

MICROBIAL BIOCHEMISTRY

3rd Year Licence — Biotechnology and health

Oxidation / fermentation of Pyruvate

1. Pyruvate: the crossroads of metabolism

Pyruvate, the end product of glycolysis, sits at the center of intermediate metabolism. Cells can reduce it (to lactate or ethanol), carboxylate it (to oxaloacetate), transaminate it (to alanine) or feed it into biosynthesis. In many organisms, however, most pyruvate is **oxidized to acetyl-CoA**, the universal two-carbon “currency” used for the Krebs cycle, fatty-acid and isoprenoid synthesis, and fermentation.

Bacteria use three main oxidative reactions, and yeasts and some bacteria add a fourth, non-oxidative route. The main difference between them is what happens to the electrons and the carbon that is released.

2. The four routes at a glance

Route	Reaction & enzyme	Side product	Typical organisms	Typical conditions
1	Pyruvate + CoA + NAD ⁺ → acetyl-CoA + NADH + H ⁺ + CO ₂ — pyruvate dehydrogenase (PDH) complex	CO ₂ , NADH	<i>E. coli</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , yeast and human mitochondria	Aerobic respiration
2	Pyruvate + CoA + 2 Fd _{ox} → acetyl-CoA + 2 Fd _{red} + CO ₂ — pyruvate:ferredoxin oxidoreductase (PFOR)	CO ₂ , reduced ferredoxin (→ H ₂)	<i>Clostridium</i> , <i>Desulfovibrio</i> , <i>Helicobacter pylori</i> , <i>Giardia</i> , <i>Trichomonas</i>	Strict anaerobiosis
3	Pyruvate + CoA → acetyl-CoA + formate — pyruvate formate-lyase (PFL)	Formate (no CO ₂ , no NADH)	Enterobacteriaceae (<i>E. coli</i>), <i>Lactococcus lactis</i> (some conditions), <i>Chlamydomonas</i>	Anaerobiosis (O ₂ -sensitive)
4	Pyruvate → acetaldehyde + CO ₂ — pyruvate decarboxylase (PDC) ; then acetaldehyde + NADH → ethanol	CO ₂ , ethanol	<i>Saccharomyces</i> , <i>Kluyveromyces</i> , <i>Zymomonas mobilis</i> , plants under hypoxia	Alcoholic fermentation (also aerobic at high sugar in yeast)

Quick rule: with oxygen → PDH. Strict anaerobes → PFOR. Facultative anaerobes without oxygen → PFL. Yeasts and ethanol producers → PDC.

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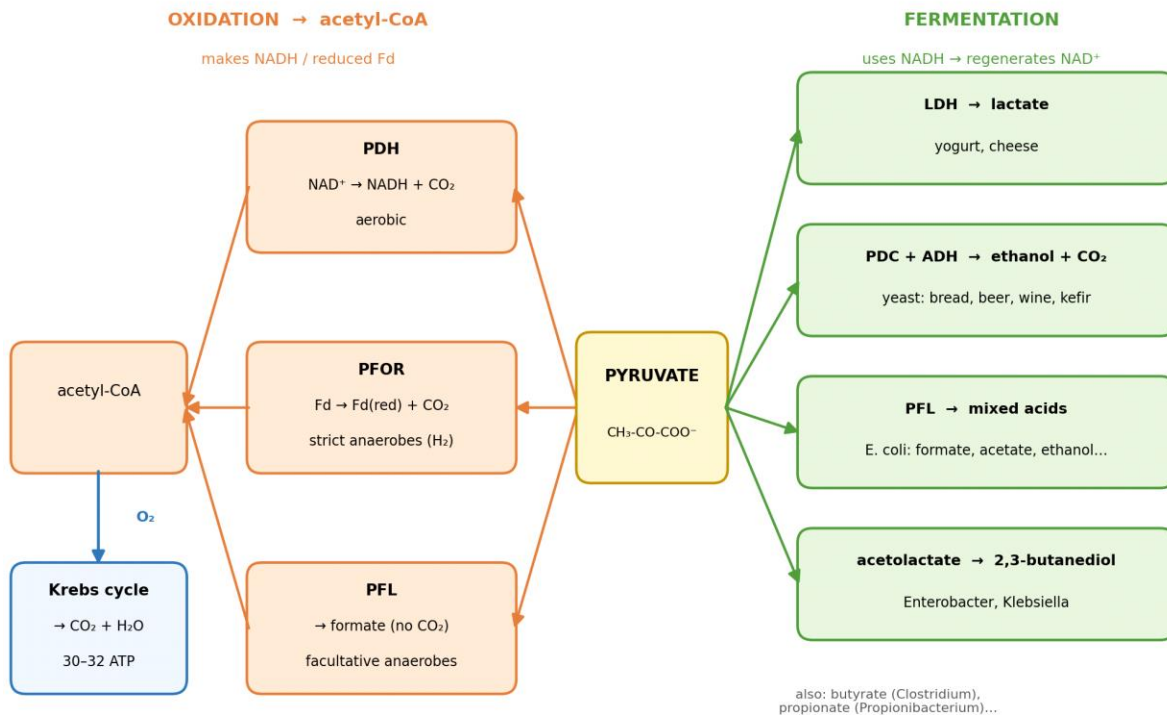
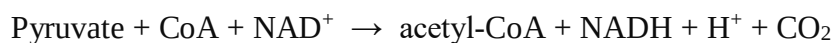


Figure 1. Pyruvate at the crossroads: oxidation routes (orange) make acetyl-CoA and NADH; fermentation routes (green) use NADH.

3. The routes in detail

3.1 Route 1 — Pyruvate dehydrogenase (PDH) complex



PDH is a large multienzyme complex made of three catalytic components, working in sequence like an assembly line:

- **E1** (pyruvate dehydrogenase, thiamine pyrophosphate – TPP): decarboxylates pyruvate.
- **E2** (dihydrolipoyl transacetylase, lipoamide): transfers the acetyl group to coenzyme A.
- **E3** (dihydrolipoyl dehydrogenase, FAD): re-oxidizes lipoamide and passes electrons to NAD⁺.

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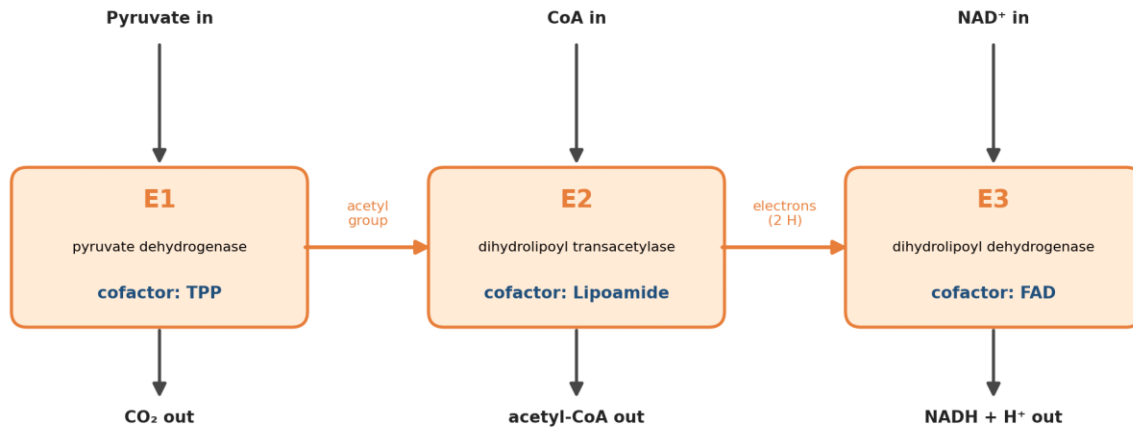


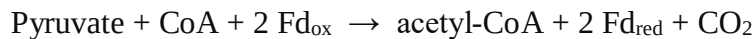
Figure 2. The PDH complex: three enzymes, three cofactors (TPP, lipoamide, FAD), and the mnemonic “Tender Loving Care For Nancy”.

It is found in all aerobic organisms. Its main role is to supply acetyl-CoA to the Krebs cycle, so that NADH can be re-oxidized by the respiratory chain.

Examples: in human cells PDH sits in the mitochondrial matrix and links glycolysis to the Krebs cycle. In *E. coli*, *Bacillus subtilis* and *Pseudomonas*, it is the main entry into the Krebs cycle during aerobic growth. In *Acetobacter* and other aerobes, it feeds respiration.

Regulation: PDH is slowed down when energy is abundant (high NADH, high acetyl-CoA, high ATP). In eukaryotes it is also switched off by phosphorylation (pyruvate dehydrogenase kinase) and switched on by dephosphorylation (phosphatase).

3.2 Route 2 — Pyruvate:ferredoxin oxidoreductase (PFOR)



PFOR is an iron–sulfur enzyme that uses TPP and transfers electrons to **ferredoxin** (Fd) instead of NAD⁺. It plays a major role in many strictly anaerobic bacteria and is highly sensitive to oxygen. Reduced ferredoxin is usually re-oxidized by a hydrogenase, which releases hydrogen gas (H₂).

Examples:

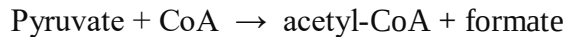
- *Clostridium* (e.g. *C. pasteurianum*, *C. acetobutylicum*): acetyl-CoA is converted to acetate, butyrate, butanol or acetone, and H₂ is released.
- Sulfate-reducing bacteria (*Desulfovibrio*) and the hyperthermophiles *Thermotoga* and *Pyrococcus*.
- Anaerobic protozoa such as *Trichomonas* and *Giardia* (in hydrogenosomes or the cytosol).

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Medical relevance: the antibiotic **metronidazole** is a prodrug that is activated when reduced by PFOR-derived ferredoxin. It therefore works against anaerobic bacteria and parasites (*Clostridioides difficile*, *Giardia*, *Trichomonas*) but not against aerobic human cells.

3.3 Route 3 — Pyruvate formate-lyase (PFL)



PFL cleaves pyruvate without producing CO₂ or NADH. It is a **glycyl-radical enzyme**: it has to be activated by a dedicated activating enzyme (which uses S-adenosylmethionine and a reduced flavodoxin), and **oxygen irreversibly destroys the radical**. For this reason PFL works only in the absence of oxygen.

It is widespread among facultative anaerobes, especially Enterobacteriaceae, and is also found in some phototrophic eukaryotes.

Examples:

- *E. coli*: under anaerobic conditions PFL drives **mixed-acid fermentation** (formate, acetate, ethanol, lactate, succinate). Formate can then be split into CO₂ and H₂ by the formate hydrogenlyase complex, which explains the gas production observed in the Durham tube or in the Voges–Proskauer / methyl red tests.
- *Streptococcus* and *Lactococcus lactis*: some strains switch from homolactic to mixed-acid fermentation (more formate, acetate and ethanol) when sugar is limiting or growth is slow. This is of interest in dairy fermentations.
- The green alga *Chlamydomonas reinhardtii* produces formate, acetate and ethanol through PFL when it is kept in the dark without oxygen.

3.4 Route 4 — Pyruvate decarboxylase (PDC): the ethanol route



Yeasts and some ethanol-producing bacteria have a fourth enzyme, PDC (TPP- and Mg²⁺-dependent), that removes CO₂ from pyruvate to form acetaldehyde. Acetaldehyde is then reduced to ethanol by alcohol dehydrogenase. This second step is essential because it **regenerates NAD⁺**, which allows glycolysis to continue without oxygen.

Examples:

- *Saccharomyces cerevisiae*: bread making (the CO₂ makes the dough rise), beer, wine and bioethanol.

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- *Zymomonas mobilis*: a bacterium that makes ethanol very efficiently (pulque, bioethanol research).
- *Kluyveromyces marxianus* and other lactose-fermenting yeasts in kefir and fermented whey: ethanol and CO₂ contribute to the light effervescence and flavor of the beverage.
- Plants such as rice and maize roots under flooding (hypoxia) switch on PDC and alcohol dehydrogenase to keep glycolysis running.

4. Oxidation vs. fermentation of pyruvate

These two terms are often confused. The simplest way to separate them is to follow what happens to **NADH**:

- **Oxidation of pyruvate** = pyruvate is converted to acetyl-CoA (it loses a carbon and electrons). It **produces** reducing power (NADH or reduced ferredoxin).
- **Fermentation of pyruvate** = pyruvate (or a compound made from it) is **reduced** by NADH. It **consumes** reducing power and regenerates NAD⁺, so that glycolysis can keep running without oxygen or any external electron acceptor.

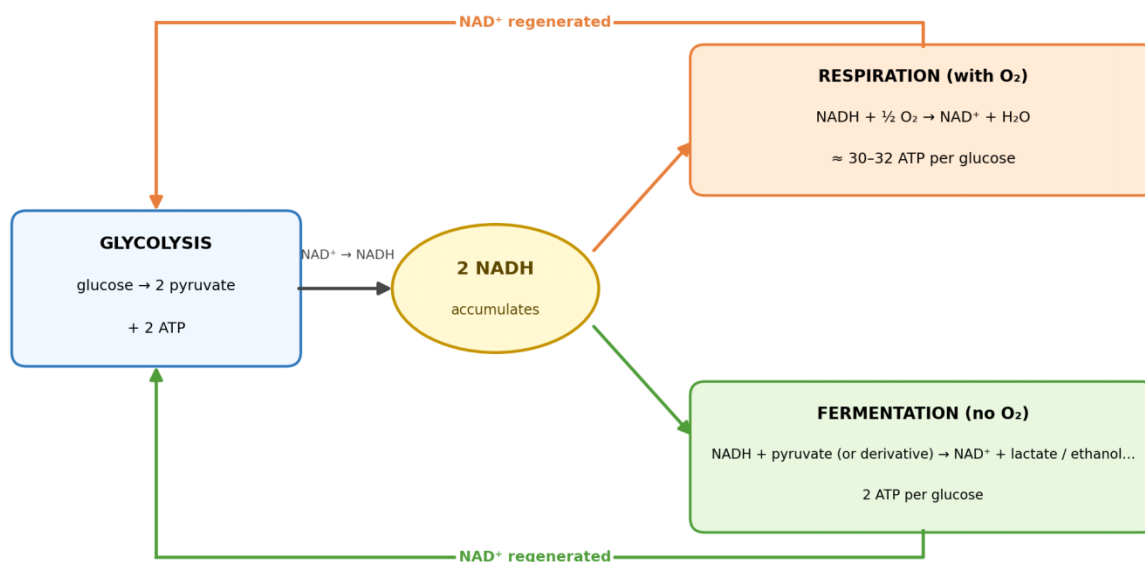


Figure 3. Redox balance: NADH made in glycolysis must be re-oxidized, either by respiration (with O₂) or by fermentation (without O₂).

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Criterion	Oxidation of pyruvate	Fermentation of pyruvate
Main goal	Make acetyl-CoA for the Krebs cycle and biosynthesis	Regenerate NAD^+ (redox balance) and keep glycolysis going
Fate of NADH	Produced (PDH: 1 NADH; PFOR: reduced Fd)	Consumed (re-oxidized to NAD^+)
Final electron acceptor	O_2 through the respiratory chain (aerobes); $\text{H}^+ \rightarrow \text{H}_2$ via hydrogenase (PFOR organisms)	An internal organic compound: pyruvate, acetaldehyde, acetyl-CoA, acetoacetate...
Oxygen	Needed for complete oxidation by PDH + Krebs cycle + respiration	Not needed (anaerobic or oxygen-independent)
Key enzymes	PDH, PFOR, PFL	Lactate dehydrogenase, PDC + alcohol dehydrogenase, acetaldehyde dehydrogenase, butyrate and propionate pathway enzymes
Fate of pyruvate carbon	Fully oxidized to CO_2 (Krebs cycle)	Only partly oxidized: products (lactate, ethanol, butyrate...) still contain much energy
ATP per glucose	High: about 30–32 with respiration	Low: 1 to 4, usually 2
Typical organisms	Aerobes, mitochondria, strict anaerobes (PFOR)	Lactic acid bacteria, yeasts, Enterobacteriaceae, <i>Clostridium</i> , <i>Propionibacterium</i>

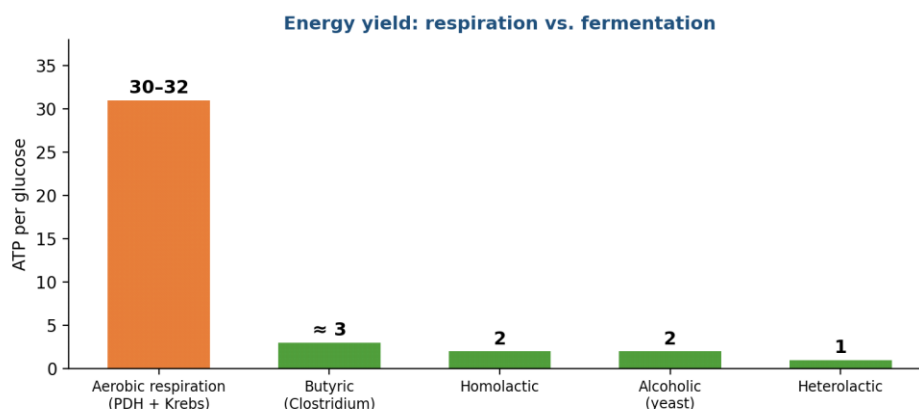


Figure 4. Energy yield per glucose: respiration gives about 15 times more ATP than fermentation.

Important nuance: PFL and PDC are not true redox reactions in the net balance (they split pyruvate without a net transfer of electrons to NAD^+). They are traditionally grouped with “oxidation” because they convert pyruvate into a two-carbon unit. What matters for fermentation is the **next** step: acetyl-CoA or acetaldehyde is reduced by NADH (for example, acetaldehyde $\rightarrow$ ethanol).

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5. Fermentation of pyruvate

5.1 The principle: redox balance

Glycolysis produces ATP but also reduces NAD^+ . The NAD^+ pool is small, so it must be regenerated:



- With oxygen: NADH is re-oxidized by the respiratory chain.
- Without oxygen: NADH is re-oxidized by **reducing pyruvate or a derivative**. This is fermentation.

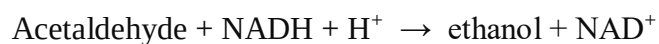
Golden rule: NADH produced = NADH consumed. Every fermentation pathway is built to balance this equation. The name of a fermentation comes from its main product (lactic, alcoholic, butyric, propionic...).

5.2 Key equations to know

Lactic fermentation (lactate dehydrogenase, LDH):



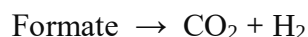
Alcoholic fermentation (PDC + alcohol dehydrogenase, ADH):



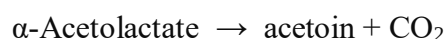
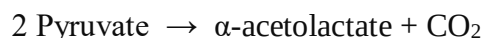
Ethanol from acetyl-CoA (mixed-acid and heterolactic fermentations; bifunctional ADHE or two enzymes):



Formate splitting in Enterobacteriaceae (formate hydrogenlyase):

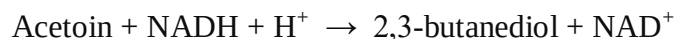


2,3-Butanediol pathway (neutral products, Voges–Proskauer positive):



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Propionic fermentation (Wood–Werkman cycle):



Why acetate and ethanol appear together: making acetate from acetyl-CoA gives an extra ATP but does **not** re-oxidize NADH. Making ethanol from acetyl-CoA consumes **2 NADH**. Cells therefore combine them to keep the redox balance (this is the case in mixed-acid and heterolactic fermentations).

5.3 Recap table of the main fermentations

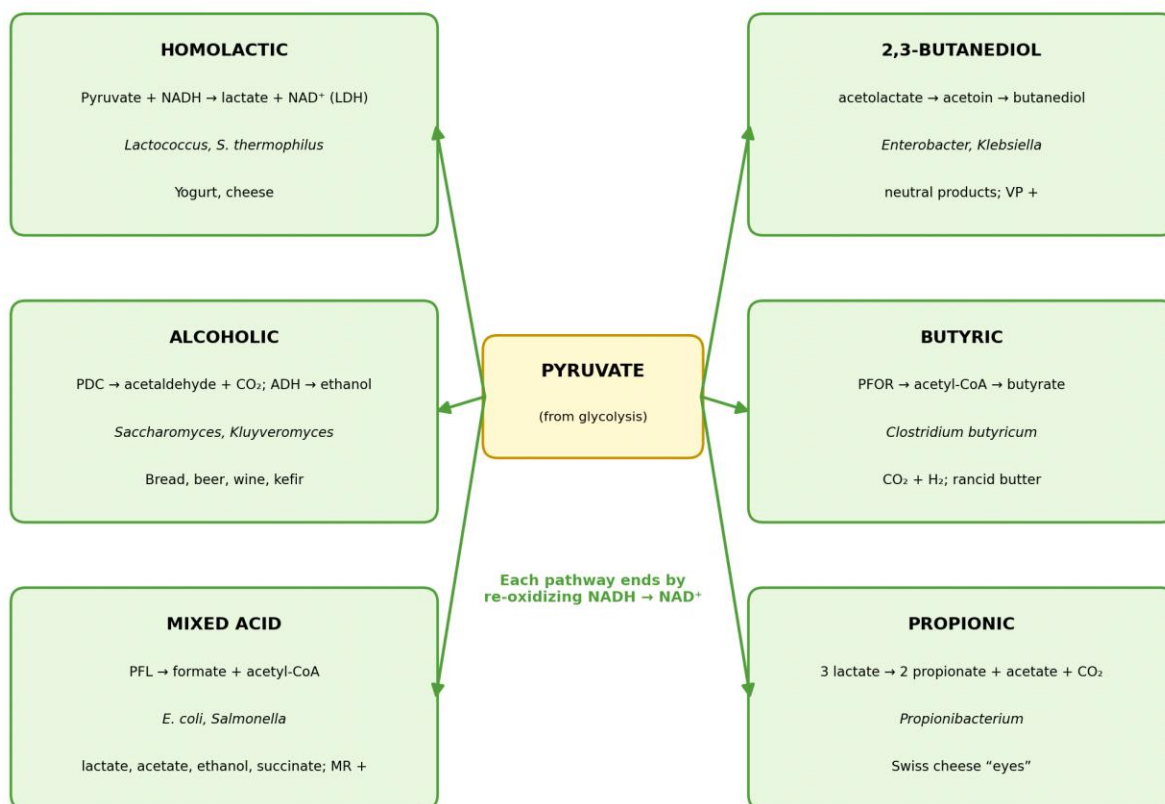


Figure 5. Map of the main fermentations: enzyme or pathway, typical organisms and examples.

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Type	Overall equation (per glucose)	Key steps	Typical organisms	Examples / tests	ATP
Homolactic	Glucose $\rightarrow$ 2 lactate	Pyruvate $\rightarrow$ lactate (LDH)	<i>Lactococcus</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i>	Yogurt, cheese, fermented milk	2
Heterolactic	Glucose $\rightarrow$ lactate + ethanol (or acetate) + CO ₂	Phosphoketolase pathway; acetyl-CoA $\rightarrow$ ethanol	<i>Leuconostoc</i> , <i>Lactobacillus fermentum</i> , kefir lactobacilli	Kefir, sauerkraut, kimchi; gas production	1
Alcoholic	Glucose $\rightarrow$ 2 ethanol + 2 CO ₂	PDC + ADH	<i>Saccharomyces</i> , <i>Kluyveromyces</i> , <i>Zymomonas</i>	Bread, beer, wine, kefir, bioethanol	2 (1 for Zymo.)
Mixed acid	Glucose $\rightarrow$ lactate + acetate + ethanol + formate ($\rightarrow$ CO ₂ + H ₂) + succinate	PFL; formate hydrogenlyase	<i>E. coli</i> , <i>Salmonella</i> , <i>Shigella</i>	Methyl red positive, VP negative; strong acidification	About 2–3
2,3-Butanediol	Glucose $\rightarrow$ 2,3-butanediol + ethanol + CO ₂ + H ₂ (+ some acids)	Acetolactate $\rightarrow$ acetoin $\rightarrow$ butanediol	<i>Enterobacter</i> , <i>Klebsiella</i>	VP positive, methyl red negative; less acid	About 2
Butyric	Glucose $\rightarrow$ butyrate + 2 CO ₂ + 2 H ₂	PFOR; hydrogenase; butyryl-CoA pathway	<i>Clostridium butyricum</i>	Rancid butter smell; gas; gut butyrate	About 3
Acetone–butanol–ethanol (ABE)	Glucose $\rightarrow$ butanol + acetone + ethanol + CO ₂ + H ₂	Two phases: acids first, then solvents	<i>Clostridium acetobutylicum</i>	Historic industrial solvent production (Weizmann)	Variable
Propionic	3 lactate $\rightarrow$ 2 propionate + acetate + CO ₂ + H ₂ O	Wood–Werkman (methylmalonyl-CoA) cycle	<i>Propionibacterium freudenreichii</i>	Swiss-type (Emmental) cheese “eyes”	Variable

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5.4 Other non-fermentative fates of pyruvate

- **Pyruvate oxidase** (pyruvate + O₂ → acetyl-phosphate + CO₂ + H₂O₂): in *Lactobacillus* and *E. coli* (PoxB), mainly in the presence of oxygen or in stationary phase.
- **Oxaloacetate** (pyruvate carboxylase or PEP carboxylase) and **alanine**: anabolic uses that refill the Krebs cycle or build amino acids.
- **Diacetyl** (buttery aroma): formed from α-acetolactate in *Lactococcus*, *Leuconostoc* and *Streptococcus thermophilus* in butter, buttermilk and some cheeses.