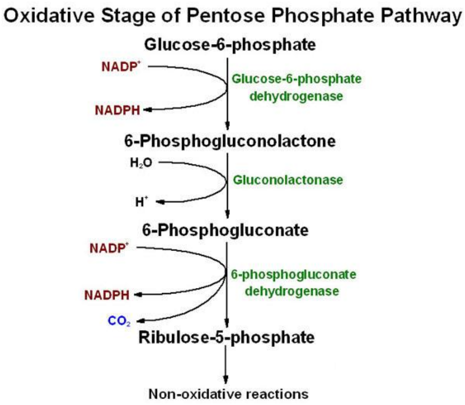
Microbial metabolism

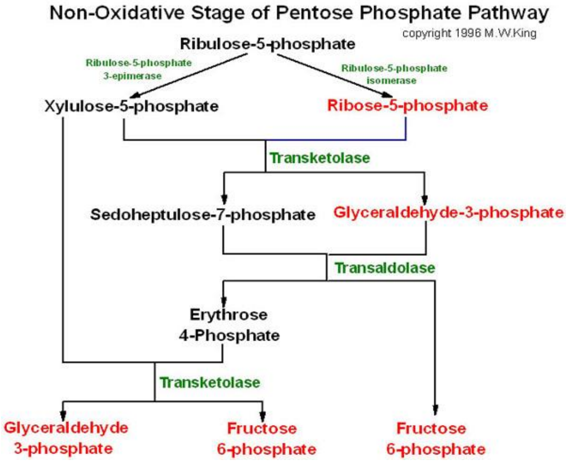
Power Point No. 4,5,6

- **Alternative to embden meyer hoff Pathway:**
- **1-The pentose phosphate pathway** (or *hexose monophosphate*
- *pathway*) operates simultaneously with glycolysis and provides a means
- for the breakdown of five-carbon sugars (pentoses) such as xylose and
- arabinose as well as glucose . A key feature of this pathway is to
- produce important intermediate pentose's (ribose-5-phosphate) used in
- the synthesis of (1) nucleic acids , (2) glucose from carbon dioxide in
- photosynthesis, and (3) certain aromatic amino acids from erythrose-4-
- phosphate. The pathway is an important producer of the reduced coenzyme
- NADPH from NADP for cytosolic biosynthesis eg. synthesis of fatty acids and
- alcohols. Pentose phosphate pathway has two phases: the oxidative phase
- gives pentose phosphate,NADPH and CO_2 from glucose-6-phosphate
- whereas the
-

- non-oxidative phase allows interconversion between a pool of
- phosphorylated sugars.The pentose phosphate pathway yields a
- net gain of only one molecule of ATP for each molecule of glucose
- oxidized. Bacteria that use the pentose phosphate pathway include
- *Bacillus subtilis, E. coli, Leuconostoc mesenteroides, Enterococcus*
- *faecalis* and yeast *D. polymorpha, Schizo. Pombe , C. shehatae* and
- *Pichia stipitis.*

1-The Pentose Phosphate Pathway





Non-Oxidative Stage of Pentose Phosphate Pathway
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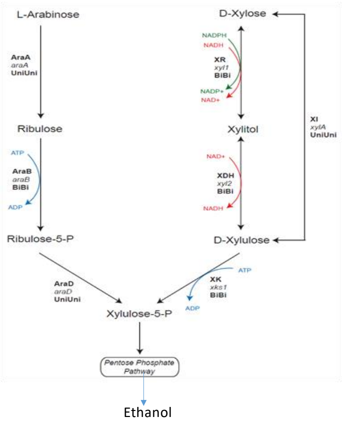
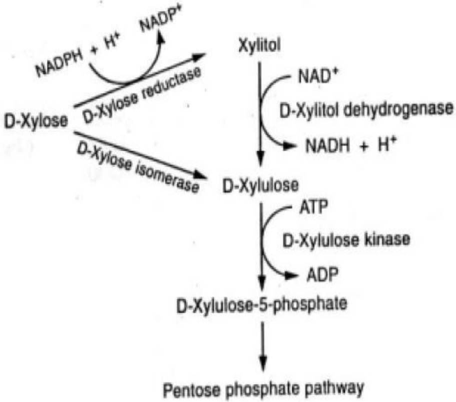
Ribulose-5-phosphate
Ribulose-5-phosphate 3-epimerase
Ribulose-5-phosphate isomerase
Xylulose-5-phosphate
Ribose-5-phosphate
Transketolase
Sedoheptulose-7-phosphate
Glyceraldehyde-3-phosphate
Transaldolase
Erythrose 4-Phosphate
Transketolase
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Fructose 6-phosphate

- Some microorganism can not follow this pathway
- for pentose sugar degradation such as xylose because they does not
- possess xylose isomerase enzyme which responsible for conversion
- of xylose to xylulose in singl step such as *Candida utilis* and
- *Rhodotorula gracilis*. Other microorganisms direct this conversion by
- two steps and required two different enzymes instead of xylose isomerase.
- (diagram below). Arabinose also utilized within 3steps into xylulose-5-p.
- Then via pentose phosphate converted both to GA and fructose-6-p
- and by glycolysis pathway under fermentation condition the pathway
- ended to the formation of ethanol.

Pathways for arabinose and xylose

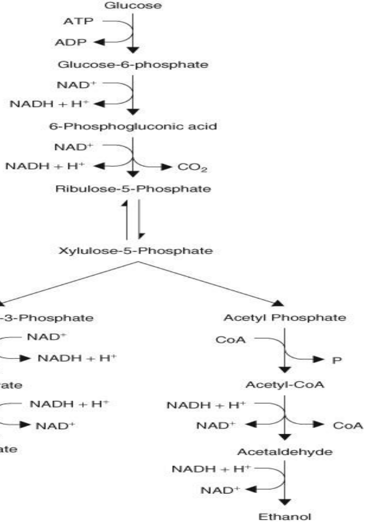
- metabolism in *S. cerevisiae*.
- AraA (isomerase),
- AraB (ribulokinase),
- AraD (epimerase),
- xylose reductase (XR),
- xylitol dehydrogenase (XDH), and
- xylulokinase (XK).




NADPH + H+
NADP+
D-Xylose reductase
Xylitol
NAD+
D-Xylitol dehydrogenase
NADH + H+
D-Xylose
D-Xylose isomerase
D-Xylulose
ATP
D-Xylulose kinase
ADP
D-Xylulose-5-phosphate
Pentose phosphate pathway

- **3- The Phosphoketolase (Heterolactic)Pathway:**
- The phosphoketolase pathway is distinguished by the key cleavage
- enzyme, **phosphoketolase**, which cleaves pentose phosphate into
- glyceraldehyde-3-phosphate and acetyl phosphate. As a rmentation
- pathway, it is employed mainly by the **heterolactic acid bacteria**,
- which include some species of *Lactobacillus* and *Leuconostoc.*
- (*Leuconostoc mesenteroides,Lactobacillus bifermentous, Leconostoc*
- *lactisn*) In this pathway, glucose-6-phosphate is oxidized to
- 6-phosphogluconic acid,

- which becomes oxidized and decarboxylated
- to form pentose phosphate. Unlike the EM pathway, NAD
- mediated oxidation take place before cleavage of the
- substrate being utilized. Pentose phosphate is subsequently
- cleaved to glyceraldehyde-3-phosphate (GAP) and acetyl
- phosphate. GAP is converted to lactic acid by the same
- enzymes as the E-M pathway. This branch of the pathway contains
- anoxidation coupled to a reduction while 2 ATP are produced by
- substrate levelphosphorylation. Acetyl phosphate is reduced in three
- steps to ethanol but does not yield ATP. The overall reaction is
- Glucose ---------->1 lactic acid + 1 ethanol +1 CO_2 with a net gain
- of 1 ATP. The efficiency is about half that of the E-M pathway.

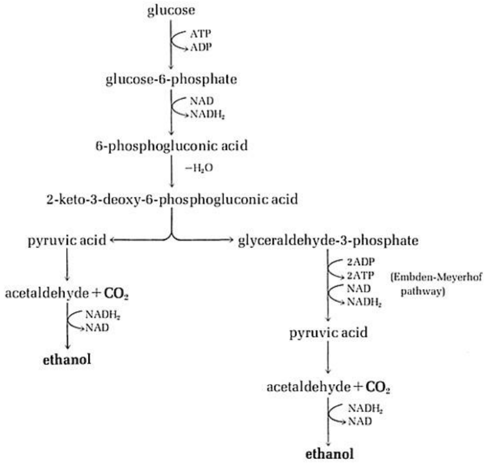

Glucose
ATP
ADP
Glucose-6-phosphate
NAD+
NADH + H+
6-Phosphogluconic acid
NAD+
NADH + H+
CO2
Ribulose-5-Phosphate
Xylulose-5-Phosphate
Glyceraldehyde-3-Phosphate
2 ADP
NAD+
2 ATP
NADH + H+
Pyruvate
NADH + H+
NAD+
Lactate
Acetyl Phosphate
CoA
P
Acetyl-CoA
NADH + H+
NAD+
CoA
Acetaldehyde
NADH + H+
NAD+
Ethanol

4-The Entner-Doudoroff Pathway:

Only a few bacteria such as *Zymomonas,* employ the Entner-Doudoroff pathway as a fermentation path. However, many bacteria, especially those grouped around the *Pseudomonas*, *Azatobacter, Xanthomonas* and *Anterobacter* use the pathway as a way to degrade carbohydrates for respiratory metabolism.The E-D pathway yields 2 pyruvic acid from glucose (same as the E-M pathway) but like the phosphoketolase pathway, oxidation occurs before the cleavage, and the net energy yield per mole of glucose utilized is one mole of ATP. In the E-D pathway, glucose phosphate is oxidized to 2-keto-3-deoxy-6-phosphogluconic acid (KDPG) which is cleaved by **KDPG aldolase** to pyruvate and GAP. The latter is oxidized to pyruvate by E-M enzymes where is 2 ATP are produced by substrate level phosphorylations.

- Pyruvic acid from either branch of the pathway is
- reduced to ethanol and CO_2, in the same manner
- as yeast, by the "yeast-like bacterium", *Zymomonas*
- (Figure below). Thus, the overall reaction is
- Glucose ---------->2 ethanol +2 CO_2,
- **The Entner-Doudoroff Pathway of Fermentation.**
- **The overall reaction is, Glucose -----→2 ethanol + 2 CO_2 + 1 ATP**
- **(net).**
- This pathway may also be induced in certain Gr+
- Organisms such as *Streptococcus faecalis* by growing
- them in a medium containing Gluconate.

4- The Entner-Doudoroff Pathway of Fermentation



- **Krebs cycle:**
- Krebs cycle (also known as **Citric Acid Cycle** or **Tricarboxylic Acid Cycle**) is a step wise cyclic process which is used to oxidize the pyruvate formed during the glycolytic breakdown of glucose into Carbon Dioxide (CO_2) and Water (H_2O). It also oxidizes acetyl CoA which arises from breakdown of carbohydrate, lipid, and protein. The first product of Krebs cycle is citric acid (citrate).Therefore, it is also known as citric acid cycle. Sometimes Krebs cycle is also referred as Tricarboxylic acid cycle or **TCA.**

- **Steps of Krebs cycle**
- **Step 1: Condensation**
- In first step of Krebs cycle, Acetyl CoA combines with oxaloacetate in the presence of condensing *enzymes* ***citrate synthetase***. CoA is released out. The product of condensation is citrate which is a tricarboxylic 6-carbon compound.

$$\text{Acetyl Co-A} + \text{Oxaloacetate} + H_2O \xrightarrow[\text{citrate synthetase}]{} \text{Citrate} + \text{CoA}$$

- **Step 2: Isomerisation**
- Citrate formed in first step is converted into its isomer isocitrate by the enzyme ***aconitase*** by two steps.
- (i) **Dehydration** : A molecule of water is released and citric acid is changed into cis-aconitate.

$$\text{Citric acid} \xrightarrow{\text{Aconitase, Fe}} \text{Cis} - \text{Aconitate} + H_2O$$

- (ii) **Rehydration** : Cis – aconitate combines with a molecule of water and form isocitrate.

$$\text{Cis} - \text{Aconitate} + H_2O \xrightarrow{\text{Aconitase, Fe}} \text{Isocitrate}$$

- **Step 3: Dehydrogenation**
- Now isocitrate undergoes dehydrogenation in the presence of an *enzyme* ***isocitrate dehydrogenase*** and changed into oxalosuccinate (6C).

$$\text{Isocitrate} + NAD^{+} \xrightarrow{\text{Isocitrate dehydrogenase}} \text{Oxalosuccinate} + NADH_2$$

- **Step 4: Decarboxylation**
- Oxalosuccinate in Step 4 undergoes decarboxylation. In the presence of ***oxalosuccinate decarboxylase*** enzyme, oxalosuccinate is changed into α-ketoglutarate.

$$\underset{(6C)}{\text{Oxalosuccinate}} \xrightarrow{\substack{\text{Oxalosuccinate} \\ \text{decarboxylase}}} \underset{(5C)}{\alpha\text{ - Ketoglutarate} + CO_2}$$

- **Step 5: Oxidative Decarboxylation**
- In this step 5-carbon compound, α – Ketoglutarate undergoes
- simultaneous dehydrogenation and decarboxylation in the presence
- of *enzyme* ***α – ketoglutarate dehydrogenase*** complex.

$$\alpha\text{ - Ketoglutarate} + NAD^{+} + CoA \xrightarrow[\textit{TPP, Lipoic Acid + transsuccinylase } Mg^{2+}]{\alpha - \textit{ketoglutaratedehydrogenase}} \text{Succinyl CoA} + NADH_2 + CO_2$$

- **Step 6: Substrate level ATP/GTP Synthesis**
- In the presence of enzyme ***succinyl thiokinase***, succinyl CoA is
- hydrolyzed. CoA and Succinate are formed. The energy liberated
- during the process is used in synthesis of ATP in Plants and
- microorganisms and GTP (Guanosine triphosphate) or ITP (Inosine
- triphosphate) in animals. CoA is released out.

$$\begin{array}{c} \text{Succinyl CoA} + \text{ADP/ GDP/IDP} \\ + H_3PO_4 \end{array} \xrightarrow[]{\text{Succinyl thiokinase}} \begin{array}{c} \text{Succinate} + \text{ATP/GTP/ITP} \\ + \text{CoA} \end{array}$$

- **Step 7: Dehydrogenation (Oxidation)**
- In this step 4–Carbon compound Succinate is oxidized to another
- 4-carbon compound fumarate with the help of enzyme ***succinate***
- ***dehydrogenase*** and hydrogen acceptor FAD (Flavin Adenine
- Dinucleotide).
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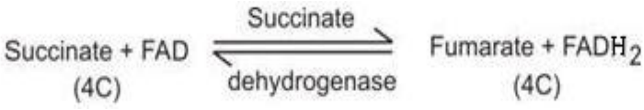


- **Step 8: Hydration**
- In step 8, Fumarate reacts with a molecule of water, in the presence of an enzyme ***fumarase*** forming another 4-carbon dicarboxylic acid called Malate.

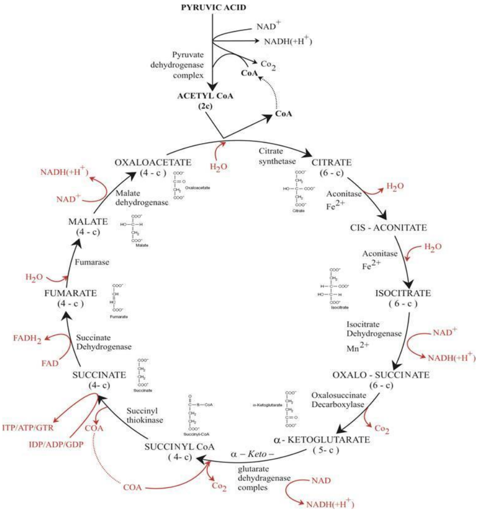
$$\text{Fumarate} + H_2O \xrightleftharpoons{\text{Fumarase}} \text{Malate}$$

- **Step 9: Dehydrogenation (Oxidation)**
- With the help of enzyme ***malate dehydrogenase***, Malate formed in step 8 is oxidized to oxaloacetate. NAD^+ reduced to $NADH_2$.
-
- Malate + NAD $\xrightarrow{\text{Malate dehydroganase}}$ Oxaloacetate + $NADH_2$

- An oxaloacetate formed in this reaction becomes available to combine with acetyl CoA to start a new cycle all over again.
- $$\text{Oxaloacetate} + \text{Acetyl CoA} \xrightarrow[\text{synthetase}]{\text{citrate}} \text{Citrate} + \text{CoA}$$
- **Note** : The overall equation of oxidative catabolism of pyruvate can be written as follows:-

$$\text{Pyruvic acid} + 4NAD^+ + FAD^+ + 2H_2O + ADP + P_i \longrightarrow 3\,CO_2 + 4\,NADH_2 + FADH_2 + ATP$$

- **$NADH_2$ & $FADH_2$** are linked to electron transport system and formation of ATP by oxidative phosphorylation.



PYRUVIC ACID
NAD+
NADH(+H+)
Pyruvate dehydrogenase complex
Co2
CoA
ACETYL CoA (2c)
CoA
OXALOACETATE (4 - c)
H2O
Citrate synthetase
CITRATE (6 - c)
NADH(+H+)
NAD+
Malate dehydrogenase
Oxaloacetate
Citrate
H2O
Aconitase Fe2+
MALATE (4 - c)
Malate
CIS - ACONITATE
Aconitase Fe2+
H2O
Fumarase
H2O
FUMARATE (4 - c)
Fumarate
Isocitrate
ISOCITRATE (6 - c)
Isocitrate Dehydrogenase Mn2+
NAD+
NADH(+H+)
FADH2
Succinate Dehydrogenase
FAD
SUCCINATE (4- c)
Succinate
OXALO - SUCCINATE (6 - c)
Oxalosuccinate Decarboxylase
Co2
α-Ketoglutarate
Succinyl-CoA
Succinyl thiokinase
ITP/ATP/GTR
COA
IDP/ADP/GDP
SUCCINYL CoA (4- c)
α – Keto – glutarate dehydragenase comples
α - KETOGLUTARATE (5- c)
COA
Co2
NAD
NADH(+H+)

- **Sites for Carbon Dioxide Production**
- Carbon Dioxide is not formed during glycolysis. **Three molecules of Carbon Dioxide** are formed during complete oxidation of each of the two pyruvates. **One** molecule is produced during link reaction when Oxidative Decarboxylation of pyruvate to acetyl CoA takes place. **Two** molecules are produced during Krebs cycle

- **Sites for Substrate level Phosphorylation**
- **Four molecules of ATP** are formed through substrate level phosphorylation in glycolysis.
- (i) Two during dephosphorylation of two molecules of 1, 3 – diphosphoglyceric acid to two molecules of 3 – phosphoglyceric acids.
- (ii) Two during dephosphorylation of two molecules of phosphoenol pyruvate to the two molecules of pyruvate.
- **Two molecules of ATP/GTP/ITP** are formed through substrate level phosphorylation which is linked to release of energy at the time of breaking thioester bonds of two molecules of succinyl CoA to succinate state.

- **Sites for Reduced Co-enzymes**
- (i) **Two molecules of NADH** are formed in glycolysis during oxidation
- of two molecules of glyceraldehydes -3- phosphate to 1, 3 –
- diphosphoglycerate state.
- (ii) **Two Molecules of NADH** are produced in link reaction or gateway
- step when two pyruvate molecules are oxidatively decarboxylated to
- the state of acetyl CoA.

- **In Krebs Cycle six molecules of NADH** and **two molecules of FADH2** are formed.

- **The overall reaction for the aerobic respiration of glucose is**

- **Glucose + 6 O_2 ----------> 6 CO_2 + 6 H_20 + 688 kcal (total)**

- Which can be written.The net number of coenzymes formed from one
- molecule of glucose are 10 NADH2 and 2$FADH_2$. There is also a gain
- of 4 ATP molecules.
- **Glucose ----------> 6 CO_2 + 10 $NADH_2$ + 2 $FADH_2$ + 4 ATP**

- In *E. coli,* **2 ATP** are produced for each pair of electrons that are introduced into the ETS by **$NADH_2$**. **One ATP** is produced from a pair of electrons introduced by $FADH_2$. Hence, the equation can be rewritten
- Glucose + 6 O_2 ----------> 6 CO_2 + 6 H_20 + 20 ATP (ETP) + 2 ATP (ETP) + 4 ATP (SLP) + 688 kcal (total)
- In *Pseudomonas* (or mitochondria), due to the exact nature of the ETS, 3 ATP are produced for each pair of electrons that are introduced into the ETS by $NADH_2$ and 2 ATP are produced from a pair of electrons introduced by $FADH_2$. Hence, the overall reaction in *Pseudomonas,* using the same pathways as *E. coli,* is
- Glucose + 6 O_2 ----------> 6 CO_2 + 6 H_20 + 38 ATP + 688 kcal (total)

Production of 4 and 5 intermediate compounds of TCA by anaerobic microorganisms

- Anaerobic microorganisms also produce intermediate compounds
- similar to that present in kreb cyle to that of aerobic or facultative
- microorganisms for the biosynthesis of amino acid and other cellular
- constituents that require four or five intermediate compounds oF
- TCA cycle.

Operation of the enzymes of the TCA cycle under anaerobic conditions