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| Tips and Tools

Demystifying anaerobic respiration: a problem-solving exercise

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ABSTRACT Anaerobic respiration reactions are of fundamental importance to global biogeochemical cycling of elements. Yet, the idea that cellular respiration can occur not only in the absence of oxygen but also involve the oxidation of inorganic substrates (e.g., AsO_3^{3-}, Fe^{2+}, H_2, H_2S, Mn^{2+}, NH_3, and S^0) is often foreign to many undergraduate students. This article describes a problem-solving exercise where students are introduced to the thermodynamic fundamentals of respiration with a particular focus on the role of redox (reduction-oxidation) potentials ($E_0{}'$). In the exercise, the students investigate how the difference in redox potential ($\Delta E_0{}'$) between different pairs of reductants and oxidants affects the range of permissible microbial metabolic reactions in natural environments when oxygen is absent.

KEYWORDS active learning, problem-based learning, anaerobic respiration, redox potential, metabolism

Microorganisms, especially prokaryotes (1), display an astounding metabolic diversity that is rarely appreciated outside the field of microbiology. Undergraduate courses in biochemistry tend to focus almost exclusively on aerobic energy metabolism (respiration) of carbohydrates and lipids. Many undergraduate students within biological subjects therefore tend to believe that respiration is synonymous with oxygen dependence and organic substrates.

Without a firm grasp of the fundamental principles that govern respiratory processes, the idea that respiration not only can occur in the absence of oxygen but can also involve inorganic substrates can at first appear alien to undergraduate students. The author teaches introductory microbiology as part of transdisciplinary programs in food science, crop science, and environmental science and has observed that undergraduate students often struggle to fully appreciate both the possibilities and constraints of microbial respiration under anaerobic conditions. Improving student understanding is clearly important since anaerobic respiratory processes play critical roles in the biogeochemical cycling of essential elements such as carbon, nitrogen, and sulfur—processes that are inexorably linked to agriculture and the natural environment.

Redox (reduction-oxidation) potentials lie at the heart of respiratory processes but only get a brief mention in most biochemistry textbooks (2). The redox potential reflects the relative affinity that a redox couple (e.g., O_2/H_2O) has for electrons, which determines whether a particular redox reaction is thermodynamically favorable or not. For example, the redox couple CO_2 (oxidized state)/CH_4 (reduced state) has a redox potential ($E_0{}'$) of –0.20 V under standard conditions (25°C and pH 7), while the redox couple O_2 (oxidized state)/H_2O (reduced state) has a redox potential of +0.82 V. In practical terms, this means that the oxidation of methane (the reductant) by O_2 (the oxidant) to produce CO_2 and water is thermodynamically favorable, while the reverse scenario (oxidation of water by CO_2 to produce O_2 and methane) is not. The change in free energy ($\Delta G°{}'$) can be approximated from the difference in redox potentials ($\Delta E_0{}'$) between oxidant and reductant using equation 1.

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$$\Delta G^{\circ\prime} = -n\,F\,\Delta E_0{}' \qquad \text{(Eq. 1)}$$

where *n* corresponds to the number of electrons transferred per molecule of oxidized reductant, and *F* represents the Faraday constant (96.48 kJ V^{-1} mol^{-1}). The relatively high redox potential of the O_2/H_2O redox couple means that any respiration reactions occurring in aerobic environments tend to use O_2 as the terminal electron acceptor (TEA) since it will yield the most energy. It is generally believed that following the acquisition of mitochondria through an endosymbiotic event, the ability of primordial eukaryotic cells to use O_2 as TEA in respiration was key to the subsequent diversification of eukaryotes (3).

The purpose of the following exercise is to introduce students to the thermodynamic fundamentals that govern respiration reactions and how this affects the range of permissible microbial respiration reactions in natural environments when oxygen is absent. The exercise also aims to familiarize students with the substances that commonly occur in anaerobic respiration reactions in nature, which can clear up common misconceptions such as confusing oxyanions with oxides (e.g., nitrite with nitrogen dioxide).

PROCEDURE

The current exercise was developed for bachelor-level students taking courses in introductory microbiology in either their first or second year as part of transdisciplinary programs in crop science, food science, or environmental science. These students had received some previous instructions on the concept of redox potentials as part of preceding courses in chemistry and biochemistry but not in the context of anaerobic respiration. The total number of students per class typically varied from 10 to 50 students. The suggested setup below is best performed with up to 20–30 students per session. For larger classes that cannot be split into smaller groups due to constraints on scheduling or teacher resources, the exercise should be possible to adapt to larger groups with some practical suggestions by the author, which is given at the end of this section.

At the start of the session, the class is divided into groups of 2–4 students per group. Each group is given print-outs of the attached student exercise kit (File S1), which consists of a reaction template sheet with instructions and formulae, a sheet with paper tokens of oxidants and reductants that students cut out with scissors, a table of redox potentials at standard conditions, and two graph paper templates (Fig. 1). The paper tokens are color-coded with respect to the element that is being oxidized or reduced. The oxidation state of the relevant element is indicated by the number in the top left corner of the token to help students keep track of which substances can act as reductants, oxidants, or both. The reaction template sheet allows students to easily swap one redox couple for another.

The teacher may begin the exercise by explaining the concept of redox potentials and how they derive from the relative electron affinity of competing oxidants. The mathematical relationship between the difference in redox potentials ($\Delta E_0{}'$) and free energy ($\Delta G^{\circ\prime}$) can be demonstrated using the archetypal example of aerobic glucose oxidation (equation 2).

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \qquad \text{(Eq. 2)}$$

Students are asked to calculate $\Delta G^{\circ\prime}$ using equation 1 and plot the result on the graph template provided. The graph template can also be projected onto a whiteboard. Students are then instructed to try other oxidants by swapping out the tokens of oxidant redox couple on the reaction template sheet, recalculate $\Delta E_0{}'$ and $\Delta G^{\circ\prime}$, and plot the results on the graph template. The linear relationship between $\Delta E_0{}'$ and $\Delta G^{\circ\prime}$ should quickly become apparent. Using further examples provided in the teacher's supplement (File S2), students are then instructed to try other combinations of reductants and

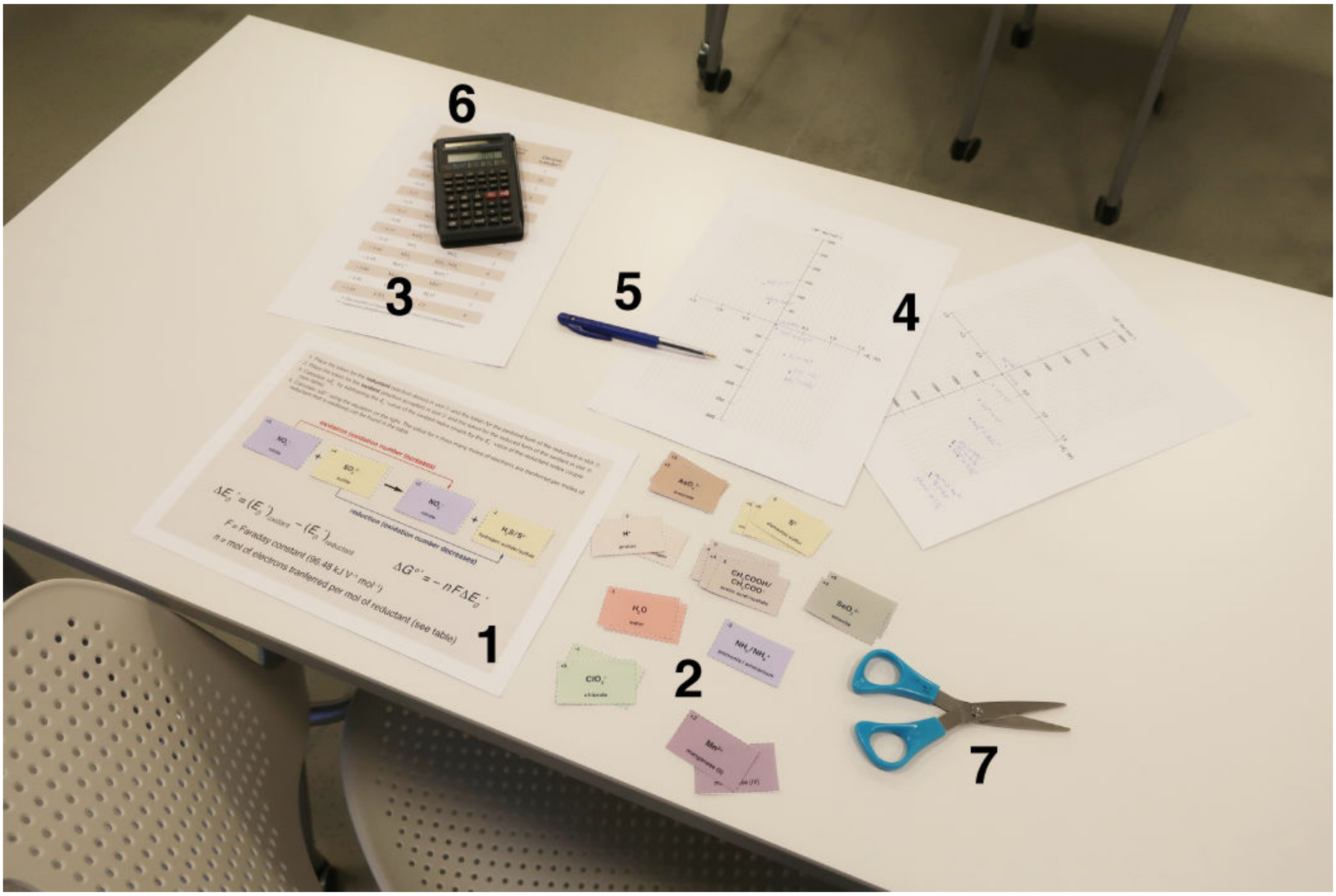

FIG 1 Overview of the printed student exercise kit (File S1), which includes a reaction template sheet with instructions and formulae (1), color-coded tokens of oxidants and reductants (2), a table with redox potentials under standard conditions (25°C and pH 7) (3), and two graph paper templates (4). In addition to the exercise kit, students will require a pen (5), a pocket calculator (6), and a pair of scissors (7).

oxidants by simply swapping tokens on the reaction template sheet, recalculate $\Delta E_0'$ and $\Delta G^{\circ\prime}$, and plot their results on the graph templates.

Finally, each group is given a set of substances that are either fully oxidized, fully reduced, or partially reduced/oxidized (solved examples are provided in File S2). The task is to determine which respiration reactions are thermodynamically favorable among the available substances by calculating the corresponding values for $\Delta E_0'$ and $\Delta G^{\circ\prime}$ for each pair of reductants and oxidants. Water, chloride, and Mn(II) are purposefully included as "red herrings" in most examples since their redox potentials are too high to serve as electron donors in most of the examples. The students are then asked to repeat the exercise but should now also include all the new substances that were formed during the first set of respiration reactions.

This final exercise illustrates how one anaerobic respiration reaction can supply new reductants and oxidants that make other respiration reactions possible, reactions that potentially are performed by completely different microorganisms. In anoxic marine sediments for example, organic carbon is oxidized by sulfate-reducing bacteria and archaea, which produce significant quantities of hydrogen sulfide, a large portion of which is then re-oxidized back to sulfate higher up in the sediment by sulfide-oxidizing bacteria and archaea that use electron acceptors such as molecular oxygen or nitrate (4).

CONCLUSION

The purpose of the present exercise is to make students reflect on the role that redox potentials play in constraining microbial chemotrophic metabolism in different environments when oxygen is absent. The author is aware that there are additional biogeochemically important redox reactions that are not covered in this exercise, most notably the latter half of the denitrification pathway. With a better understanding of redox potentials, students will also be able to appreciate why only some substances (e.g., AsO_3^{3-}, Fe^{2+}, NO_2^-, and S^0) can be used as electron donors for photosynthesis by bacteriochlorophyll-containing purple and green bacteria, while only chlorophyll-containing organisms such cyanobacteria and photosynthetic eukaryotes can generate sufficient redox potential (>+1.25 V) to be able to oxidize water and thereby produce O_2 (5).

The author has not yet conducted a formal assessment of how the current exercise format employing physical manipulation of paper tokens improves understanding of the thermodynamic principles of anaerobic respiration compared to a previous exercise format where students were limited to using just notepaper, a printed table of redox potentials, and a pocket calculator. However, the author has noted that students require fewer attempts at handing in correct answers following the introduction of the current exercise format, which would suggest that the students appear to grasp the principles faster.

For larger classes where time or resources do not permit working in smaller groups, teachers can modify the basic elements of the exercise by, for example, using on-line tools where students can submit answers, and the collective results are then visualized in real time. However, the author would still recommend that the physical exercise kit is distributed to individual students or groups of students. One possible approach is to use real-time online polling plug-ins within lecture slides for calculations of $\Delta E_0'$ and $\Delta G°'$. Such applications allow every student to submit their individual answers to a given problem, and the distribution of results for the entire class can instantly be plotted on the lecture slide. This has the added benefit of allowing the teacher to monitor what proportion of the class has grasped how to correctly perform the calculations for $\Delta E_0'$ and $\Delta G°'$. Another possibility is to use shared on-line documents for plotting graphs of $\Delta E_0'$ vs $\Delta G°'$, where every student or group of students could be responsible for a single data point each.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental file 1 (jmbe00044-24-s0001.pdf). Student exercise kit, which consists of a reaction template sheet with instructions and formulae, a sheet with paper tokens of oxidants and reductants (that students cut out with scissors), a table of redox potentials at standard conditions, and two graph paper templates.

Supplemental file 2 (jmbe00044-24-s0002.pdf). Teacher's supplement that contains detailed descriptions of suggested exercises.

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