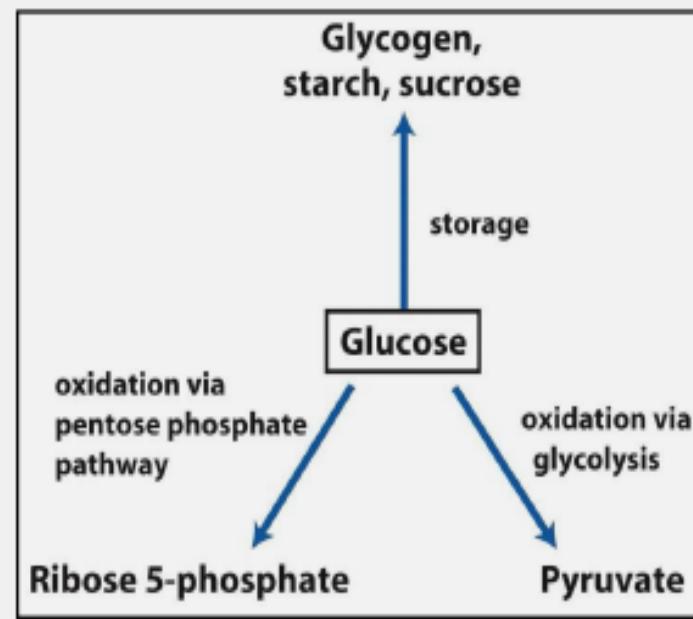
## Anabolism

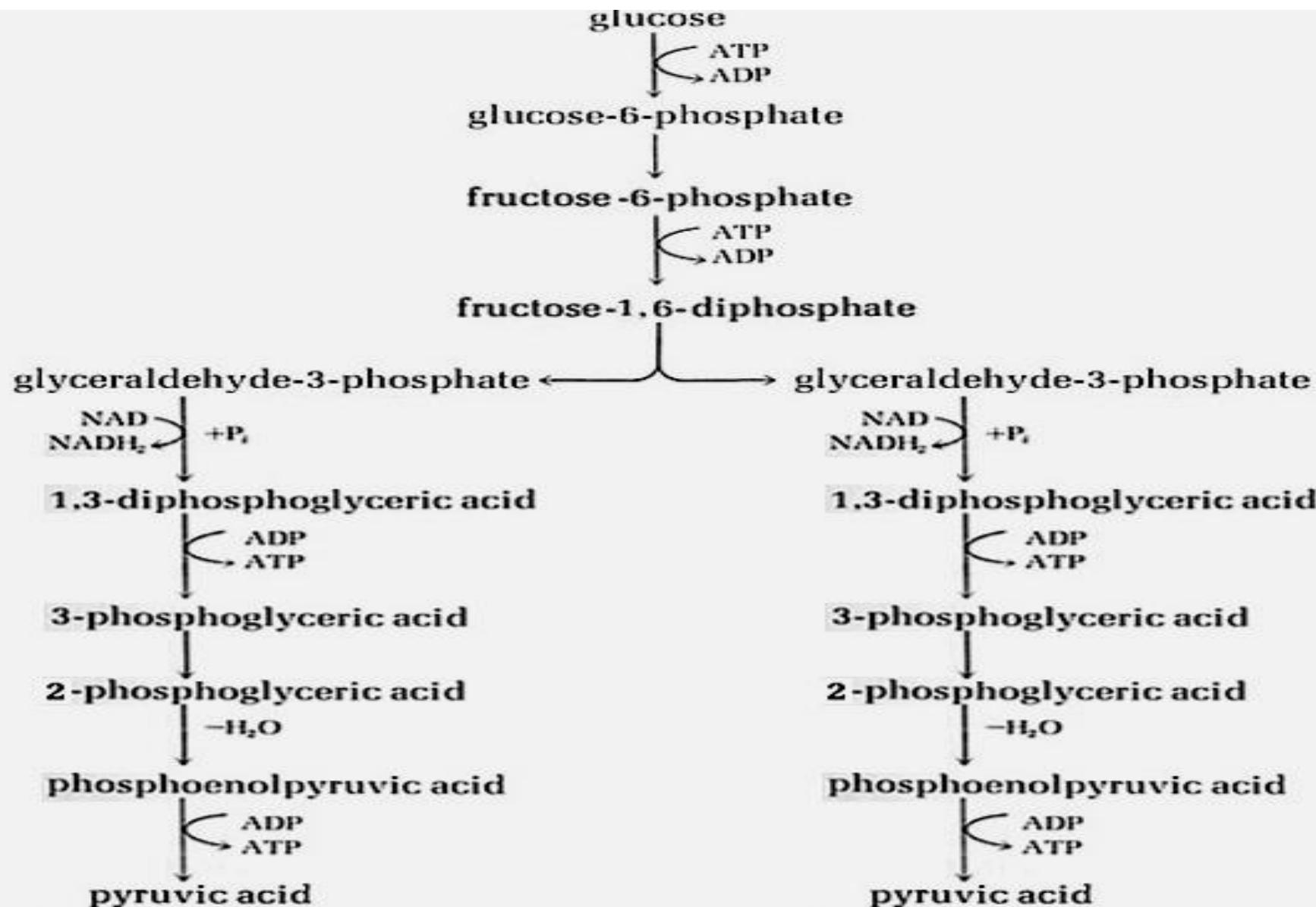
Biosynthetic metabolism



# A central pathway: glycolysis

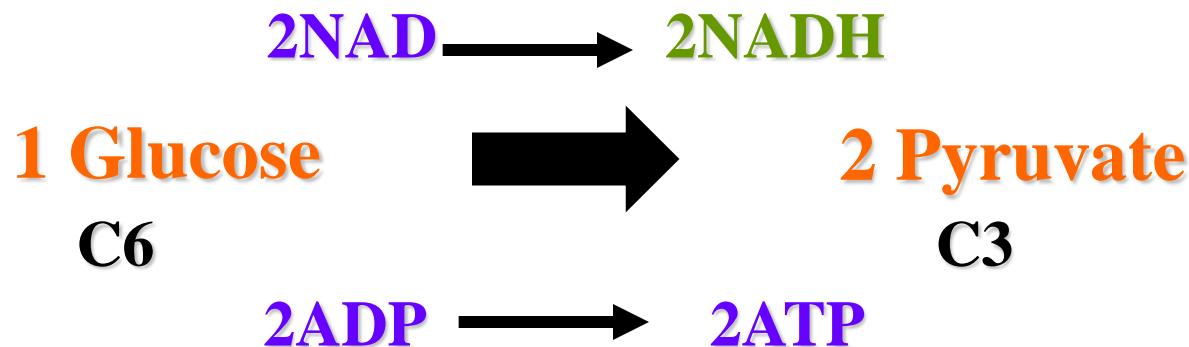
- **Glycolysis** (from the Greek *glykys* meaning “sweet” and *lysis* meaning splitting)
- Glucose is the major fuel for most organism > it's rich in potential energy: its complete oxidation to CO<sub>2</sub> and water has a standard free-energy change of -2840 kJ/mol
- Glucose is stored in big polymers like starch or glycogen
- When energy demand increases, it can be released quickly and used to produce ATP either aerobically (using oxygen) or anaerobically (fermentation)
- Glucose is also a versatile molecule for building the basic carbon skeleton of other molecules like fatty acids, amino acids etc.





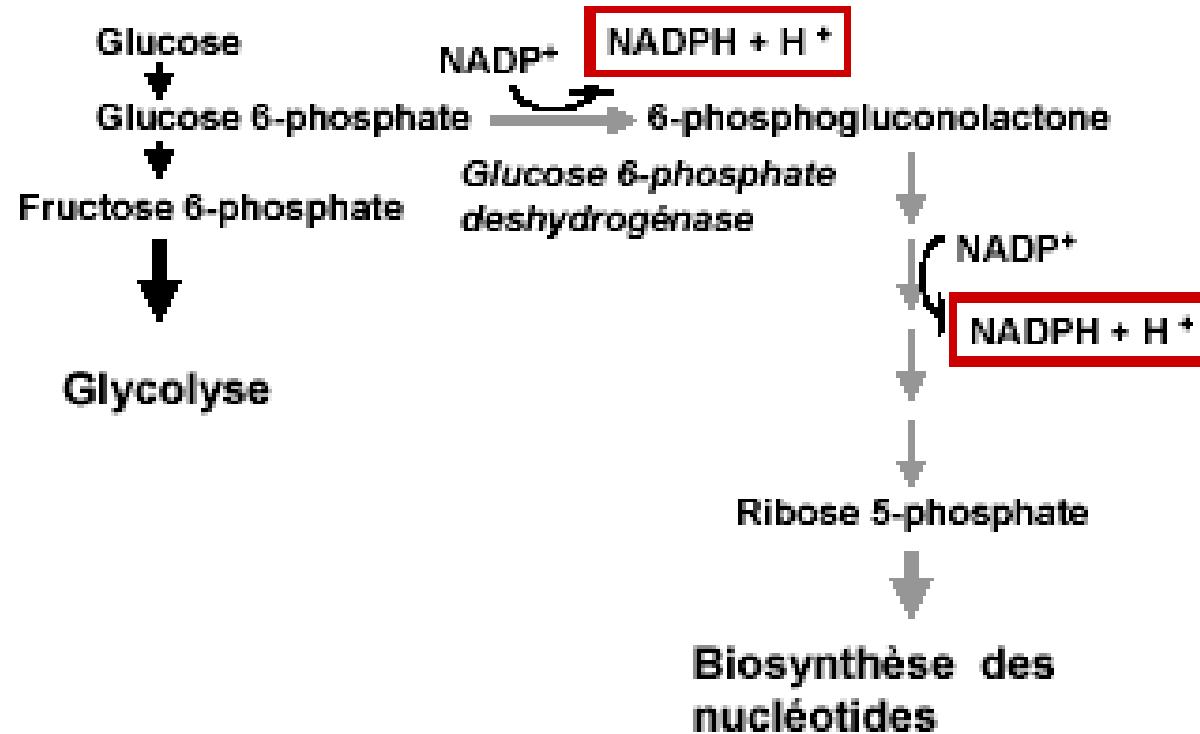
The Embden Meyerhof pathway for glucose dissimilation. The overall reaction is the oxidation of glucose to 2 pyruvic acid. The two branches of the pathway after the cleavage are identical, drawn in this manner for comparison with other bacterial pathways of glycolysis.

# Glycolyse

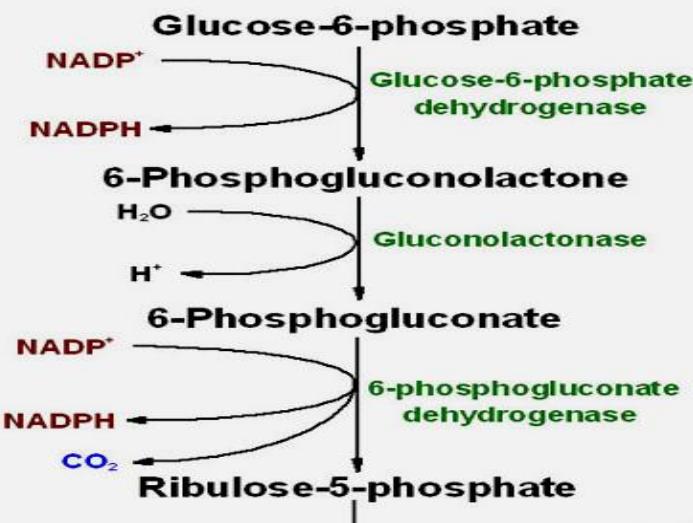


## VOIES METABOLIQUES ESSENTIELLES ET SITES DE CONTRÔLE

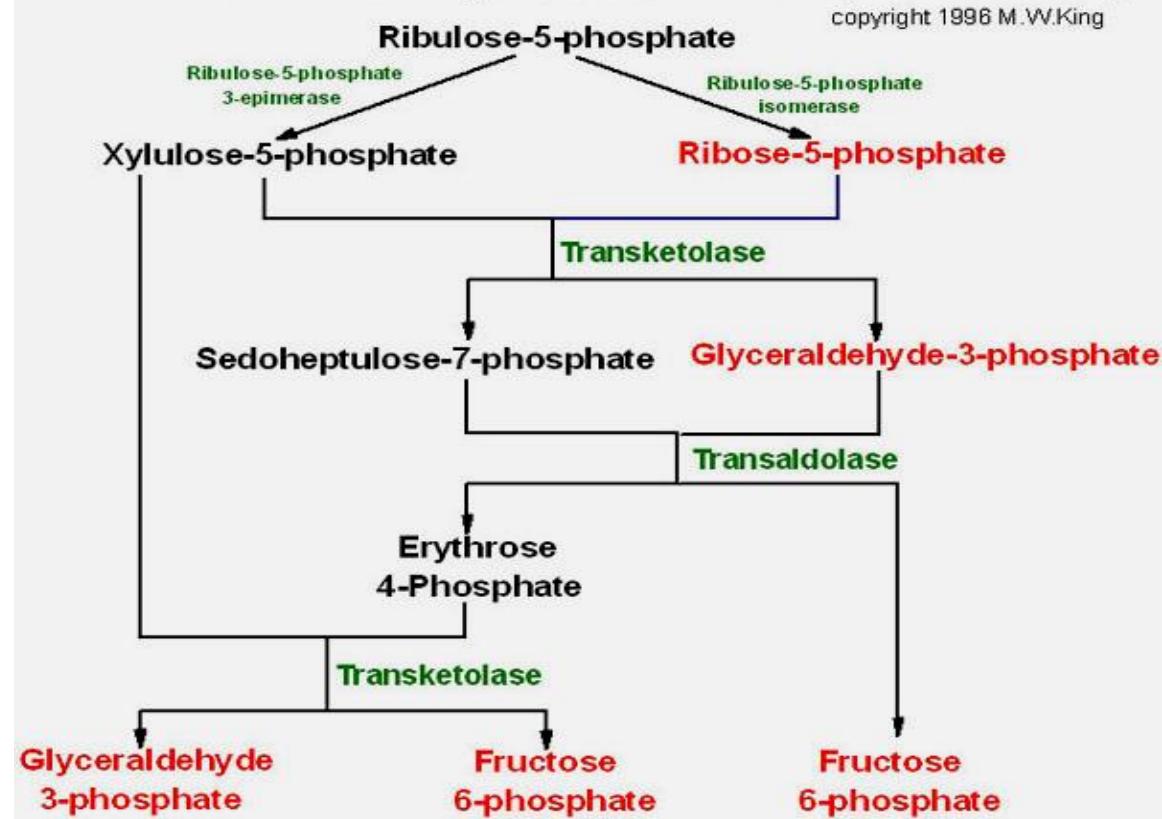
### LA VOIE DES PENTOSES PHOSPHATE



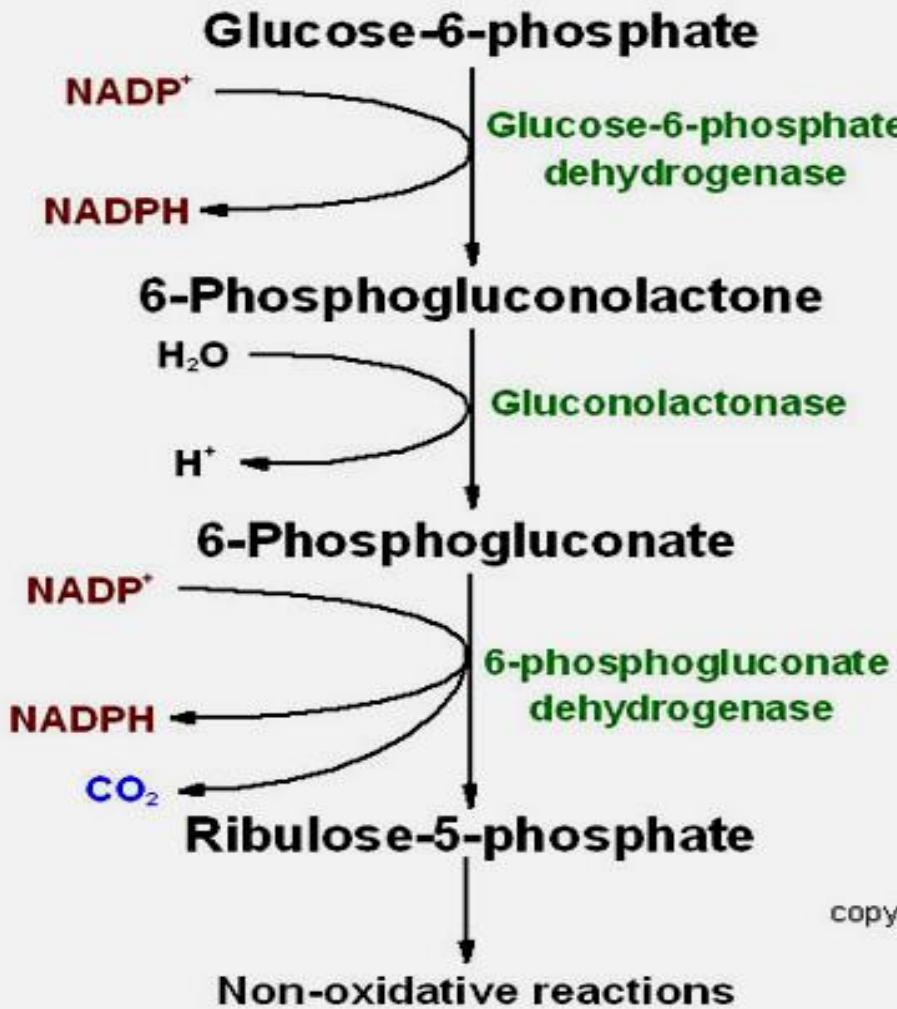
## Oxidative Stage of Pentose Phosphate Pathway



## Non-Oxidative Stage of Pentose Phosphate Pathway



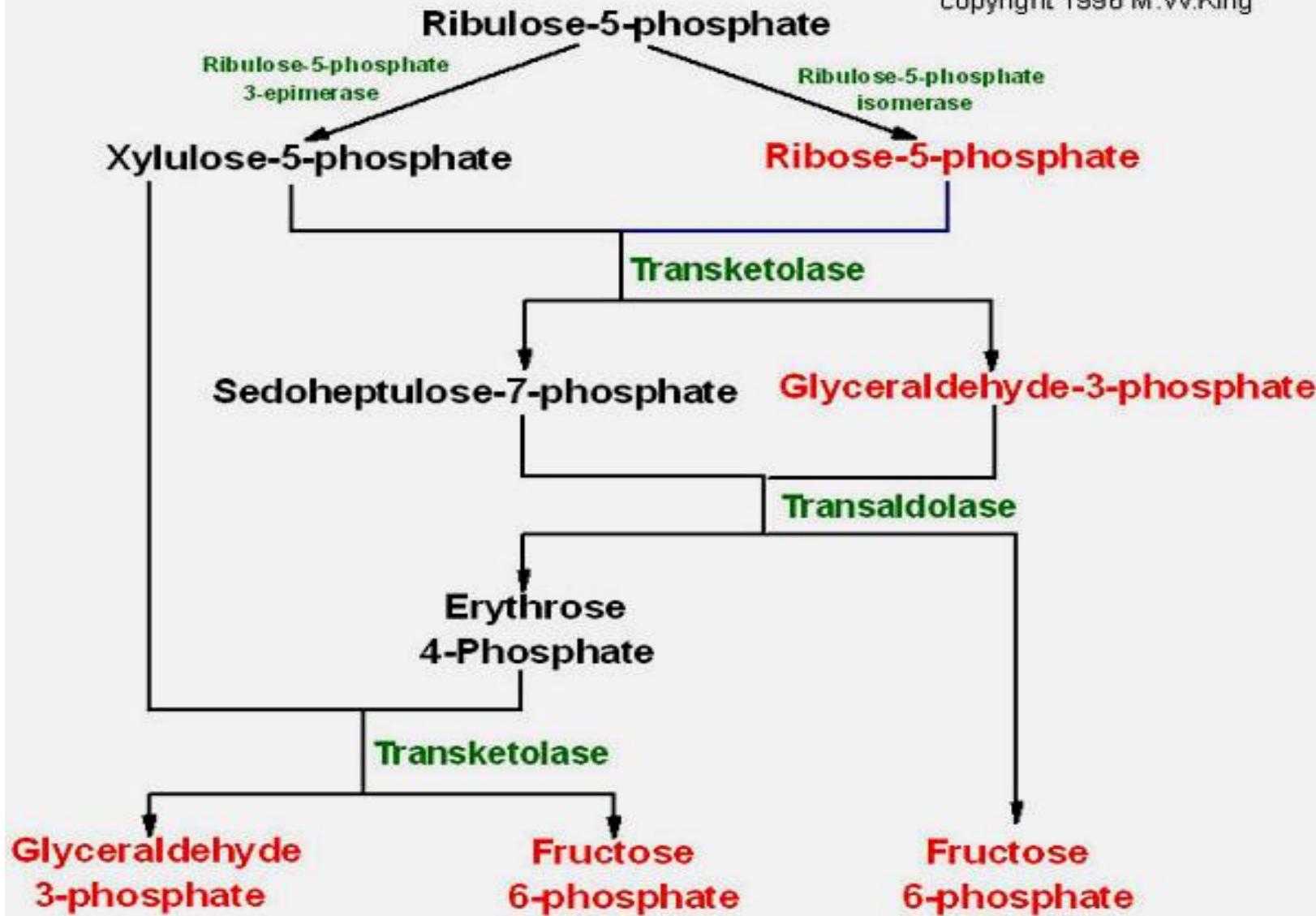
## Oxidative Stage of Pentose Phosphate Pathway

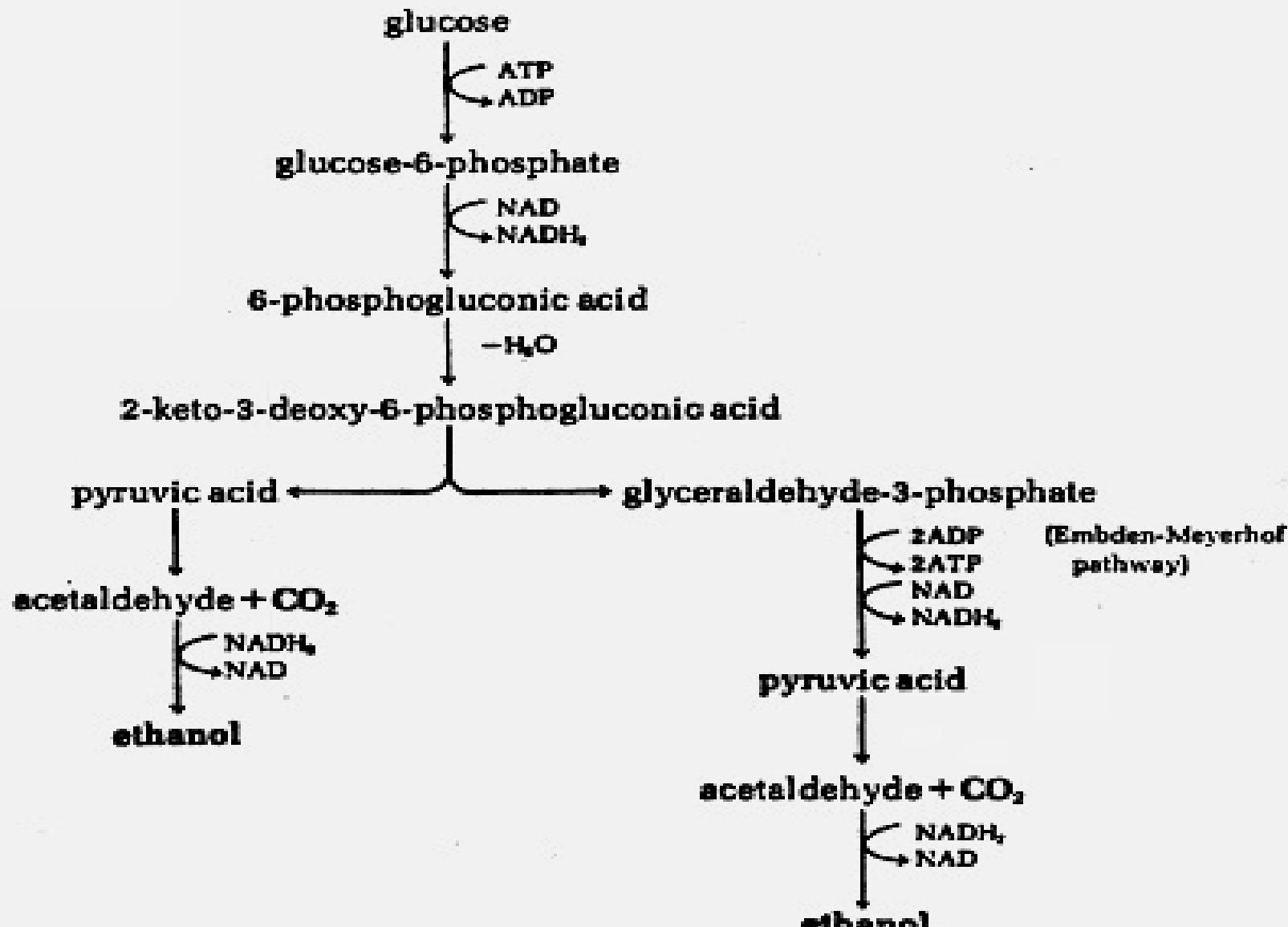


copyright 1996 M.W.King

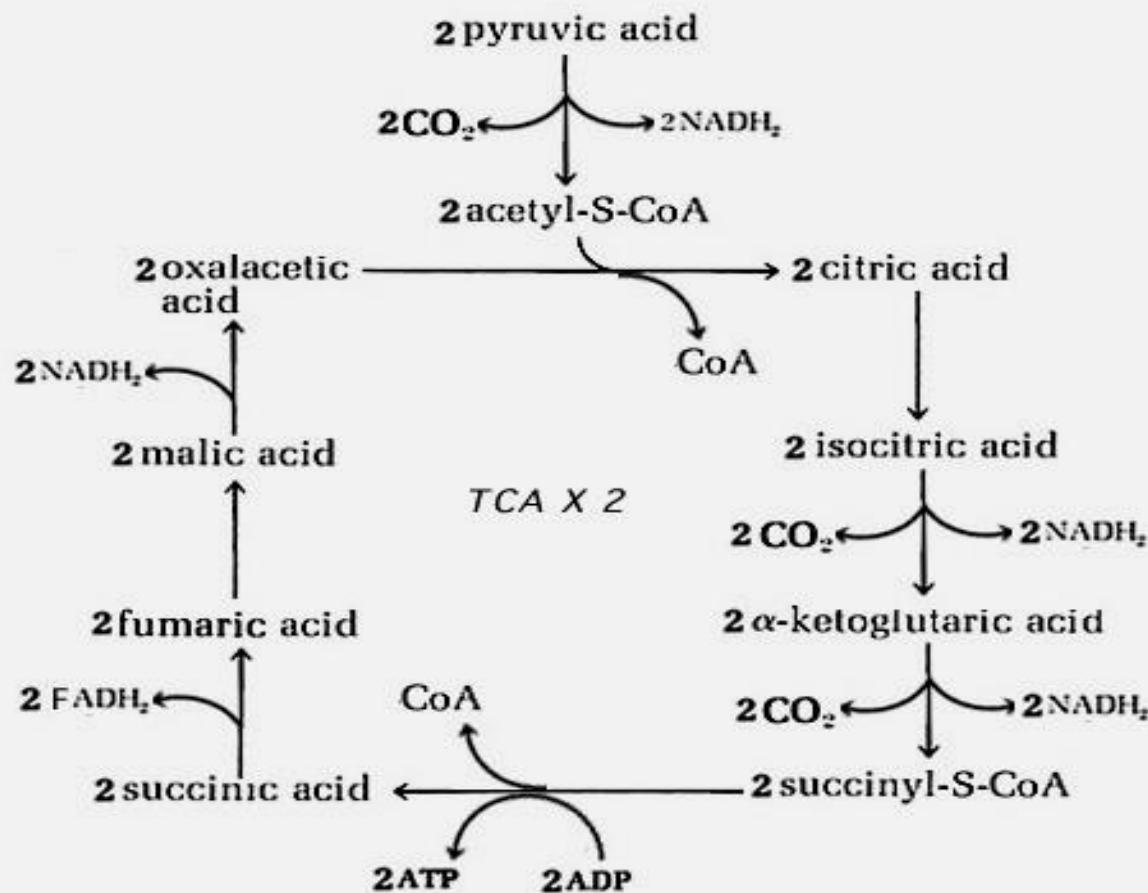
## Non-Oxidative Stage of Pentose Phosphate Pathway

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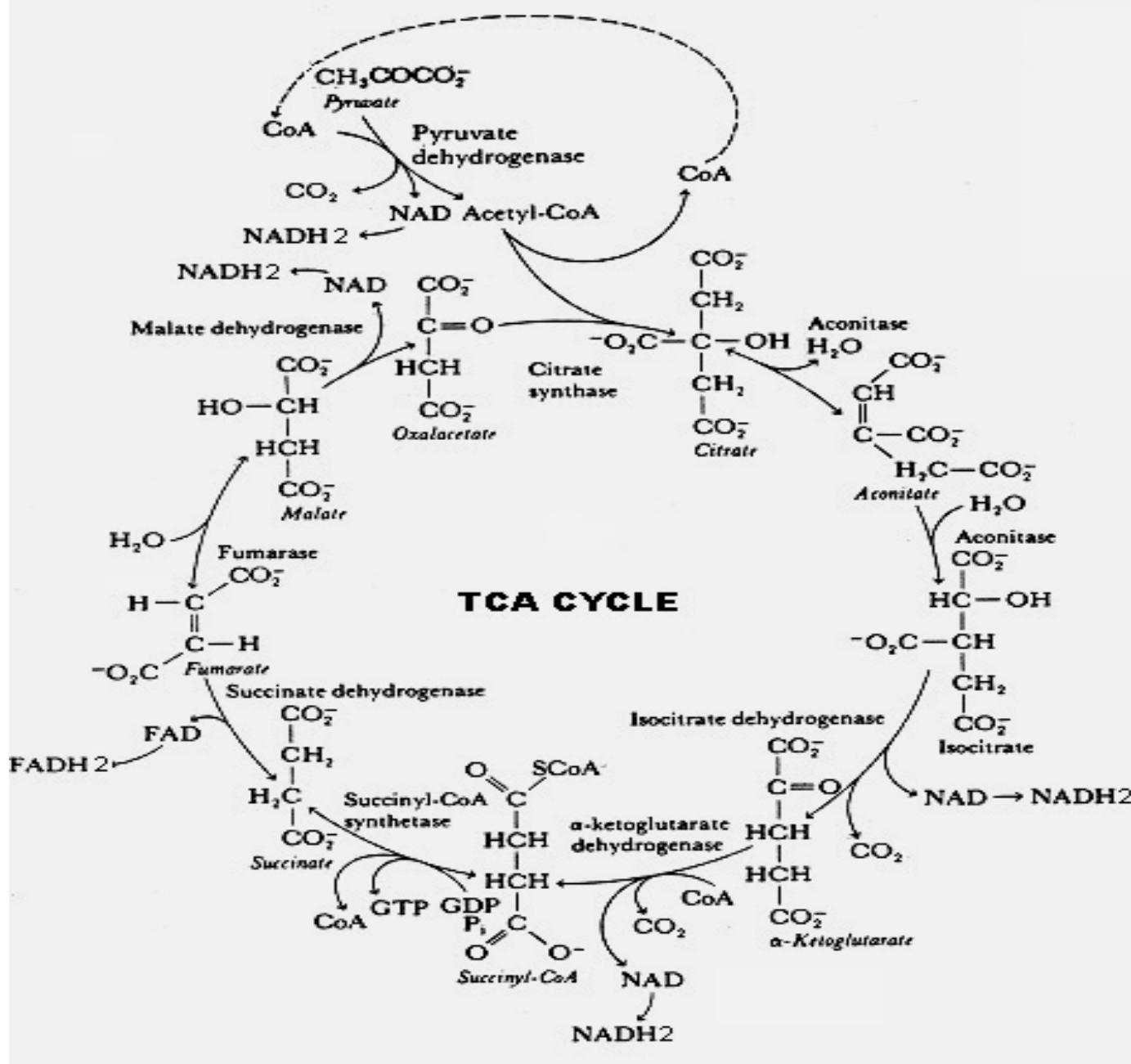




The Entner-Doudoroff Pathway of Fermentation

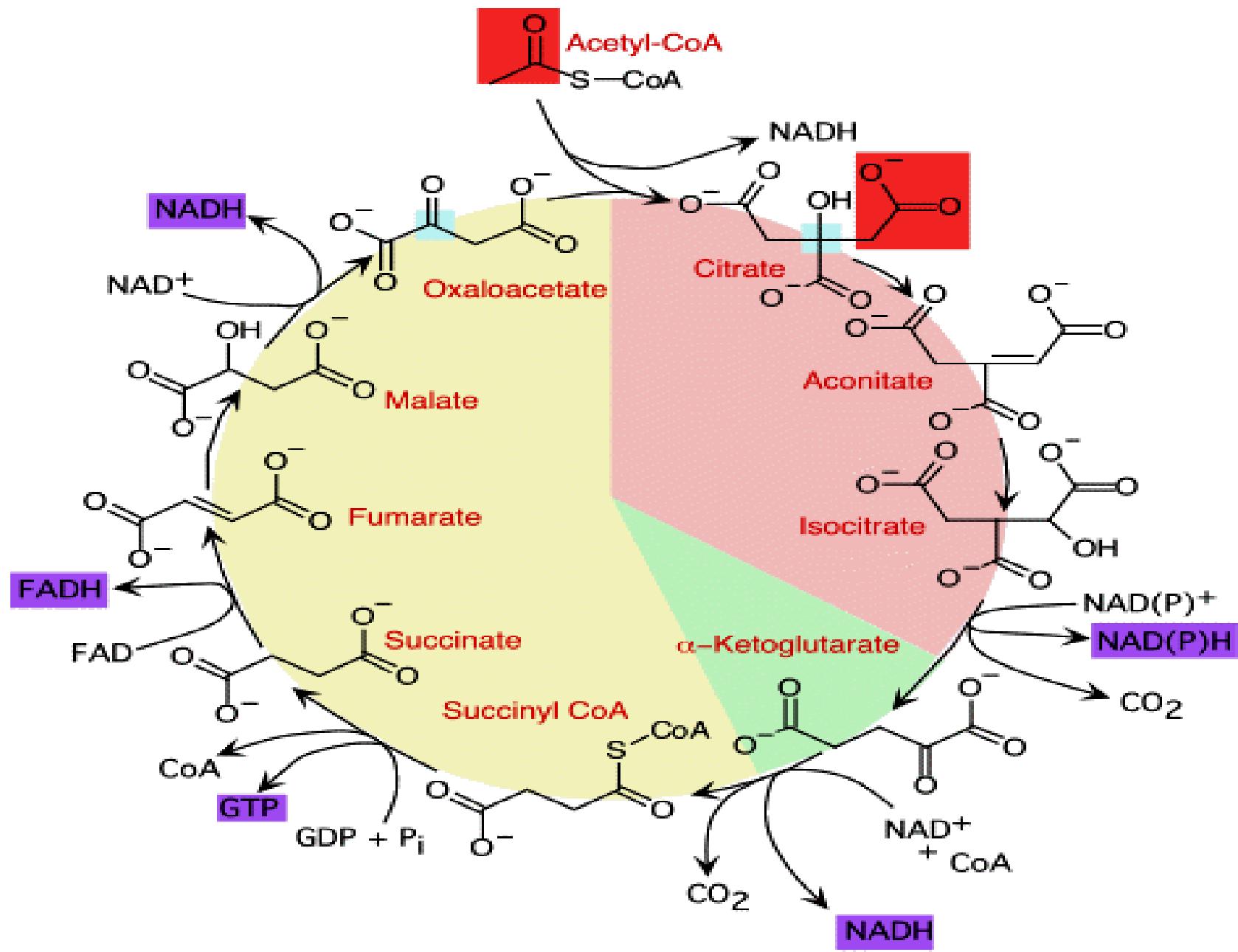


**Glucose + 6 O<sub>2</sub> -----> 6 CO<sub>2</sub> + 6 H<sub>2</sub>O + 38 ATP**

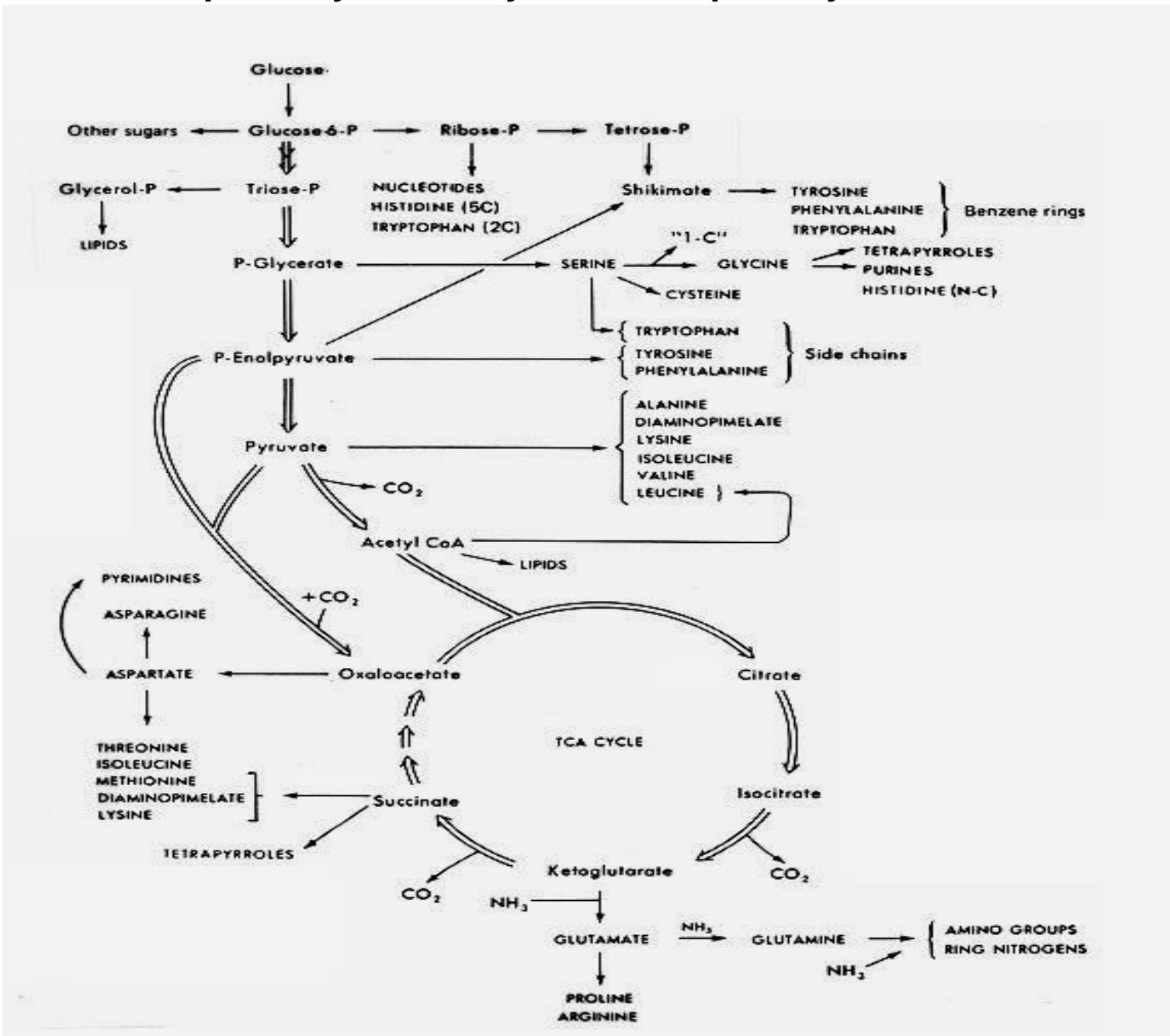


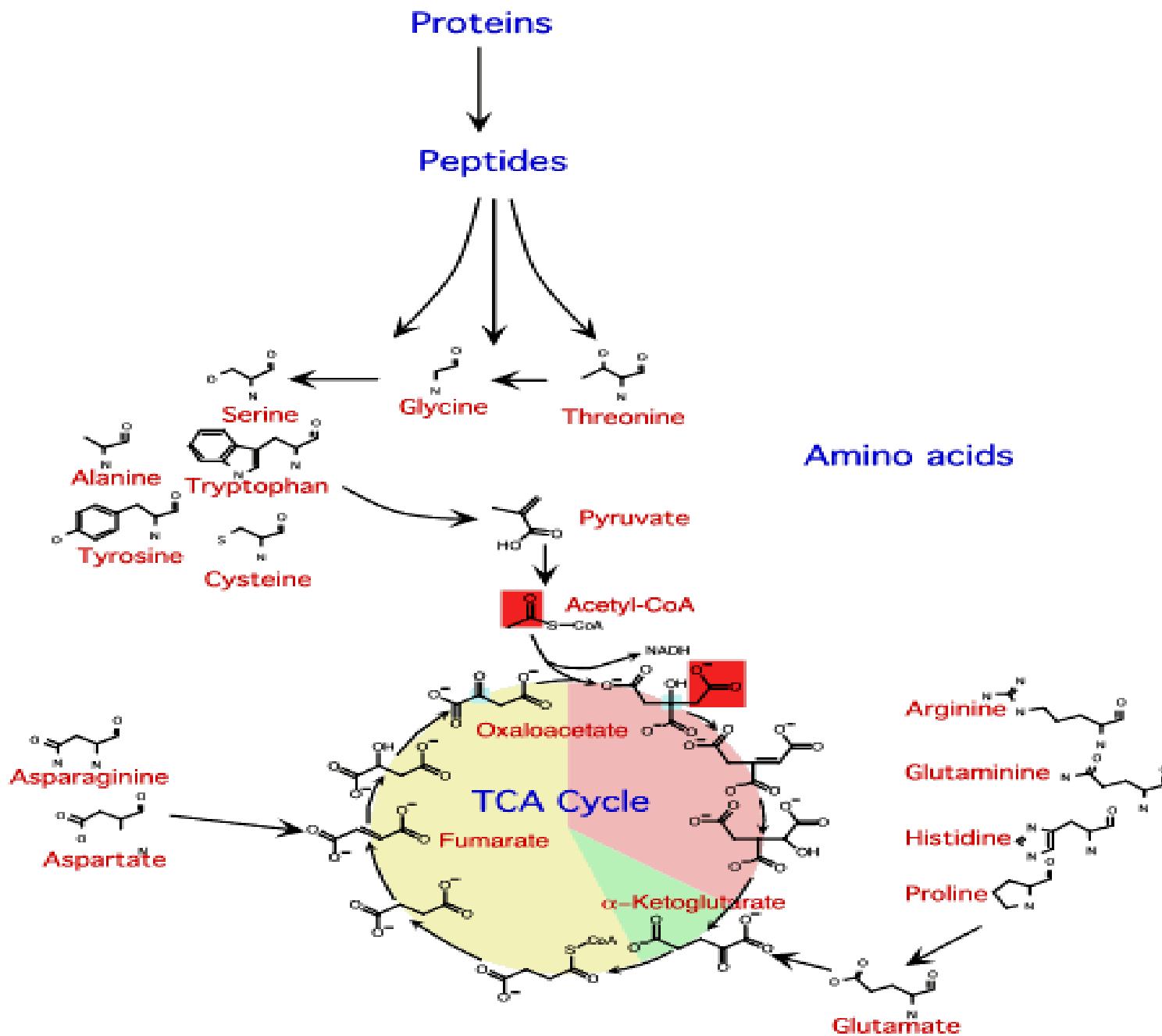
The tricarboxylic acid (TCA) or Kreb's cycle

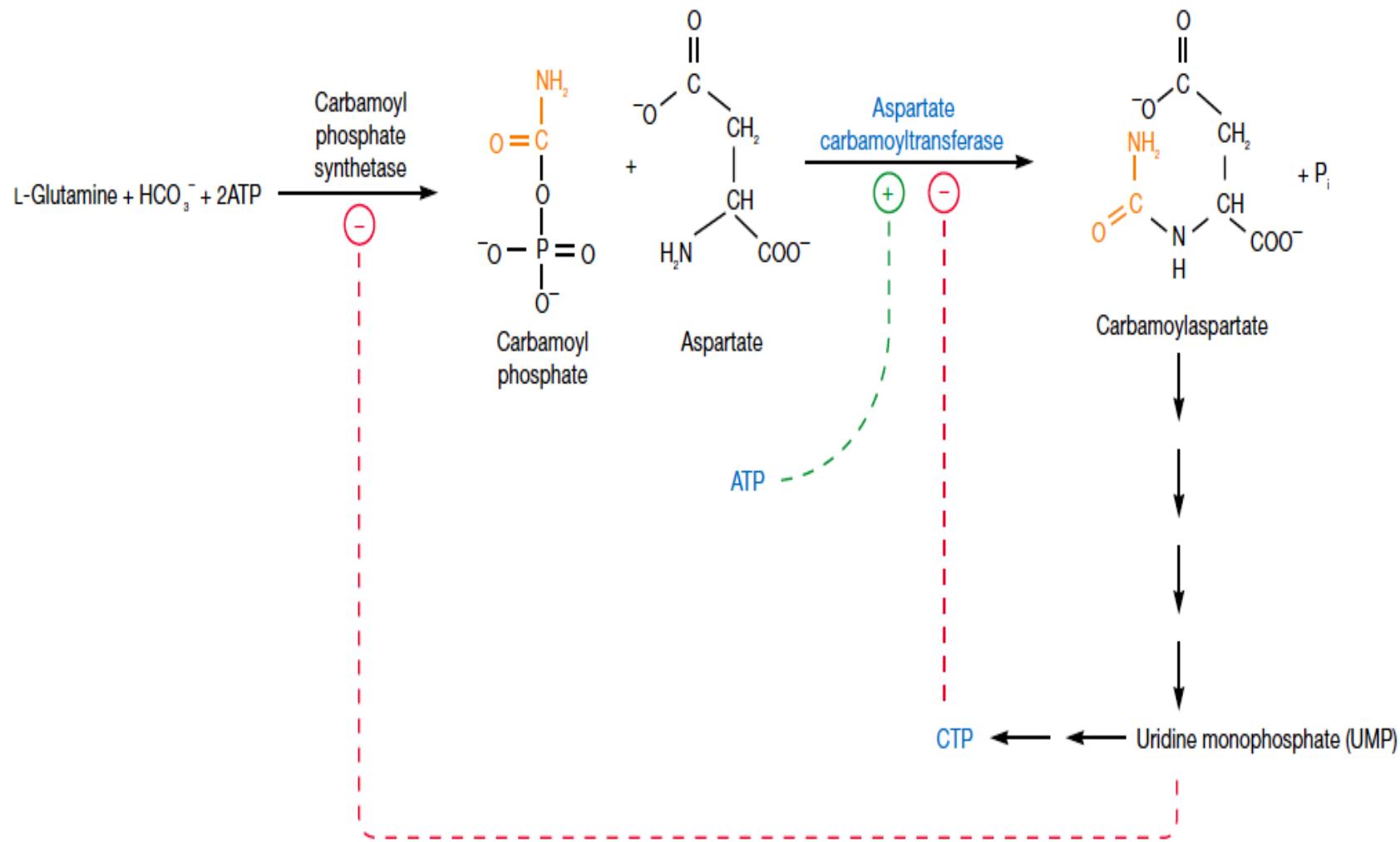
## CYCLE DE KREBS



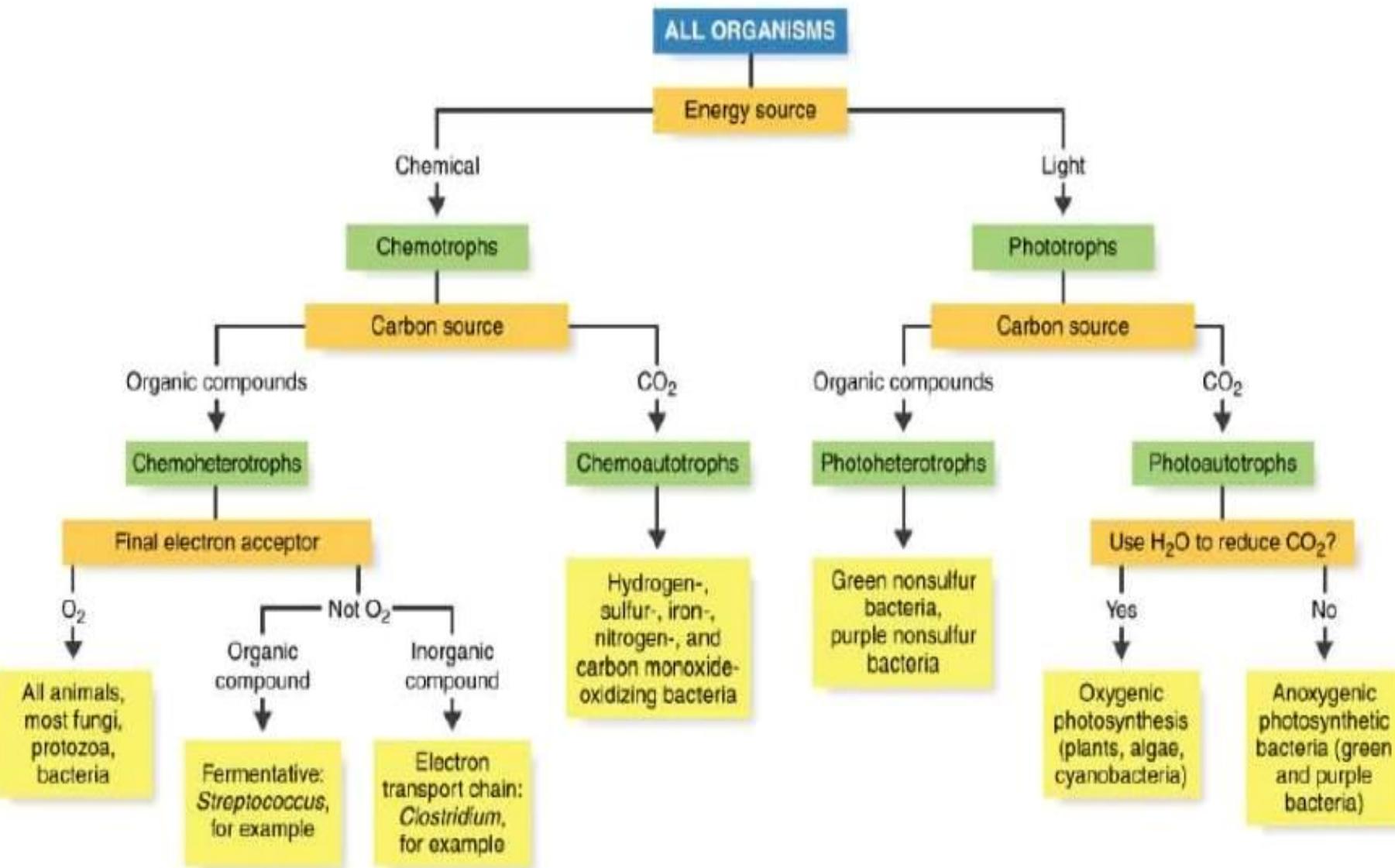
# The main pathways of biosynthesis in prokaryotic cells



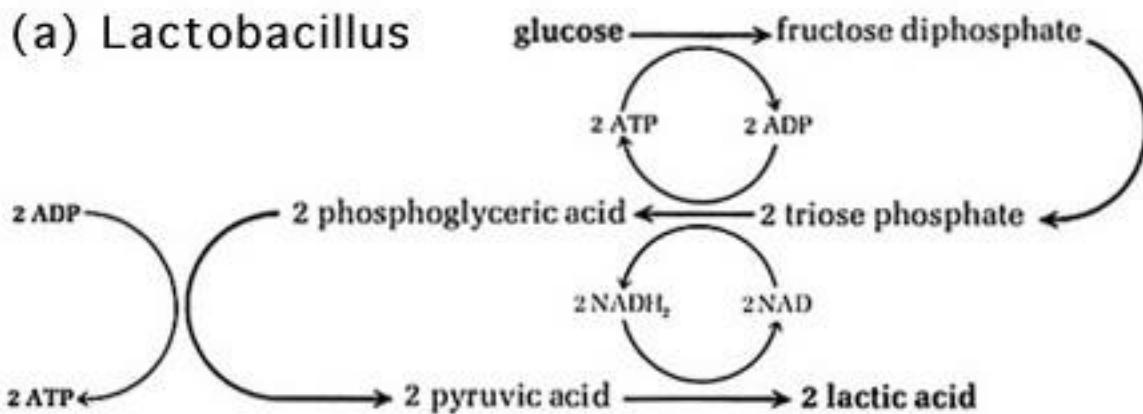




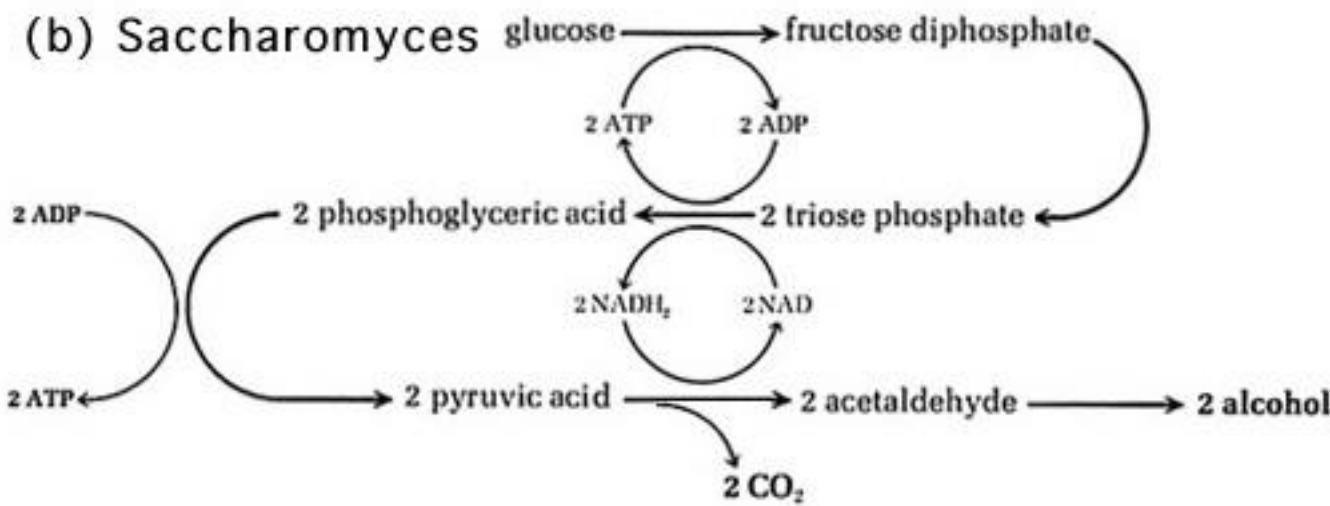
**Figure 8.23 ACTase Regulation.** The aspartate carbamoyltransferase reaction and its role in the regulation of pyrimidine biosynthesis. The end product CTP inhibits its activity (−) while ATP activates the enzyme (+). Carbamoyl phosphate synthetase is also inhibited by pathway end products such as UMP.

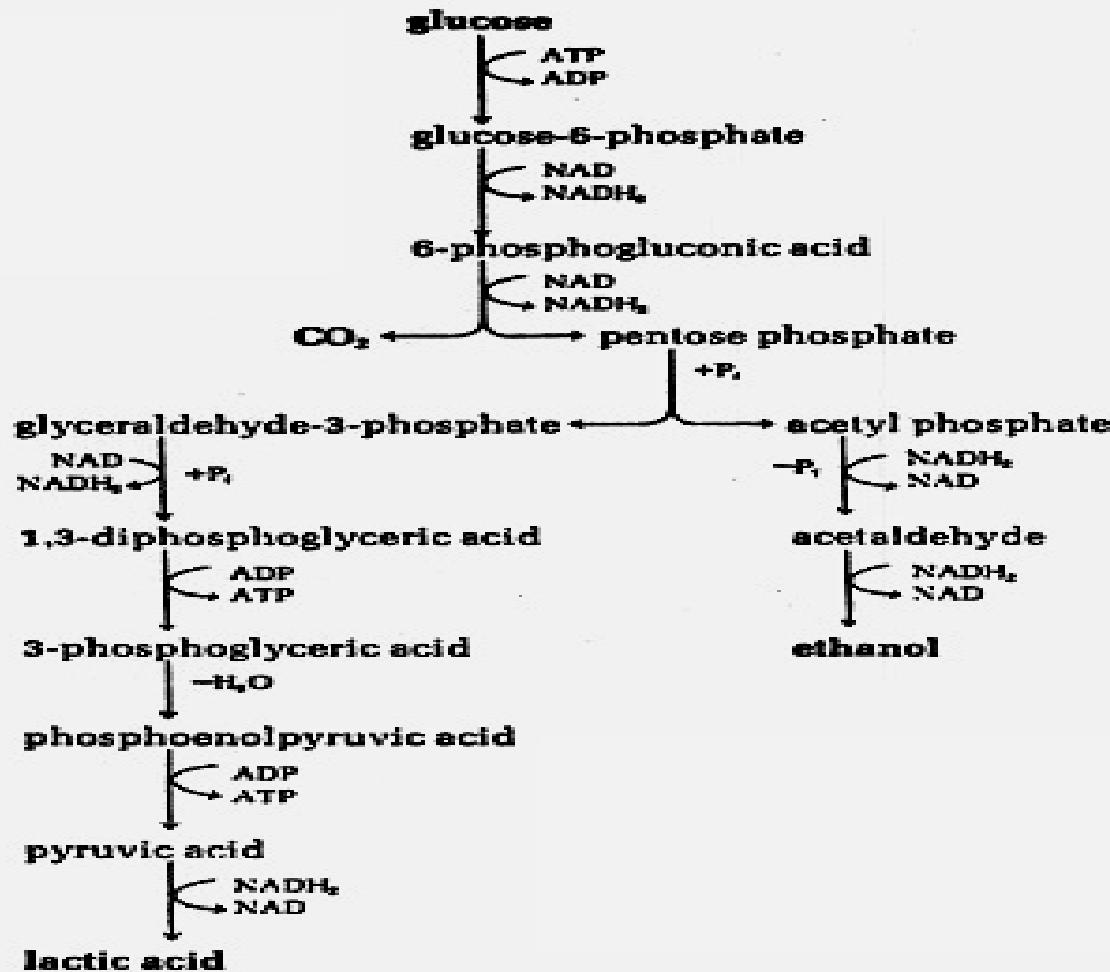


(a) *Lactobacillus*



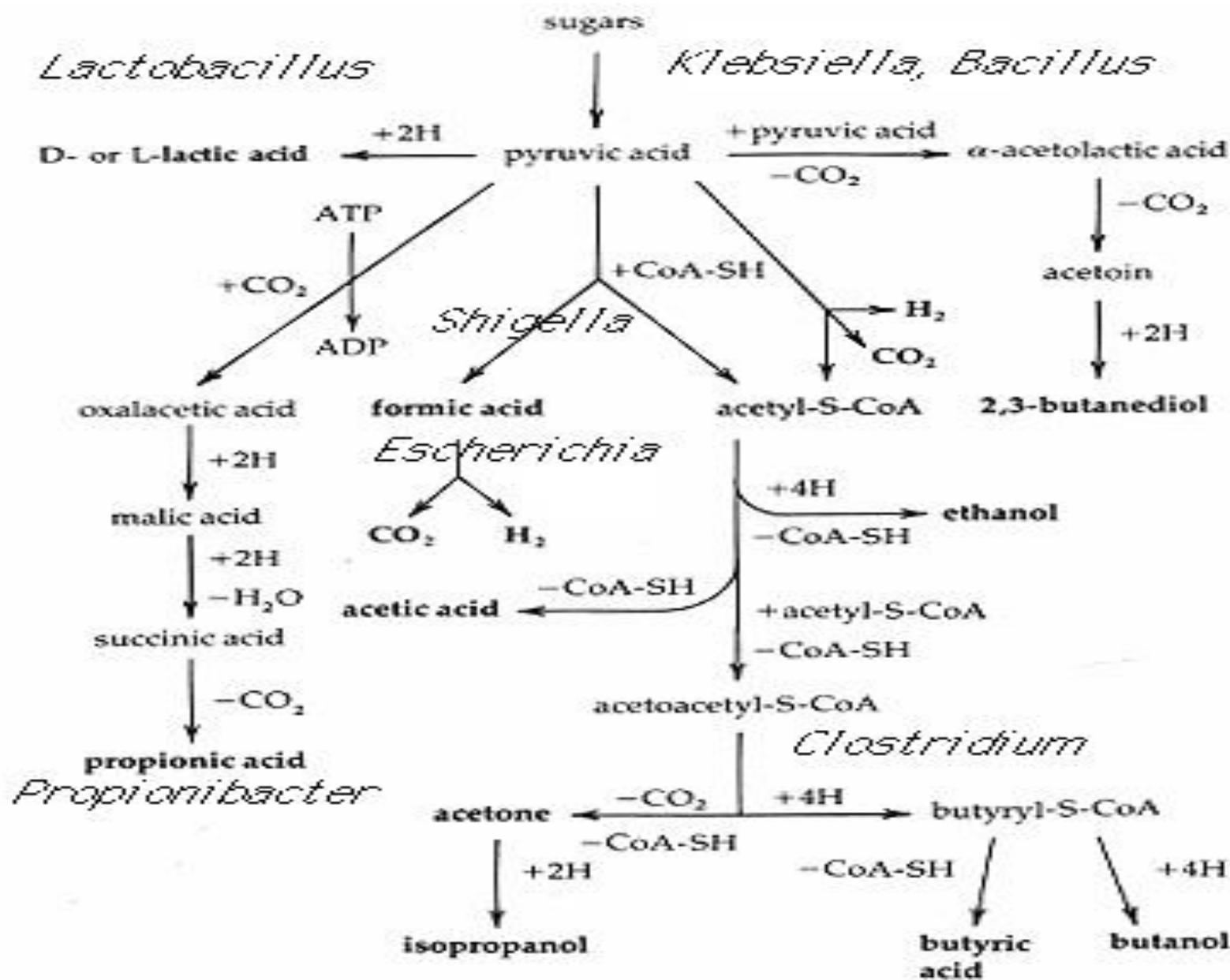
(b) *Saccharomyces*



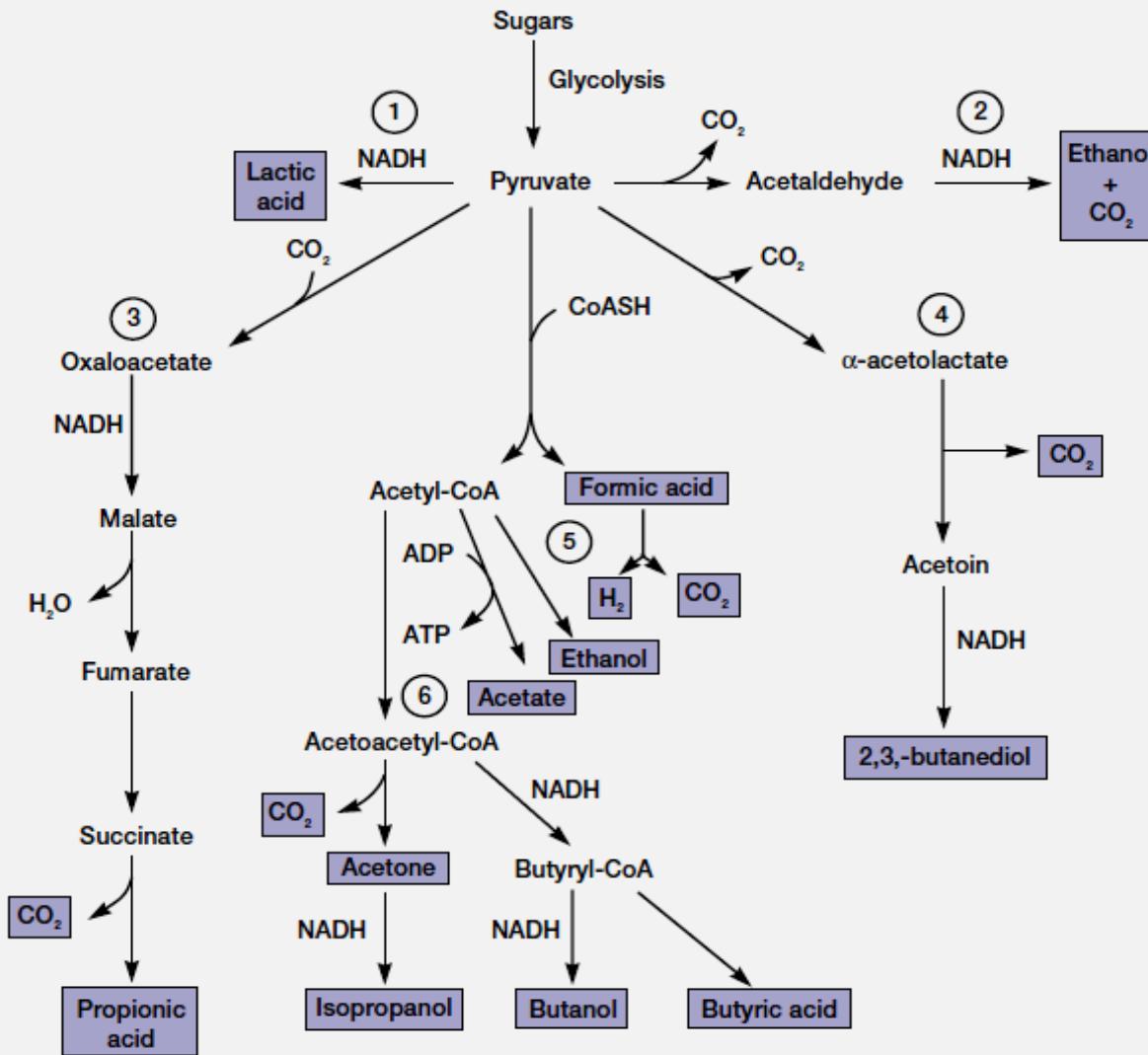


The heterolactic (phosphoketolase) pathway of fermentation

# Fermentations in bacteria that proceed through the Embden-Meyerhof pathway

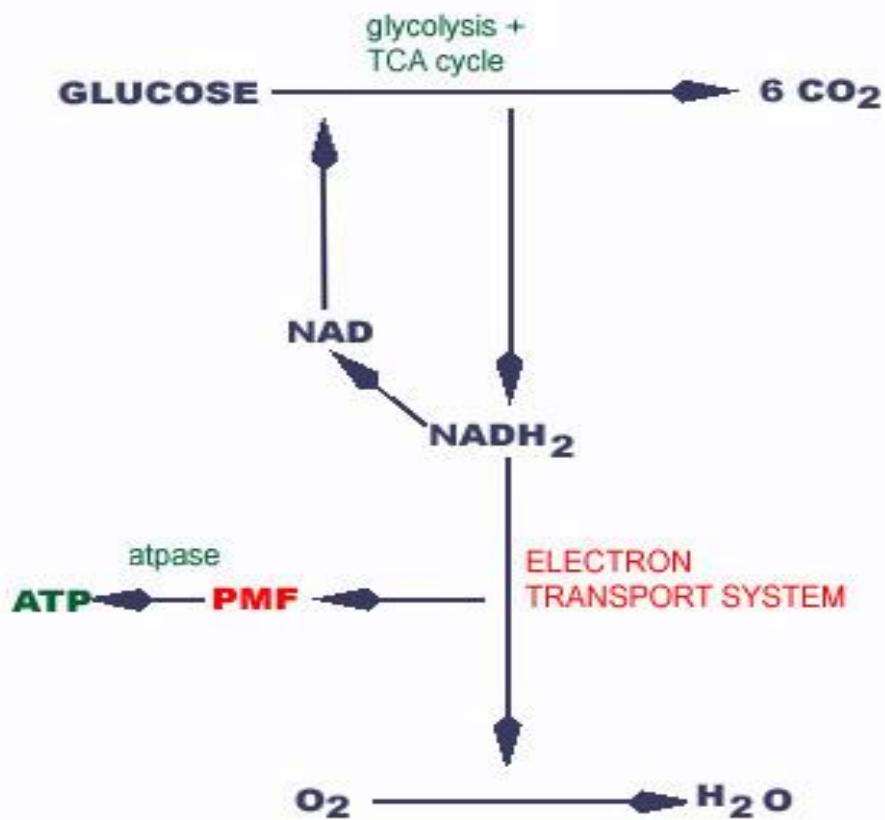


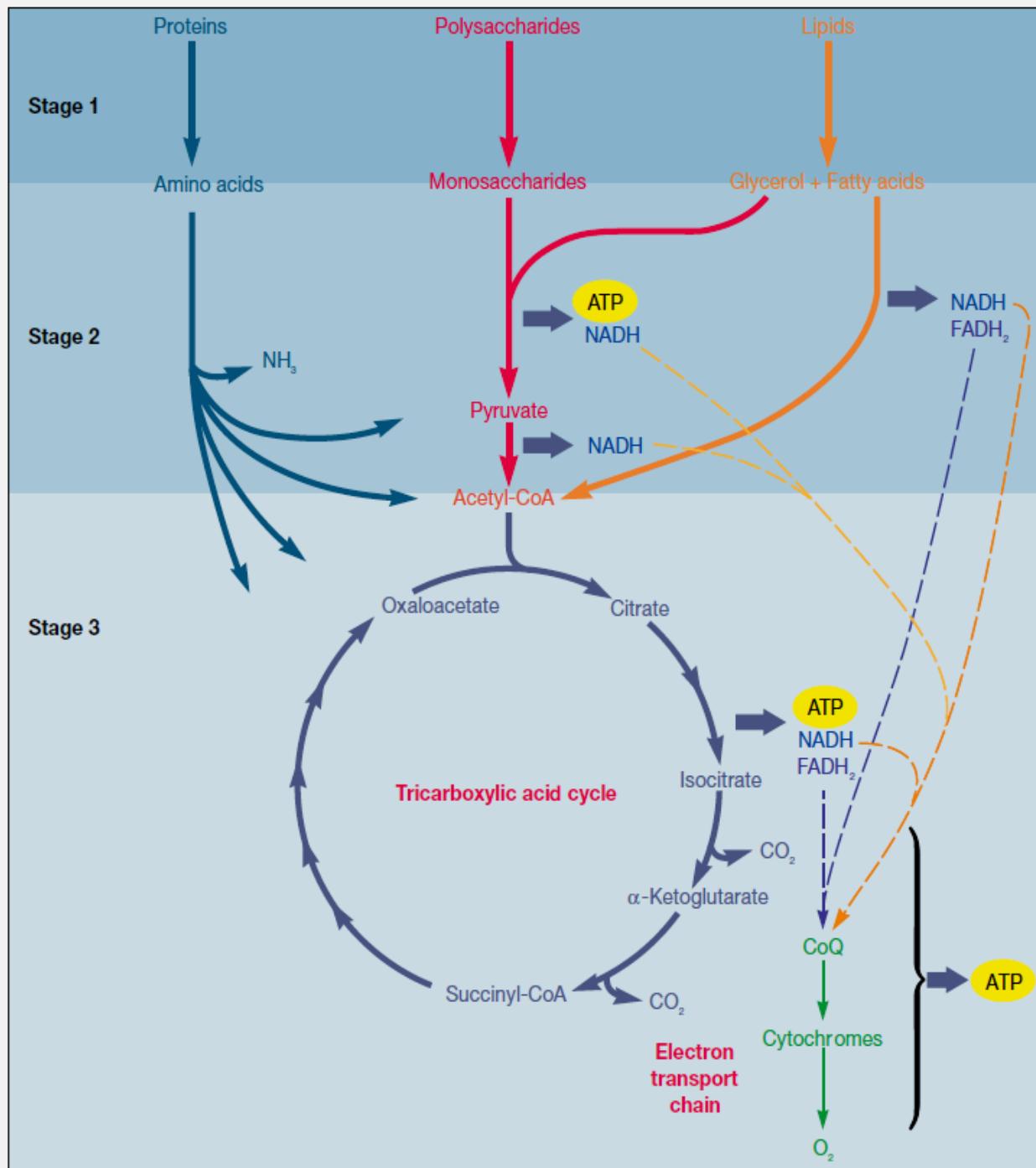
**Figure 20.1 Outline of Major Fermentation Pathways.** Microorganisms produce various waste products when they ferment glucose. The by-products released (shaded boxes) are often characteristic of the microorganisms and can be used as identification tools.



1. Lactic acid fermentation. Lactic acid bacteria (*Streptococcus*, *Lactobacillus*).
2. Alcoholic fermentation. *Zymomonas*, *Saccharomyces*.
3. Propionic acid fermentation. Propionic acid bacteria (*Propionibacterium*).
4. 2,3-butanediol fermentation. *Enterobacter*, *Serratia*, *Bacillus*.
5. Mixed acid fermentation. Enteric bacteria (*Escherichia*, *Enterobacter*, *Salmonella*, *Proteus*).
6. Butyric acid fermentation. *Clostridium*.

## AEROBIC RESPIRATION

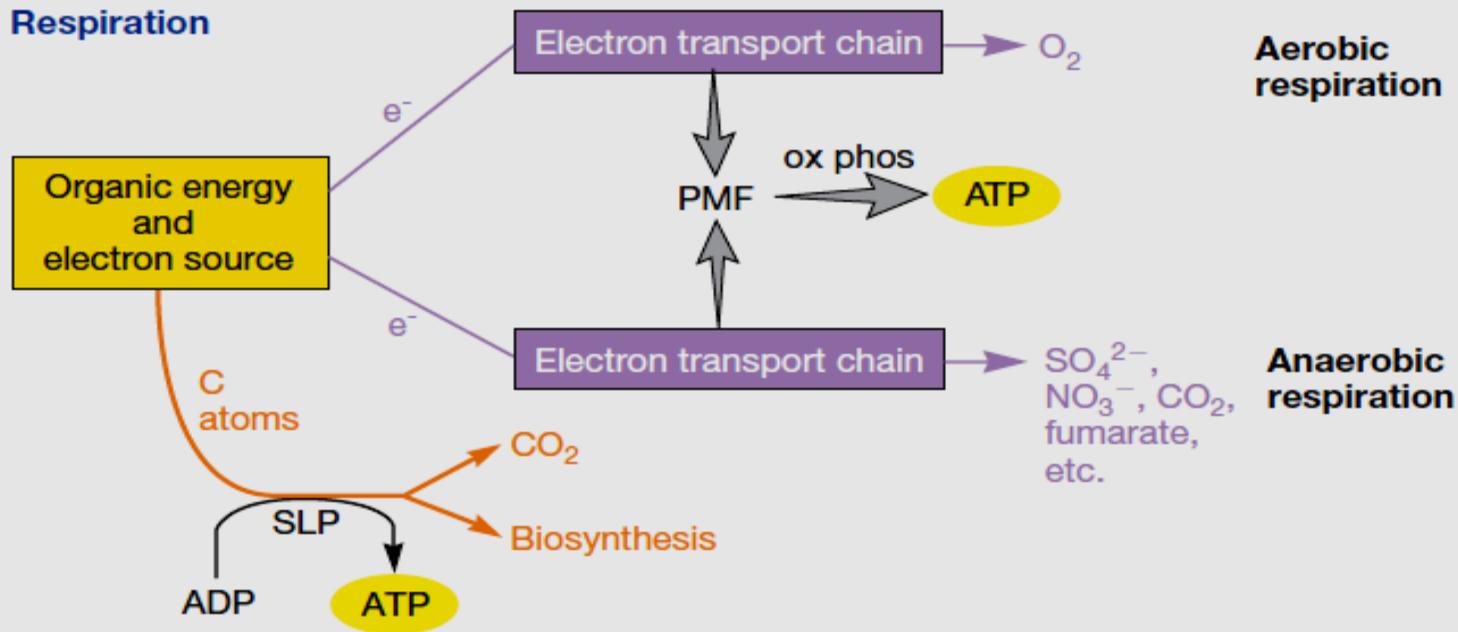




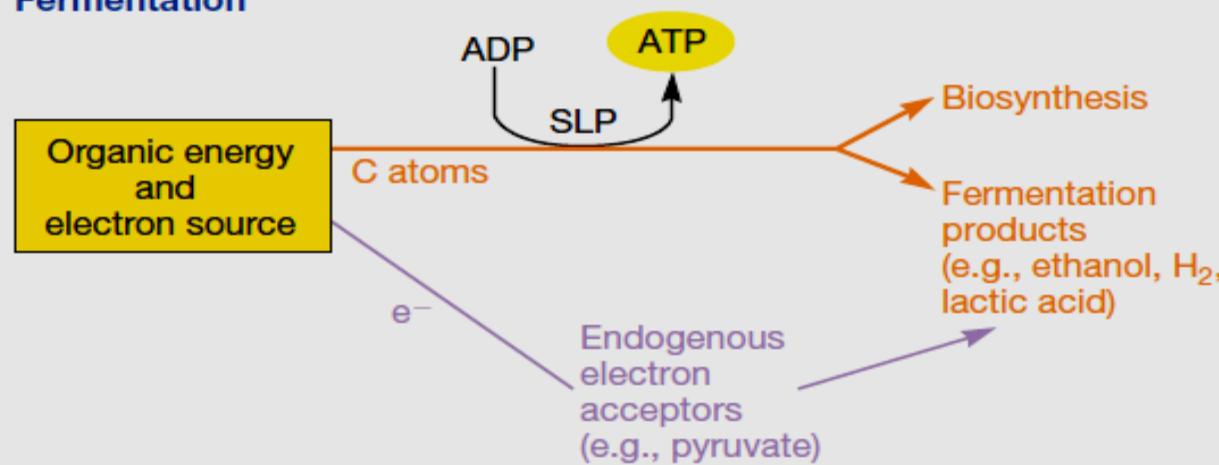
**Figure 9.3 The Three Stages of Aerobic Respiration.** A general diagram of aerobic respiration in a chemoorganoheterotroph showing the three stages in this process and the central position of the tricarboxylic acid cycle. Although there are many different proteins, polysaccharides, and lipids, they are degraded through the activity of a few common metabolic pathways. The dashed lines show the flow of electrons, carried by  $\text{NADH}$  and  $\text{FADH}_2$ , to the electron transport chain.

## Chemoorganotrophic Fueling Processes

### Respiration



### Fermentation

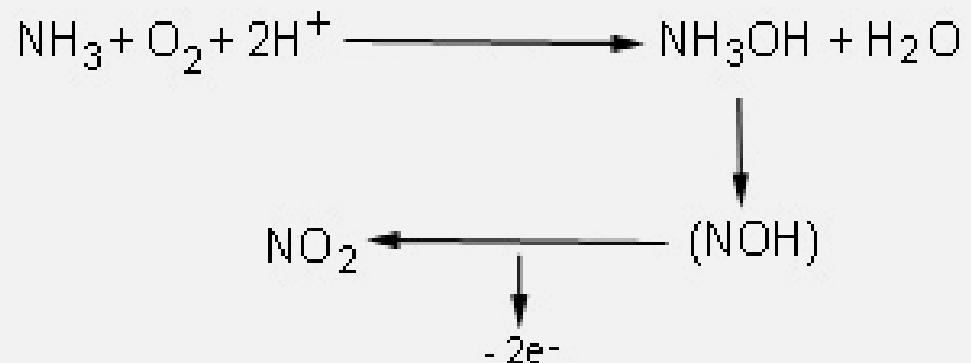


## Electron acceptors for respiration and methanogenesis in prokaryotes

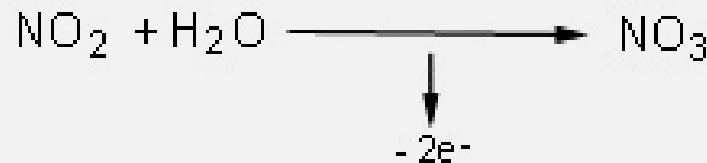
Electron acceptor	Reduced end product	Name of process	Organism
O <sub>2</sub>	H <sub>2</sub> O	Aerobic respiration	Escherichia, Streptomyces
NO <sub>3</sub>	NO <sub>2</sub> , NH <sub>3</sub> or N <sub>2</sub>	Denitrification	Bacillus, Pseudomonas
SO <sub>4</sub>	S or H <sub>2</sub> S	Sulfate reduction	Desulfovibrio
Fumarate	succinate	Anaerobic respiration using an organic e- acceptor	Escherichia
CO <sub>2</sub>	CH <sub>4</sub>	Methanogenesis	Methanococcus

## Lithotrophic oxidations

*Nitrosomonas*



*Nitrobacter*



*Thiobacillus*



# PHYSIOLOGICAL GROUPS OF LITHOTROPHS

PHYSIOLOGICAL GROUP	ENERGY SOURCE	OXIDIZED END PRODUCT	ORGANISM
Hydrogen bacteria	H <sub>2</sub>	H <sub>2</sub> O	Alcaligenes, Pseudomonas
Methanogens	<u>H<sub>2</sub>, CO<sub>2</sub></u>	H <sub>2</sub> O	Methanobacterium
Carboxydobacteria	CO	CO <sub>2</sub>	Rhodospirillum, Azotobacter
Nitrifying bacteria*	NH <sub>3</sub>	NO <sub>2</sub>	Nitrosomonas
Nitrifying bacteria*	NO <sub>2</sub>	NO <sub>3</sub>	Nitrobacter
Sulfur oxidizers	H <sub>2</sub> S or S	SO <sub>4</sub>	Thiobacillus, Sulfolobus
Iron bacteria	Fe ++	Fe+++	Gallionella, Thiobacillus

\* The overall process of **nitrification**, conversion of NH<sub>3</sub> to NO<sub>3</sub>, requires a consortium of microorganisms

## Distribution of Bacteria in a Lake

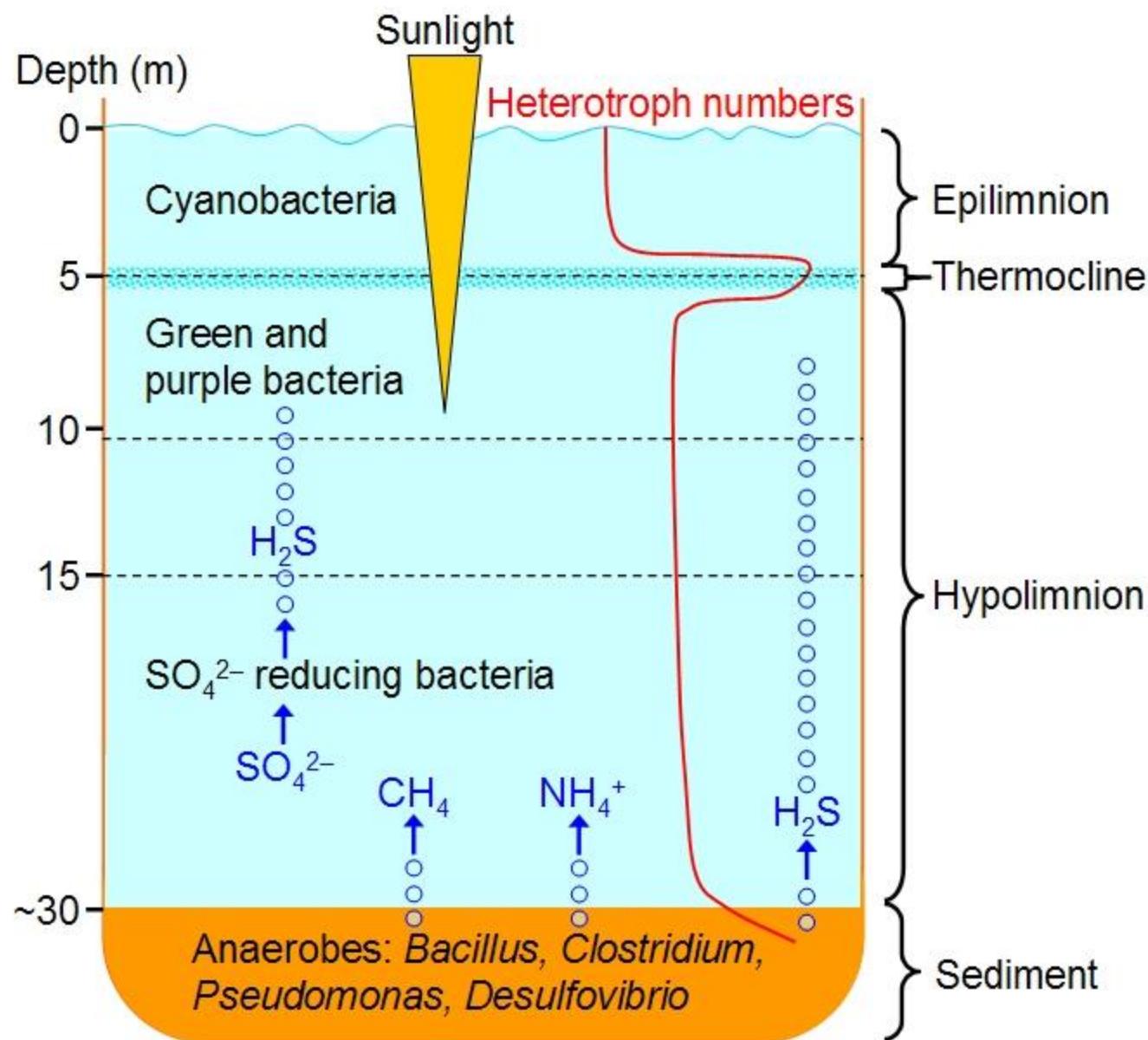


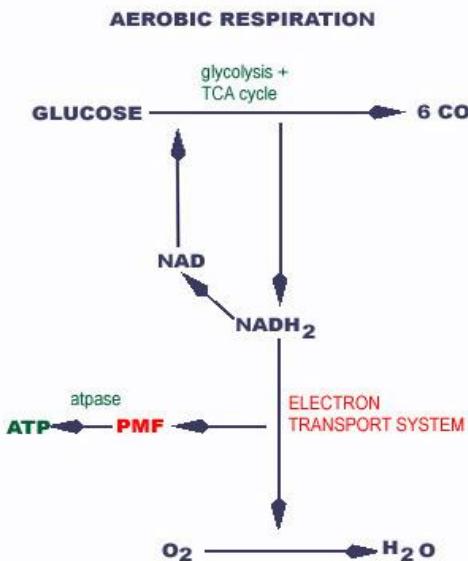
Tableau III.3 – Types d'interactions entre deux micro-organismes A et B.

Relation	Germe A	Germe B	Nature
Prédation	+	-	B détruit A pour se développer
Parasitisme	+	-	B se développe au détriment de A sans le détruire
Compétition	-	-	A et B s'inhibent par limitation de substrats
Amensalisme	-	0	B nuit à A, sans être influencé d'une manière
Neutralisme	0	0	A et B évoluent indépendamment
Syntrophie ou commensalisme	+	0	B est bénéfique à A, sans être influencé
Synergie ou protocoopération	+	+	A et B profitent l'un à l'autre, sans obligation
Symbiose ou mutualisme	+	+	A et B ont besoin obligatoirement l'un de l'autre

# METABOLISME PHOTOTROPHIQUE:

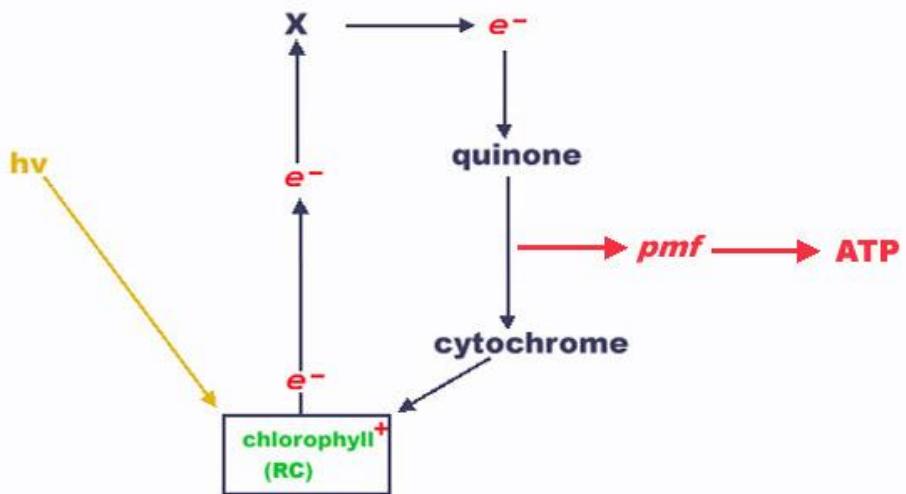
- Acquisition de l'énergie à partir de la lumière et son utilisation pour fixer le CO<sub>2</sub> et production de O<sub>2</sub> photosynthèse vraie comme chez les plantes, les algues et cyanobactéries.
- Ou utilisation de cette énergie pour les besoins énergétiques de bactéries sans fixation de CO<sub>2</sub> sans production de carbohydrates se novo.

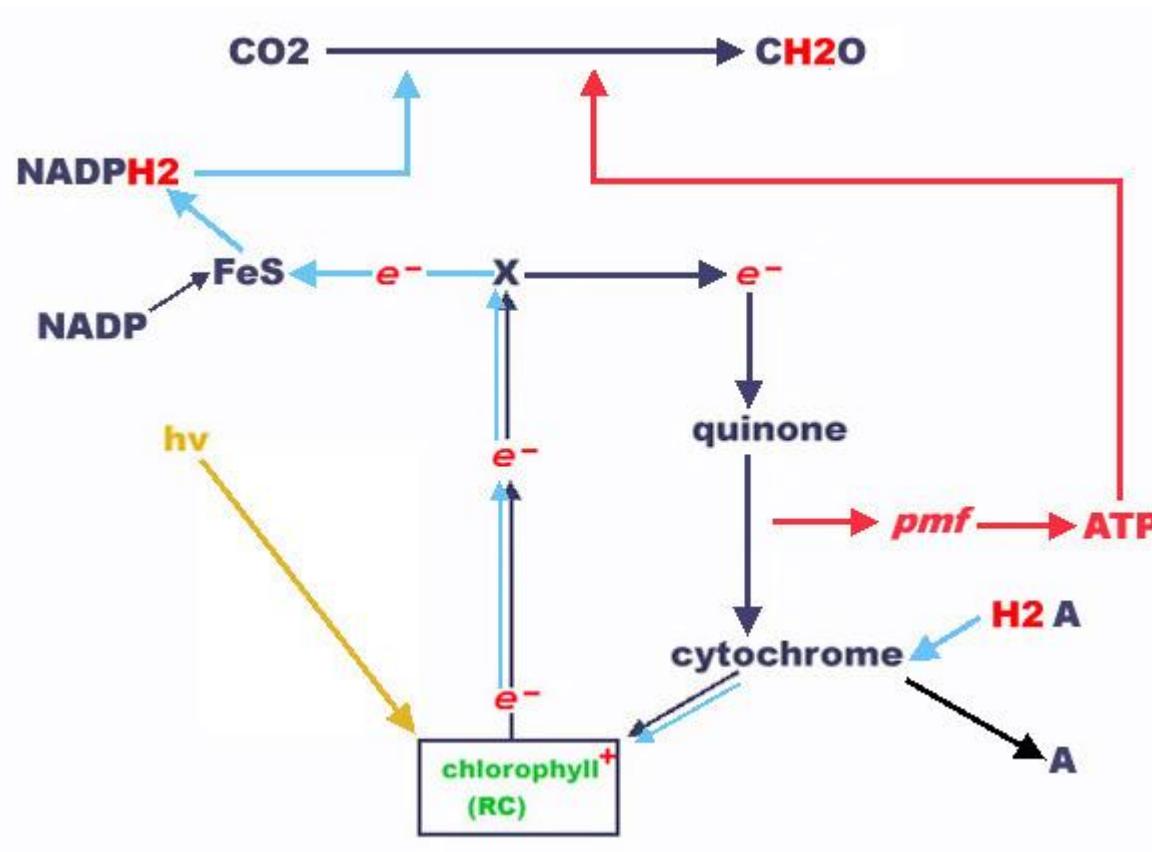
## Comparaison du photosystème I (photophosphorylation cyclique) à la respiration aérobie



Aerobic respiration

Photosystem I: cyclical electron flow coupled to photophosphorylation

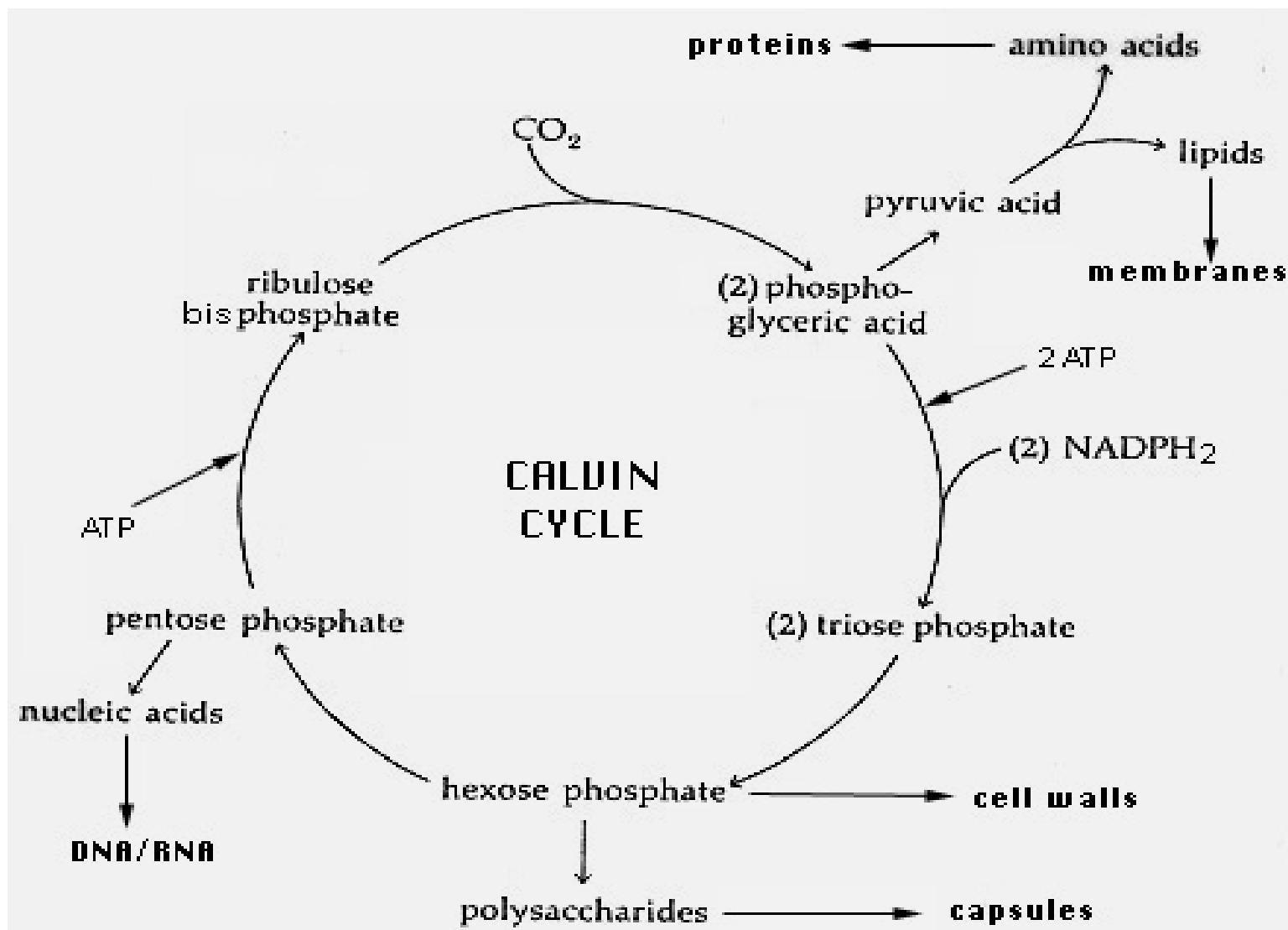




Coupling the light and dark reactions of photosynthesis

## Differences between plant and bacterial photosynthesis

	<b>PLANT PHOTOSYNTHESIS</b>	<b>BACTERIAL PHOTOSYNTHESIS</b>
<b>ORGANISMS</b>	Plants, Algae, Cyanobacteria	purple and green bacteria
Type of chlorophyll	chlorophyll a absorbs at 650-750nm	bacteriochlorophyll absorbs at 800-1000nm
Photosystem I (cyclic photophosphorylation)	present	present
Photosystem II (noncyclic photophosphorylation)	present	absent
Produces O <sub>2</sub>	yes	no
Photosynthetic electron donor	H <sub>2</sub> O	H <sub>2</sub> S, other sulfur compounds or certain organic compounds



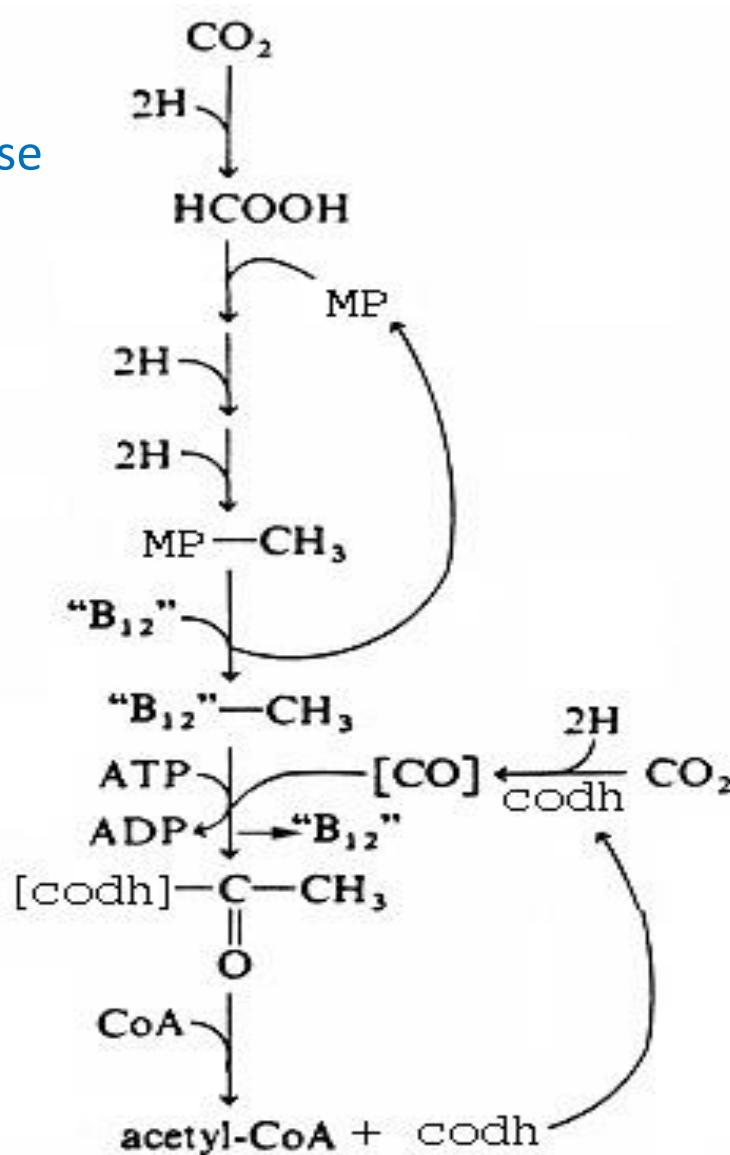
**The Calvin cycle and its relationship to the synthesis of cell materials**

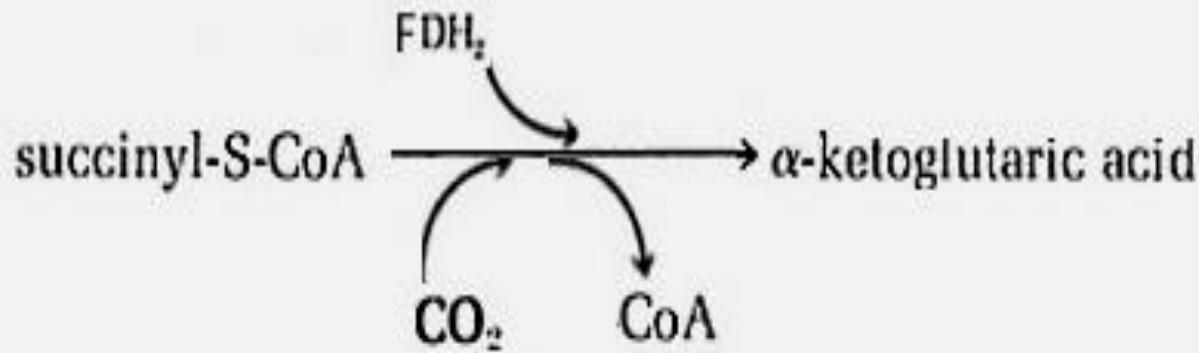
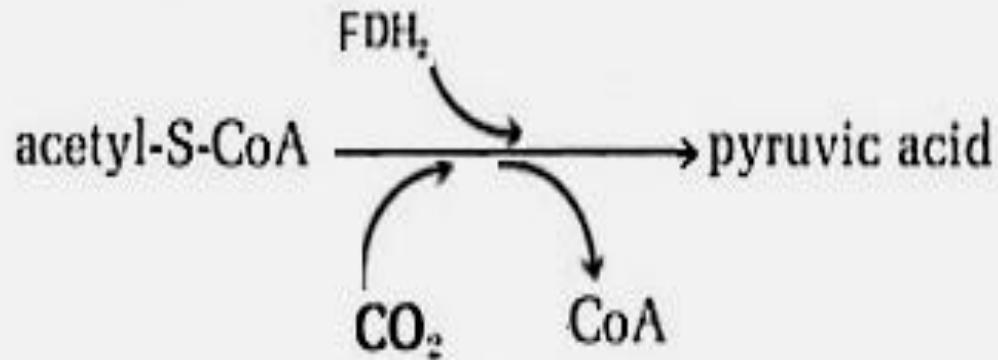
## The CODH or acetyl CoA pathway of CO<sub>2</sub> fixation in the methanogens

### Carbon monoxide dehydrogenase/acetyl-CoA synthase

The bifunctional enzyme carbon monoxide dehydrogenase (CODH)/acetyl-CoA synthase is a key enzyme involved in the Wood–Ljungdahl pathway of carbon fixation that operates in anaerobic bacteria.

As such, it is a major player in the global carbon cycle. The CODH component of the enzyme catalyzes the reversible reduction of CO<sub>2</sub> to CO, which is then channeled to the ACS active site where it reacts with CoA and a methyl group provided by the corrinoid iron–sulfur protein (CFeSP) to form acetyl-CoA





The two ferredoxin (FD)-mediated reactions used for CO<sub>2</sub> uptake in the green bacteria are a reversal of the oxidation of keto acids mediated by NAD and CoA

# The main pathways of biosynthesis in prokaryotic cells

