Microbes play a big role for Wisconsin

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Microbes play a role in making cheese and beer, as well as in growing soybeans. The tiny organisms can clean some older industrial sites with contaminated soils (bottom left). Researchers are studying whether microbes joined to form a film also may be used to prevent mussels from attaching to lake equipment, as in these quagga mussels (bottom middle) attached to a line.

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Microbes, those tiny organisms like bacteria and fungi, don't just make you sick. In fact, the vast majority of microbes are not harmful to humans at all. There is practically a species of bacteria for everything, from those that help grow our food to those that are required to make it. Microbial research is at the cutting edge of helping solve some of our worst environmental problems. So put down that anti-bacterial soap for a second and take a look at how we depend on microbes in Wisconsin.

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Microbe brews, cheesy bacteria

If you cannot imagine Wisconsin without beer and cheese, you cannot imagine a state without microbes. Beer without yeast is nonalcoholic and has much less flavor, and cheese without bacteria is at best curdled milk.

Beer-making depends on yeast, which gets energy by fermenting sugar.

During the brewing process, the starches in barley malt are mixed with water and heated to break them down into more simple sugars. This sugar water is sterilized, hops are added for flavor and aroma and the whole mix - wort - is cooled down.

This is where yeast, a single-celled fungus, comes in, converting the sugary wort into an alcoholic beverage.

"Yeast start reproducing and they start eating sugar," said Andy Jones, quality manager at Lakefront Brewery in Milwaukee. "They'll eat about 75% of the sugar in there and convert it to alcohol, carbon dioxide and other flavors."

The alcohol and flavors stay, but carbon dioxide, which gets added back later in a lesser amount to give beer its fizz, is released. "Or the tanks would explode," Jones explained.

After this fermentation step, the liquid is separated from the yeast, often fermented a second time and then aged, filtered and carbonated.

Jones said depending on the beer being brewed on a particular day, the brewers will add different yeasts, such as ale or lager yeasts.

"There are well over 100 strains of yeast," Jones said.

And it is hard to run out of yeast. It gets its energy by fermenting sugar, which means each time the wort is fermented, more and more yeast is produced. Jones said the brewery can reuse the yeast for 10 to 12 cycles.

While it may be difficult to argue that beer is healthy, it certainly will not make you sick (at least not in small quantities). Beer is acidic, contains alcohol, is free of oxygen and the hops have anti-microbial properties, all qualities that make it nearly impossible for bacteria, especially most pathogens, to survive.

Cheese, on the other hand, relies on the survival of bacteria.

According to Mark Johnson, interim director of the Wisconsin Center for Dairy Research in Madison, bacteria provide two main, critical services during cheese-making.

"One, they make acid, and two, they break down the (milk) components into more flavorful compounds," Johnson said.

Starter bacteria, including the *Lactococcus* and *Lactobacillus* genera, are responsible for producing acid, which starts the cheese-making process. The acid creates cheese curds and starts to give the cheese some of its body and texture.

"For most cheeses, it's a very simple process," Johnson said. "You add a starter (bacteria), add rennet, cut (the curd) into smaller pieces and then cook it." He said the bacteria-derived acid also helps to remove some of the water and dissolve the calcium in milk.

After cooking at no more than 120 degrees, the curd is separated from the whey and formed into a block, awaiting the flavor ripening that only bacteria and some molds can do.

Johnson said that much of the flavor in cheese comes from the bacteria naturally in milk. This is one reason a cheddar cheese made the same way with milk from two different farms will always taste slightly different.

Depending on the cheese, the ripening process may rely solely on those natural bacteria, but occasionally bacteria or other microbes are deliberately added. To make blue cheese, mold is added to the curds. For Camembert, a cheese wheel is formed first and then molds are added to the outside.

"Without micro-organisms, we wouldn't have cheese nor the great diversity of flavors we see in cheese," Johnson said. "If you want to have a stronger flavor, a real diverse flavored cheese that tastes different than buttery flavored milk, you've got to have bacteria."

High-microbe plant diets

Just like humans, plants can acquire diseases from bacteria or fungi. And, just like humans, plants often require other microbes to live in them or on them to harvest required nutrients from their food source - soil and air.

Legumes such as soybeans, the second most abundant crop in Wisconsin, are a perfect example.

"Legumes don't need fertilizer. They just need microbes," said Jean-Michel Ané, assistant professor of agronomy at the University of Wisconsin-Madison.

Most plants get their required nitrogen from soil in the form of molecules called nitrates. Legumes, however, can take in gaseous nitrogen from the air - but only if their roots are colonized with bacteria called *Rhizobia*. These bacteria convert nitrogen gas, which plants cannot use directly, into ammonium, which the plants can use.

"Some bacteria form nodules on roots, which are new organs on plants," Ané said. "The microbes and roots are in very close contact, so the nutrients can be exchanged."

To grow soybeans or alfalfa, farmers merely have to inoculate the soil with *Rhizobia*, a practice that has been going on for more than 100 years.

Ané said there is no environmental cost to adding bacteria to the soil, and the cost of purchasing the bacteria is minimal - inoculating one hectare of soybeans costs approximately $1.60, with typical profits of $57.

Outside the legume family, *Rhizobia* have no effect on plant nutrition. That's where other microbes, the mycorrhizal fungi, come in.

"More than 80% of plants, and almost all crops, form these mycorrhizae," the name for the nodules formed on roots by the fungus, Ané said.

Mycorrhizae allow plants to take up phosphate that often exists in soil in a form inaccessible to plants. They also protect the plants against many pathogens.

"If you have mycorrhizae, you limit the amount of fertilizer needed," Ané said. "But the technical problem is that making it on such a large scale is expensive."

Then again, making fertilizer is expensive. It takes 1 ton of gas to produce 1 ton of fertilizer, and Ané said that as the price of gas goes up, fertilizer will become more expensive.

Luckily for the Midwest, the soil is abundant in plant-accessible phosphate. It is, however, lacking in nitrate. Growing soybeans without fertilizer is simple, then, as the soil, air and *Rhizobia* produce all the nutrients the legumes need. In fact, the plants even deposit nitrates in the soil that other plants can use.

Without mycorrhizal fungi, though, corn cannot get the nitrates it needs without fertilizer. Ané said that crop rotation or even mixing soybeans and corn in the field is one way farmers lessen their need for fertilizers.

Spot-cleaning soil

Milwaukee's rich industrial history has left a legacy of chemicals stored and sometimes dumped by manufacturers, contaminating some of the surrounding soils and groundwater with toxins.

While chlorinated compounds such as PCE, commonly used by dry cleaners and manufacturers, might cause cancer in humans, those same chemicals are harmless to and often required by certain species of bacteria. Thus, the field of bioremediation was born, in which the microbes clean up our messes.

"Before, the only choice was to dig (contaminated soils) up and move the contamination somewhere else," said Terry Evanson, a hydrogeologist with the Wisconsin Department of Natural Resources. She said that not only can bacteria convert the toxins to harmless products, it is often less expensive to use bacteria than it is to haul tons of soil to containment sites.

Evanson said that often the bacteria needed to degrade a particular chemical are already living in the soil, although occasionally a particular bacterial species needs to be introduced. After ensuring that the correct bacteria are present, then it is just a matter of tweaking conditions to allow those bacteria to thrive.

At a PCE-contaminated site on the north side of Milwaukee, Mafizul Islam, a civil engineer with Sigma Environmental Services Inc., said his group is using bacteria to rid the ground of pollutants.

"We were very lucky the site has plenty of natural bacteria. We just had to add a food source."

Islam's consulting group was hired by the property owners after discovering the contamination, the source of which is unknown but suspected to be a "midnight dumping" that occurred on the then-abandoned property in the 1970s.

The food source needed was carbon, which could be injected into the contaminated soil in the environmentally safe forms of soybean oil and corn syrup. Islam said that his team monitored levels of PCE as well as bacterial counts over time, and that as the bacterial population grew up to 10,000 times in size, the concentration of contaminants dropped proportionately.

"When we breathe in oxygen, we breathe out water and carbon dioxide," Evanson said.

And in a sense that's what's happening in the soil.

In this case, the bacteria, called *Dehalococcoides*, use the chlorinated compounds the same way we use oxygen. These bacteria cannot survive if oxygen is around or if the chlorinated compounds are absent.

Dehalococcoides can breathe in PCE, which has four chlorine atoms, remove one of those chlorines to make the three-chlorine TCE, and breathe out a chloride ion and the TCE. Then, the bacteria can start the cycle again, stripping TCE of a chlorine atom until the known carcinogen vinyl chloride is made.

"This is the problem with vinyl chloride," Evanson said. "If there is PCE around, (the bacteria) are not going to be using the vinyl chloride."

The good news, Evanson added, is that if the vinyl chloride can be moved out of the oxygen-deprived zones to oxygen-rich zones, other bacteria can degrade it rapidly.

While bioremediation has its benefits - bacteria are, for example, primarily responsible for the relatively rapid degradation of oil from the Gulf spill - it is not a perfect fix for all toxic spills.

The Fox River cleanup project, the largest remediation project in the state, requires dredging and digging up river sediments contaminated with other chlorinated chemicals, PCBs, that the PCE-degrading bacteria cannot use.

"People have been able to develop bacteria in the laboratory that consume PCBs," said Jim Hahnenberg, remedial project manager with the Environmental Protection Agency. "But when you try to apply it to the field, it doesn't work."

Evanson added that dense clay soils through which liquids cannot move are also not good candidates for in-soil bioremediation.

"(Microbes) are great coworkers, but they're not going to fix everything," Evanson said.

A filmy union

When a bunch of bacteria get together, they can congregate into a biofilm, an amorphous mass of cells that essentially work together and lessen their susceptibility to harm, such as antibiotic treatments.

A biofilm that shows up as plaque on our teeth or scum in a shower drainpipe can become a health problem. But a biofilm that, say, prevents hundreds of quagga mussels from attaching to objects in Lake Michigan, might just be a solution.

Quagga and zebra mussel larvae require a hard surface to attach to during their maturation. They sink buoys, damage boats and spur millions of dollars in expense to prevent them from blocking water flow through pipes at water treatment plants.

According to Jim Maki, associate professor of biology at Marquette University, biofilms of bacterial species that are naturally occurring in Lake Michigan are harmlessly coating these same surfaces.

"Initially what we were looking at was, what is the role of these natural biofilms in terms of invertebrate attachment," Maki said. It was unclear whether biofilms promote or inhibit attachment, and it may depend on which bacteria are in the biofilm.

Using several species of bacteria found in Lake Michigan, Maki and his research team have been trying to identify different surfaces, such as glass or polystyrene, that allow the formation of biofilms but inhibit mussel larvae attachment.

While the research is preliminary, Maki said they could not identify one species that always inhibited attachment on one particular surface. Interestingly, the surface coating created by the biofilm itself does not seem to inhibit mussel attachment. Instead, it seems that the bacteria in the biofilm are releasing chemicals, called extracellular polymers, in response to their attachment to a particular surface. It is likely those polymers that are inhibiting mussel attachment. He said his group is now trying to identify exactly what this chemical is.

"If we can stop them from blocking pipes or attaching to buoys . . .&enspor stop them from attaching to dams or locks such that the mechanisms don't work, that's great," Maki said. He hopes that the identification of inhibitory biofilm chemicals leads to the development of a slow-release coating that can be applied to these surfaces and prevent or limit mussel attachment.

What cannot be prevented, at least not easily, is the spread of the mussels.

"We're not affecting their life cycle," Maki said. "There are plenty of rocks out there that they can attach to."