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Evolutionary causes and consequences of metabolic division of labour: why anaerobes do and aerobes don't

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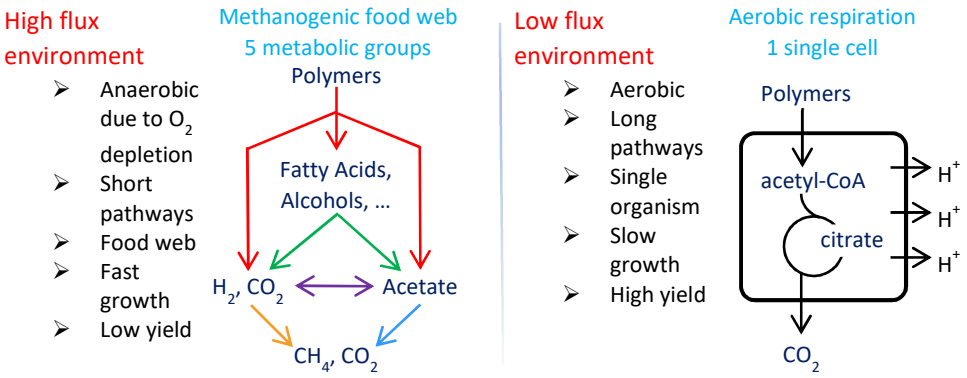
Running title: Evolution of metabolic division of labour

Key words: pathway length; rate yield trade-off; kinetic theory; overflow metabolism; evolution; thermodynamics; complete ammonia oxidation

Highlights

- **Catabolic division of labour is prevalent in anoxic, but not in oxic environments**
- **Anoxic environments are oxygen depleted because of a high influx of organic matter**
- **Such high flux favours evolution of shorter, incomplete pathways**
- **Aerobes are complete oxidizers because low flux, long pathways suffice and respiration makes more ATP at the end**
- **Bioreactors are high flux environments so microbes must be engineered to resist evolution to shorter pathways**

Graphical abstract



Abstract

Metabolic division of the labour of organic matter decomposition into several steps carried out by different types of microbes is typical for many anoxic – but not oxic environments. An explanation of this well-known pattern is proposed based on the combination of three key insights: (i) well studied anoxic environments are high flux environments: they are only anoxic because their high organic matter influx leads to oxygen depletion; (ii) shorter, incomplete catabolic pathways provide the capacity for higher flux, but this capacity is only advantageous in high flux environments; (iii) longer, complete catabolic pathways have energetic happy ends but only with high redox potential electron acceptors. Thus, aerobic environments favour longer pathways. Bioreactors, in contrast, are high flux environments and therefore favour division of catabolic labour even if aeration keeps them aerobic; therefore, host strains and feeding strategies must be carefully engineered to resist this pull.

Introduction

Methanogens 'sit at the end' of the food web for anaerobic degradation with CO_2 and methane as end products (Fig. 1). Why are methanogens not using sugars as substrates? Sugars would provide more energy. If methanogens used sugars themselves, there would be no anaerobic food web. Instead, they would reap all the benefits from doing all the catabolic work themselves. Mechanistic explanations such as lacking transporters or bottlenecks in metabolism cannot answer such questions because any such mechanistic details can change during evolution.

Aerobic environments, in contrast, have selected for microbes that oxidize their substrates completely (Fig. 1). That is, catabolic division of labour is not as ubiquitous as often claimed [1–4]. It is usual in anaerobic but unusual in aerobic environments. There are, however, apparent exceptions to this general pattern that we will examine later. First, we present our three-pronged argument that is, to our knowledge, the first explanation of this general pattern. Equipped with this conceptual framework, we will briefly explain what this means for biotechnological applications.

I. Shorter pathways can run faster if substrate supplies support it

The first prong of our argument is based on the kinetic theory of optimal pathway length developed by Heinrich, Holzhütter and Schuster [5]. For a longer overview see [6,7]. This theory makes three key assumptions: (1) Catabolic pathways evolved to maximise the rate of ATP production. This is equivalent to maximizing the specific growth rate, as ATP requirements for biomass formation (Y_{ATP}) are quite independent of the catabolic pathway. Note, however, that this common assumption is not correct for all environments, for example, the growth rate of biofilms is determined by growth yield rather than specific growth rate [8]. (2) The total concentration of all enzymes in a pathway is constrained because making enzymes costs ATP and precursors and the capacity of the cell to synthesize proteins is limited. (3) The total concentration of metabolites in a pathway is likewise constrained, e.g., because metabolites, especially at higher concentrations, can leak out of the cell or lead to side reactions or toxicity [9].

It follows from these assumptions that the concentrations of substrates and enzymes for each step have to be reduced if the pathway is lengthened. Since reaction rates increase with both increasing enzyme and increasing substrate concentrations, lengthening pathways strongly lowers substrate flux. Assuming that ATP gain in a pathway is proportional to pathway length (every step provides a little ATP, a simplification we address later), the rate of ATP production will be optimal at a certain, usually short pathway length [5]. Shortening a pathway can thus increase the specific growth rate, which would be an important fitness advantage under well-mixed conditions.

Kinetic theory of optimal pathway length explains acetate cross-feeding and incomplete ammonia oxidation

The theory has been applied to explain the evolution of cross-feeding via acetate in chemostat cultures of *Escherichia coli* [6] and the division of labour in nitrification [7]. Nitrification is the oxidation of ammonia via nitrite to nitrate, carried out by two different types of organisms in contrast to the chemically similar oxidation of methane to CO_2, which is carried out by a single organism with a complete oxidation pathway (Fig. 2). Since kinetic theory predicted that the shorter pathway of incomplete ammonia oxidation to nitrite increases specific growth rate but reduces growth yield, it was proposed that complete ammonia oxidation, nicknamed comammox, should have a fitness advantage in biofilms where substrate influx is slowed down and yield is crucial [7]. Comammox was then independently discovered – in biofilms – by two groups in 2015 [10,11]. After obtaining a pure culture, comammox growth parameters could be measured [12], confirming predictions by Costa *et al*. [7] that the specific growth rate should be lower and growth yield higher in comammox than in incomplete ammonia oxidizers. As expected, long solids retention times, or systems including an attached growth phase that also leads to long solids retention times, favour slower growing microbes such as comammox while high ammonia concentrations do not [13].

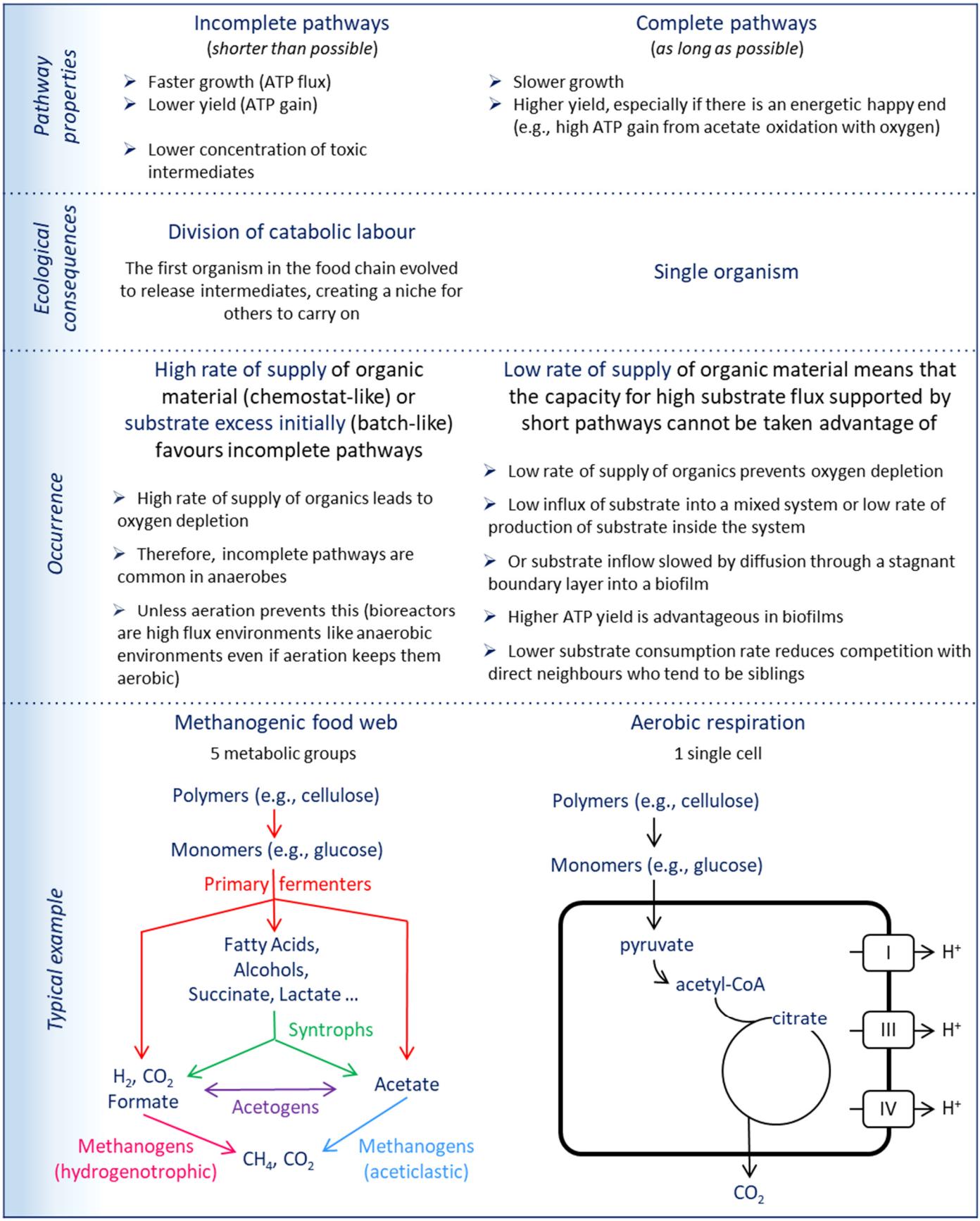


Figure 1. Why anaerobic conditions are coupled with high substrate flux and shorter pathways, leading to catabolic division of labour, in contrast to aerobic conditions.

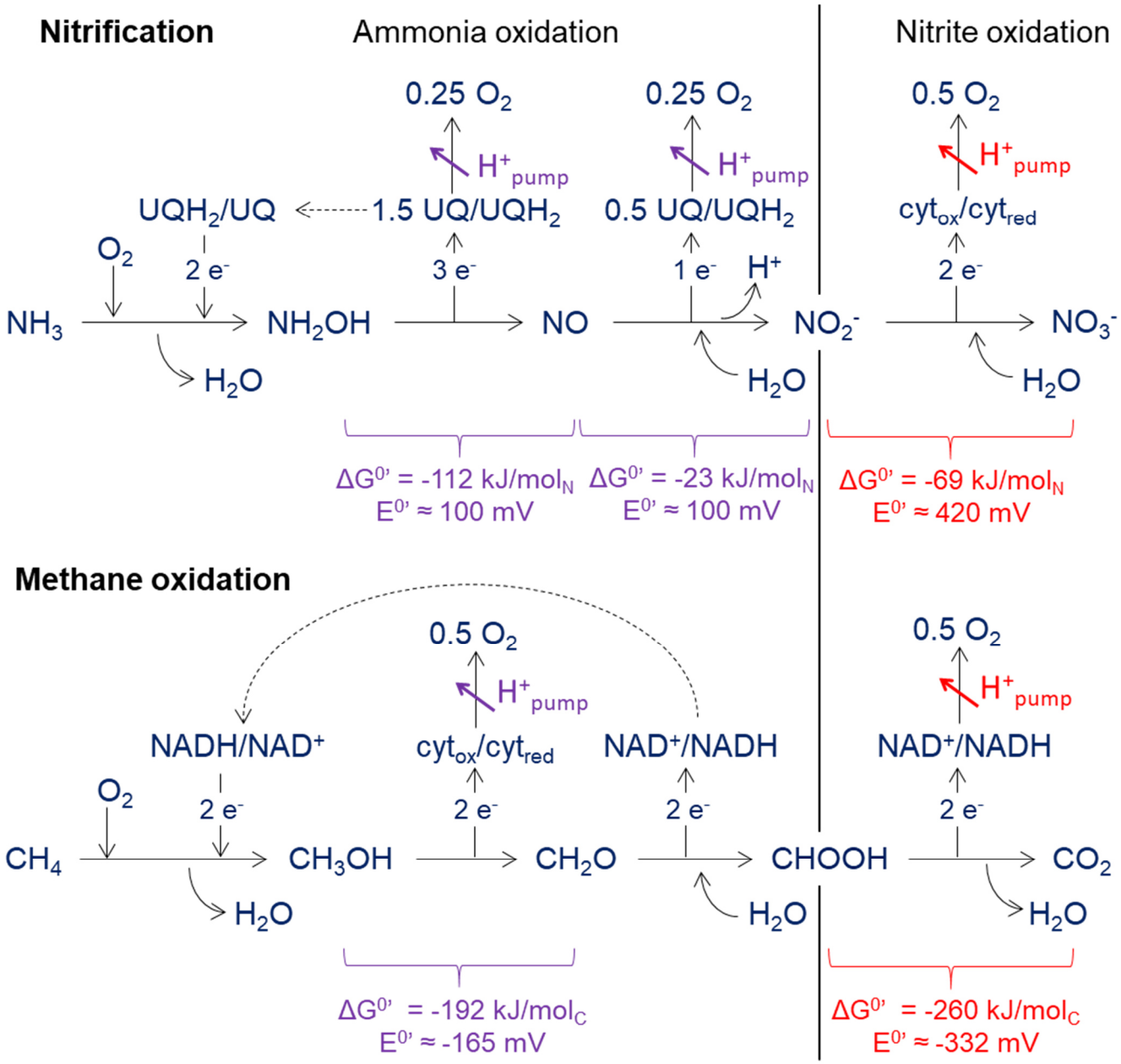


Figure 2. Compared to nitrification, the biochemically similar methane oxidation has an energetic happy end. The last oxidation step releases much more Gibbs free energy in methane oxidation than in nitrification, selecting for complete oxidation in methanotrophs and reducing the yield advantage for complete oxidation of ammonia. Simplified schematic.

Substrate affinity – important for fitness but ill understood

High substrate affinity is advantageous in biofilms [14] as well as in oligotrophic environments [15] that are mimicked in chemostats [16]. Hence, it is not surprising that the pure strain of comammox, *Nitrospira inopinata*, has a higher substrate affinity for ammonia than incomplete ammonia oxidizing bacteria. However, measurements so far do not cover the diversity of comammox and substrate affinity is even higher in one species of incomplete ammonia oxidizing archaea, which have a less ATP consuming CO_2 fixation pathway [12]. Comammox can also utilize nitrite, but has a low affinity for this substrate [12]. Costa et al. [7] did not attempt to predict substrate affinity due to our lack of understanding of substrate affinity from first principles that would be required to predict affinities of unknown organisms. This is an important gap to fill. While unfilled, dynamic kinetic models such as the theory of optimal pathway length have at least the potential to consider the effect of assumed or measured affinity, in contrast to stoichiometric flux-balance models. Disregarding affinity effects, recent stoichiometric modelling of optimal resource allocation across metabolic pathways came to the same conclusions as the theory of optimal pathway length, but recast this in terms of protein costs and limited solvent capacity of the cell [17–20].

Substrate concentration versus flux

We frame the discussion of optimal pathway length in terms of substrate influx into the environment, i.e., in terms of rate of substrate supply to consumers, rather than in terms of substrate concentration. This may seem an unimportant detail, but it is fundamentally important because a low substrate concentration in the environment could be the result of very high rates of production and consumption of this substrate. For example, hydrogen, which for thermodynamic reasons is maintained at very low concentrations, is nevertheless rapidly exchanged in interspecies hydrogen transfer between juxtaposed producers and consumers [21–23]. The advantage of shorter catabolic pathways is their capacity for higher substrate flux, which is only beneficial in environments where the rate of substrate supply is high, regardless of whether the substrate concentration is high or low. This is analogous to the advantage of a sports car with its capacity for high speed, which is only beneficial when speed is not limited by traffic and road conditions. However, its disadvantage of a low mileage or yield always exists.

Alternative explanations for division of labour in anoxic environments

Dolfing [22] has argued that the division of labour characteristic for the methanogenic food web can be explained by thermodynamics alone. However, converting glucose to methane and CO_2 is not a problem thermodynamically. It is more exergonic ($\Delta G^{\circ\prime} = -418\ kJ \cdot mol^{-1}$, calculated after [24]) than any partial conversion observed in the methanogenic food web. Consider splitting a pathway, converting S to P, into several steps. First, the overall thermodynamics from S to P would not change but the multi-step process would be more constrained thermodynamically as each step has to be exergonic enough to conserve ATP and all organisms would have to grow at the same specific growth rate to maintain the consortium long term. Second, extracellular transport of intermediates would require concentration (and Gibbs free energy) gradients and become slower with distance, imposing additional constraints on intermediate concentrations and fluxes. Third, intermediates could get lost before being taken up. Hence, thermodynamic limitations simply cannot be overcome by catabolic division of labour. Therefore, thermodynamics alone would not shed light on the pros and cons of complete versus incomplete oxidation of ammonia or organic matter under aerobic conditions, nor sugar metabolism via Embden-Meyerhof-Parnas (EMP) versus Entner-Doudoroff (ED) pathways [25,26].

Reduced overhead for regulating short catabolic pathways and narrow utilizable substrate ranges have also been suggested to explain the division of labour in anaerobes [21]. However, costs of regulating gene expression are far lower than costs of producing large numbers of catabolic enzymes [27]. Moreover, costs of regulating many pathways are likely higher than costs of regulating a single pathway whether long or short, since regulation is organized into operons.

II. Anoxic conditions result from high organic matter influx

The second prong of our argument is the recognition that those anaerobic environments we know about are anaerobic because they receive a high influx of organic matter. Examples are the cow rumen, anaerobic digesters, rice paddies or lake sediments. These environments are embedded in an oxic world, produced by oxygenic photosynthesis. They are only anoxic because any oxygen entering is rapidly depleted by the activity of aerobic microbes around the periphery and facultative anaerobes within the system. Here again what matters is the high rate of influx of degradable organic material, surpassing the rate of influx of oxygen required for its oxidation, not the concentration of the material in the environment as such. There is nothing magical about anaerobic metabolism *per se* that explains the prominence of short catabolic pathways, it is the high substrate flux that matters, combined with a low ATP gain at the end of the complete pathway, which we will discuss next.

III. Aerobic but not anaerobic catabolism has an energetic happy end

The third prong of our argument considers differences in the distribution of ATP conserving steps between anaerobic and aerobic pathways. Complete aerobic oxidation of glucose is highly exergonic ($\Delta G^{\circ\prime} = -2{,}870\ kJ \cdot mol^{-1}$) while its conversion to methane, using CO_2 as electron acceptor, supplies only 15% of this Gibbs free energy change ($\Delta G^{\circ\prime} = -418\ kJ \cdot mol^{-1}$) [24]. Other anaerobic food chains use terminal electron acceptors with redox potentials similar to CO_2 (around -250 mV) [24]. Not only is the total possible ATP gain much reduced, but also the distribution of ATP conserving steps over the methanogenic food chain gives the primary fermenters the poor man's lions' share while syntrophs and methanogens get even less [28,29]. This is in marked contrast to the energetic happy end in aerobic respiration, where about 63% of ATP is conserved by oxidizing acetyl-CoA compared to 37% from glucose to acetyl-CoA. Thermodynamically, denitrification yields 94% of the Gibbs free energy of aerobic respiration [24,30], but biochemically, fewer coupling sites are used so the ATP gain is only about 60% [31], though this is still much higher than other anaerobic respiratory chains. Moreover, there is still a happy end as more NADH is produced in the citric acid cycle than upstream and the oxidation of NADH in the respiratory chain is energetically equivalent regardless of where in the pathway NADH has been formed.

In summary, the anaerobic environments we know about harbour food chains where catabolic labour is divided into several short steps. This is because their conditions of high substrate influx, resulting in oxygen depletion, and low ATP gain in the final steps of substrate conversion, make short pathways produce ATP at a higher rate, but at the cost of a reduced growth yield. In contrast, aerobes usually oxidize their substrates completely as the capacity of short pathways for high flux is not needed in low influx environments and forsaking the happy end of aerobic respiration would be too costly (Fig. 1).

Examples and exceptions – how generally valid is our explanation?

The empirical support for what is typical in aerobic versus anaerobic environments summarized above comes from a range of examples that are shown in Box 1. More elucidating than supportive examples, however, are those cases which appear to contradict our reasoning. They are also discussed in Box 1. When compiling evidence, we need to be aware that our knowledge is heavily biased as better studied organisms were enriched in batch cultures, which select for fast growth under substrate excess, indeed yielding the typical short pathway organisms. Exceptional microbes with complete metabolism were discovered later.

From understanding pathway evolution to biotechnological applications

The above discussion aims to explain the driving forces for pathway evolution in natural microbial communities and why microbes either completely convert a substrate or divide the catabolic labour. Regarding biotechnological applications, one needs to recognize that there are many different applications with contrasting constraints and objectives. For example, bioprocesses to convert substrate to non-biomass product require complete conversion without any overflow metabolism. Here, product yield is paramount and biomass a waste product. Thus, single strains with long pathways are desired as they are far easier to engineer and easier to work with under controlled conditions [32,33]. Such organisms and processes are not necessarily engineered to completely oxidize substrates to CO_2 but to have a maximal product (but not biomass) yield, produced at sufficient (but not necessarily maximal) rates to be economical.

Since single "superbugs" are engineered against natural tendencies, it is important for the metabolic engineer to understand the evolutionary forces and metabolic trade-offs and how they depend on the environmental conditions. Bioreactors have high substrate influx like anaerobic environments typically do. Indeed, if bioreactors need to be kept aerobic, a massive aeration effort is required. Under these conditions, kinetic theory of optimal pathway length explains why engineering a process to increase specific growth rate above some critical rate may favour overflow metabolism and thus reduce product yield. Other theories that are also based on trade-offs in resource allocation, but do not consider enzyme kinetics, come to the same general conclusion [2,18]. The evolutionary tendency for an organism in well-mixed environments to maximize growth rate at the expense of making the desired product must be engineered against. This can be done by removing the genes that allow for overflow metabolism and carefully controlling substrate feeding rates. Including a "happy end" in terms of driving force in the product forming pathway will also improve the push to its completion. Industrial processes occur over much shorter time periods than communities evolve in nature, but biotechnology strains still must be stable through the required seed trains and large-scale operations, let alone the engineering cycles themselves.

Another type of application are processes to recover resources or energy from complex 'waste' materials, whether crop plant residues or sewage. Product yield is again crucial, but now the substrate is a complex mixture of unknown and variable composition of complex materials. In this case, a natural community of microorganisms dividing catabolic labour has advantages, in addition to faster conversion, due to its self-organized reconfiguration to the ever changing substrate composition [34]. Also, a diverse natural community would be more robust and resilient under environmental perturbations [32,35]. Indeed, this very robustness makes it difficult to engineer the environment in such a way that microbes with specific metabolic characteristics would be favoured [36]. For a recent analysis of mechanisms that have been proposed to improve the evolutionary robustness of metabolic cooperation, see [3,4,19,37].

Conclusions

Pathway length and distribution of ATP conserving steps are crucial for understanding the pros and cons of catabolic division of labour in particular pathways. In nature, aerobic catabolism usually oxidizes substrates completely because a higher capacity for substrate turnover would remain unused in the low substrate influx environments that remain aerobic. Moreover, the final stage of aerobic catabolism, transferring electrons from acetyl-CoA oxidation to a high redox potential terminal electron acceptor, is an energetic happy end too good to miss. We show that most exceptions can be explained within our three-pronged conceptual framework. In biotechnological applications to convert substrate to non-biomass product, single strains without overflow metabolism are required and engineering strategies must be devised to increase flux through the product forming pathway without decreasing product yield.

Box 1. Examples of catabolic pathways, exceptions and explanations.

The rules	Example(s) confirming rule	Exception(s)	Explanation(s) of exception(s)
Long pathways in aerobic microbes (and denitrifiers using high redox potential electron acceptors)	Complete oxidation of sugars to CO_2 [25,38]	Crabtree positive yeasts, cancer cells, Acetic acid bacteria	Overflow metabolism; Boost of specific growth rate if substrate supply rate is high; Spoilage
	Methane oxidation	—	Energetic happy end (Fig. 2)
	Complete ammonia oxidation (comammox)	Incomplete ammonia oxidation	Unhappy end so comammox less advantageous (Fig. 2)
	Complete denitrification	Partial denitrification [39,40]	All NO_x reductions have equivalent ATP gain. Shorter pathway has rate advantage if electron acceptor supply not limiting [31]
Short pathways in anaerobic microbes (using low potential electron acceptors)	Methanogenic food chain consists of several steps [23]	—	A hypothetical sugar consuming methanogen would have a tiny fitness advantage in yield-favouring biofilms with very low substrate influx
	Incomplete oxidation of fermentation products such as lactate to acetate by some sulfate reducers; oxidation of acetate to CO_2 by other sulfate reducers [41]	Sulfate reducing hyperthermophilic archaeon *Archaeoglobus fulgidus* oxidizes starch to acetate (less incomplete) and lactate to CO_2 [42]	Unclear
	Anaerobic methane oxidation with sulfate as electron acceptor in two steps [43]	Anaerobic methane oxidation with nitrate as electron acceptor in one step [44]	Nitrate reduction to nitrite has much higher redox potential (+433 mV, cf. O_2 +818 mV) than sulfate reduction (-210 mV)
	Demethylation and aromatic ring degradation by two different metabolic types	Demethylation and aromatic ring degradation by *Holophaga foetida* [45]	Higher growth yield is advantageous in biofilms where substrate influx is limited [8]
	Incomplete reductive dehalogenation of tetrachloroethene to dichloroethene	Complete reductive dehalogenation of tetrachloroethene [46]	Unclear. Maybe requiring others to remove toxic dichloroethene might disadvantage incomplete dehalorespirers when they are sparsely distributed

Explanations expounded

- Overflow metabolism: Mechanistic explanations for overflow metabolism that invoke insufficient capacity for respiration [47] or other bottlenecks do not address fitness advantages of pathways since such bottlenecks could evolve away if there were a fitness advantage.
- Boost of specific growth rate if substrate supply can support it: This type of explanation of overflow metabolism invokes trade-offs in resource allocation or the similar macromolecular crowding/solvent capacity constraint [17,48], which are fundamental so cannot be overcome by evolution. Demonstrated to explain incomplete oxidation of glucose in *Acetobacter methanolicus* [49], acetate overflow in *Escherichia coli* [17], Crabtree effect in budding yeast and the Warburg effect in cancer and other rapidly propagating mammalian cells [48,50].

- Spoilage: In environments with initially high substrate concentration enabling rapid consumption, such rapid consumption of the substrate coupled with producing toxic metabolites (acids and alcohols) reduces the share that competitors can get from the substrate [48]. This could explain why some organisms use pathways that increase substrate consumption rates without also increasing specific growth rates. For example, *Zymomonas mobilis* grows at about the same rate as *Saccharomyces cerevisiae* but produces ethanol at twice the rate and half the ATP yield, resulting in roughly equal specific growth rates [26]. This requires producers of toxic metabolites to have a high tolerance for the toxins to make sense.

References and recommended reading

1. Brenner K, You L, Arnold FH: Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol 2008, 26:483–489.

2. Bernstein HC, Paulson SD, Carlson RP: Synthetic *Escherichia coli* consortia engineered for syntrophy demonstrate enhanced biomass productivity. J Biotechnol 2012, 157:159–166.
   ●Shows that overflow metabolism reduces biomass yield, which can be avoided by a second scavenging strain in batch, chemostat and biofilm systems.

3. Stump SM, Johnson EC, Klausmeier CA: How leaking and overproducing resources affect the evolutionary robustness of cooperative cross-feeding. J Theor Biol 2018, 454:278–291.

4. Stump SM, Johnson EC, Sun Z, Klausmeier CA: How spatial structure and neighbor uncertainty promote mutualists and weaken black queen effects. J Theor Biol 2018, 446:33–60.

5. Heinrich R, Schuster S, Holzhütter HG: Mathematical analysis of enzymic reaction systems using optimization principles. Eur J Biochem 1991, 201:1–21.
   ●●Seminal paper showing that there is an optimal length for a metabolic pathway.

6. Pfeiffer T, Bonhoeffer S: Evolution of cross-feeding in microbial populations. Am Nat 2004, 163:E126–E135.

7. Costa E, Pérez J, Kreft J-U: Why is metabolic labour divided in nitrification? Trends Microbiol 2006, 14:213–219.

8. Kreft J-U: Biofilms promote altruism. Microbiology 2004, 150:2751–2760.
   ●Demonstrates the crucial importance of growth yield rather than rate for fitness in biofilms.

9. Bar-Even A, Noor E, Flamholz A, Buescher JM, Milo R: Hydrophobicity and charge shape cellular metabolite concentrations. PLOS Comput Biol 2011, 7:e1002166.

10. Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, et al.: Complete nitrification by *Nitrospira* bacteria. Nature 2015, 528:504–509.
    ●●One of two independent discoveries of single organisms oxidizing ammonia completely to nitrate.

11. van Kessel MAHJ, Speth DR, Albertsen M, Nielsen PH, Op den Camp HJM, Kartal B, Jetten MSM, Lücker S: Complete nitrification by a single microorganism. Nature 2015, 528:555–559.
    ●●One of two independent discoveries of single organisms oxidizing ammonia completely to nitrate.

12. Kits KD, Sedlacek CJ, Lebedeva EV, Han P, Bulaev A, Pjevac P, Daebeler A, Romano S, Albertsen M, Stein LY, et al.: Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. Nature 2017, 549:269–272.
    ●●Measurements of kinetics and yields of the first pure culture of comammox compared with other ammonia oxidizers.

13. Cotto I, Dai Z, Huo L, Anderson CL, Vilardi KJ, Ijaz U, Khunjar W, Wilson C, Clippeleir HD, Gilmore K, et al.: Long solids retention times and attached growth phase favor prevalence of comammox bacteria in nitrogen removal systems. bioRxiv 2019, doi:10.1101/696351.

14. Vannecke TPW, Volcke EIP: Modelling microbial competition in nitrifying biofilm reactors. Biotechnol Bioeng 2015, 112:2550–2561.

15. Button DK: Biochemical basis for whole-cell uptake kinetics - specific affinity, oligotrophic capacity, and the meaning of the Michaelis constant. Appl Environ Microbiol 1991, 57:2033–2038.

16. Jannasch HW: Estimations of bacterial growth rates in natural waters. J Bacteriol 1969, 99:156–160.

17. Basan M, Hui S, Okano H, Zhang Z, Shen Y, Williamson JR, Hwa T: Overflow metabolism in *Escherichia coli* results from efficient proteome allocation. Nature 2015, 528:99–104.
    ●●Demonstrates that overflow metabolism starts above a threshold specific growth rate for various substrates and strains.

18. Carlson RP, Beck AE, Phalak P, Fields MW, Gedeon T, Hanley L, Harcombe WR, Henson MA, Heys JJ: Competitive resource allocation to metabolic pathways contributes to overflow metabolisms and emergent properties in cross-feeding microbial consortia. Biochem Soc Trans 2018, 46:269–284.

19. Schepens D, Carlson RP, Heys J, Beck AE, Gedeon T: Role of resource allocation and transport in emergence of cross-feeding in microbial consortia. J Theor Biol 2019, 467:150–163.

20. Zeng H, Yang A: Modelling overflow metabolism in *Escherichia coli* with flux balance analysis incorporating differential proteomic efficiencies of energy pathways. BMC Syst Biol 2019, 13:3.

21. Schink B: Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 1997, 61:262–280.

22. Dolfing J: The microbial logic behind the prevalence of incomplete oxidation of organic compounds by acetogenic bacteria in methanogenic environments. Microb Ecol 2001, 41:83–89.

23. Müller N, Timmers P, Plugge CM, Stams AJM, Schink B: Syntrophy in methanogenic degradation. In (Endo)symbiotic Methanogenic Archaea. Edited by Hackstein JHP. Springer International Publishing; 2018:153–192.

24. Thauer RK, Jungermann K, Decker K: Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 1977, 41:100–180.

25. Fuhrer T, Fischer E, Sauer U: Experimental identification and quantification of glucose metabolism in seven bacterial species. J Bacteriol 2005, 187:1581–1590.
●●Seminal paper showing that the Entner-Doudoroff pathway is more common than glycolysis in non-model organisms.

26. Jacobson TB, Adamczyk PA, Stevenson DM, Regner M, Ralph J, Reed JL, Amador-Noguez D: ^{2}H and ^{13}C metabolic flux analysis elucidates in vivo thermodynamics of the ED pathway in *Zymomonas mobilis*. Metab Eng 2019, 54:301–316.

27. Kalisky T, Dekel E, Alon U: Cost–benefit theory and optimal design of gene regulation functions. Phys Biol 2007, 4:229–245.

28. Regueira A, González-Cabaleiro R, Ofiţeru ID, Rodríguez J, Lema JM: Electron bifurcation mechanism and homoacetogenesis explain products yields in mixed culture anaerobic fermentations. Water Res 2018, 141:349–356.

29. Smeaton CM, Van Cappellen P: Gibbs Energy Dynamic Yield Method (GEDYM): Predicting microbial growth yields under energy-limiting conditions. Geochim Cosmochim Acta 2018, 241:1–16.

30. González-Cabaleiro R, Ofiţeru ID, Lema JM, Rodríguez J: Microbial catabolic activities are naturally selected by metabolic energy harvest rate. ISME J 2015, 9:2630–2641.

31. Chen J, Strous M: Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution. Biochim Biophys Acta BBA - Bioenerg 2013, 1827:136–144.
●Excellent comparison of the energetics and kinetics of denitrification with aerobic respiration and why simultaneous use can make sense.

32. Kleerebezem R, van Loosdrecht MC: Mixed culture biotechnology for bioenergy production. Curr Opin Biotechnol 2007, 18:207–212.

33. Pachapur VL, Kutty P, Pachapur P, Brar SK, Le Bihan Y, Galvez-Cloutier R, Buelna G: Seed pretreatment for increased hydrogen production using mixed-culture systems with advantages over pure-culture systems. Energies 2019, 12:530.

34. Agler MT, Wrenn BA, Zinder SH, Angenent LT: Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol 2011, 29:70–78.

35. Carballa M, Regueiro L, Lema JM: Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr Opin Biotechnol 2015, 33:103–111.

36. Rombouts JL, Mos G, Weissbrodt DG, Kleerebezem R, Van Loosdrecht MCM: Diversity and metabolism of xylose and glucose fermenting microbial communities in sequencing batch or continuous culturing. FEMS Microbiol Ecol 2019, 95.

37. Wood KE, Komarova NL: Cooperation-based branching as a mechanism of evolutionary speciation. J Theor Biol 2018, 445:166–186.

38. Klingner A, Bartsch A, Dogs M, Wagner-Döbler I, Jahn D, Simon M, Brinkhoff T, Becker J, Wittmann C: Large-scale ^{13}C flux profiling reveals conservation of the Entner-Doudoroff pathway as a glycolytic strategy among marine bacteria that use glucose. Appl Environ Microbiol 2015, 81:2408–2422.

39. Carlson CA, Ingraham JL: Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *Paracoccus denitrificans*. Appl Environ Microbiol 1983, 45:1247–1253.

40. Lilja EE, Johnson DR: Segregating metabolic processes into different microbial cells accelerates the consumption of inhibitory substrates. ISME J 2016, 10:1568–1578.
●Engineered partial denitrification with nitrite as intermediate, demonstrating that toxic intermediates favour incomplete pathways similar to ammonia oxidation.

41. Widdel F, Pfennig N: A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum (emend.) acetoxidans*. Arch Microbiol 1977, 112:119–122.

42. Labes A, Schönheit P: Sugar utilization in the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324: starch degradation to acetate and CO_2 via a modified Embden-Meyerhof pathway and acetyl-CoA synthetase (ADP-forming). Arch Microbiol 2001, 176:329–338.

43. Scheller S, Yu H, Chadwick GL, McGlynn SE, Orphan VJ: Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. Science 2016, 351:703–707.

44. Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW: Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 2013, 500:567–570.

45. Kappler O, Janssen PH, Kreft JU, Schink B: Effects of alternative methyl group acceptors on the growth energetics of the O-demethylating anaerobe *Holophaga foetida*. Microbiology 1997, 143:1105–1114.

46. Seshadri R, Adrian L, Fouts DE, Eisen JA, Phillippy AM, Methe BA, Ward NL, Nelson WC, Deboy RT, Khouri HM, et al.: Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. Science 2005, 307:105–108.

47. Postma E, Verduyn C, Scheffers WA, Dijken JPV: Enzymic analysis of the Crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae*. Appl Env Microbiol 1989, 55:468–477.

48. Goel A, Wortel MT, Molenaar D, Teusink B: Metabolic shifts: a fitness perspective for microbial cell factories. Biotechnol Lett 2012, 34:2147–2160.

49. Müller RH, Babel W: Oxidative capacity determines the growth rate with *Acetobacter methanolicus*. Acta Biotechnol 1993, 13:3–11.

50. Shlomi T, Benyamini T, Gottlieb E, Sharan R, Ruppin E: Genome-scale metabolic modeling elucidates the role of proliferative adaptation in causing the Warburg effect. PLoS Comput Biol 2011, 7:e1002018.