Is There Life Without Oxygen?

By Payal Patel, Senior Majoring in Biological Sciences

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For the past two years as an undergraduate student, I have been doing research with Wiebke Ziebis, an assistant professor for BISC 120 who focuses on marine microbiology and marine biogeochemistry, which is the study of the cycles of chemical elements and their interaction with living things and biological systems. Dr. Ziebis's love for the ocean stems from her upbringing in Germany, a country located between the Baltic Sea and the North Sea. She set her sights on becoming an oceanographer, and thus spent her undergraduate years pursuing a degree in biological oceanography at the University of Kiel, an institution fittingly seated near the Baltic Sea. She received her Master's at the Institute for Marine Research in Kiel, a world renown institute for biological, physical, and chemical oceanography. Her independent research project included SCUBA diving and exploring the Mediterranean Sea. As a Ph.D. student at Max-Planck Institute for Marine Microbiology, one of the most prestigious research institutes in Germany, she began to concentrate on the seafloor, a topic that remains her current interest, and graduated with *summa cum laude*, which are the highest honors. She continued post-doctoral education working in the Mediterranean Sea on oxygen transport and its effects on the biogeochemistry and microbiology of the ocean floor with a European Union Project. She has worked with hydrothermal vents and methane seeps in research areas involved in understanding the role of methane in the global carbon cycle and how it affects every field spanning from plate tectonics to microbial ecology. An example of one of Ziebis's published studies includes the exploration of the relationship of methane-influenced pore water and its affect on foraminifera distributions and carbonate isotope geochemistry.

Ziebis concentrated on methane cycling in the marine environment at the Scripps Institution of Oceanography in La Jolla, California for three years and has been a part of the University of Southern California since 2004, where she continues her research in biogeochemistry and microbial ecology of the ocean floor. She is heavily involved in a newly established National Science Foundation (NSF) funded Center for Dark Energy Biosphere Investigations at USC, where she serves as a leader for a team that focuses on the “Activity in the deep subseafloor biosphere: function & rates of global biogeochemical processes,” one of the four themes of this center. The other three themes include “Extent of life: biomes and the degree of connectivity (biogeography & dispersal),” “Limits of life: extremes and norms of carbon, energy, nutrient, temperature, pressure, pH,” and “Evolution and survival: adaptation, enrichment, and repair.” Her research projects have spanned from coastal areas to the deep sea and deep subsurface. Ziebis is a certified research diver, an elected member of the Deep Submergence Science Committee, and has participated in a drilling expedition in the South Pacific Gyre, the Earth’s biggest system of ocean currents located south of the equator between South America and Australia, in 2010. She will join yet another drilling expedition from September to November to collect even more samples from the seafloor. A newfound area of interest lies in the deep subsurface biosphere (>1m depth). Current research indicates that there are abundant populations of microorganisms in the deep-sea environment, but we know very little about their metabolism. In my undergraduate research, I play a role in unveiling more about the metabolism of this deep biosphere.

The seafloor, which constitutes about 70% of our planet is a dynamic environment covered with sediment ranging in depth from a few meters to hundreds of meters. The average cell numbers of microorganisms in the sediment (10^6 to 10^{10} cells per cm^3) far exceeds the average prokaryotic abundance per volume in the ocean’s water column (10^4 to 10^6 per cm^3), which makes it the largest biome on Earth. Extremely little is known abut this hidden biome, which is generally thought to rely on the electron donor and acceptor supply of the overlying water or via settling of material to the seafloor. Oxygen, the most favorable electron acceptor, is thought to penetrate only a few millimeters into coastal seabeds. This means that most of the seafloor does not contain oxygen, making it an anoxic environment, and is dominated by microorganisms that gain energy through anaerobic processes which do not require oxygen. Only a thin layer of the surface is oxic, containing molecular oxygen. So, aerobic microorganisms, which are organisms that respire oxygen, are confined to this thin zone close to the sediment surface.

In a recent expedition to the center of the Atlantic Ocean, Ziebis’s research team discovered that oxygen actually penetrated up to nine meters into the seafloor. This is the deepest penetration of oxygen that has ever measured in the Atlantic and represents a much deeper penetration depth than anyone had predicted (Ziebis et al. submitted). This is significant because it goes against general research findings which demonstrates that most of the seafloor is anoxic. Oxygen transport into the sea floor and its effect on geochemistry has been a large focus of Ziebis since the 1990s. She found that processes other than molecular diffusion to transport oxygen into the deeper layers of the seabed exist, and carry a large impact on subsurface biogeochemical processes of the microbial committees. So this discovery of oxygen penetration to such a deep level opened up doors to much investigation. We are currently studying the microbiology and biogeochemistry in samples that were collected during this expedition. When we incubated sediment samples under oxic conditions we came across a surprising finding. As we were measuring nitrogen fixation in the gas chromatograph, we found that there was methane being produced in the sediment even though it was oxic. Methanogens are believed to be strict anaerobes because the enzyme involved in the reaction is inhibited by oxygen. But we repeated the incubation experiments, and found that there is definitely methane being produced, meaning that the general belief that methanogens are anaerobes could be wrong.

Methane is produced anaerobically by Archaea. There are two metabolic pathways that methane can be produced, via CO_2 reduction and acetate fermentation.

- CO_2 reduction: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (4)
- Acetate fermentation: $CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$

Both of these reactions were believed to be strictly anaerobic, until we came across the production of methane in the samples we collected. The production of methane through carbon dioxide reduction uses an oxygen-sensitive enzyme, which means methanogens that use this mechanism would not be able to tolerate oxic environments, but acetoclastic methanogens, which form methane through acetate fermentation, can actually tolerate oxic conditions. Whether or not they can be metabolically active is unknown.

Using radioisotopes, Ziebis's lab labeled ^{14}C of acetate and of carbon dioxide to follow which carbon was being incorporated into methane. This allowed them to determine that sediments did in fact contain organisms that produced methane via acetate fermentation, and not the oxygen-sensitive mechanism, carbon dioxide reduction. A metabolic process called methanotrophy uses methane and the oxidation of oxygen to gain energy. In most environments, oxygen and methane production are completely separated, but in the samples collected, Ziebis hypothesizes that there is a cycle of methane production and methane consumption. If in fact, these microbes have the ability to undergo methanotrophy, we will be able to demonstrate the existence of a very rare, but extremely efficient metabolic process. The methane process requires further investigation, and that is the basis of my research as an undergraduate. My research project includes investigation of this methane production and consumption in sediment incubation from the deep seafloor of the Atlantic. Measurements involve monitoring oxygen concentrations using microelectrodes, chemical analysis of methane using gas chromatography, and measurement of bicarbonate (CO_2) via a flow injection machine. In order to estimate the number of microorganisms at different stages of incubation, microbiological analysis is also being done. This includes the filtering, staining, and counting of cells. I have already found that all the samples do in fact contain oxygen, proving the environment is oxic and other metabolic trends involving methane and carbon dioxide are currently being determined.

Even though our research focuses on investigation at the microscope level, it actually applies to the human population a lot more than most people think. The majority of all biological processes like nitrogen fixation, recycling of nutrients, or breaking down of organic matter depend on microbes. The ocean makes up majority of Earth and contains an enormous flora of microbes that have yet to be discovered. The discovery of new metabolic processes provides us with an insight on how our atmosphere is being used and gives us further clues to evolutionary beginnings and how organisms work together in an ecosystem. Methane is a highly potent greenhouse gas, and thus plays an integral role in the control of Earth’s climate. Discovery of oxic methanogenic organisms opens up many more doors for investigation. With this discovery, the level of contribution and the number of contributors changes. While the effects of our lab’s research on global warming are still unknown, it is a topic that needs to be further analyzed. The detection of a new metabolic pathway that invalidates previous research on methanogens demonstrates that much of our accepted knowledge about biology has exceptions, and that new discoveries will never cease!