

Chem*4570 Applied Biochemistry Lecture 4

Production of butyric acid and butanol

The energetic basis for the anaerobic metabolism of glucose is **rearrangement of the dispersed single-bonded O into carboxylate groups and carbon dioxide**. This can be achieved by reducing the remain C atoms to hydrocarbon.

Three closely related *Clostridium* species follow this principle to generate products with potential for human exploitation:

Clostridium butyricum makes **butyric acid**

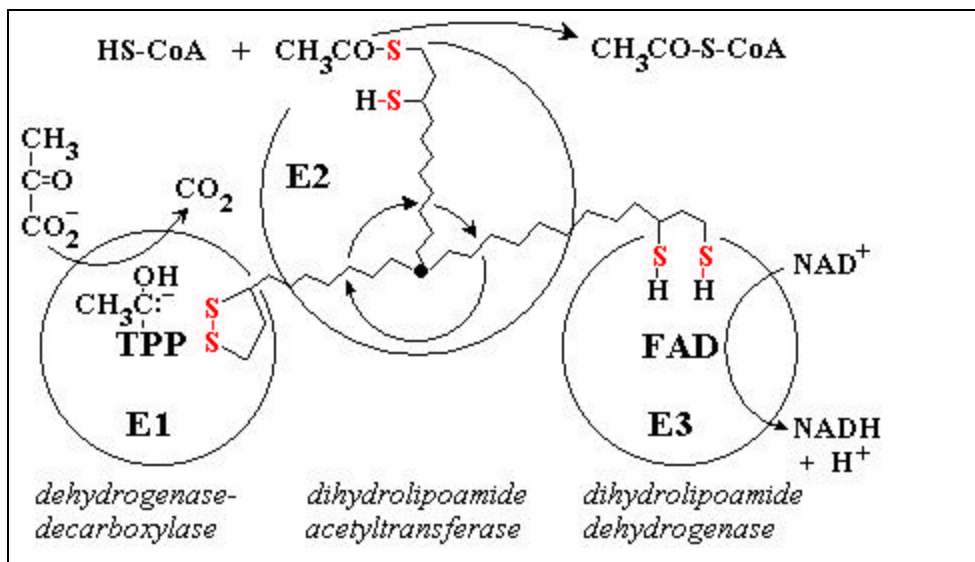
Clostridium butylicum makes **butanol**

Clostridium acetobutylicum makes **butanol and acetone**.

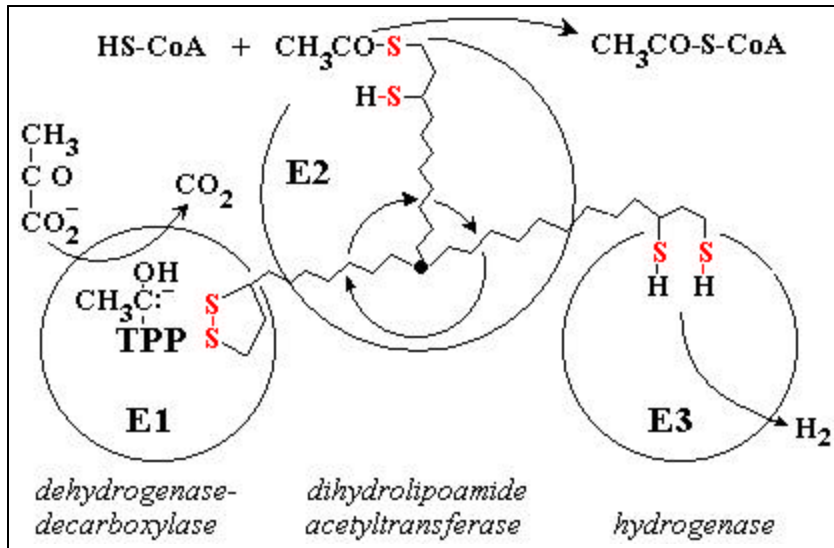
These organisms possess a novel *pyruvate dehydrogenase* coupled to *hydrogenase*.

The common form of *pyruvate dehydrogenase* is a three-component multienzyme complex E1-E2-E3 containing the internally bound coenzymes thiamine pyrophosphate (TPP), lipoamide, and FAD. In E1, pyruvate is decarboxylated, in E2, the bound acetaldehyde forms an adduct with lipoamide, effectively being oxidized to an acetyl radical which can be transferred to CoA. In E3 the resulting dihydrolipoamide is reoxidized using NAD^+ as hydrogen acceptor.

The overall equation is



These *Clostridium* species, which are highly adapted to anaerobic growth, have a version of pyruvate dehydrogenase that reoxidizes dihydrolipoamide by coupling to **hydrogenase** which releases H₂ gas.



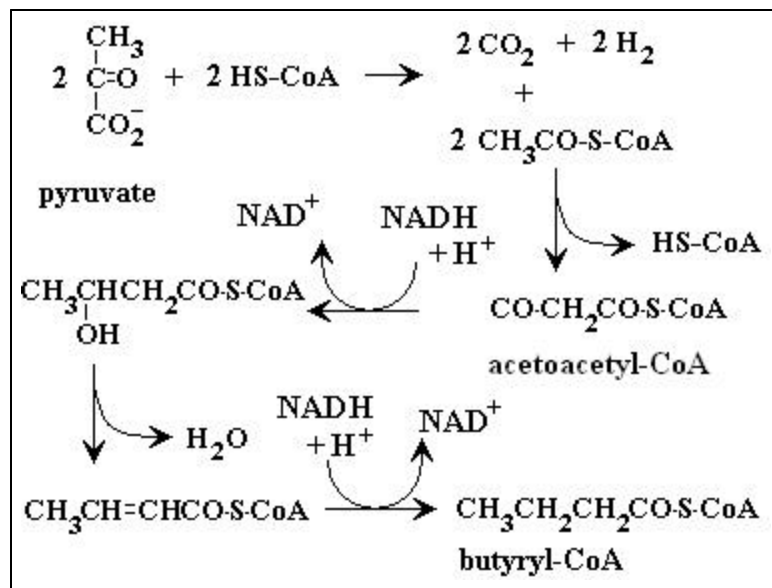
Further oxidation of pyruvate is normally difficult under anaerobic it shifts NAD⁺ even further towards NADH. Hydrogenase circumvents the NAD⁺ requirement at the cost of a slightly smaller energy change, and allows acetyl-CoA to be produced without consuming NAD⁺.

This opens up the way to other reactions that consume NADH and restore NAD⁺ :

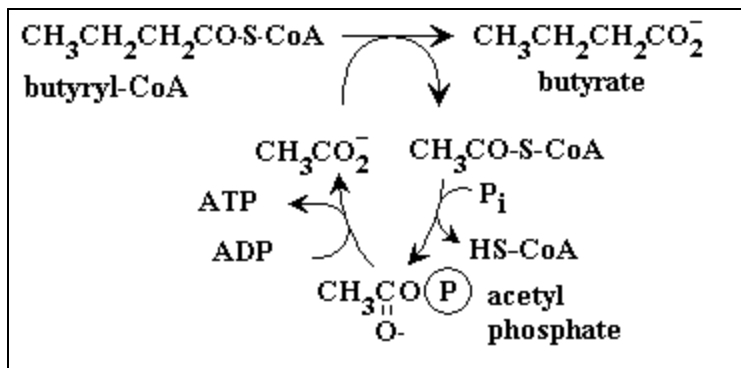
2 moles of pyruvate are made from one glucose with net gain of 2 ATP as usual and producing 2 NADH which needs to be recycled.

Two moles of acetyl-CoA are produced, releasing H₂ instead of additional NADH.

The acetyl-CoA molecules condense to acetoacetyl-CoA, and this uses 2 moles of NADH in steps resembling β-oxidation in reverse to be reduced to butyryl-CoA. This balances the NADH made earlier.



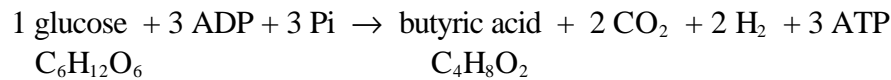
Butyryl-CoA contains a high energy bond which can now be used to generate an extra ATP.



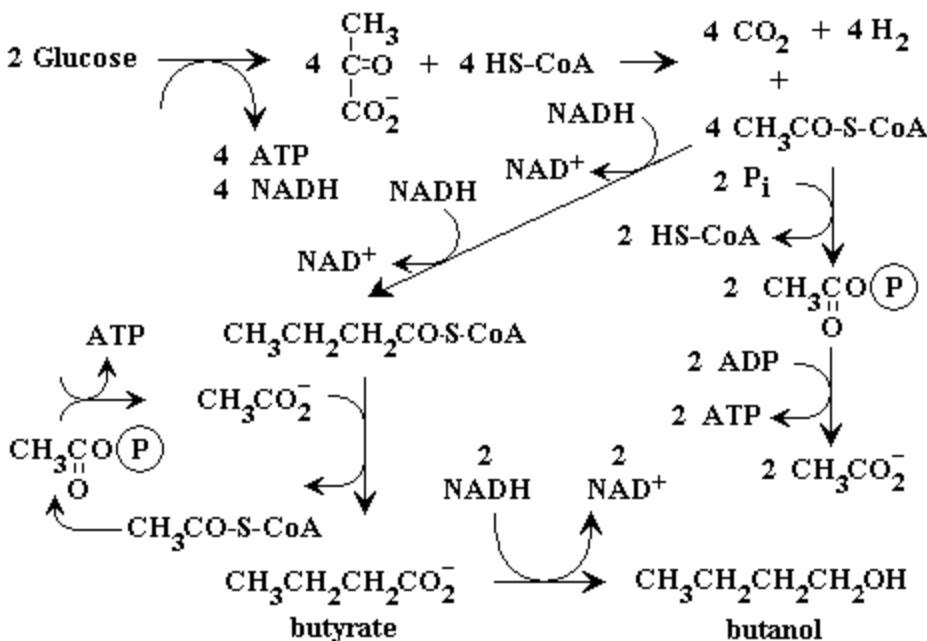
Butyryl CoA exchanges with acetate to release butyrate to the medium and conserving the high energy bond as acetyl CoA.

Acetyl-CoA then exchanges its high energy bond to make acetyl phosphate. Finally acetyl phosphate can act as a phosphate donor to make ATP.

The overall anaerobic process in *Clostridium butyricum*

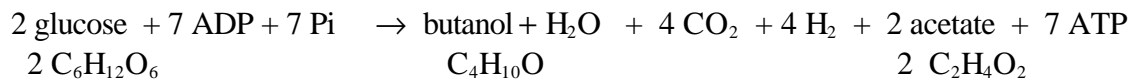


In *Clostridium butylicum*, the butyrate is reduced to butanol, consuming 2 additional NADH.

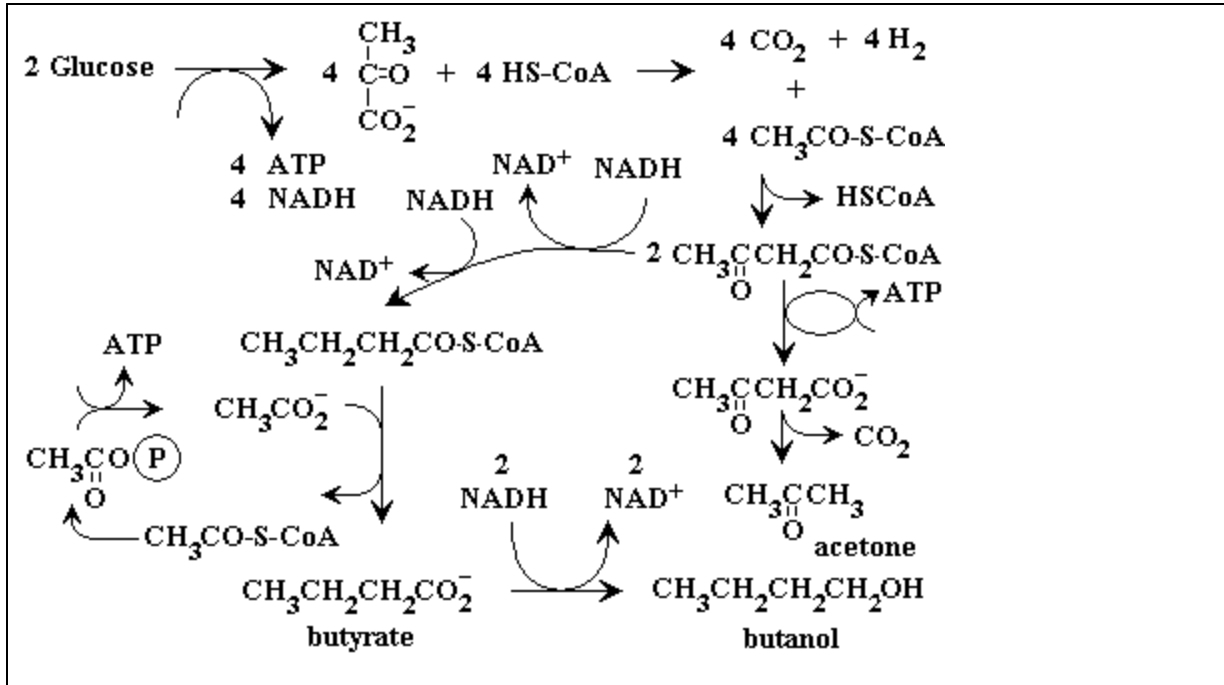


This allows for a second molecule of glucose to be converted to 2 pyruvates, and to 2 acetyl CoA, each of which can yield ATP

The overall process yields 3.5 ATP per glucose



In *Clostridium acetobutylicum*, butanol is produced as above, but all four molecules of acetyl-CoA condense to two molecules of acetoacetyl-CoA. One molecule of acetoacetyl-CoA is then reduced to butanol, consuming all the NADH, while the other is decarboxylated to yield acetone. One ATP can be produced from each branch by the acetyl phosphate loop mechanism.



Although acetone is produced, the disadvantage is that it is mixed with butanol, requiring distillation to separate it out.

The overall process yields 3 ATP per glucose

