Electron-Based Bioscience and Biotechnology
Masaharu Ishii • Satoshi Wakai
Editors

Electron-Based Bioscience and Biotechnology

Springer
Preface

Living organisms use electron in various biological reactions for energy conversion and signal transduction. For example, electron transport chain in respiration is well characterized, and biological component, which can transfer electrons, is also identified. In addition, various technologies using these electron-transferring properties have been developed, for example, microbial fuel cells and electrochemical biosensors.

On the other hand, novel phenomena related to living organisms and electron have recently been discovered, e.g., novel types of electron bifurcation processes, electrochemically active microorganisms, electrotrophic metabolisms, and metal corrosion by electron-consuming microorganisms. To deeply understand these novel phenomena, we must study using not only traditional biochemical methods, which are biologist friendly, but also electrochemical methods, which are non-friendly for biologists.

This book covers the novel findings and latest knowledge related to “living organisms and electron” as reviewed by experts consisting of senior and young scientists. Especially, this book is composed of three parts: Part I, Electron-Based Bioscience; Part II, Electron-Based Biotechnology; and Part III, Electron-Based Biocorrosion. Each part contains some chapters, and these chapters describe historical information, latest knowledge, and future application of each research field.

We believe that this book will help in deep understanding of relationship between living organisms and electron through detailed description of the electron flow during metabolic process in (micro)organisms and introduction of the development toward resolution of social issues and probable application. We named eBioX as a subtitle of this book. It means electron-based Bioscience, Biotechnology, and Biocorrosion. We hope that many scientists and students connect to each other through this book, and each research field would be fused and advanced in the near future.

Bunkyo-ku, Tokyo, Japan  
Masaharu Ishii
Yokosuka, Japan  
Satoshi Wakai
Contents

Part I  Electron Based Bioscience

1  Latest Knowledge of Electromicrobiology  .............................................. 3
   Satoshi Wakai

2  Importance of Electron Flow in Microbiological Metabolism  .................. 13
   Masafumi Kameya, Hiroyuki Arai, and Masaharu Ishii

3  Extracellular Electron Transfer in Bioelectrochemically Active
   Microorganisms  ................................................................................. 33
   Takashi Fujikawa and Kengo Inoue

4  Extracellular Electron Uptake Mechanisms in Sulfate-Reducing
   Bacteria  .......................................................................................... 43
   Xiao Deng and Akihiro Okamoto

5  Conversion of Electrical Energy into Life Energy  ................................. 61
   Norio Matsumoto

Part II  Electron Based Biotechnology

6  Electrochemical Interactions Between Microorganisms
   and Conductive Particles  ..................................................................... 73
   Souichiro Kato

7  Bioelectrochemical and Reversible Interconversion in the Proton/
   Hydrogen and Carbon Dioxide/Formate Redox Systems
   and Its Significance in Future Energy Systems  ................................. 81
   Yuki Kitazumi and Kenji Kano

8  Application of Enzymatic Reactions Involving Electron Transfer
   and Energy Supply for the Production of Useful Chemicals  ............... 101
   Jun Ogawa, Michiki Takeuchi, Akinori Ando, Ryotaro Hara,
   Makoto Hibi, and Shigenobu Kishino
9 Fatty Acid Production from Xylose by Xylose-Assimilating Thraustochytrid and Cellular NADPH/NADP⁺ Balance .................................. 121
Masahiro Hayashi, Ayako Matsuda, and Aya Nagaoka

10 Control of Microbial Metabolism by Electrochemical Cultivation Method .................................................. 129
Shin-ichi Hirano

Part III Electron Based Biocorrosion

11 Microbiologically Influenced Corrosion .................................................. 145
Satoshi Wakai

12 Electrochemistry on Corrosion Engineering ........................................ 159
Nobumitsu Hirai

13 Microorganisms Inducing Microbiologically Influenced Corrosion .................. 169
Takao Iino

14 Electron Flow Rate in Microbiologically Influenced Corrosion and Its Applications ........................................ 193
Satoshi Wakai

15 Biocorrosion and Souring in the Crude-Oil Production Process ........ 207
Kazuhiko Miyanaga

16 Effect of Metallurgical Factors on Microbial Adhesion and Microbiologically Influenced Corrosion (MIC) ........ 217
Yasuyuki Miyano and Sreekumari Kurissery
Part I

Electron Based Bioscience
Chapter 1
Latest Knowledge of Electromicrobiology

Satoshi Wakai

1.1 Introduction

Electrons and living cells are strongly related, particularly in terms of the electron transport chain of respiration and photosynthesis. In the electron transport chain, electrons move via various biomolecules from an electron donor to an electron acceptor. For example, in the mitochondrial electron transport chain, NADH dehydrogenase, quinone, cytochrome $b_{c_1}$ complex, cytochrome $c$, and cytochrome $c$ oxidase are involved in electron transfer from NADH as an electron donor to oxygen as an electron acceptor. Such electron transfer among biomolecules is effective for biological energy conversion because the process is directly connected to each other without diffusion of the reacting substances into water fraction, cytosol and extracellular solvent. Electron transfer in the field of biology is common. However, little is known about inter- and intramolecular electron transfer, although many studies in related fields have been performed.

Recently, novel types of electron bifurcation systems and electron transfer systems such as extracellular electron transfer have been reported. The first study of an electron bifurcation system was performed using cytochrome $b_{c_1}$ complex, which is a member of the electron transport chain in respiration (Mitchell 1975, 1976); additionally, novel types of flavin- and metal-based electron bifurcation systems in various energy metabolism processes were recently discovered (Li et al. 2008; Peters et al. 2019; Yuly et al. 2019). Similarly, various extracellular electron transfer systems such as outer membrane multi-heme cytochrome, electron conductive pili, and diffusible mediator have recently been characterized. Studies of microbial fuel cells would contribute to the understanding and utilization of these microbial energy
metabolism processes. Thus, “electron-based bioscience” and “electron-based biotechnology” have progressed together.

Recent progress in the fields of electron-based bioscience and biotechnology may be related to increased studies of electromicrobiology in microbiology. This chapter focuses on the progression of electromicrobiology studies in microbiology field.

1.2 Brief History of Electromicrobiology

Although studies related to electromicrobiology have greatly advanced in recent years, conceptual research was performed a long time ago. In 1911, Potter reported that yeast and bacteria can produce electric currents during their metabolism (Potter 1911). After that, many studies have been conducted for a long time, but the production of electric currents by microbial metabolism remained small (Fig. 1.1). In 1988, two different research groups reported two different iron- and manganese-reducing bacteria, Geobacter sp. and Shewanella sp., which are currently used as model microorganisms in the electromicrobiology field (Myers and Nealson 1988; Lovley and Phillips 1988). Both bacteria can consume organic acids and reduce iron oxide or manganese oxide; electrons liberated from the oxidation of organic acids are transported to extracellular insoluble materials as terminal electron acceptors. These studies were the first demonstration of extracellular electron transport.

This discovery of extracellular electron transport established the field of “electromicrobiology” and greatly contributed to the progression of microbial fuel cell research, as the current produced by electrochemically active microorganisms was notably higher than previously known (Kim et al. 1999; Logan et al. 2006; Lovley 2006). In these microorganisms, electrons flow from the cytosol outward to the extracellular space. Inward electron flow also occurs. A simple model of electron flow is iron and sulfur oxidation in the acidophile Acidithiobacillus ferrooxidans (Wakai 2019). Although this electron flow connects extracellular molecules and intracellular metabolism, studies have not focused on A. ferrooxidans as an electrochemically active microorganism for a long time. However, this microorganism contributed to the development of an electrochemical cultivation method. In this system, iron-oxidizing bacterium uses ferrous ion reduced by an external power supply and can grow to higher densities compared to in conventional batch culture (Matsumoto et al. 1999). Although this simple approach involved indirect electrochemical cultivation, the situation changed dramatically in 2015.

Ishii et al. demonstrated that the iron- and sulfur-oxidizing A. ferrooxidans can grow by consuming electrons directly from an electrode, known as electrolithoautotrophic growth (Ishii et al. 2015). The chemolithotrophic growth of this bacterium was demonstrated in 1951 (Temple and Colmer 1951), prior to the discovery of Shewanella and Geobacter. Extracellular electron transfer during metabolism in A. ferrooxidans has not been recently studied, but can be used as a model of electrotrophic metabolism. The term “electrotrophy” was firstly used in 2011 (Lovley 2011), although electrotrophic metabolism has not been well-defined.
Previously, electrotrophy-like metabolism was reported in iron-corrosive microorganisms (Dinh et al. 2004). Although the term electrotrophy was not evaluated by Dinh et al. (2004), it has been reported that novel types of sulfate-reducing bacteria and methane-producing archaea can corrode metallic iron (Fe\(^0\)) by directly consuming electrons in solid-state Fe\(^0\). In 1934, cathodic depolarization theory, which is the concept of iron corrosion by hydrogen-consuming sulfate-reducing bacteria, was reported (von Wolzogen Kühr and van der Vlugt 1934); since then, it has been thought that hydrogen-consuming microorganisms can corrode metal materials by consuming atomic and molecular hydrogen generated chemically on the cathodic area. However, almost all hydrogen-consuming microorganisms excepting for part of iron-corrosive microorganisms do not accelerate corrosion (Mori et al. 2010). In addition, Mori et al. demonstrated the presence of true iron-corrosive microbe from clearly accelerated iron dissolution (2010). Namely, the cathodic depolarization theory cannot explain corrosion mechanism by hydrogen-consuming bacteria, and
the reports of iron-corrosive microorganisms as electrotrophs have changed current thinking about the corrosive microorganisms.

Studies of electromicrobiology have progressed in various fields. Various types of electrochemically active microorganisms and energy metabolism processes have been discovered, and these discoveries can contribute to the development of new technology such as electrobiosynthesis, that is, established by combined research microbial fuel cells and electrotrophy.

1.3 Biological Molecules of Electrochemically Active Microorganisms

Microorganisms capable of transferring directly electrons to extracellular substances or acquiring electrons from extracellular substances are referred to as electrochemically active microorganisms. This unique ability is named as extracellular electron transfer, which differs from the electron transport chain that is completely on the inner membrane. The extracellular electron transfer system can be broadly divided into two modes, direct electron transfer and mediated electron transfer (Fig. 1.2).

*Shewanella* and *Geobacter* use direct electron transfer which involves biological molecules, with multi-heme cytochromes and cytochrome network as the key components (Nealson and Rowe 2016). This electron transfer process has been well-studied (Santos et al. 2015; Edwards et al. 2017). Recently, in addition to *Shewanella* and *Geobacter*, direct electron transfer systems were demonstrated in the iron-corrosive, sulfate-reducing bacteria *Desulfovibrio ferrophilus* and *Desulfobacterium corrodens* (Deng et al. 2015; Beese-Vasbender et al. 2015). Additionally, iron-corrosive methanogen *Methanococcus maripaludis* KA1 can corrode metallic iron by acquiring electrons from a metal surface (see Chap. 14), but does not contain a cytochrome system and likely possesses other biological molecules for this purpose.

*Shewanella oneidensis* MR-1 uses not only a direct electron transfer system but also a mediated electron system (Nealson and Rowe 2016; Gralnick 2012; Gross and El-Naggar 2015). In the mediated electron transfer in *Shewanella*, multi-heme cytochromes are cellular components, and diffusible flavin is an extracellular component. In addition to flavin, a menaquinone derivative and phenazine derivative have been reported as biological electron mediators (Newman and Kolter 2000; Rabaey et al. 2005). Flavin-based extracellular electron transfer systems are found in gram-positive bacteria (Light et al. 2018), which have a thick cell wall compared to gram-negative bacteria such as *Shewanella, Geobacter*, and iron-corrosive sulfate-reducing bacteria. Thus, their extracellular electron transfer system remained unknown for a long time. These findings further extend the distribution of extracellular electron transfer abilities in microbiology.

Furthermore, the concept of electron conductive biofilms has been reported (Lovley 2008). Direct extracellular electron transfer is ineffective because only
single-layered cells on the substrate surface acquire electrons. Mediated extracellular electron transfer proves ineffective because electron mediators are discrete in the natural environment. Also, several microbial cells survive by forming biofilms. The concept of electron conductive biofilms, comprising a combination of direct and mediated extracellular electron transfer systems, is reasonable because these systems effectively use limited substrate surface.

---

**Fig. 1.2** Various electron transfer systems. (a) Mitochondrial electron transport chain in respiration. This is not extracellular transfer system because electron transfer complete on the inner membrane. (b, c) Outward and inward electron transport chains in gram-negative bacteria. Outward type is multi-heme cytochrome type of extracellular electron transfer. Inward type is iron-oxidizing model in *A. ferrooxidans*. (d) Extracellular electron transfer using flavin in gram-positive bacteria.
1.4 Application of Electrochemically Active Microorganisms

Studies of electrochemically active microorganisms have mainly focused on *Shewanella* and *Geobacter*. These bacteria can transfer electrons from organic matter to electrodes in microbial fuel cells. Specifically, the energy metabolism of *Shewanella* and *Geobacter* can generate electric current, and hence these bacteria are known as “electric bacteria,” “electricigens,” and so on. However, the production of electric current is insufficient to replace thermal and nuclear power generation, and the technology of microbial fuel cells has been applied to waste water treatment. Its application in waste water treatment contributes to the reduction of solid waste materials and methane during operation.

On the other hand, these bacteria can transfer electrons in the opposite direction by controlling the potential. Specifically, electrons from an external power source can be transferred to molecules in the microbial cells. This allows the production of a variety of chemicals from CO₂ and small volatile fatty acids; this technology is known as electrobiosynthesis and microbial electrosynthesis. Recently, similar studies were carried out, and various volatile fatty acids such as acetate, propionate, butyrate, valerate, caproate, and succinate were successfully produced (Andersen et al. 2015; Prochaska et al. 2018; Prévot et al. 2019). Although low productivities and sources of external power in these systems pose challenging, this technology would contribute to reduce the emission of carbon dioxide.

In addition to microbial fuel cells and chemical production, there have been recent developments in the field of bioremediation using electrochemically active microorganisms. For example, soluble, toxic metal ions such as chromium and uranium could be reduced and deposited as nontoxic metal hydroxides (Hsu et al. 2012). Similarly, the possibility of electrobiochemical reduction of arsenate, selenate, and selenite has been reported (Kato 2015). Furthermore, the effective removal of nitrobenzene by the combination of *Shewanella* sp. and a mediator-modified matrix has been reported (Wang et al. 2013). The applications of electrochemically active microorganisms and their biological components are expected to expand.

1.5 Important Techniques in Modern Microbiology

1.5.1 Development of Sequencing Technologies

In the recent development of microbiology, next-generation sequencing technology has made a significant contribution. Microbial community analysis by meta-16S analysis has revealed several uncultured microorganisms and contributed to our understanding of microbial dynamics in the field of ecology. Metagenomic analyses have revealed the genomic structure of uncultured microorganisms in natural environments, including extreme environments, and discovered several novel and useful
enzymes by heterologous expression of homologous genes and unknown function genes. Metatranscriptomic analyses have revealed the functional dynamics of environmental microorganisms, including uncultured microorganisms. In the past decade, the equipment for next-generation sequencing has become a familiar research tool, and many researchers have been able to use the technology and related services on a daily basis. It is suggested that this technology has accelerated research on environmental microorganisms.

Although next-generation sequencing is a technique for analyzing a large amount of short reads, the technique of long-read analysis has become widespread recently and has become an indispensable technique for whole-genome analysis (Loman and Pallen 2015). Furthermore, as one of the nanopore sequencers that can perform long-read analysis, MinION has been developed, which can be used for fieldwork and can be carried out in the field of view; it is also called the fourth-generation sequencer. Its versatility is also being reported, including applications of whole-genome analysis performed using data from only this nanopore sequencer (Loman et al. 2015), metagenomic analysis (Moss et al. 2020), meta-16S analysis (Gonçalves et al. 2020), and metatranscriptome analysis (Semmouri et al. 2020). I has also succeeded in analyzing a tandem repeat structure (50–100 kbp in length), which is difficult for short-read sequencer analysis, by using it for genome resequencing analysis of genetically engineered filamentous fungi (unpublished).

The handy-type nanopore sequencer is claimed to be useful in field work, and will be in demand for onboard analysis in long-term research cruises. In addition, in technical fields using microbial communities such as microbial fuel cells, it may be possible to use on-site microbial communities for quality control.

1.5.2 Novel Cultivation System

In the field of electromicrobiology, the technology of electrochemical cultivation has been established, and with developments in the field of microbial fuel cells, the electrochemical activities of various microorganisms have been revealed. The concept of electrotrophy as the third energy acquisition system of life has also emerged from this field. Electrochemical cultivation systems have become an essential tool for the isolation of electrotrophs and understanding of their physiology. Therefore, the departure from Koch’s established culture technology and the design of a novel culture technology needs to be explored further for the development of microbiology. In fact, several microorganisms are still uncultivable at present, and a technique for culturing them is required for discovering new biochemical phenomena and analyzing functions of genes with unknown functions.
1.6 Concluding Remarks

The relationship between life phenomena and electrons is not a novel discovery in the history of biology. In the field of microbiology, extensive research and development has occurred in electromicrobiology. This may be owing to contributions from applied research on the development of microbial fuel cells and the basic science of proposing a new concept of electrotrophy. In addition, the recent development of genome analysis tools has made a significant contribution to visualizing the whole picture of microorganisms. However, despite the revelation of the phylogenetic position of novel microorganisms and their genomic information, several aspects remain unelucidated. For further development of microbiology in the future, it is necessary to link vast genomic information to biological functions. For this, it will be necessary to stably culture huge amounts of uncultured microorganisms. On the other hand, culturing these dark matter microorganisms would remain impossible if we rely on traditional culture techniques alone; there is a need to establish a culture method based on a novel concept.

References


Chapter 2
Importance of Electron Flow in Microbiological Metabolism

Masafumi Kameya, Hiroyuki Arai, and Masaharu Ishii

2.1 Introduction

Many types of oxidative and reductive reactions occur within individual microbial cells to mediate intracellular metabolism and biological activities. These reactions are diverse, with more than 1800 Enzyme Commission (EC) number entries categorized in the oxidoreductases class (EC 1) in the ENZYME database (Bairoch 2000) till date (October 2019). These redox reactions coordinate to accomplish various physiological demands, such as energy conservation, anabolic biosynthesis, maintenance of cellular redox homeostasis, and antioxidant defense. Intracellular electrons flow through coordinated redox reactions arranged in complicated chains and networks. Understanding the regulated electron flow can form the basis for studies on electron-based bioscience, biotechnology, and biocorrosion as discussed in this book.

With this premise, we elaborate on the biological electron carriers operative in cellular metabolism (Sect. 2.2) and the redox reactions and pathways that govern the total electron flow in microbes (Sect. 2.3). Further, we discuss the ubiquitous energy synthesis system through the respiratory chain (Sect. 2.4). Finally, we will shed light on the unconventional mechanisms of electron transfer carried out by electron bifurcation systems (Sect. 2.5).
2.2 Biological Electron Carriers

Biological electron transfers are mediated by many kinds of biomolecules, including quinone/quinol, cytochromes, flavins (FMN and FAD), and disulfides. In this section, nicotinamide nucleotide coenzymes (Sect. 2.2.1) and ferredoxin (Sect. 2.2.2) are reviewed as primary electron carriers.

2.2.1 Nicotinamide Nucleotide Coenzymes:

\textit{NAD(H) and NADP(H)}

Among the various biological electron carriers, NAD(H) and NADP(H) serve as the primary electron acceptor/donor and contribute ubiquitously to most metabolic pathways. Because these two share a similar structure except for the presence/absence of an additional phosphate group, their redox potentials are comparable to each other \(E_0^0 = -320 \text{ mV}\). The oxidized forms, NAD\(^+\) and NADP\(^+\), get reduced by the transfer of a hydride ion (H\(^-\)) onto the nicotinamide ring. Due to this reaction mechanism, their oxidation and reduction require a simultaneous transfer of two electrons. The importance of this feature would be discussed in further detail for understanding two-electron/one-electron switch in the flavin-based bifurcation system (Sect. 2.5).

Several physiological differences exist between NAD(H) and NADP(H). One of them is their redox ratio; while NADP(H) is generally maintained in a reduced state, NAD(H) remains in a more oxidized state. It is reported that in bacterial cells, NADPH/NADP\(^+\) ratio ranges from 1.1 to 59, whereas NADH/NAD\(^+\) ratio is lower (between 0.032 and 0.27) (Spaans et al. 2015). Another difference occurs in the metabolic pathways in which these two coenzymes work; while NAD(H) is involved in catabolism and a wide range of metabolisms, NADPH works as an electron donor in anabolic and biosynthetic pathways such as photosynthesis and fatty acid synthesis (Spaans et al. 2015; Agledal et al. 2010). The highly reduced state of NADP(H) can promote biosynthetic metabolism by driving the NADPH-dependent reduction reactions. The difference in the redox balance between NAD(H) and NADP(H) also forms a key factor for an electron bifurcation system (NADH-dependent reduced ferredoxin: NADP\(^+\) oxidoreductase in Sect. 2.5.3) in which NADPH and NAD\(^+\) serve as an electron donor and acceptor, respectively.

2.2.2 Ferredoxin

Ferredoxin (Fd) is a small metalloprotein harboring Fe–S cluster(s) as the redox center. Fd can be classified based on the structure of its Fe–S cluster, such as [2Fe–2S], [4Fe–4S], [3Fe–4S], and [7Fe–8S]. In contrast to NAD(H) and NADP(H), an Fe–S
cluster in Fd physiologically transfers only one electron at a time. Due to its simple structure and wide distribution among organisms, Fd is presumed to be one of the evolutionarily oldest proteins and to be even more primitive than NAD(H) (Eck and Dayhoff 1966; Hall et al. 1971; Daniel and Danson 1995).

Another characteristic of Fd as an electron carrier is its low redox potential. Although Fds are diverse in their redox potentials ranging from $-500$ to $-340$ mV, many of them are around $-420$ mV (Valentine 1964; Tagawa and Arnon 1962; Buckel and Thauer 2018b), significantly lower than those of NAD(P)H. Thus, reduced Fd can provide a reducing power strong enough to drive energetically unfavored reactions that cannot be driven by NAD(P)H, such as H$_2$ production, CO$_2$ fixation, and nitrogen fixation.

2.3 Redox Reactions in Metabolism

2.3.1 Carbon Metabolism

2.3.1.1 Glycolysis

Conversion of one molecule of glucose to two pyruvate molecules is accompanied by the generation of reducing equivalents ($4e^-$). In the Embden-Meyerhof pathway in many bacteria, the reducing equivalents are transferred to NAD$^+$ by glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), followed by the NADH re-oxidation coupled to the respiratory chain (see Sect. 2.4). In bacteria where respiration is not functional, NADH is oxidized by the donation of electrons to glycolysis products, resulting in fermentation products such as ethanol and lactate. Another type of glycolytic pathway, the Entner-Doudoroff pathway, is operative in some microbes (Chen et al. 2016). This pathway donates electrons not only to NAD$^+$ but also to NADP$^+$, providing one NADH and one NADPH per one glucose oxidized. Instead of these pathways, glucose can be metabolized also through the pentose phosphate pathway (Stincone et al. 2015). It should be noted that the provision of NADPH for cellular biosynthetic reactions is one of the important roles of the pentose phosphate pathway because electrons are donated not to NAD$^+$ but only to NADP$^+$ in this pathway.

In archaea, some NAD-reducing reactions in glycolysis are replaced by Fd-reducing reactions. Glyceraldehyde-3-phosphate Fd oxidoreductase (EC 1.2.7.6) substitutes (or coexists with) NAD-dependent glyceraldehyde-3-phosphate dehydrogenase with the concomitant reduction of ferredoxin in archaeal species, including *Pyrococcus furiosus*, *Pyrobaculum aerophilum*, and *Methanococcus maripaludis* (Reher et al. 2007; Mukund and Adams 1995; Costa et al. 2013). As another example, pyruvate:Fd oxidoreductase (POR; EC 1.2.7.1) substitutes NAD-depending pyruvate dehydrogenase complex (PDH; EC 1.2.4.1, 2.3.1.12, 1.8.1.4), catalyzing oxidative decarboxylation of pyruvate to acetyl-CoA (Furdui and Ragsdale 2000). Fd reduced
by these reactions is used for hydrogenation as an electron sink or for driving methanogenesis in these archaea living in anaerobic environments.

### 2.3.1.2 Tricarboxylic Acid Cycle

The tricarboxylic acid (TCA) cycle is a major source of NADH and electron equivalents in many aerobic organisms. In this cycle, NAD$^+$ is reduced to NADH by isocitrate dehydrogenase (EC 1.1.1.42), 2-oxoglutarate dehydrogenase (OGDH) complex (OGOR; EC 1.2.4.2, 2.3.1.61, 1.8.1.4), and malate dehydrogenase (EC 1.1.1.37). In the other oxidative reaction catalyzed by succinate dehydrogenase (EC 1.3.5.1), electrons are transferred not to NAD$^+$ but to quinone because the reducing power provided by the oxidation of succinate to fumarate is weak ($E_0' = 33$ mV) (Thauer et al. 1977).

The TCA cycle produces reducing equivalents (8e$^-$ per 1 acetyl-CoA oxidized), but an electron sink is often unavailable in organisms grown in anaerobic environments. In these organisms, an incomplete TCA cycle functions in a “horseshoe” structure, being divided into two halves: an oxidative half cycle leading to 2-oxoglutarate and a reductive half cycle to fumarate (Jahn et al. 2007; Marco-Urrea et al. 2011). This incomplete cycle still provides TCA cycle metabolites as important precursors for various biosynthetic processes without producing excess electrons.

### 2.3.1.3 Carbon Fixation

To date, six metabolic pathways have been found to fix CO$_2$ in autotrophic organisms (Montoya et al. 2012; Berg and Ivanovskii 2009; Berg et al. 2010). All of the fixing pathways require reductants to convert CO$_2$ into metabolites. While Fd is used in three pathways (including the acetyl-CoA pathway and the reductive TCA cycle) out of the six, the other three pathways (including the Calvin cycle) rely only on NAD(P)H. Because the reduced Fd can more strongly drive the reaction due to its low redox potential than that of NAD(P)H, the Fd-dependent pathways require less ATP equivalents than the Fd-independent pathways.

The reductive TCA cycle is known as a “reversed” version of the TCA cycle, and the two cycles share homologous enzymes and reaction steps. However, the difference between them surfaces especially when they are analyzed from the perspective of electron carriers. Whereas PDH and OGDH in the TCA cycle use NAD$^+$ as the electron acceptor, the reverse reactions in the reductive TCA cycle are catalyzed by POR and OGOR using reduced Fd as the electron donor (Ikeda et al. 2010; Yamamoto et al. 2010). This difference is feasible because the carboxylation reactions catalyzed by POR and OGOR are energetically unfavorable and require strong reductants with a low redox potential. In addition, while succinate oxidation in the TCA cycle donates electrons to quinone, *Hydrogenobacter thermophilus* is reported...
to reduce fumarate using NADH as the reductant and not quinol (Miura et al. 2008). Considering that the redox potentials of NAD+/NADH are significantly lower than those of fumarate/succinate and quinone/quinol, the use of NADH is advantageous to drive the fumarate/succinate conversion irreversibly in the direction of the reductive TCA cycle.

### 2.3.2 Nitrogen Metabolism

Nitrogen forms a constituent of inorganic compounds with various redox states: +5 in nitrate (NO$_3^-$), +3 in nitrite (NO$_2^-$), +2 in nitric oxide (NO), +1 in nitrous oxide (N$_2$O), 0 for dinitrogen (N$_2$), −1 in hydroxylamine (NH$_2$OH), and −3 in ammonium (NH$_4^+$). Organisms utilize these compounds/intermediates as electron donors or acceptors in energy synthesis processes known as denitrification, anammox, and nitrification, as already reviewed elsewhere (Canfield et al. 2010; Stein and Klotz 2016; Kuypers et al. 2018).

Besides energy synthesis, reduction of inorganic nitrogen compounds occurs in cellular anabolism. Organisms can assimilate nitrogen only in the form of NH$_4^+$, thereby necessitating the reduction of the oxidized forms of nitrogen to NH$_4^+$ before assimilation. Assimilatory nitrate reductase (aNar) and nitrite reductase (aNir) catalyze the reduction of NO$_3^-$ into NH$_4^+$ in their coupling reaction. NH$_4^+$ is incorporated into Glu by the coupling reaction of Gln synthetase and Glu synthase (GOGAT) (Kameya et al. 2006). While many bacterial aNar and GOGAT are NAD(P)H-dependent, cyanobacteria and chloroplast possess Fd-dependent aNar and GOGAT, which had been considered to be “plant-type” enzymes. However, recent studies discarded this paradigm by reporting Fd-dependent aNar and GOGAT in non-phototrophs, such as hydrogen-oxidizing bacteria and haloarchaea (Martínez-Espinosa et al. 2001; Kameya et al. 2007; Zafrilla et al. 2011; Pire et al. 2014; Kameya et al. 2017).

Nitrogen fixation is a process where N$_2$ is reduced to NH$_4^+$ by nitrogenase (EC 1.18.6.1) with the aid of ATP hydrolysis and electron supply from Fd (Buckel and Thauer 2018b). Because ammonification of N$_2$ is energetically unfavorable, the use of Fd with a low redox potential is the most suitable resort to drive the reaction, as for the carboxylation reactions discussed above.

### 2.3.3 Sulfur Metabolism

As with nitrogen, sulfur forms various inorganic species at different redox states, for instance, −2 in thiosulfate (S$_2$O$_3^{2-}$), 0 in elemental sulfur, +4 in sulfite (SO$_3^{2-}$), and +6 in sulfate (SO$_4^{2-}$). Many microbes use sulfur species for electron transfer during energy synthesis and redox metabolism.