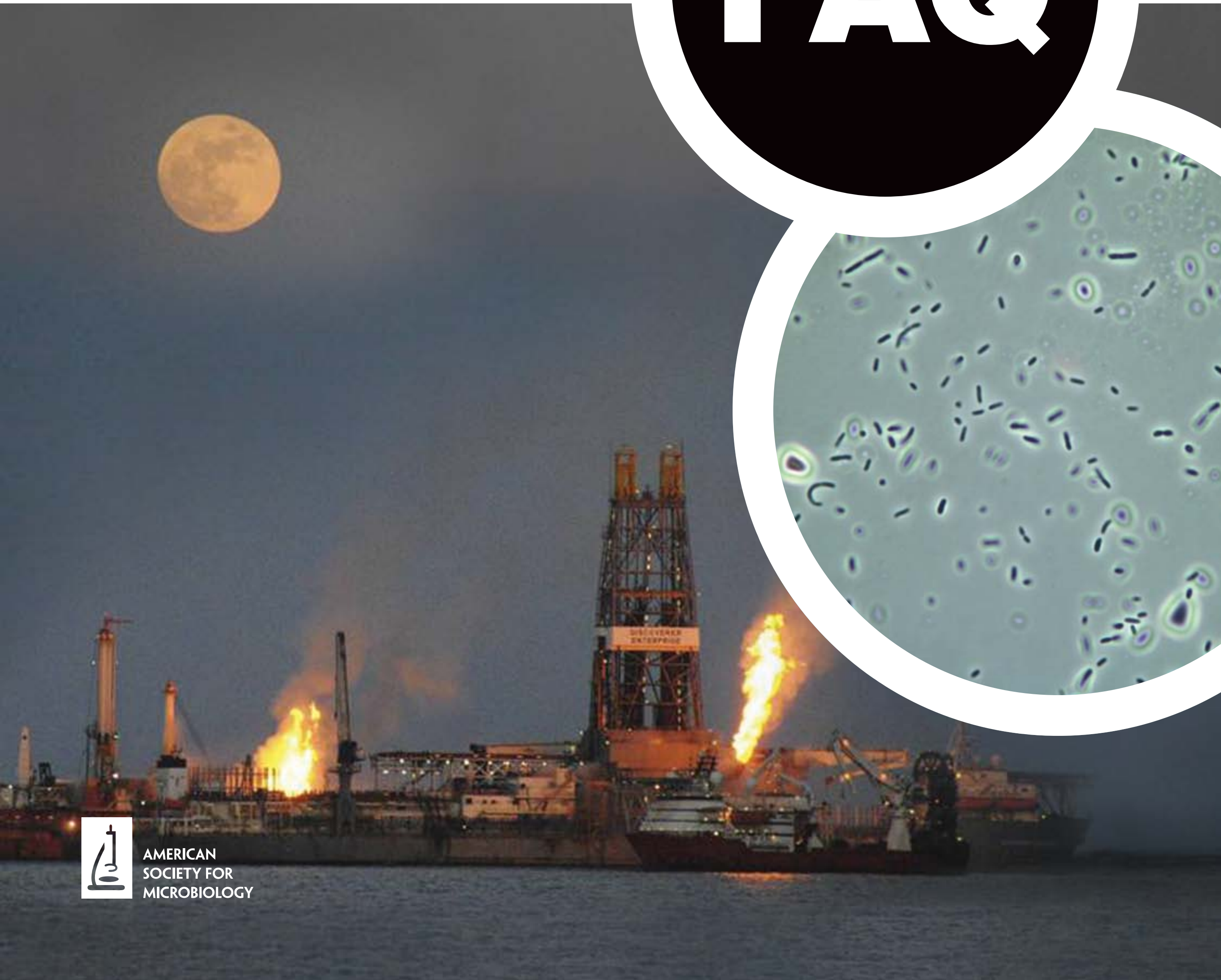
A REPORT FROM
THE AMERICAN ACADEMY
OF MICROBIOLOGY

MICROBES & OIL SPILLS

FAQ



AMERICAN
SOCIETY FOR
MICROBIOLOGY

FAQ

ABOUT ASM FAQs

The American Society for Microbiology (ASM), the world's oldest and largest life science organization, provides support for the FAQ series. The **American Academy of Microbiology** (Academy) manages the FAQ program. The Academy is the honorific leadership group within ASM; its mission is to recognize scientists for outstanding contributions to microbiology and provide microbiological expertise in the service of science and the public.

The FAQ series provides science-based information about important topics in which microbes play an important role. The reports are based on the deliberations of a group of Academy Fellows and other experts who come together for a day to develop clear answers to frequently asked questions about the FAQ topic. The Academy thanks the scientists listed below for their participation.

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1. What does it mean to say that microbes can 'clean up' an oil spill?

Let's begin by defining some terms – microbes, clean-up, and oil. First, what do we mean by microbes? When we talk about microbes that are able to clean up oil, we're talking primarily about bacteria and fungi. Bacteria can break down oil to carbon dioxide and water. However, no single organism can break down all the components of crude oil or refined fuels spilled into the environment. The tens of thousands of different compounds that make up oil can only be biodegraded by communities of microorganisms acting in concert. Some bacteria can degrade several hydrocarbons or a class of hydrocarbons. The combined action of the community can degrade almost all of the components **(BOX 1)**.

Second, what is oil? Crude oils—oils that are found in natural reservoirs—are principally derived from ancient algae and plant material. In other words, oil is a natural product, generated from organisms that long ago used sunlight as their energy source through the process of photosynthesis. The algae were buried deep in the Earth and heated at great pressure over millions of years. The resulting material is oil, in which is stored the energy generated by that ancient photosynthetic activity. Therefore, the components of crude oil are a great source of energy, not only as fuel to power internal combustion engines, but also as food for microbes. It is not surprising that microbes have evolved the ability to use oil as their food source—in other words, to metabolize or biodegrade the compounds for energy and use them as raw material for growth.

Much of the Earth's crude oil is trapped in underground reservoirs, but some leaks to the surface, and has been doing so for millions of years. It is estimated that about half of the oil entering the world's oceans today comes from these natural seeps and the rest from human

BOX 1: Eat, consume, metabolize, degrade, break-down… what are the microbes doing to the oil anyway?

Photosynthetic organisms use energy from sunlight to convert carbon dioxide and water into carbohydrates, proteins, and fats, with oxygen as a byproduct. Energy is stored in the newly formed chemicals. When the organisms die and are buried in sediment, these chemicals are not destroyed, but rather the heat and pressure deep underground convert them into a huge variety of different hydrocarbons – compounds that contain carbon and hydrogen. The energy stored in the chemical bonds can be released by burning (that's what happens in your car's engine), or by a more precise chemical reaction carried out by enzymes inside a living cell. These enzymes enable microorganisms to "combust" the hydrocarbons at much lower temperatures than burning.

The genetic machinery needed to make these oil-degrading enzymes is most commonly found in bacteria (although many fungi and some other organisms can also degrade oil). The bacterial cell then harnesses the energy released by degrading the compounds to support its own life processes. The situation is exactly analogous to the way our bodies break down the chemical energy in food to provide the energy and raw materials for maintenance, growth, and repair of our tissues. There are many different varieties of hydrocarbons, and over millions of years, bacteria have evolved catalytic machines (enzymes) that are specific for particular degradation reactions. Some of the simpler compounds can be degraded by a very wide variety of bacteria, but the ability to degrade other compounds (aromatic hydrocarbons, for example) is found in fewer species. No one bacterium can make all the different enzymes – instead, each kind of bacterium specializes in a few hydrocarbons as preferred food sources.

Most microbial oil degradation occurs by aerobic respiration, in other words, the oil-degrading microbes "breathe" oxygen and burn oil hydrocarbons just as humans breathe oxygen and burn food for energy. In the absence of oxygen, microbes have other mechanisms to degrade hydrocarbons for energy. Biodegradation of oil constituents without oxygen (i.e., under anoxic conditions) is much slower but anoxic processes may be relevant to the long-term restoration efforts (e.g., in oil-contaminated salt marsh environments).

From the human point of view, what the microbes are doing is degrading—or breaking down—the oil, which results in cleaning up the environment that has been contaminated by the spill. From the microbial point of view, what they are doing to the oil is "eating"—or metabolizing—or consuming it to provide the energy and materials needed to live and grow.

activities. Oil enters waterways not only when an offshore rig blows up, a pipeline ruptures, or a tanker runs aground, but also when it is rinsed off roads and parking lots, spilled at marinas and discharged by outboard motors, released during offshore oil operations, or washed out of ships' ballast tanks.

The Deepwater Horizon rig explosion and subsequent leak of the Macondo Well in the Gulf of Mexico released light crude oil composed of a variety of compounds, with varying degrees of biodegradability **(BOX 2)**. Crude oils vary from source to source, containing different proportions of hydrocarbons ranging from methane (natural gas), to light materials similar to gasoline, to heavy materials that resemble asphalt. Refineries convert crude oils to products ranging from gasoline and aviation fuel to the heavy fuel oils used for ship engines and the asphalts used for roofing tiles and roads. Microbes can biodegrade up to 90% of some light crude oil, but the largest and most complex molecules—like the ones that make up road asphalt—are not significantly biodegradable. When refined petroleum products are spilled, their fate depends on their composition. Gasoline, kerosene, and diesel fuel are so volatile and easily biodegradable that they rarely persist in marine environments, although they can remain longer if buried in sediment, soils, groundwater, or marshes where oxygen levels are very low. Heavy fuels oils, such as those spilled in the Prestige spill off the coast of Spain in 2002, contain a large proportion of heavy components that biodegrade very slowly.

Defining what we mean by 'cleaned up' can also be challenging. One possible measure of successful cleanup might be that the oil can no longer be seen, smelled or tasted. Human senses, however, are relatively unreliable. Another measure might be toxicity – a spill could be considered cleaned up when the concentrations of its components are no longer toxic to humans, other animals, or plants. Other chemical or physical measures of cleanliness are established by regulatory agencies. Under the Oil Pollution Act of 1990 , after an oil spill or hazardous substance release, response agencies like the U.S. Environmental Protection Agency (EPA) or the U.S. Coast Guard (USCG) oversee clean up efforts with the goal of eliminating or reducing risks to human health and the environment. Cleanup efforts may not be able fully to restore impaired natural resources or address their loss for public Use. Studies are conducted through the Natural Resource Damage Assessment (NRDA) process to identify the extent of resource injuries, the best methods for restoring those resources, and the type and amount of restoration required. Clean-up activities themselves can have ecological consequences, and these must be balanced against the desire to remove every vestige of contamination. Therefore a Net Ecological Benefit Analysis (NEBA) is often used—an approach that was first applied to the Exxon Valdez spill in Prince William sound. The most rigorous definition of successful clean-up would be that the oil is no longer detectable by any means and the area has returned to its pre-spill condition. As detection technologies become more sophisticated, this most rigorous definition of cleanliness becomes increasingly difficult or impossible to achieve. Since many marine ecosystems naturally contain a certain amount of crude oil because of seepage, and many others also include varying levels of oil from human activities, defining when an ecosystem has returned to its pre-spill state is not straightforward. A comprehensive overview of cleanup efforts in the Gulf of Mexico after the Deepwater Horizon rig explosion can be found at: http://www.gulfspillrestoration.noaa.gov/.

Biological mechanisms are not the only factor involved in cleaning up an oil spill. A variety of physical and chemical processes are also at work, such as:

■ EVAPORATION

The volatile hydrocarbons evaporate quickly into the atmosphere when they reach the water surface. Under controlled experimental conditions, about 50% of a typical light crude oil evaporates within 20 hours. The characteristics of the spilled oil and environmental conditions make a big difference; light oil on a calm sea will evaporate much faster than heavier oil that has been churned into the water by heavy waves. Evaporation will also be faster at warmer temperatures.

■ DISSOLUTION

Some components of crude oil dissolve in water. These compounds are the most likely to be acutely toxic to

BOX 2: A brief introduction to oil

Crude oil (or petroleum): a liquid mixture of a variety of hydrocarbon compounds derived from ancient algal and plant remains and found in reservoirs under the Earth's surface. Nitrogen and sulfur containing molecules ("resins") are common constituents of some crude oils.

Crude oil components:

Volatile compounds - low molecular weight compounds, like methane (natural gas) or propane, that are normally gaseous or evaporate very quickly at room temperature.

Saturated hydrocarbons - compounds with carbon and hydrogen atoms connected only by single bonds. Saturated hydrocarbons can be arranged in straight or branched chains of up to about 25 carbon atoms. Saturated hydrocarbons are readily biodegraded although degradability decreases with chain length.

Aromatic compounds - compounds that contain rings of carbon atoms held together with double bonds between the carbon atoms. The smallest aromatic compounds in petroleum have six carbons in such a ring structure (e.g. benzene and toluene), but other compounds contain multiple rings. These are known as polycyclic aromatic hydrocarbons, often abbreviated 'PAH'. Most aromatic molecules in petroleum have multiple attached hydrocarbon chains. The smallest aromatic molecules (one- and two-rings) are both volatile and readily biodegraded, even with attached side-chains, but four-ring and larger aromatic compounds are more resistant to biodegradation. They are, however, susceptible to photooxidation. Some larger PAHs are of concern because they are potentially carcinogenic; 16 different PAHs are designated as priority pollutants by the EPA. The percentage of PAHs in crude oil varies, but the 'priority pollutants' are present at low levels in crude oils; they are much more common as a byproduct of burning carbonaceous materials such as fuel, coal, wood, tobacco and other materials. Asphaltenes (used in making roads and roofing products) are examples of high molecular weight (heavy) PAHs that have additional chemical side chains attached to their aromatic rings. Asphaltenes are not soluble in water and most organic solvents.

sea life, but they are also among the most volatile and readily biodegradable under most conditions. Not all toxic compounds are lost through evaporation—some, like heavy polycyclic aromatic hydrocarbons (PAHs), are poorly soluble in water and more likely to adhere to particles and thus remain in the water or sediment. PAHs can be broken down by microbes over time. However, this process is often slow enough that these hydrocarbons can accumulate in such invertebrates as shellfish. Fish and other vertebrates metabolize them rapidly.

■ DISPERSION

Dispersion is the process by which oil is broken up into small droplets and spread through the water. This is the same physical process that is at work when we whisk oil and vinegar together to make salad dressing. Just as with salad dressing, the stability of the resultant emulsion can vary. Physical dispersion can only happen in the presence of adequate mixing energy (e.g. by wave action or high pressure leaks)—under turbulent conditions, dispersion can prevent oil from reaching the surface where it might otherwise evaporate. Dispersion can also drive floating oil into the water column and largely prevent it from forming surface slicks that can threaten birds and mammals. One advantage of dispersion is that oil is broken up into tiny droplets with more surface area, which facilitates microbial degradation. A potential disadvantage is that it might increase exposure of some inhabitants of the ecosystem to the oil. Dispersion can be enhanced by the addition of chemical dispersants, which will be discussed later.

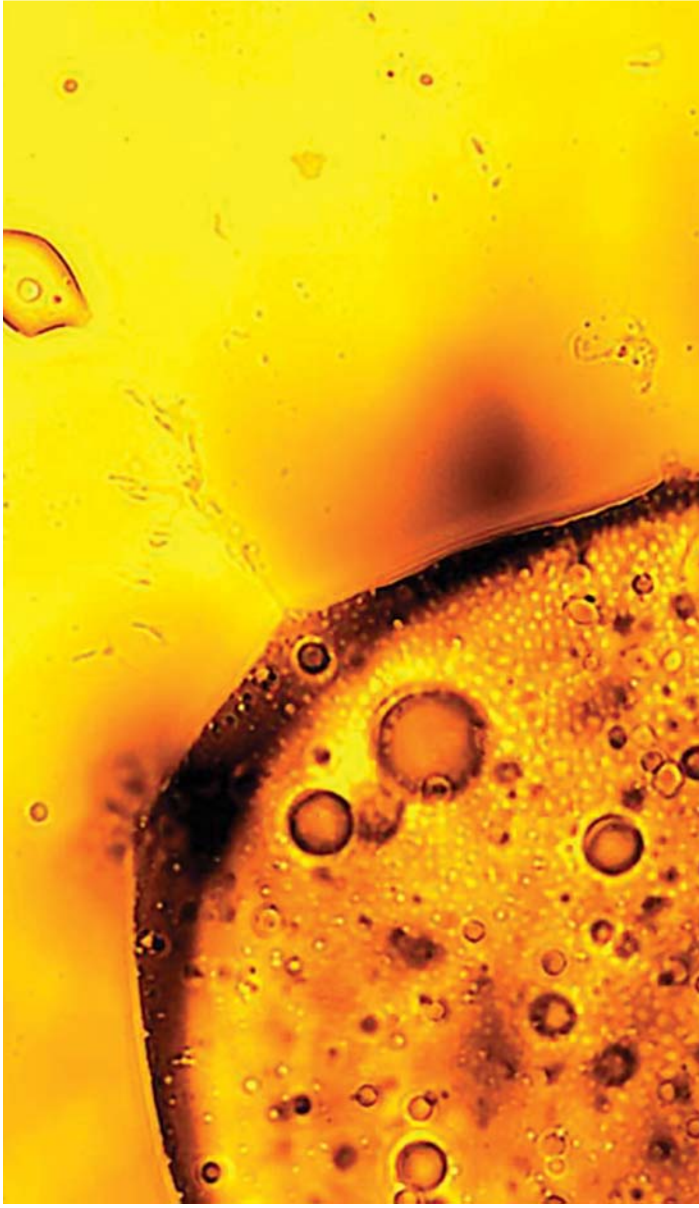
■ PHOTO-OXIDATION

Sunlight reacts with some oil constituents, especially the polycyclic aromatic hydrocarbons (PAHs). The process, known as photolysis, is important because by breaking aromatic ring structures, it enhances the availability of such compounds to microbes and hence microbial degradation. On the other hand, the photo-oxidized PAHs have been shown to be substantially more toxic to water-dwelling organisms.

The physical processes of evaporation, dissolution, dispersion, and photo-oxidation begin as soon as oil is spilled or reaches the surface. Prompt human action (like skimming and burning) can also physically remove some of the oil. When the danger of an oil spill reaching shorelines or other sensitive environments is imminent, physical removal, via skimming or burning, can be a critically important means of minimizing damage. With the exception of burning, which comes with its own set of risks and limitations, these physical processes, however, do not destroy the oil. They do not break it down into harmless carbon dioxide and water. Only living organisms or high temperature combustion (i.e., burning) can do that.

2. Where do the 'oil-eating' microbes come from? Are they everywhere? Does that mean we don't need to worry about oil spills because microbes will always clean them up? What are they doing when there isn't any oil?

Microbes that use oil as their source of energy have been around for hundreds of millions of years—indeed, for as long as this energy-rich substance has been available. Where oil is naturally present, for example, on the floor of the Gulf of Mexico, the community of microbes that collectively feeds on all the different compounds contained in the oil is well established and diverse. Even where the background levels of oil are low, a few microbes with the capability of degrading oil always seem to be present **(BOX 3)**.

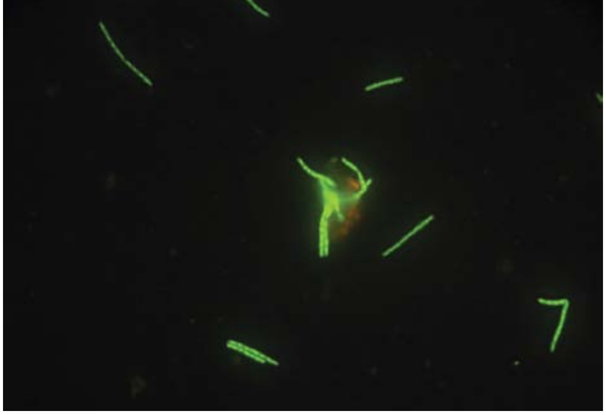


When there is a spill of crude or refined oil, the bacteria capable of degrading hydrocarbons proliferate quickly. Microbial cleanup can be considered in terms of "supply and demand." The local community of microbes is already adapted to the background supply of oil. It takes a certain amount of time—a lag time—for their populations to increase in response to the influx of new resources. The surge of oil from a leak or spill can temporarily outpace the capacity of the local oil-degrading microbes. The oil remains until demand catches up to supply. Eventually, though, along with the physical and chemical processes discussed above, the microbes will "take care" of the problem by consuming the oil compounds that are biodegradable.

The ability to metabolize oil is displayed by many different types of microbes—some more versatile than others. Certain microbes highly prefer oil hydrocarbons over other energy sources and their numbers will increase faster than others in the community in response to an oil spill. Other bacteria are capable of using many different food sources and oil constituents are just one of many compounds these bacteria can utilize for growth. Such microbes can 'turn on' the necessary metabolic machinery in the presence of "edible" oil hydrocarbons to switch over to the newly abundant food source.

Microbes can also evolve enhanced capabilities for degrading oil. One of the most rapid ways this can happen is by horizontal gene transfer (HGT). HGT is a mechanism whereby microbes can share genes with each other—with HGT, a microbe that has the genetic instructions for producing oil-degrading enzymes can transfer copies of those genes to other microbes—even microbes of different species previously incapable of degrading oil components. In this way, microbes that were unable to use oil as a food source acquire that capability. The ability to share genes can greatly promote a local microbial community's capacity to clean up an oil spill. Although the process is well established, the extent to which it takes place after an oil spill is unknown.

Microbes can be counted on to biodegrade oil over time. However, the process may not be fast enough to prevent ecological damage. Immediate containment or physical removal of the oil is therefore an important first line of defense. Even though oil-degrading microbes are found everywhere, their mere presence does not mean that environmental conditions are ideal for oil biodegradation. Environmental conditions, as well as the location, duration, and form of an oil spill strongly affect how quickly biodegradation will occur.



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BOX 3: New ways to find oil-eating microbes

For many years, laboratory experiments have indicated that microbes isolated from many different environments are able to biodegrade oil constituents. Recently developed techniques suggest that such microbes are even more widespread in the environment than previously suspected. The recent dramatic decline in the cost of DNA sequencing has made it possible to detect microbes with the ability to degrade oil even when they make up only a small percentage of the initial microbial community. Scientists can now routinely extract and sequence all the DNA from an environmental sample—such as soil, seawater, or sediment—and find out exactly "who is there?" Just as the Human Genome Project deciphered the sequence of the entire human genome, this technique—known as metagenomics—determines the collective "genome" of a microbial community. Computer programs have been designed that allow scientists to search metagenomes for gene sequences associated with the enzymes involved in oil degradation. And, indeed, everywhere we have looked, oil degraders have been found. Hydrocarbons are everywhere on this planet, and not just from petroleum sources (e.g., the resins made by pine trees and the waxes made by plant leaves are also hydrocarbons). Because microbes can grow rapidly and have only a single copy of DNA, mutations and gene transfer events that improve fitness have an immediate effect. This gives microbes a profound, innate ability to evolve the genetic machinery that lets them use almost any form of carbon as a food and energy source, and since hydrocarbons are present in all environments, it is not surprising that hydrocarbon degraders also are ubiquitous in nature.

3. What do the microbes need in order to biodegrade oil and how long does it take? What are the end-products of microbial degradation? How is biodegradation measured in the environment?

Microbes are able to consume oil because they have the genetic instructions to produce oil-degrading enzymes. But just as crops grow faster with the right amount of light, water, and fertilizer, microbes can degrade oil much more quickly when environmental conditions are optimal. Some of the important factors are:

- **Physical nature of the oil:** If the oil is in a single large slick, there is less surface area for the microbes to gain access to the oil, so degradation is slower. Furthermore, if the oil is heavy and viscous, the biodegradable components must first diffuse through that thick matrix to the oil-water interface so that the microbes can access them. The lighter the oil, the faster this diffusion, making the biodegradable compounds more available to the microbes.

- **Chemical nature of the oil:** Biodegradation rates vary depending on the particular hydrocarbons that make up the spilled oil. Oil is composed of thousands of different compounds—some may be preferred food sources and be consumed very quickly, others are degraded more slowly or not at all. In a marine environment like the Gulf of Mexico, hydrocarbons in which carbons are arranged in an unbranched chain can degrade quickly, in a matter of days or weeks. Hydrocarbons that have a branched structure, or those in which the carbons are arranged in multiple rings (known as polycyclic aromatic compounds), can be far more difficult to biodegrade and therefore persist longer. The most recalcitrant fractions of crude oil including resins and asphaltenes can last for millennia.

- **Availability of nutrients:** Like all living organisms, microbes have many nutritional requirements including nitrogen, phosphate and other nutrients. These substances are found in nature but may be present in limiting quantities. When food levels are high (for example, after an oil spill), the microbes can only degrade the hydrocarbons as fast as the availability of other nutrients allows. If nitrogen and phosphate levels are very low, biodegradation of oil constituents will take place slowly.

- **Availability of oxygen:** The enzymatic process of breaking down oil is usually most rapid in the presence of oxygen. Theoretically, given enough oil and other nutrients, microbial populations could grow so quickly that they exhaust the oxygen from the water in the vicinity of an oil spill. In practice, oxygen has not proven to be as important a limiting factor as nutrients in restricting oil degradation in the ocean, although degradation rates could be slow if a spill occurred in a location where oxygen levels are low.

- **Water temperature:** Generally, oil is degraded more quickly in warmer waters. The problem is not that microbes cannot live in cold water— plenty of oil-degrading microbes are fully adapted to life at cold temperatures—but oil metabolism proceeds more slowly in those habitats for the same reason that milk spoils more slowly in a refrigerator than on the kitchen counter. However, in environments that are always cold, e.g. In the arctic or deep ocean, cold-loving microbes have adapted so that they can degrade oil as quickly as warm water-adapted microbes do in their normal habitat. But colder temperatures also have physical effects on the speed of degradation—oil evaporates more slowly, so there is more oil left in the water for the microbes to degrade. The oil is also more viscous, so it spreads out and disperses less readily—providing less surface area for the microbes to access.

The microbes that consume the oil are themselves consumed as part of the local food web, and thus the carbon and energy contained in the oil are eventually distributed through the food web.

- **Pressure:** The Deepwater Horizon oil spill released oil at a depth of over 1500 meters, where the temperature is low and the pressure high. These are conditions where degradation might have been expected to be quite slow. However, early results show a high number of oil-degrading microbial species adapted to life even in these extreme conditions.

- **pH and salinity:** In most of the ocean, pH and salinity do not vary enough to make a big difference in oil degradation rates. Some specific environments, like salt marsh sediments, exhibit not only high salinity, but also rapid fluctuations in salinity, oxygen, and pH—all characteristics that typically slow oil degradation.

- **Other microbes:** Natural microbial communities are diverse, with many different types of microbes that both compete and cooperate. The complex interactions that characterize healthy, natural microbial communities are only beginning to be understood, but interdependence is the norm. This is one reason why adding microbes to oil spills in the hope of speeding degradation is challenging; outsiders (i.e., artificially introduced microbes) have a hard time breaking into the existing community structure and competing with the local species that have evolved together over the millennia in a particular habitat.

Given all the above variables, it is not surprising that the rates at which oil is degraded can vary enormously. Examples include estimates that natural processes, including biodegradation, removed 99.4% of the crude oil spilled in Alaska's Prince William Sound in 10 years (NOAA), whereas oil spilled in the Kuwait desert is expected to remain for centuries.

When aerobic (oxygen-using) microbes completely degrade various oil constituents, the end-products are carbon dioxide and water. Some oil constituents are only partially degraded and the identities of these partially degraded compounds are not always known. Some of the intermediates may be toxic to marine organisms, although they are not likely to accumulate to levels that cause greater harm than the original oil components. The microbes that consume the oil are themselves consumed as part of the local food web, and thus the carbon and energy contained in the oil are eventually distributed through the food web.

There are many ways to measure clean up of an oil spill. The ultimate goal of scientists or responders measuring biodegradation is to document the mass loss of hydrocarbons attributable to microbial activity. Traditionally, such measurements have focused on the disappearance of particular oil constituents, the appearance of products of biodegradation (like carbon dioxide) and the depletion of oxygen. Decline in the amount of specific oil components, or changes in the ratios of compounds, can be taken as evidence of biodegradation processes. Declining oxygen and nutrient levels are also indicative of biodegradation. Additional measurements include detailed chemical analyses of petroleum components, assessments of toxicity levels, and detection of increases in specific oil-degrading microbial populations. In the open ocean, it can be particularly challenging to quantify biodegradation and multiple approaches are required. Recently, such new strategies as computational modeling and molecular tools for assessing changes in microbial communities have become available for evaluating the progress of biodegradation.

One question that scientists measuring oil degradation rates want to answer is "how fast is the oil being consumed?" Another is "when will the oil be gone?" The two questions are linked, of course, but unlike gasoline consumption in a car's gas tank, microbial degradation does not proceed linearly over time. So, if one-half of the oil is gone in one week, it does not mean that all of the oil will be gone in two weeks. Generally, degradation rates slow as the oil concentrations decrease, making it difficult to calculate a certain end-point. They also slow as the more readily degradable components are used up, leaving behind the more recalcitrant components.

4. How do dispersants and nutrients affect oil biodegradation, and what are the ultimate fates of these additives?

DISPERSANTS

Dispersants work on the same principle as dishwashing detergents—they break the oil into small droplets that disperse in water. Dispersants help surface oil slicks mix into the water, reducing the immediate risk to seabirds and shorelines. At the Deepwater Horizon spill, dispersants were added close to the broken wellhead with the goal of dispersing the oil at its source and preventing much of it from reaching the surface close to the wellhead where many ships were busy trying to stop the leak. By themselves, dispersants do nothing to get rid of the oil—they merely change its physical form. The decision to use dispersants involves trading off the advantages of changing the form of the oil with the risks of moving the oil from the surface of the water into the water column. Generally, dispersants are used to mitigate immediate danger to sea life and shorelines. The dispersants may themselves have harmful effects; however, they are added in low concentrations relative to the oil and are less toxic than the oil itself.

In principle, dispersants enhance biodegradation by increasing the surface area and availability of the oil to the microbes. Studies of the effect of dispersants specifically on biodegradation rates are difficult to design and execute because conditions vary so widely depending on the type of spill, the local environment, and weather conditions.

Based on their chemical structures, the dispersants that have been used on oil spills should themselves be fully biodegradable, but biodegradation rates have not been extensively studied. A number of bacterial species produce compounds that function as dispersants and these compounds are now produced commercially for use in the food, cosmetic, crop treatment, and bioremediation industries. Biosurfactants, as the bacterial products are known, have not yet been used to treat an oil spill, but their use raises most of the same questions as that of chemically synthesized dispersants: do they speed up biodegradation of the oil? do the microbes that feed off the dispersants compete with the oil-consuming microbes for scarce nutrients and possibly slow down oil degradation? The question ultimately is whether there is a net benefit to dispersant use, beyond the immediate physical impact of dispersing and thereby diluting the oil. In the Gulf of Mexico, the use of dispersants appears to have significantly reduced the amount of oil reaching the shore, which reduced the immediate threat to beaches and salt marshes. The effects of dispersants on the biodegradation in the deep plumes are unknown.

NUTRIENTS

Nutrients including nitrogen, phosphate, and iron are essential to any biological process and crude oils are naturally deficient in these major nutrients. Many marine ecosystems are naturally nutrient-poor. Thus, when an oil spill results in a sudden increase in available food (oil hydrocarbons), there may not be enough nutrients in the water to support microbial growth. Nutrient addition to relieve this limitation is one tool to enhance bioremediation and various strategies have been employed to provide nutrients in a suitable form. One of the best understood examples of a large scale addition of nutrients was in response to the Exxon Valdez oil spill, where nutrient addition indeed enhanced oil degradation in the ecosystems of Prince William Sound. Theoretically, nutrient addition could have unintended consequences that upset the natural ecosystem balance, but no such consequences have been reported.

5. Does adding bacteria to the contaminated environment speed the cleanup of an oil spill or improve the effectiveness of biodegradation? Is it possible to engineer microbes so they work even better?

Because bacteria that can degrade oil constituents are ubiquitous, to date there is little convincing evidence that bioaugmentation (addition of more bacteria) significantly enhances either the rate or the extent of oil biodegradation in most environments. Bioaugmentation has not been shown to speed the mitigation of oil spills on marine shorelines, freshwater wetlands, salt marshes, or soil.

While seemingly not useful for oil spills, addition of oil-consuming microbes may be useful in engineered systems (e.g., tanks of contaminated water). Bioaugmentation also has been helpful for biodegradation of man-made organic chemicals that have entered the biosphere recently. Chlorinated solvents and transformer fluids (PCBs) generally are not found in the natural environment. There are microbes capable of degrading chlorinated solvents but their numbers are low and they grow slowly; microbial communities evolved to use these compounds as sources of food and energy are not found everywhere. Bioaugmentation with competent degrading strains of bacteria can stimulate the rate and extent of biodegradation of these compounds in appropriate environments.

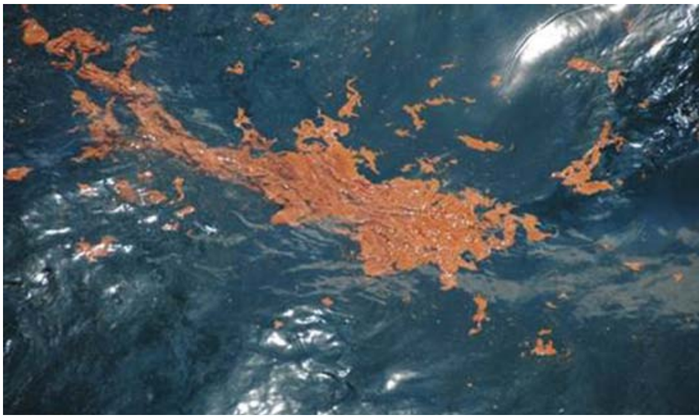
It is possible to engineer microbes that show enhanced oil degrading capabilities in the laboratory, but such microbes are unlikely to have a major impact in environmental settings. First of all, from the moment the oil enters the environment, local microbes begin colonizing the oil droplets. A laboratory strain would have to be able to displace the indigenous microbes which are well-adapted to local conditions. Designing microbes that are both more efficient at degrading oil and well adapted to the particular environmental conditions is a daunting challenge, which has not yet been achieved.

It is important to remember that microbial communities, not individual microbes, are involved in degrading all the various constituents of oil. The level of metabolism in each individual microbe (which is what theoretically could be increased in an engineered microbe) is not what limits the rate of biodegradation. Even the odds of significantly increasing the rate of oil metabolism in individual microbes are not good. Natural selection has been acting on microbes for billions of years, honing the metabolic pathways for degradation of hydrocarbons to a highly efficient level. Collectively, the natural communities have evolved remarkable capabilities to capitalize on every bit of energy that can be extracted from oil constituents.

Microbes might be selected or engineered to function in particular habitats with extremes of pH, temperature, salinity, or other conditions. It is possible to design microbes that are better at degrading oil, but given the ubiquity of oil-degrading microbes, the difficulty in predicting when and where the next oil spill will occur, and, most importantly, safety and public acceptance considerations surrounding the release of genetically-modified organisms, oil spill bioengineering is not the most promising research target.

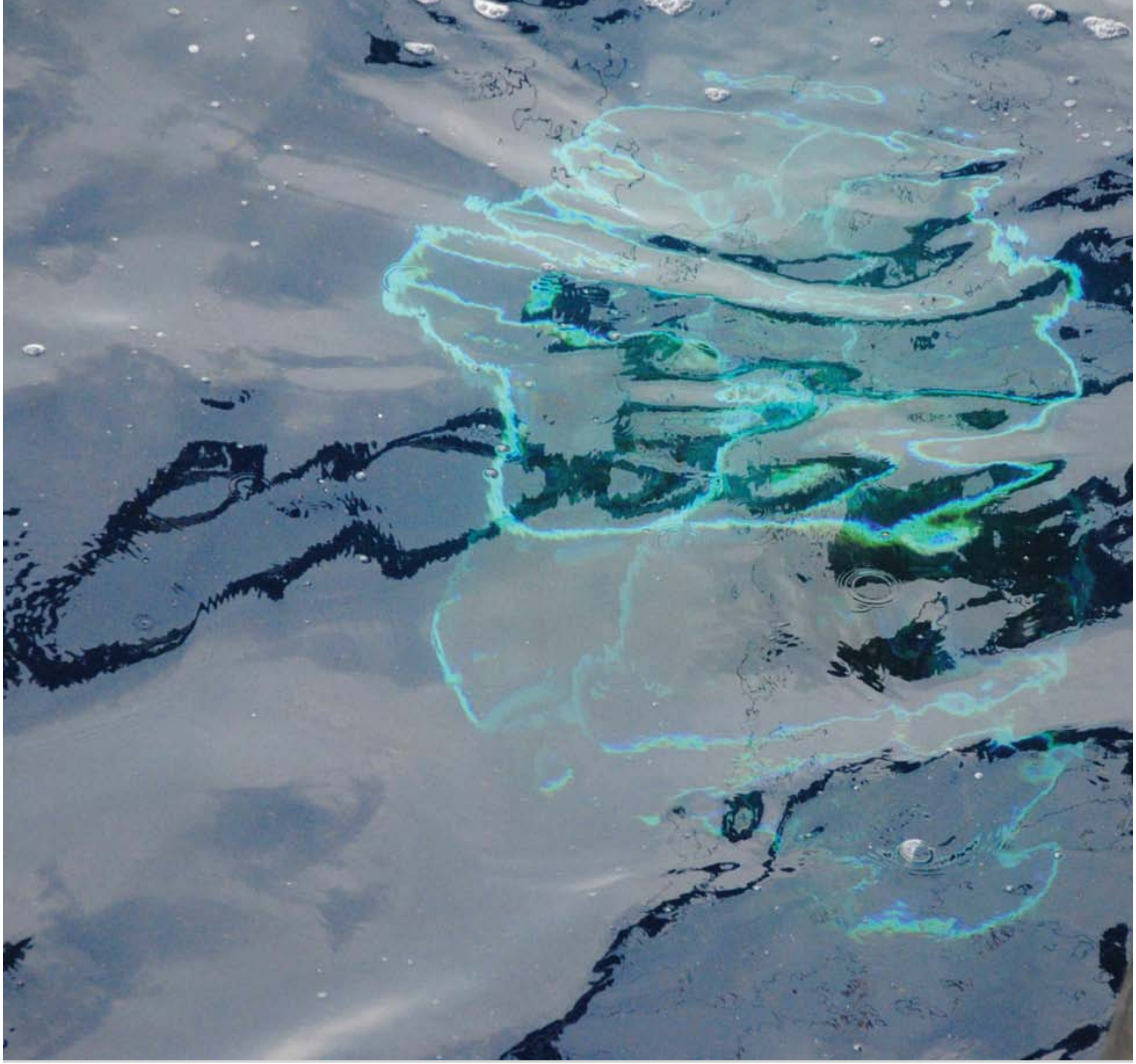
6. What happens to the oil after the microbes degrade it? Does it go up the food chain like DDT ? What happens to all the other animals in the area while the oil-eating microbes are at work? Does an oil spill increase the risk of dangerous pathogen 'blooms'?

Oil does not build up in a microbe any more than salad oil builds up in our stomachs. Just as our digestive tracts break food down, microbes break oil down into simple carbon compounds that are used to make the sugars, fats, and proteins needed for growth and energy production, with the ultimate byproducts being carbon dioxide and water. The simple carbon compounds are incorporated into new cellular constituents—in other words, they are used to make more microbes! (Formally, we say that the carbon has been used to produce additional biomass.) The new microbes continue eating oil, unless they themselves are eaten by natural predators. The important fact is that biodegradable hydrocarbons do not accumulate on or inside cells and thus do not go up the food web. It is possible that oil spills might contain some recalcitrant oil components that are only poorly degraded by microbes. Such compounds might bioconcentrate through the food chain, but this phenomenon has not been observed in large ecosystems like the Gulf of Mexico.



Marine animals will leave the vicinity of a spill (if they can). Some of the compounds in crude oil are toxic, others are irritating to membranes like eyes and nostrils; they taint the water and are generally actively avoided by motile marine animals. Organisms that cannot swim away could be affected if the microbial oil degradation is using up all the available oxygen. But there is no evidence that in open systems like the ocean, oxygen depletion due to oil degradation would be so great as to affect other animals in the system.

It is possible to calculate how much oxygen microbes would need to break down all of the oil and those calculations suggest that most spills are unlikely to result in dangerous decreases in dissolved oxygen (DO) levels. DO was one of the major variables monitored during the Deepwater Horizon response, and although some small declines in DO were measured in the area where the dispersed oil plume was found (1000- to 1300-meter depths), levels of oxygen that would be dangerous to marine life were never approached.



There are certain relatively closed ecosystems, like estuaries, salt marshes, or wetlands, where oxygen depletion from oil degradation is sufficient to harm organisms that live there and require oxygen. Oxygen depletion is more likely to affect such ecosystems as the seafloor, salt marshes, or beach sands, where the natural supply of oxygen tends to be limited. A notable example of an area with limited oxygen is the dead zone in the Gulf of Mexico where the Mississippi River empties into the Gulf—the zone was not caused by the oil spill but by the regular influx of nutrients from the Mississippi, which carries agricultural fertilizer run-off from Midwestern fields and lawns into the Gulf. There is no evidence that a one-time spill of oil—even a large one—into the open ocean could create persistent dead zones, although localized and temporary areas of low oxygen are possible.

The microbes that degrade oil are generally not human pathogens. Even oil-degrading microbes that are related to human pathogens do not cause disease (and their disease-causing relatives cannot degrade oil). Even though their numbers increase significantly after a spill, oil-degrading microbes pose no threat to human health. No evidence has been found that stress from an oil spill on organisms like oysters or shrimp causes them to harbor more human pathogens. No case of a pathogen outbreak has been documented as the result of an oil spill, even though thousands of spills occur annually.

7. What happens to the microbes when the oil is all gone? Does an oil spill result in a permanent change in the mix of microbial species that live in the area?

The microbes that degrade oil are part of the local food web; they are consumed by other microbes that are, in turn, consumed by larger predators. When the oil is gone, the oil-degrading microbes, with less food available, stop dividing so rapidly and eventually return to pre-spill abundance.

However, determining whether the species composition is exactly as it was pre-spill is complicated at the microbial level, because microbial communities are dynamic systems. It is difficult to determine exactly what the community looked like before the oil spill and we know little about the dynamics of natural populations, even in the absence of obvious disturbances. Further complicating matters, several kinds of bacteria may carry out similar functions. The relative numbers of these 'redundant' groups may change after an oil spill, but the overall functionality of the community largely remains the same. The functional stability of microbial communities makes it difficult—and perhaps unnecessary—to determine whether a community is exactly the same as it was before the spill. However, no evidence has been found that a bloom of oil-degrading microbes crowds out other microbial species and drives them to extinction. Even at the height of oil-degrading activity, oil-degrading microbes make up only a small percentage of the total microbial community.

Modern molecular methods are providing new ways of measuring the composition and function of microbial communities. High-throughput analytical technologies make it possible to obtain the genetic sequence of the entire community **(see BOX 3)**. It is also possible to measure change over time in the abundance of genes that code for oil-degrading enzymes. The Deepwater Horizon spill was one of the first where advanced high-throughput approaches were applied comprehensively and much is likely to be learned about what happens within the local microbial community at the genetic level during and after such a spill.

FAQ

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