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MICROBES AND THE BIOECONOMY:

GREASING THE GEARS OF SUSTAINABILITY

AMERICAN
SOCIETY FOR
MICROBIOLOGY

microcosm SPRING 2024

Microbes have long greased the gears of the bioeconomy. But the story of how microbiology drives vital processes that support the bioeconomy often goes untold. The Spring 2024 issue of *Microcosm* takes an in-depth look at the critical role of microbes in creating sustainable fuel alternatives, remediating wastewater, fighting foodborne pathogens and beyond.

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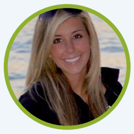

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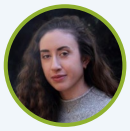

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Bioeconomy 101: Making Microbial Connections

BY MADELINE BARRON, PH.D., LEAH POTTER, M.S., AND EMILY READY

Did you know that the tiniest parts of an ecosystem play massive roles in driving the economy? In fact, microbes are at the heart of energizing agriculture, transportation and pharmaceutical development worldwide.

This collective system—a cog in the global economic machine—is known as the bioeconomy. But what is the bioeconomy? What is the relationship between microbiology and the bioeconomy? And why is the bioeconomy relevant to One Health?

The bioeconomy is a facet of the global economy that uses biological resources to produce goods or services across a diverse network of industries. Source: American Society for Microbiology.

WHAT IS THE BIOECONOMY?

Capturing a single definition of the bioeconomy is challenging, and the U.S. has yet to adopt an official definition. In general, the bioeconomy is a facet of the global economy that uses biological resources to produce goods or services across a diverse network of industries. This encompasses a variety of fields, from food production to the development of biofuels. This often involves reusing or repurposing existing materials and products to reduce waste and minimize environmental impacts (i.e., contributing to an economy that is circular). The bioeconomy yields economic benefits by creating new jobs, advancing capital toward sustainable systems and more. Although defining the bioeconomy is complex, illustrating the importance and impact is imperative, and microbiologists—and the field of microbiology at large—will play a key role.

WHAT IS THE ROLE OF MICROBIOLOGY IN THE BIOECONOMY?

Microbes have many features that make them integral to the bioeconomy. In addition to being a renewable resource, they have varied metabolic capabilities and physiologic traits that can be harnessed to generate high-value, sustainable products.

Food Security

For example: fermentation is a microbial process critical to crafting dairy products like cheese and beverages, including wine and kombucha. The capabilities of lactic acid bacteria not only support the overall making of fermented foods and drinks, but also prolong a product's shelf life and bolster food security in the long run.

Agricultural processes, too, benefit from microbial functions. Soil-dwelling microbes produce compounds that improve plant growth, which promotes the growth of healthy crops and contributes to feeding a growing population.

Biofuel Development and Electricity

Beyond food and agriculture, microbes can be employed to generate electricity. For instance, the emerging industry of bioelectrochemical systems leans on electrogenic bacteria that produce and sustain an electrical current. The production of biofuels, like biodiesel and biogas, similarly leverages the metabolic functions of microbes to power cars, heating and cooling systems, farming equipment and more.

Antimicrobial and Pharmaceutical Production

Pharmaceutical development is another microbiology-driven sector vital to global public health and economic growth. With the rising threat of antimicrobial resistance (AMR), microbiologists are using biological resources to design and test novel therapeutics that ultimately will prevent the overuse of antimicrobial drugs. This includes genetically engineering microbes to produce new antimicrobial compounds, as well as developing vaccines to combat diverse pathogens. New therapeutics will benefit patients worldwide, and, by improving health outcomes, lower health care spending overall.

Waste Management

Across sectors, the ability of microbes to degrade and transform pollutants and waste into useable products is groundbreaking. Reflecting circular economic practices, microbial processes continue to be relied on to reduce waste—for example, when used to help us reclaim our wastewater, remove radioactive waste and transform waste into products like bioplastics (i.e., bioremediation).

WHY IS THE BIOECONOMY IMPORTANT?

Advancing the bioeconomy is key for addressing some of the most pressing challenges of our time, like climate change and food security. Bioeconomic development eases our reliance on fossil fuels, reduces environmental pollution, informs development of drugs to improve human and animal health, promotes sustainable agricultural practices and expands the number of jobs available across diverse sectors. Ultimately, by leveraging bioeconomic processes, we can maintain and enhance the collective well-being of people, animals and the planet (i.e., support One Health)—now and in the future.

Interested in learning more about the role of microbiology in the bioeconomy?
Attend a session from the Bioeconomy Curated Itinerary at ASM Microbe 2024.

Register Now

How the Bioeconomy Sustains People and the Planet

BY ASHLEY HAGEN, M.S.

Penicillin, the first true antibiotic, originally produced by Penicillium moulds, is an early example of a life-changing bioeconomy product. Photo taken at Nobel Museum. Source: Flickr

Does money really make the world go round, or could it be the consortia of microorganisms that surround and inhabit us? Microbes are naturally responsible for some of the most fundamental processes influencing human and environmental health. But where does the economy fit into this equation? Or, perhaps more pertinently, where do microbes fit into the economy?

Looking to organisms that can biologically convert materials considered to be of low or lesser value (e.g., waste, pollutants) to products that are more highly valued (e.g., food, energy)—and to do so in a repeatable or sustainable fashion—not only generates revenue but is also integral to the future of our planet and society. It is this life-changing and innovative thinking that motivates scientists, manufacturers and policymakers alike to seek methods to use raw materials and renewable resources to power a biobased economy.

But what is a bioeconomy? Can we adequately (and ethically) attribute monetary value to biological resources? How can microbes grease the gears of innovation, ingenuity and sustainability and, ultimately, offer solutions to humanity's most pressing challenges? Answering these questions will be crucial to fully comprehend the power of biological resources and the plausibility of using them to create sustainable systems.

HOW IS THE BIOECONOMY DEFINED?

Using Biological Resources to Produce Goods and Services

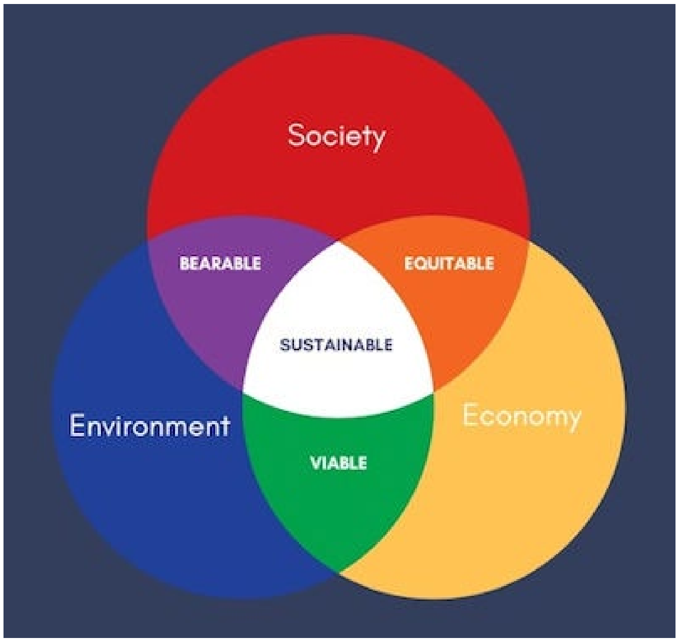
According to Timothy Donohue, Ph.D.—ASM Past President, University of Wisconsin Foundation Fetzer Professor of Bacteriology and Director of the Great Lakes Bioenergy Research Center (GLBRC)—"The bioeconomy is a relatively new term." Yet, the practice of using biological resources to produce goods or services has been around for millennia. "We depend on the bioeconomy every day. When you go to the store and buy a loaf of bread, you're buying a product that was made by yeast," Donohue explained. The oldest evidence of bread-making was discovered in a 14,500-year-old Natufian site in the Jordan desert. "We're surrounded by the bioeconomy," Donohue continued. "If you like wine or beer or soy sauce, those are all bioeconomy products. [For each of these items] there's a biological catalyst that's making the product from a biological material."

Many well-loved food and beverage items are bioeconomy products.
Source: iStock.com/fcafotodigital.

Still, when it comes to defining the term "bioeconomy," things get more complicated. While a number of countries have adopted formal definitions or frameworks for the term, the U.S. has yet to do so.

Michèle Friend, Ph.D., is an associate professor at The George Washington University who teaches philosophy of logic, mathematics, science and the environment. She also has an appointment at the University of Lille Nord de France, where she teaches bioeconomics and is working with a group of biochemists who are evaluating how to use enzymes to break down polymers and produce biofuels and bioplastics. Friend explained that, at its core, the economy is a social construct. Neoclassical economics looks strictly at the flow of money within a system, and, as a result, many people understand only that the bioeconomy involves the flow of money when biological elements are involved. "When biology goes into the factory, [many think only about] how the economics play out," she elaborated.

Indeed, this is part of it. The bioeconomy uses biotechnology and biomass (renewable organic matter that comes from plants and animals) to produce goods, services and energy. This requires an understanding of how to manipulate and apply genetic, genomic and molecular processes and mechanisms to improve industrial processes and develop new products. Yet, official definitions for the bioeconomy vary based on differences in vision surrounding biotechnology, bioresources and bioecology, with some favoring broader definitions and others favoring more narrow ones (e.g., excluding food, beverage and forestry and focusing more on biological innovations).



A Venn diagram shows the intersection of the social, environmental and economic factors that make up the bioeconomy. Source: Ashley Hagen, M.S.

Preserving Harmony Between Society, the Economy and the Environment

Friend favors a broader definition and promotes taking our understanding of the bioeconomy a step further by introducing the concept of ecological economics, an interdisciplinary field that underpins the bioeconomy. Ecological economics considers societal factors (e.g., human health and education) and natural resources (e.g., land, soil and crops) to be natural capital and seeks to develop effective policies that evenly distribute these resources to create sustainable ecological systems.

The field ultimately highlights the dependencies between human economies and natural ecosystems. Without humans, the economy would not exist, and money would not flow. Yet, humans are more directly dependent on the environment than money to survive. If we cannot breathe clean air, or access fresh water, we will die, and the economy will die with us. As Earth's climate is changing, and humans are consuming significantly more global resources and producing more waste now than in the past (the World Bank predicts global waste will grow to 3.4 billion tonnes by 2050), an alternative to current patterns of economic growth and environmental degradation is urgently needed.

According to Friend, understanding the link between economics of human and natural systems can help us manage our natural resources appropriately. "If we have a sense of health of an ecosystem, we have a sense of health of society, and we have a sense of health of the economy," she said, adding that we must think about the ways in which the decisions made in one sphere of influence positively or negatively impact the health and well-being of the others and recognize that decisions about how we manage resources have far-reaching consequences.

Fortunately, if we take a page out of the book of microorganisms, which have cornered the market on resourcefulness and adaptability, the bioeconomy can provide an alternative to exhausting available resources until the gears of society—and thus the economy—come to a grinding halt.

HOW CAN MICROBES GREASE THE GEARS OF INNOVATION, INGENUITY AND SUSTAINABILITY?

Microbes Are the Ultimate Biochemists

Microbes are the most abundant organisms on Earth. They also have existed on our planet for billions of years and possess relatively simple and adaptable genomes, which means that over time and with exposure to unlikely energy sources and changing environmental factors, microorganisms have evolved the ability to perform numerous metabolic feats. If scientists can harness that power to engineer renewable microbial systems that break down harmful chemicals, make new molecules and, ultimately, manufacture innovation, the economic potential becomes limitless.

As Donohue pointed out, "Microbes have been doing chemistry longer than people. And they have the ability to make a lot of molecules that chemists already make. If we can put genes and pathways together, I can see a day where microbes are making molecules that cannot be made easily or cost effectively by existing synthetic chemistry routes today."

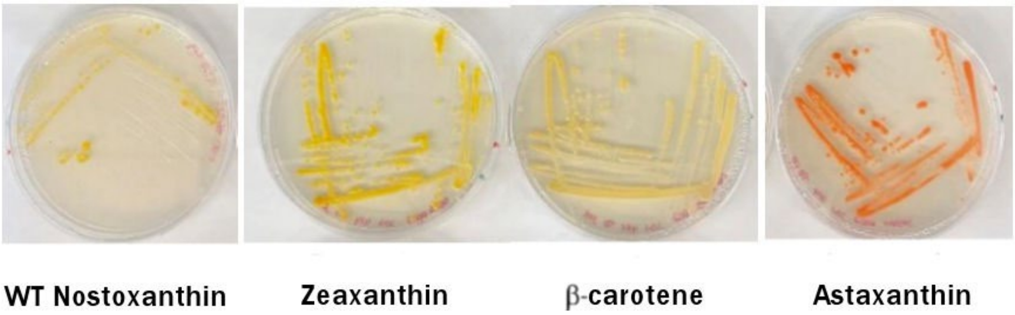
Genomics Opens the Door for Innovation

When it comes to the practical application of such techniques, Donohue credits the genomic revolution for facilitating major strides in research and development. He noted there is a lot of excitement around the idea that genomics opens new doors for using biology to make products. "Think of a gene as a Lego block," he said. "We now have the ability to take the genomic blueprints from microorganisms, mine those and put 'Lego blocks' from 1 organism into another one to make products or mix and match 'Lego blocks'—a yellow one from 1 organism, a blue one from another—to allow microbes to make things that they don't normally make."

Sustainability Means Making the Most of a Biological System

The Department of Energy Great Lakes Bioenergy Research Center (GLBRC), which is lead by Donohue, is mixing and matching genetic material from microbes to create economically viable and environmentally sustainable biofuels and bioproducts. One microbe that the team is particularly interested in is *Novosphingobium aromaticivorans* (Novo), a bacterium that was isolated in the early 1970s by the U.S. Department of Energy from a Superfund site (a location polluted by hazardous waste) in the Savannah River Basin. What made Novo particularly interesting at the time of its discovery was its ability to metabolize polyaromatic hydrocarbon pollutants that were all over the watershed area.

Today, Donohue and his team have brought Novo into the lab and found that its appetite for aromatics makes it useful for breaking down other difficult-to-metabolize compounds, including lignin, an aromatic heteropolymer that provides support and structure to plant cell walls, and converting them to biochemical precursors that can be used to create products with high economic value. "We can now take that [lignin] polymer apart and feed it to Novo, and Novo will secrete dicarboxylic acids that are precursors for nylon and biobased plastics that have a market value of trillions of dollars worldwide," Donohue explained.



Genetic engineering allows GLBRC scientists to produce lucrative products, including beta-carotine and astaxanthin from wild type nosoxanthin. Source: Timothy Donohue, Ph.D.

The innovation doesn't stop there. Novo is yellow in color, tipping off scientists to the fact that the bacterium possesses the biosynthetic pathway to make carotenoids. With some genetic engineering, researchers turn this natural ability into a sustainable system that creates lucrative organic compounds. With a single gene change, GLBRC scientists have been able to get Novo to produce beta-carotene, a vibrant red-orange pigment that is used as an antioxidant and to color many food products, including margarine. By bringing in a gene from another organism, scientists have also been able to engineer Novo to produce astaxanthin, a pink-colored compound that Donohue explained is "another multi-billion-dollar-a-year product for the aquaculture industry." Salmon farmers feed astaxanthin to farm-raised fish to help produce the desirable pink color in their flesh.

In the aquaculture industry, astaxanthin is currently made from food grade sugars, but GLBRC has found an incredibly effective way to make this highly valued product from renewable resources.

"Now we can actually take Novo and get it to make—in the cell—carotenoids, astaxanthin or beta-carotene, and secrete a nylon precursor in the intermediate. So now industry, in 1 pot, can make 2 products and make money on each one, so that we are generating as much value from 1 fermentation run as possible."

CAN YOU PUT A PRICE ON A BIOLOGICAL RESOURCE OR SYSTEM?

Once such knowledge is available, and a viable process like the Novo fermentation described above has been created, the basic science needs to move from academia to industry in a manner that is cost-effective. That means the idea must be scalable, and industries must be willing and able to invest the required resources to execute and roll out a usable bioproduct.

But recognition of the dependencies between society, the economy and the environment makes assessing the cost and value of biological resources and products more complicated than a bottom-line calculation. If something is extremely beneficial to the environment, but is of high economic cost with little to no monetary gain, is it worth the investment? What if the use of a biological resource would be extremely lucrative, but may ultimately have a negative impact on some facet of society?

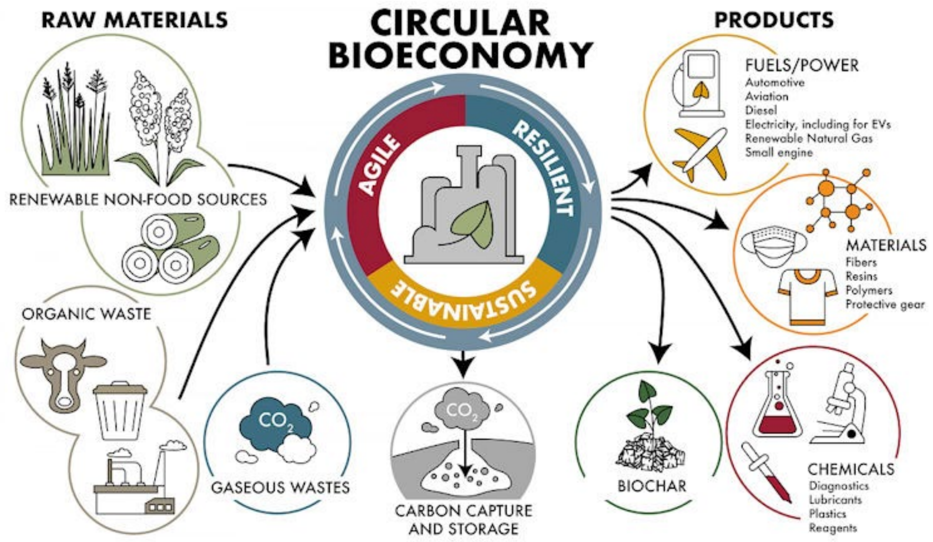
An Institutional Compass. Source: Michèle Indira Friend.

Friend is frequently called in to consult on these types of questions. She explained that many of the scientists she works with are looking for expert guidance to evaluate the moral implications of their work. "They want to have a philosopher of the environment come and help them with that more global philosophical, sociological evaluation," she said.

When asked how she makes these assessments, Friend highlighted the fact that evaluating whether a particular action or product is "worth the investment" is really more of a philosophical question and pointed to a model that she created called the "Institutional Compass."

The Institutional Compass is a tool that helps institutions recognize and make decisions based on desired qualities within their system or environment. This decision aid is both qualitative and objective and helps institutions comprehensively evaluate whether a particular action or investment will move their system in the direction of harmony (everything is going well, status quo), discipline (things are hard, there are constraints and obstacles) or excitement (things are in flux, people are investing and things are on the move). The compass takes into consideration social, political, cultural, environmental and economic factors—everything that might be important to and/or impact the institution at a given time—and uses data points and mathematical justifications to determine the outcome of each assessment.

USING RENEWABLE RESOURCES TO POWER A CIRCULAR ECONOMY



All resources and products in a circular economy are renewable and sustainable, allowing them to be reused and recycled into the system. Source: Wisconsin Energy Institute/Chelsea Mamott.

Ultimately, both Friend and Donohue emphasize the importance of managing natural resources and creating bioproducts that are environmentally sustainable. "The bioeconomy is a very large enterprise. A future circular economy is potentially a big piece that fits into the bioeconomy, [in which] things are going to be in a circle—everything's going to be renewable," explained Donohue. "So, we want to use renewable resources as the raw materials or feedstocks that power a potentially multi-trillion dollar per year circular bioeconomy." With microbes, nature's ultimate biochemists, at the helm, the possibilities are not only inspiring, but may also be life-changing—preserving the future of the society and our planet.

Are you interested in hearing more about how experts are using genetic and synthetic engineering to improve industrial processes, develop new products and elucidate exciting bioeconomic applications for microbes? Browse our Bioeconomy Curated Itinerary and join us at ASM Microbe 2024 in Atlanta in June.

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Fueling the Future: How Microbes Will Power the Bioeconomy

BY MADELINE BARRON, PH.D.

From scents to solvents, fuels to fertilizers, the world runs on chemicals. Many of the compounds underlying the humdrum of civilization are unsustainable (e.g., fossil resources) and destructive to both people and the planet. Finding alternatives that are replenishable, mitigate risks and limit (or, better yet, clean up) environmental and atmospheric pollution requires thinking big—and bitty.

Despite being a limited resource with harmful environmental effects, fossil fuels make up a majority of the fuels used to power society. Source: iStock.com/mladenbalinovac.

FOSSIL FUELS ARE DWINDLING, MICROBES CAN HELP

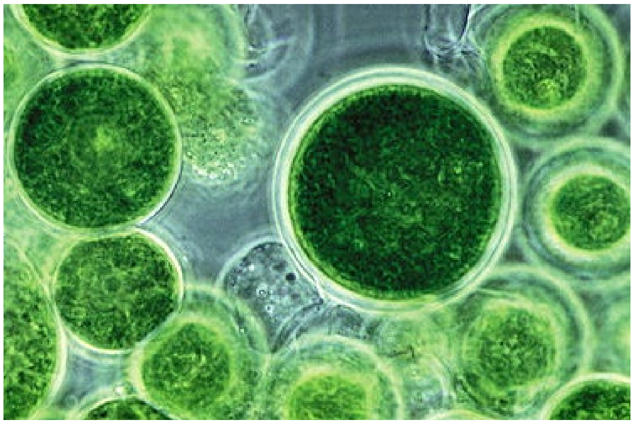
Society is powered by the chemical remnants of prehistoric, organismic death (i.e., fossil fuels). Here's the problem: fossil fuels are finite, they are drivers of climate change, and, despite the preceding facts in this list, they are everywhere (96% of transportation fuels worldwide are fossil resources). This dependence on a diminishing fuel source is a conundrum that scientists think biofuels—those derived from or produced by living organisms, like plant and microbial biomass—could help solve. "Renewable energy is our future," said Bing-Jie Ni, Ph.D., a professor of civil and environmental engineering at University of South Wales. "And I think biomass is one of the key pathways for producing renewable energy because it is so abundant on Earth."

Expanding the global biofuel landscape is a massive endeavor, with microbes as central characters. But leveraging microbial life to produce biofuel is both forward-thinking and old news.

The 10% ethanol added to gasoline to improve engine performance and reduce greenhouse gas emissions? Most of that is generated via fermentation of plant material by the yeast *Saccharomyces cerevisiae*. Feedstock fermentation by *Clostridia* bacteria yields butanol, another alcohol blended into gasoline, as well as used to generate plastics, solvents, fibers and more. These gasoline mixers are well-integrated into the current fuel economy. The future, however, will include harnessing the fuel-generating powers of microbes in new, more comprehensive ways.

ALGAE: A RICH SOURCE OF FUEL PRECURSORS

Take algae as an example. Among the many microbes with biofuel-producing potential, including cyanobacteria, fungi and yeasts, these photosynthetic organisms are near the front of the pack. Part of their appeal is that algae are physiologically rich in extractable biofuel precursors. In a sense, algae are not simply metabolic workhorses, they are also biofuel feedstocks themselves.



Algae are rich in biofuel precursors, and can be used to produce fuels like biodiesel. Source: Flickr.

Ni's lab is exploring how to use algae to produce high-value biofuel building blocks, specifically medium chain carboxylic acids (MCCAs) and alcohols. There is no current pathway for large-scale production of MCCAs. This is a desirable goal, however, as MCCAs are more easily processed than related fuel-precursors and have additional applications as additives in agricultural feedstocks and pharmaceuticals. Algae could be the way forward.

"We are currently looking at different operational conditions to regulate the process and produce more alcohol or MCCA," Ni shared. He and his team recently found that altering the pH in algae fermentation reactions changed the products the algae generated—a higher pH led to a greater abundance of MCCAs, while lower pH resulted in more alcohols. These findings will be useful for customizing the fermentation process "to produce the products you want," Ni noted, highlighting that the lab has also looked at how factors like temperature and microbial community structure and function influence product generation.

From a bioeconomic standpoint, there is a lot to love about algae. For one, they are more energy-dense than conventional crops: if corn can yield 600 L/hectare/year of ethanol, algae fermentation can produce 15 times that. Moreover, unlike fossil resources, algae do not add new carbon into an already overloaded atmosphere. Instead, they use atmospheric carbon to grow. Combustion of fuel derived from algae (and other biomass) releases that same carbon back into the environment.

In Ni's eyes, the fact that algae do not occupy arable land and can be cultivated in wastewater and marine environments is a particularly exciting feature. This differs from traditional bioethanol production, which relies on feedstocks derived from food crops, like sugar beets and corn (though non-edible feedstocks, like lignocellulose waste or crops, are emerging as viable alternatives). An added benefit: algae remove pollutants from wastewater during growth. Thus, the algae clean the water while the water provides a nutrient source for the algae to proliferate. The goal of the Ni Lab is to capitalize on these growth dynamics to engineer a system where the microbes double as wastewater-cleaning, fuel-producing machines.

"We propose, in the future, to use algae as a major pathway to treat wastewater," Ni shared, in which case algae will be cultivated in wastewater to produce even more algae. "Then, [using] those algae as a [fuel] feedstock, we will produce a lot of biofuels, which will return back to society," he continued. "Those algae are a renewable resource—that's how this whole system will be working toward sustainability." In other words, wastewater—which is never in short supply—provides a medium to support algae fermentation reactions, from which biofuel precursors can be extracted. The bioremediation features of the whole system are a bonus.

The team is also working on cultivating a circular bioeconomy in other ways, including engineering strategies to convert different biowaste (e.g., sewage sludge, food waste) into fuels.

Learn About a Career Using Microbes to Produce Biofuels

BEYOND FUEL: LEVERAGING WASTE TO PRODUCE HIGH-VALUE CHEMICALS

Ni's vision of chemically capitalizing on waste is applicable beyond the fuel realm. Indeed, fuels comprise only a subset of chemicals that are produced by human activities and can be found belching out of smokestacks, blanketing agricultural land or seeping into the ocean. With the help of microbes, scientists are finding ways to repurpose the often-problematic chemicals humans already make.

For instance, scientists demonstrated that the yeast *Yarrowia lipolytica* (known for its biotechnological potential) can convert hazardous waste from leather tanneries into amino-acid-rich supernatants, which can be repurposed as plant growth stimulants. There are also microbes that degrade and transform plastic into compounds that form the basis of products like nylon and biofuel. The options for microbe-driven waste conversion are vast and varied.

Gas emissions from steel mills can turned into ethanol with the help of the gas-fermenting bacterium, *Clostridium atuoethanogenum.* Source: iStock.com/simonkr.

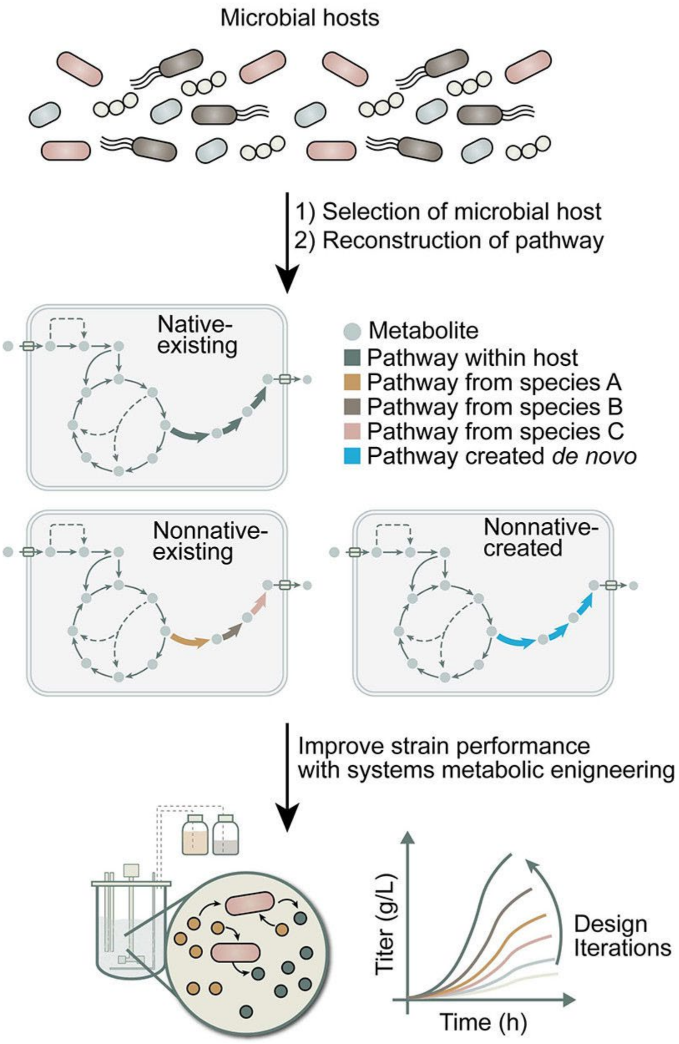
They also span all states of matter: liquid, solid—and gas. The biotechnology company LanzaTech leverages the gas-fermenting bacterium, Clostridium *atuoethanogenum*, to turn carbon-laden gas emissions from places like landfills or steel mills into ethanol, the chemical foundation for everything from fuel to perfume. The company's technology involves "bolting on a brewery next to a steel mill," explained Zara Summers, Ph.D., Chief Science Officer at LanzaTech, during a scientific session at ASM Microbe 2023. Gaseous waste is compressed, cleaned to remove toxins that could kill the bacteria and then fermented within a massive bioreactor (the "brewery"). The company can also "gasify" solid waste to feed into the system.

LanzaTech has 6 commercial plants with the capacity to produce 310,000 tons of ethanol from carbon emissions every year—preventing half a million tons of carbon from wafting into the atmosphere. "Humanity wants change for the climate, but [they] still buy stuff," like fuel and clothes, Summers said. "We're trying to let society live…but let people make a climate-smart choice."

Researchers also recently engineered a strain of *C. atuoethanogenum* to produce isopropanol and acetate, 2 chemicals with applications in diverse industries, thus expanding the potential products and their capabilities. But the key word here? Engineered.

GOT A MICROBE, WANT A CHEMICAL? CALL IN THE METABOLIC ENGINEERS

Most of the time, microbes are not inherently equipped to pump out chemicals at the efficiency and scale needed for industrial use, particularly if the goal is to "feed" them renewable carbon sources or waste material. Reaching that point requires engineering on the microscopic scale.



Microbes can be engineered to produce chemicals of interest. Sometimes they already produce the chemical natively, other times genes from other microbes must be introduced. View larger image. Source: ACS Publications.

Metabolic engineering involves "taking existing organisms and leveraging their metabolism in nature to enhance production of bioproducts," explained Ying Zhang, Ph.D., an associate professor of cell and molecular biology at the University of Rhode Island. The practice, which involves altering existing genetic pathways in microbes, or introducing new ones, is central to the bio-based chemical enterprise nestled within the bioeconomy.

While metabolic engineering is a ubiquitous practice (most studies related to microbial chemical synthesis use it in some capacity), there are various approaches scientists can take. "Some organisms, by nature, already have the capacity to produce products that we desire, like ethanol," Zhang said. In that case engineering efforts are focused on improving synthesis performance through tactics like overexpressing, downregulating or mutating endogenous genes. "But, in other cases, we take genes or enzymes we identify from elsewhere [e.g., other microbes], and introduce them into a new organism so that they gain the capacity to produce some useful products."

This approach is required if the "new organism" has desirable characteristics for chemical synthesis (e.g., survives at high temperatures, grows rapidly, withstands toxins used during production), but doesn't naturally produce the chemical of interest, or produces a precursor to the chemical but not the final product. Scientists then express genes from a single microbe that does manufacture the chemical, or engineer genes from multiple bacteria into a single biosynthetic pathway, in the host organism.

The engineering strategy researchers use ultimately depends on whether there are well-established engineering tools for the target organism, what physiological traits the microbe offers, whether those traits are conducive to chemical production and how much is known about the organism's native metabolism.

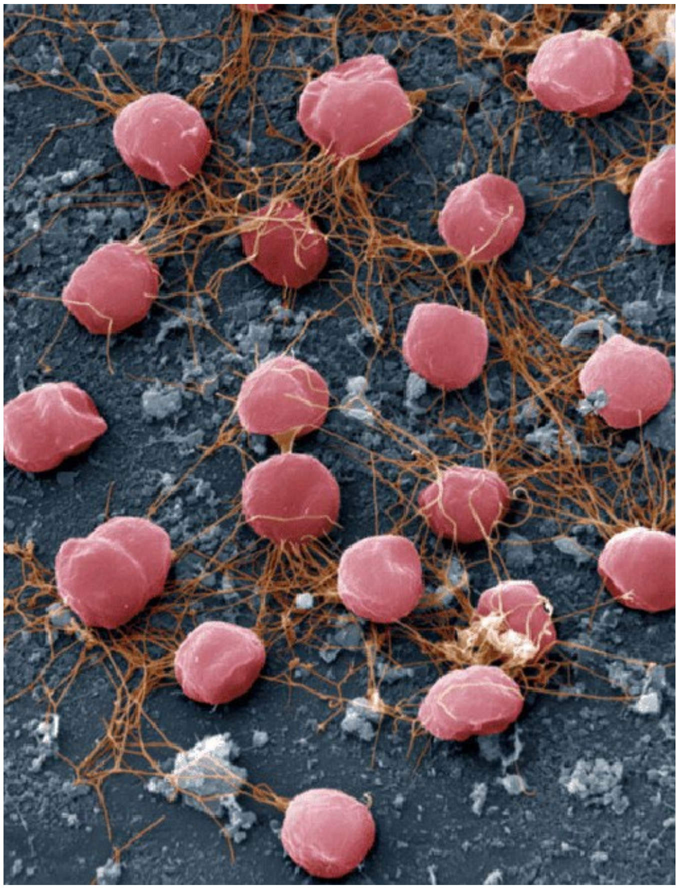
MEET THE MICROBIOLOGIST

099: Microbial engineering for biofuels and beyond with Wayne Curtis

METABOLIC ENGINEERING AND TECHNOLOGY GO HAND-IN-HAND

According to Zhang, the engineering approach a scientist takes also depends on uniting experimental data with computational power. Her lab builds genome-scale metabolic models—computational networks of all known metabolic pathways in an organism and how they interact—to inform engineering efforts. The models, which integrate diverse data (e.g., transcriptomics, metabolomics), serve as manipulatable blueprints, giving scientists a systematic view of a microbe. It allows them to delete or modify genes to determine how mutations might impact chemical output—all without touching a pipette.

With a model in hand, "our collaborators, who are geneticists or biochemists, don't need a search in the dark and randomly change things to see what works," Zhang said. "Rather, they have a more guided approach where we say, 'Okay, out of the 100 targets, maybe 10 of them will be optimal targets and you should try [them] first.'"



The hyperthermophilic archaeon, *Pyrococcus furiosus*, has been engineered to produce a range of chemicals. Source: Wikimedia Commons.

Zhang and her colleagues have demonstrated the value of this modeling-first approach for biofuel production. The team generated the first metabolic model of the hyperthermophilic archaeon *Pyrococcus furiosus*, an organism engineered to produce different fuels and chemicals, including ethanol. Previous research yielded a strain of *P. furiosus* that makes ethanol at 95° C—a valuable characteristic, as high-temperature fermentation can prevent contamination during production—but it does so inefficiently. Zhang's team wanted to fix that. They tested a panel of different mutations with their model to determine which combination (and simulated culture conditions) yielded the best benefit in ethanol production. "We found a bunch of mutants that could be useful for further testing, and some are now being examined in the lab," Zhang shared. "We're excited to see the next steps." The study highlights how technology begets strategic experimental action.

With that, the more scientists understand about the biosynthetic pathways of microbes, the greater the potential to harness and/or manipulate those pathways to produce valuable chemical products. Gaining such knowledge leans heavily on the growing spate of technological and analytical tools (e.g., omics, machine learning, modeling) that allow researchers to tease apart the intricacies of microbial life. Using these tools to profile microbial communities and/or metagenomic content in diverse environments—from swamps to hydrothermal vents—can also expand the repertoire of organisms scientists use to make chemicals and diversify the chemicals they make.

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WE'RE ALL IN THIS TOGETHER

The widespread implementation of microbial-produced or derived chemicals, particularly those involving the metabolic breakdown and conversion of waste, is still in its infancy. Cost is a primary limitation. While there are long-term economic benefits to investing in biofuels and biochemicals, non-renewable resources (fossil fuels) and existing production processes cost less in the short-term. This has limited the commercial expansion of renewable fuels and chemicals. Facilitating such expansion requires buy-in from government and industry partners, something that is happening at the level of individual companies and projects.

For example, LanzaTech, which is already operating in the commercial space, received a $200 million award from the U.S. Department of Energy (DOE) in March 2024 for a new project with their partner, Technip Energies, to produce sustainable ethylene (an ethanol-derived building block for chemicals and materials). Ni is working with various industry partners to scale up his team's algae-wastewater-biofuel system, and it's looking "very promising." Moreover, the DOE awarded $118 million in 2023 to various projects to accelerate domestic biofuel production.

The path forward is ultimately a multidisciplinary one, pulling scientists and stakeholders from a network of disciplines and industries. Zhang emphasized that her research would be impossible without the foundational work done by other scientists, and that bringing microbially generated chemicals to market requires collaboration. "We all bring in very different expertise and perspectives," she said. "I don't think a single person or lab can pull off all aspects involved in this." From Zhang's perspective, we must approach the future of the biochemical economy like we are all in it together—because we are.

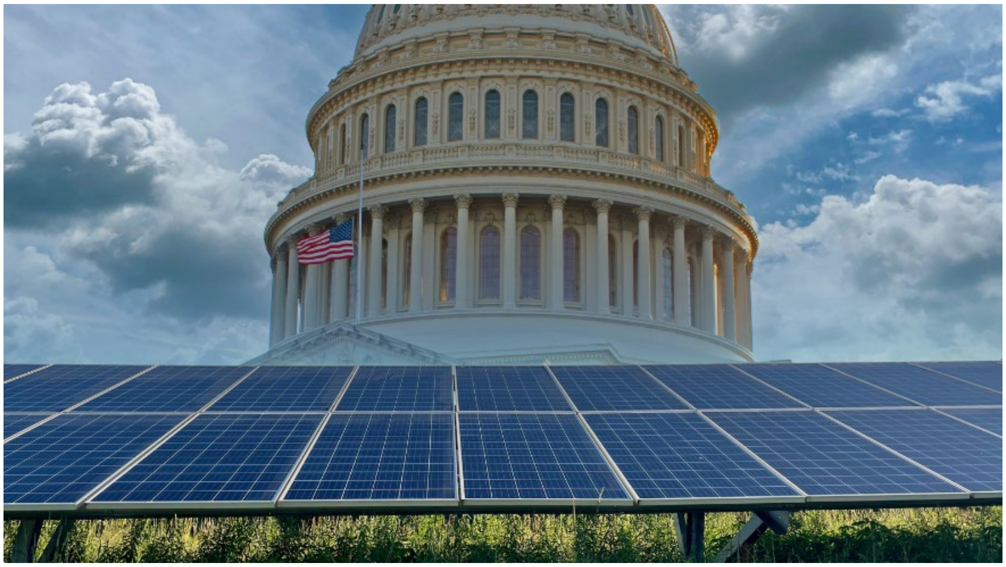
Heading to ASM Microbe 2024? Check out this curated itinerary of sessions on the bioeconomy, including those discussing the use of algae for bioproduction and synthetic biology for natural product discovery.

Register Now

A Call for Microbiologists to Influence Bioeconomy Policy

BY AMALIA CORBY, M.S.

Microbiologists are positioned to play a pivotal role in further developing a robust bioeconomy. As policymakers, funders and the public gain awareness of bio-based technologies and products, it is incumbent upon microbiologists to communicate the fundamental role of microbes.



Microbiologists are positioned to play a pivotal role in further developing a robust bioeconomy through policy. iStock.com/Douglas Rissing.

WHY SHOULD MICROBIOLOGISTS CARE ABOUT THE BIOECONOMY?

Microbial processes are the foundation of the bioeconomy. Microbes are the source of industrial catalysts and underpin the production of everything from food to fuel to antimicrobials. As U.S. bioeconomic policy is developed, those policies may impact your work, whether you consider your research part of the bioeconomy, or not. As policymakers aim to carefully balance promotion and protection of the bioeconomy, data security and ever-evolving biological threats, microbiologists should have a seat at the table and play a prominent role in discussing the risks and benefits of biotechnology research, development and applications.

A BRIEF HISTORY OF BIOECONOMY POLICY IN THE U.S.

In contrast to other countries, the U.S. lacks official mechanisms for developing, implementing and evaluating our bioeconomy. Although the Obama Administration published a National Bioeconomy Blueprint in 2012 that identified key strategic objectives, including strengthening research and development, advancing from the lab to the market, reducing regulatory barriers and expanding workforce and partnerships, no implementation plan was developed. Congress has also been slow to act, only recently passing legislation that includes provisions directing the White House to coordinate. With the passage of the CHIPS and Science Act in 2022, which included provisions to support federal investment in the bioeconomy, and the subsequent Executive Order on Advancing Biotechnology and Biomanufacturing Innovation for a Sustainable, Safe and Secure American Bioeconomy, there is renewed hope for increased coordination of federal agency bioeconomic investments and a more strategic approach to the U.S. bioeconomy. Reports released by the Biden Administration and various think tanks echo the 2012 Blueprint, which provides a key opportunity for interested stakeholders from research and industry to make a stronger case for implementation.

In addition to regulatory hurdles and the absence of coordination, federal funding remains a challenge that agencies having the foresight to see where research dollars are needed have tried to fill. While Congress does not have line items targeted to "bioeconomy" in its annual appropriations bills, these executive branch agencies have directed existing agency funds toward bioeconomy-related initiatives. Here are some examples:

- The Department of Defense is investing $1 billion over 5 years in domestic bioindustrial manufacturing infrastructure, as well as another $270 million for research and development into bio-based materials for defense supply chains.
- The U.S. Department of Agriculture (USDA) released a plan to boost biomass supply chain resiliency, announced a new $500 million grant program to support independent, innovative and sustainable American fertilizer production, and is supporting investments in bioeconomy research and workforce development through the National Institute of Food and Agriculture's Agriculture and Food Research Initiative.
- The National Science Foundation's BioFoundries initiative anticipates awarding up to $144 million over a 6-year period to enable access to infrastructure and resources to advance biotechnology. The CHIPS and Science Act also codified NSF's new Technology, Information and Partnerships directorate to advance use-inspired and translational research in all fields of science and engineering.
- In 2022, the Department of Energy (DOE) committed up to $100 million for research and development into converting biomass to fuels and chemicals, and an additional $60 million to de-risk the scale-up of biotechnology and biomanufacturing. This will lead to commercialization of biorefineries that produce renewable chemicals and fuels. More recently, DOE released a report outlining how the U.S. can sustainably produce more than 1 billion tons of biomass per year.
- Since 2021, the Advanced Research Projects Agency-Energy has awarded over $30 million in grants through the Energy and Carbon Optimized Synthesis for the Bioeconomy programs, which promote the use of advanced synthetic biology tools to engineer novel biomass conversion platforms and systems.

HOW IS ASM ENGAGING IN THE BIOECONOMY?

ASM was a key supporter of provisions in the CHIPS and Science Act that support the bioeconomy and the federal science agencies. We continue to work closely with Congress and the White House to facilitate an open dialogue with the scientific community, forging relationships with the National Security Commission on Biotechnology and connecting microbiologists in industry, academia and government to the Office of Science and Technology Policy.

ASM's Public Policy and Advocacy team continues to advocate for funding for key federal science agencies that fund basic, translational and applied research. Source: iStock.com/Ivan Bajic.

ASM's Public Policy and Advocacy team continues to advocate for funding for key federal science agencies that fund basic, translational and applied research. Additionally, the team monitors policy changes and regulatory developments that affect microbiologists and their work.

FIVE OPPORTUNITIES FOR MICROBIOLOGISTS IN THE BIOECONOMY

How can you get involved in the bioeconomy? If your work involves bioproducts, their underlying processes or foundational science, you are already part of it! If you want to do more, we've identified several additional opportunities to consider:

1. Become a Microbiology Ambassador

Biliteracy and public perception of science and technology will make or break the bioeconomy. ASM offers a variety of opportunities to improve your science communication skills and get people excited about the potential for microbe-based innovation!

2. Identify Challenges

The National Security Commission on Emerging Biotechnology is actively seeking input from the scientific community to help them identify biotech products that currently do not have a clear regulatory path to market and what makes them unique compared to products that do have a regulatory path. Now is the time to communicate what an ideal regulatory environment would look like.

3. Prevent Misuse and Promote Biosafety Norms

The responsibility to conduct research ethically and safely lies at all levels and is particularly important in today's environment. We encourage you to sign up for bioeconomy-related updates from ASM and to weigh in with your expertise when opportunities arise.

4. Shape the Future of Microbiology and Society

Weigh in on ASM's new strategic framework. As ASM embarks on a transformative journey to a generative, science-centric and globally focused organization that serves the needs of society at large, including the growth of the bioeconomy, we encourage you to send us your feedback.

5. Think Globally!

Microbes know no borders. Policymakers and the public need to see the benefits of international and interdisciplinary collaboration. Consider ways you can engage in global bioeconomy efforts or act as a connector between scientific disciplines.

Interested in connecting with microbiologists who are passionate about the bioeconomy?
Attend sessions from the Bioeconomy Curated Itinerary at ASM Microbe 2024.

Register Now

Fighting Foodborne Pathogens With Natural Antimicrobials

BY KANIKA KHANNA, PH.D.

This article, originally published in September 2022, was updated for inclusion in the Spring 2024 issue of Microcosm, "Microbes and the Bioeconomy: Greasing the Gears of Sustainability."

In April 2022, the World Health Organization traced ~150 cases of multidrug-resistant *Salmonella* Typhimurium infection in 11 countries to chocolate produced in Belgium, resulting in one of the largest chocolate product recalls to date. This is not an isolated incident. Every year, about 1 in 10 people fall prey to foodborne illnesses, as a result of food contaminated with harmful microorganisms or chemical substances. Contamination can occur at different stages of food preparation—processing, storage, distribution and/or handling—and is a severe burden on public health and the economy. Hence, preserving food is important for ensuring food safety and reducing wastage.

Every year, about 1 in 10 people fall prey to foodborne illnesses, as a result of food contaminated with harmful microorganisms or chemical substances. Source: iStock.com/Manjurul.

The food industry has now started exploring natural alternatives for preserving food to reduce the dependency on chemical preservatives, some of which are linked to obesity and metabolic syndrome. Specifically, natural antimicrobials produced by plants and microorganisms like bacteria and fungi can kill foodborne pathogens like *Salmonella* Typhimurium, *Escherichia coli, Listeria monocytogenes* and *Clostridium botulinum.* They also can target food spoilage bacteria like *Brochothrix thermosphacta*, *Lactobacillus spp., Bacillus spp.* and *Weissella spp.*, among others. Foodborne pathogens and spoilage microbes pose a serious health concern for consumers and destroy the appearance, texture and sensory characteristics of the food, affecting the food industry and consumers alike.

ESSENTIAL OILS—ESSENTIAL PLANT-BASED ANTIMICROBIALS

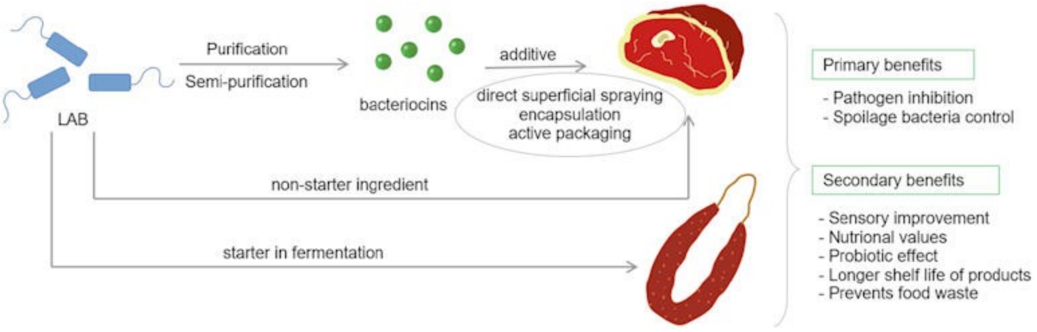
Herbs like oregano, thyme and rosemary are not only great flavoring options, but they also possess a treasure trove of antimicrobial potential against pathogens. Plants produce aromatic and volatile liquids called essential oils that have a wide spectrum of antimicrobial activity toward both gram-positive and gram-negative pathogens. Essential oils are important arsenals of plants' defense against pathogenic bacteria, fungi and insects, and the food industry is leveraging this knowledge to ward off food-borne pathogens.

Antimicrobial properties of essential oils can be mostly attributed to the presence of phenolic compounds like carvacrol, thymol and eugenol. The mechanism of action of these antimicrobial compounds is not understood completely, but evidence suggests they can make the bacterial cell membrane permeable, release intracellular contents or may interfere with membrane function by interacting with bacterial membrane proteins.

In addition to possessing antimicrobial activity, essential oils of different herbs and spices like oregano, thyme, cloves, rosemary and turmeric are also safe for human consumption. They are considered Generally Regarded As Safe (GRAS) by the U.S. Food and Drug Administration (FDA). Their antimicrobial activity against foodborne pathogens or spoilage bacteria depends on a number of factors, including their concentration and method of extraction, as well as the pH and temperature of food, to name a few. In a 2019 study, researchers showed that a low dose of tree tea essential oil (1.5% volume by weight) inhibits *L. monocytogenes* growth in ground beef. Another study in the same year demonstrated that the essential oil of garlic inhibited the growth of fungi *Aspergillus niger* and *Aspergillus flavus* in plum fruit.

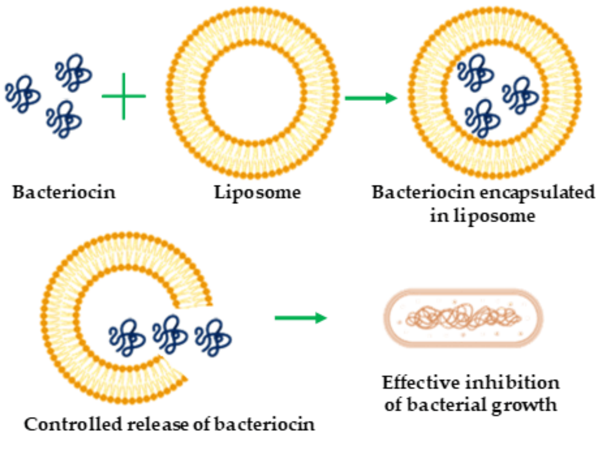
BACTERIOCINS—WEAPONS OF BACTERIAL WARFARE

Just like essential oils help plants fight pathogens, some bacteria produce small peptides with antimicrobial properties against closely related bacteria, which becomes advantageous when competing for resources in shared environments. These small peptides are called bacteriocins and help bacteria to establish their niche in an ecosystem. Bacteriocins are considered safe for human use as they are easily degraded by enzymes in the human gastrointestinal tract. Many of them are produced by bacteria belonging to Lactic Acid Bacteria (LAB) group that have a GRAS status. They can be used for food preservation in different ways—e.g., as purified products or by addition of bacteriocin-producing bacteria directly to the food.



Application of lactic acid bacteria and bacteriocins in meat products. (Click to view larger image.) Source: Sciencedirect.com.

One of the most well-studied bacteriocins is nisin, an antimicrobial peptide produced by *Lactococcus* and *Streptococcus* species. As of now, nisin is the only bacteriocin licensed by the FDA as a food preservative. Nisin inhibits bacterial growth by forming pores in cell membranes and blocking cell wall synthesis. It is used in many foods including dairy, meat and juices, either alone or in combination with other biopreservatives. For instance, nisin is used in the cheese industry to control the growth of *Clostridium spp.* and in the meat industry to reduce levels of *L. monocytogenes*. However, more research and clinical studies to test the immunogenicity and toxicity of other bacteriocins are needed for approval by regulatory authorities.



Schematic representation of bacteriocin encapsulation with liposome for antibacterial activity. Source: Scholarly Community Encyclopedia.

DELIVERING ANTIMICROBIALS IN NANOCAPSULES

While the idea of using essential oils extracted from herbs and spices and bacteriocins from LAB sounds great in theory, several factors limit practical applications. For example, the intense aroma (and flavor) of essential oils in food may not please everyone. Additionally, both essential oils and bacteriocins suffer from poor solubility and stability, reducing their efficacy.

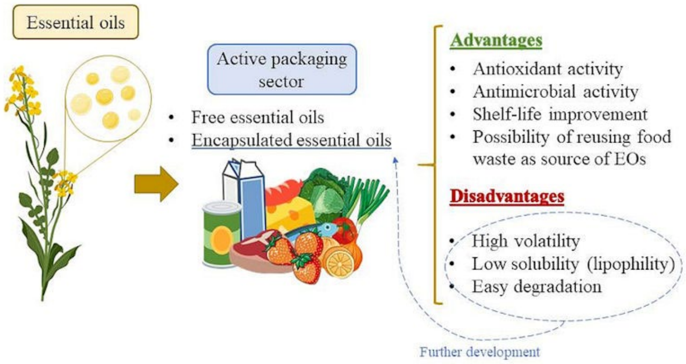
Technology to overcome these limitations is being investigated. One possibility is to deliver these antimicrobial sources by encapsulating them in nanoparticles, which allow them to remain stable in food items under different pH and temperatures. Such a system would ensure a slow and gradual release of antimicrobials during the shelf life of the food source, ensuring food preservation for longer durations. This is especially useful for controlled application of essential oils that may otherwise modify the sensory properties of the food.

Scientists are researching several biopolymers (e.g., chitosan, dextran, starch and alginate) that are non-toxic and eco-friendly to encapsulate essential oils and bacteriocins with promising results. For instance, nanoemulsions of clove essential oil in chitosan demonstrated improved antifungal activity against *A. niger*, while encapsulating nisin with alginate/resistant starch increased its efficiency in controlling *Clostridium* growth in cheddar cheese, compared to nisin treatment alone.

DELIVERING ANTIMICROBIALS IN EDIBLE COATINGS AND PACKAGING

If you have ever picked fruit on a farm, you will easily spot the difference between the fruit growing on trees and that which is sold in a grocery store. Often, the latter is treated with food-grade wax or edible films that give fruits their glossy appearance. The wax can be made up of chemicals or natural sources that protect the produce from moisture and spoilage.

Incorporating active ingredients like antimicrobials in food packaging can hit 2 targets with 1 stone—inhibiting the growth of spoilage microbes and extending the shelf-life of fresh produce. For instance, essential oils of oregano and/or thyme in soy protein isolate films reduced the populations of *Pseudomonas spp*. and coliforms in fresh ground beef patties, and chitosan coating, combined with nisin and a synthetic surfactant lauric arginate, reduced the growth of *L. monocytogenes* in sliced turkey deli meat.



Application of essential oils in food packaging. Source: mdpi.com.

In June 2022, scientists developed a low-cost and high-throughput food packaging system that wraps antimicrobial fibers around the food like a spiderweb. The system uses pullulan, a naturally occurring polysaccharide as the fibroid backbone, which is mixed with natural antimicrobials like nisin, citric acid and thyme oil. Using this system, scientists showed that avocados wrapped with antimicrobial pullulan fibers had longer shelf-life, better moisture retention and less natural microflora, compared to uncoated avocados. The wrapping is biodegradable and can be easily washed away, making it a promising method to package perishable products.

CONCERNS AROUND ANTIMICROBIAL RESISTANCE

When deploying any antimicrobial in the field, it is essential to address issues associated with the emergence of resistant pathogens. We don't know much about the development of antimicrobial resistance in foodborne pathogens or spoilage microbes when essential oils and bacteriocins are used as food preservatives.

However, there are some lab-based studies that reveal why some bacteria are resistant to certain types of bacteriocins or essential oils. For instance, an *S. enterica* outbreak in 2007 was traced to fresh basil leaves. Basil leaves are rich in phenolic compounds with antimicrobial activities, so this came as a surprise. Studies in the lab showed that *S. enterica* was able to develop resistance to the active ingredient linalool in basil. Other studies have shown that some bacterial strains of *B. subtilis* and *L. monocytogenes* that are resistant to nisin have higher levels of ABC transporters that expel nisin from the membrane and make bacteria immune.

It is critical to understand the mechanism of resistance to help minimize the emergence of resistance in the long term. Diving into these mechanisms, may reveal possible avenues to chemically synthesize derivatives of natural antimicrobials and/or use a combination of antimicrobials to overcome resistance.

FUTURE CONSIDERATIONS FOR NATURAL ANTIMICROBIALS

As the demand for fresh produce rises among health-conscious consumers, so does the need to prevent their spoilage by pathogenic microbes. Natural antimicrobials offer a safer alternative to chemical preservatives for food preservation. However, some concerns need to be addressed.

One major concern is determining the concentration of natural antimicrobials in food. Many studies addressing the effect of essential oils and bacteriocins on pathogenic microbes are performed in vitro on isolated bacterial species. However, they don't translate well when these antimicrobials are added to food items, presumably due to complex underlying interactions between the antimicrobials, the chemical structure of the food and the environment. Often, a higher concentration of antimicrobial is needed in food compared to in vitro studies, and regulatory authorities need to ensure these concentrations remain safe for human health.

Methods of application and delivery of antimicrobials also need to be optimized for different foods and different kinds of pathogens, without disturbing the sensory characteristics of the produce. Some potential solutions are delivering natural antimicrobials in nanoencapsulations and eco-friendly coatings, as well as testing the synergistic efficacy of a combination of antimicrobials.

Most importantly, the food industry and regulatory authorities have a moral obligation to actively involve consumers in the process in a transparent manner. Additional studies are needed to ensure that these systems preserve the chemical, biological and sensory properties of the food, without causing any harmful side effects to consumers' health.

Interested in learning more about what microbes span the clinical lab...and your grocery list? This next article details 3 microorganisms that, while opportunistic and capable of causing disease, are also being used for important functions, including development of food products.

Read the Article

Ruminant Methanogens as a Climate Change Target

BY MARY ANN BRUNS, PH.D

This article, originally published in June 2023, was updated for inclusion in the Spring 2024 issue of Microcosm, "Microbes and the Bioeconomy: Greasing the Gears of Sustainability."

Reducing atmospheric concentrations of methane—the potent, yet short-lived greenhouse gas—is critical for slowing the rise of global temperatures. Dairy and beef cattle, the world's most numerous ruminants, belch out about 100 teragrams (Tg) of methane (CH4) every year. Globally, enteric methane emissions rival those from the oil and gas industry.

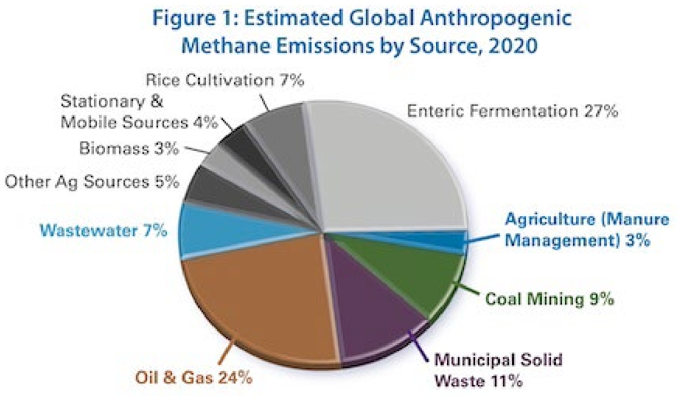
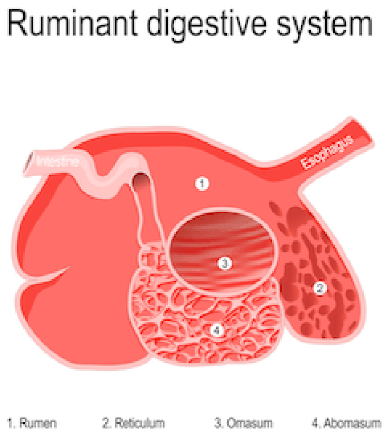


Figure 1: Estimated Global Anthropogenic Methane Emissions by Source, 2020

Estimated global anthropogenic methane emissions by source in 2020 were 27% from ruminant enteric emissions and 24% from the oil and gas industry.
Source: U.S. EPA/globalmethane.org

A quest to lower emissions of CH4 from ruminants has led to identification of 3-nitrooxyypropanol (3-NOP), a feed additive that specifically interferes with the final step of methanogenesis. Obtaining a product with an enzyme-specific mode of action is just one of many efforts over the past 75 years, along with development of vaccines and other feed additives, to reduce methanogenesis in the rumen and to understand its influence on animal health and productivity. Development of 3-NOP, now a commercial product, exemplifies the application of microbiological knowledge to mitigation of greenhouse gas emissions from agriculture. The compound, produced by the Dutch company DSM, has been patented and approved for use with dairy cows in Brazil, Chile and the European Union.



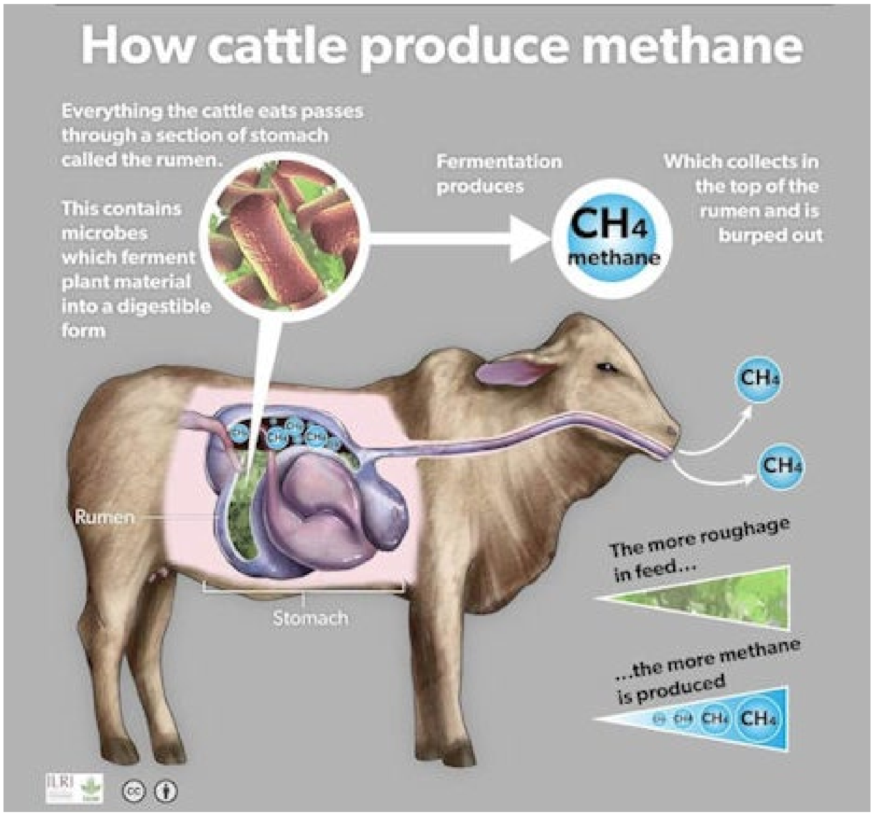
Ruminants' stomachs have 4 compartments: rumen, the primary site of microbial fermentation; the reticulum; the omasum, which receives chewed cud and absorbs volatile fatty acids; the abomasum, which is the true stomach.
Source: iStock.com/ttsz.

MICROBES AND METHANE IN THE RUMEN

Methane is produced by archaea that make up a small proportion (up to 4%) of microbial biomass in the rumen. As the largest of the cow's 4 stomach compartments, the rumen makes up 12-15% of the animal's body mass and houses an anaerobic community comprised of diverse bacteria, protists and fungi. These microbes degrade and ferment lignocellulosic roughage that the animal cannot digest.

Major microbial fermentation products consist of short-chain fatty acids that are absorbed by the animal, as well as CO2 and H2, which are converted by methanogens to CH4 waste gas. Some undigested material is regurgitated into the buccal cavity, or mouth, where the cud is chewed and swallowed again. Other undigested material passes into the abomasum, where mammalian digestive processes take over before entering the lower intestinal tract.

A typical dairy cow emits about 160 kg of CH4 per year. A minor proportion of CH4 (10-15%) from ruminants is produced in the intestinal tract and exits from their hind ends. The majority of CH4 (> 80%) exits from the mouth during eructation, or belching. The amount of methane produced within the rumen depends on many factors, including feed digestibility, total quantity of carbohydrate fermented, the ratios of fatty acids formed and H2 concentrations.



Cattle produce methane as a byproduct of microbial fermentation. Source: Flickr.

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METHANOGENS IN RUMEN MICROBIOMES

Most rumen methanogens have hydrogenotrophic metabolisms, meaning they use electrons from H2 to reduce CO2 to CH4, an efficient way to reduce H2 concentrations in the rumen. In a global study of rumen microbiomes from 32 ruminant species, 74% of archaea belonged to just 2 hydrogenotrophic clades representing *Methanobrevibacter gottschalkii* and *Methanobacterium ruminatium*. Two other known methanogen groups that produce CH4 from either acetate or methyl-group compounds are far less abundant in the H2-rich rumen habitat.

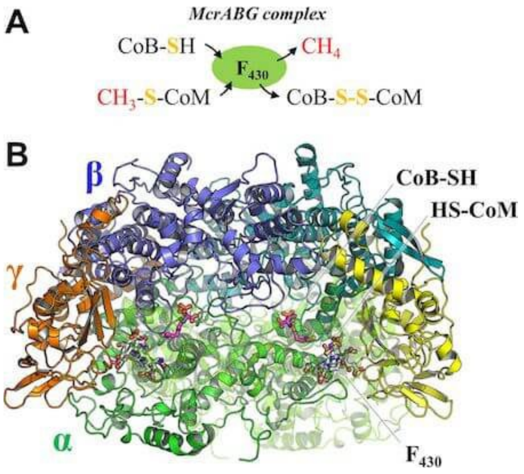
MOTIVATIONS FOR REDUCING CH4

Methanogenesis can be considered a symbiotic process because it pulls fermentation reactions forward, thereby assisting in continued production of fatty acids for the animal. However, it also represents an energy loss for milk and meat production. Percentages of gross energy intake lost through methane eructation have been estimated at 2-12%, with greater losses associated with forage-rich diets. For decades, improved feed efficiency was the goal for research on lowering ruminant methane through dietary modifications. Greenhouse gas mitigation became a stronger impetus for reducing livestock methane with the first Intergovernmental Panel on Climate Change (IPCC) report in 1992. The IPCC report described the rise in atmospheric CH4 concentrations from 750-1800 parts per billion (ppb) over the previous 100 years. It recognized that global population increase and demand for animal protein would drive greater livestock production, with 1.3 billion cattle estimated to account for 12% of global methane emissions in 1995.

METHANE INHIBITION

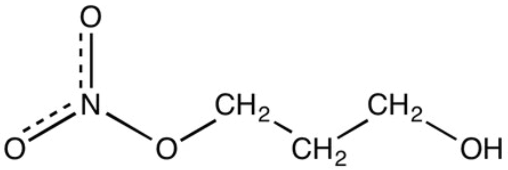
FEEDING 3-NOP

More recently, a targeted, biochemical approach to methanogen inhibition was based on crystal structures of enzymes responsible for methane production (methyl coenzyme M reductase, or MCR) in hydrogenotrophic methanogens like *Methanobacter thermoautotrophicum* and *Methanothermobacter marburgensi*. Computational screening identified a group of small molecules, the nitrooxy carboxylic acids, as potential inhibitors that could fit into the MCR active site. Enzyme inactivation was hypothesized to occur when a compound like 3-NOP fit into the MCR active site, inhibiting its ability to carry out a key step in CH4 formation. More specifically, 3-NOP binds to MCR near coenzyme F430, a prosthetic group containing a critical Ni(I) atom. Proximity of the nitrate group of 3-NOP is postulated to cause oxidation of Ni(I) so that it can no longer carry out the final reduction step of CH4 formation.



Chemical reaction and quaternary structure of MCR.
Source: Wagner T. et al./Journal of Bacteriology, 2017.

Feeding trials of 3-NOP to sheep, lactating cows and beef cattle in feedlots have resulted in an average 30% reduction of CH4 emissions, confirming that 3-NOP works in vivo. In the laboratory, however, inhibition by 3-NOP on growth of different methanogens in pure cultures varies widely. Very low (micromolar) concentrations prevent growth of hydrogenotrophic methanogens like *M. ruminantium*, while 100 times higher concentrations are needed to inhibit other hydrogenotrophs like *Methanomicrobiium mobile* and *Methanosarcina barkeri*. Phylogenetic and physiological diversity among methanogens may make it difficult to bypass the symbiotic relationship established between ruminants and archaeal consumers of H2 in the rumen.



Chemical structure of 3-NOP.
Source: Wikipedia.

Next-generation sequencing of extracted microbial DNA and RNA from rumens has become a boon for more comprehensive understanding of the effects of 3-NOP on the microbiome. Concomitant with reduced CH4 emissions, for example, supplementation of 60 mg 3-NOP per kg of feed dry matter in dairy cows over 4-12 weeks resulted in a decline of dominant *Methanobrevibacter* spp. and increases in unclassified *Methanobacteriaceae* and *Methanosphaera* spp. compared to controls. Because non-methanogen members of rumen microbiomes were not significantly affected, alterations in methanogen composition by 3-NOP appeared to explain the increases in propionic acid (a short chain fatty acid) and H2 that were measured during the study.

Notably, as methanogens inhibited by 3-NOP die, H2 concentrations may accumulate and interfere with carbon and energy flow before other methanogens can take over. This may necessitate the addition of other electron acceptors to assist efficient fermentation. Whether 3-NOP will be used more extensively will depend on its acceptance, cost and research confirming that animal health and productivity are not affected in the long term.

FEEDING *ASPARAGOPSIS TAXIFORMIS*

Most recently, feeding ruminants with the red seaweed *Asparagopsis taxiformis* to reduce methane emissions has attracted attention in the industry. In vitro studies using these algae have shown methane inhibition of up to 99% without adverse health effects. The proposed mode of action is introduction of a bioactive brominated compound (bromoform) contained within the seaweed biomass, which is a halogenated methane analogue that inhibit methanogenesis. For the specific purpose of climate mitigation, considerable venture capital is being directed toward production of adequate supplies of red seaweed in locations that would lower transportation costs.

LACK OF SUCCESS WITH ANTI-METHANOGENIC VACCINES

In addition to many dietary supplements tested for methanogen suppression, anti-methanogenic vaccines have been developed based on the recognized dominance of relatively few hydrogenotrophic species. To that end, vaccines have been derived from either whole cells or cell components of mixed cultures of methanogens. However, in vivo studies measuring methane emissions, mainly in sheep, following inoculation with mixed-culture vaccines have reported little or no reductions, possibly reflecting greater breadth of methanogen diversity. To address this challenge, antibody cross-reactivity using immunocapture beads can be tested in vitro with diverse methanogens in rumen fluids prior to in vivo trial. Still, with a lack of vaccine success, dietary modifications remain the most common approach to reducing CH4.

LOWERING METHANE EMISSIONS VIA BREED SELECTION

Consistent patterns in the magnitude of methane emissions are observed among species and breeds of domestic ruminants. Some breeds of cattle emit consistently lower levels of methane than others, with beef cattle generally emitting less methane than dairy cows. Evidence that host genetics affect microbiome composition and energy flow through the rumen is leading to efforts that integrate animal genetics and microbiome analyses. Buccal sampling of saliva and mouth contents are being evaluated for biomarkers that reflect animal traits for faster and more frequent data collection.

GLOBAL PROSPECTS

In some countries, the rise of confined feedlot systems for raising and finishing cattle has resulted in increased feeding of concentrates containing more digestible carbohydrates and more protein than high-roughage forages. Methane emissions from cattle fed with concentrate are lower than from cattle fed high-roughage diets. Genetic selection of cattle can also result in lowered methane emissions, with high-efficiency feeders producing less methane than low-efficiency feeders.

However, concentrate feeding, use of selected breeds and feed additives are not as readily implemented in under-resourced nations, in part due to limited research infrastructure. Still, options are available for agriculturalists in under-resourced countries to lower methane emission from ruminants. Raising ruminants not only provides milk and meat, but also provides crucial ecosystem and economic services of converting low-quality cellulosic materials to high-quality protein on land that cannot otherwise be cultivated. And, as we look toward supporting a circular economy, it will be critical to ensure equitable global access to multiple agricultural innovations that reduce methane emissions and protect human, animal and environmental health worldwide.

Learn more about current knowledge gaps and research priorities related to reducing methane emissions from ruminant methanogens.

View Our Research Roadmap

How Microbes Help Us Reclaim Our Wastewater

BY BRIAN LOVETT, PH.D.

This article, originally published in June 2023, was updated for inclusion in the Spring 2024 issue of Microcosm, "Microbes and the Bioeconomy: Greasing the Gears of Sustainability."

A key challenge to setting up a civilization is the plumbing. This is one reason why major cities, particularly those with a long history, are conspicuously placed along rivers; pressurized water and the kind of plumbing we have today would not be unfamiliar thousands of years ago to civilizations like the Mayans. Despite its long and pivotal role in our success, once wastewater is done swirling down our toilet bowls, most of us are blissfully unaware of what happens next. It may not come as a surprise that microbes are the heroes of this untold story.

SEPARATING THE WASTE FROM THE WATER

Before we examine these little heroes of sanitation, let's establish the big picture of wastewater management. The ultimate goal is to take water rendered unusable by waste and purify it sufficiently to restore it to the environment. Waste removed during the process is digested by microbes, and what remains is dried and disposed of in landfills, incinerators or applied to soil as a conditioner, depending on the source and process. Large-scale operations manage the bulk of our wastewater and follow a process called activated sludge. Invented a little over 100 years ago, this process incorporates the following basic steps: filtration, activation (aeration), clarification (settling) and disinfection.

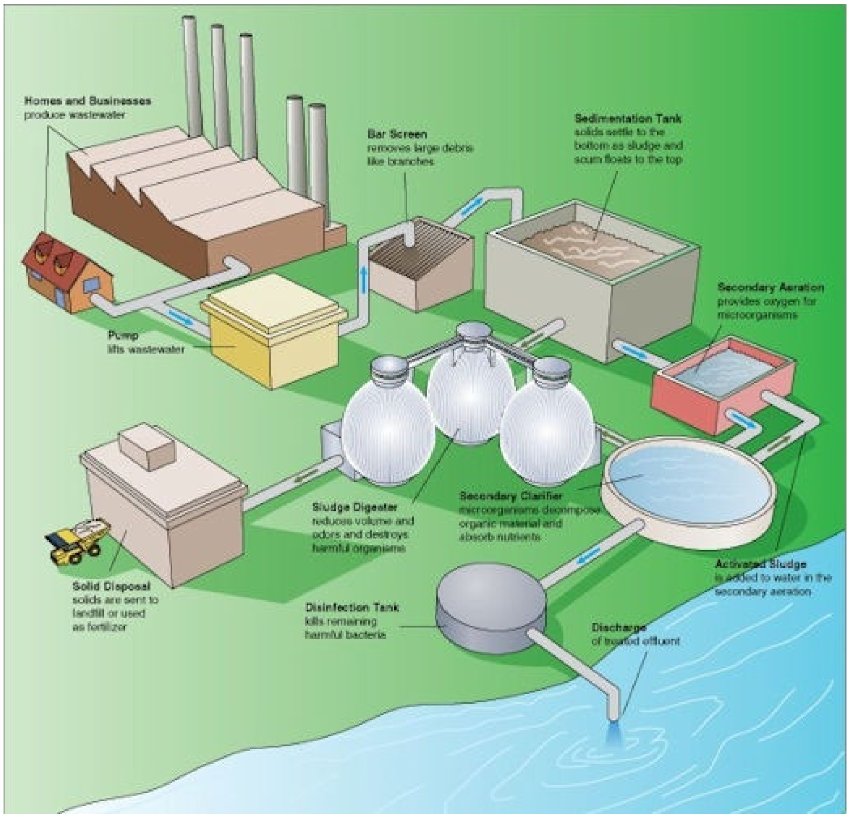


Illustration of the main steps in the activated sludge process used by large-scale wastewater management facilities. Source: Wikimedia Commons.

Sludge is the euphemistic term used to describe the brown, viscous liquid that results after raw sewage has been filtered to remove grit. The sludge itself is inhabited by a diverse community of microbes, including bacteria, protozoans and even some eukaryotes like tardigrades, that have hitched a ride (perhaps through us) along the sewers connecting our homes to the waste management facility. Sludge comprises an incredibly rich medium, full of organic matter that we find unappetizing, but bacteria find delicious. Once this sludge has been processed by bacteria, it is called activated sludge, which can refer to both the material itself and the waste management process.

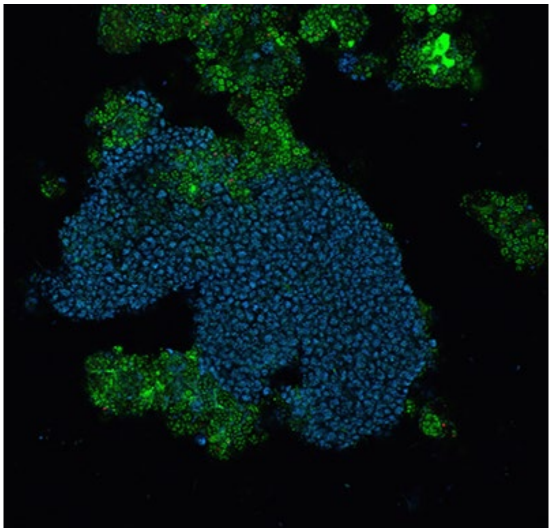
The cast of characters varies in each waste management facility, but a recent global survey of the microbiome of wastewater activated sludge found that there are 28 core bacterial members of healthy activated sludge. The most abundant of these are *Dokdonella kunshanensis*, *Zoogloea* species and Nitrospira species. These are all aerobic, gram-negative bacteria. We know little about *D. kunshanensis*, other than it can be readily isolated from activated sludge. We know more about the other 2 species. The name *Zooglea* means "living glue" because of the species' proclivity to form sticky biofilms. *Nitrospira* species help oxidize nitrite to nitrate, and are important for cycling aquaria because nitrate is much less toxic to fish than the ammonia they excrete. As we shall see, these traits facilitate the activated sludge process. However, these most abundant bacteria still represent only a small percentage (~3% of total abundance) of the diverse bacteria present in activated sludge microbiomes.

AERATION

After passing through filtration, key bacteria are alive, but are not thriving like we need them to, so we "activate" them through aeration. Stirring or bubbling the sludge introduces oxygen throughout, which encourages air-loving microbes to begin to actively grow and reproduce, while discouraging the growth of other kinds of microbes. This simple selection, inherent to the process, is similar to how home microbiologists cultivate a specific, useful subset of microbes for composting or for a sourdough starter. In fact, facilities sometimes prime incoming sludge with activated sludge to ensure bacterial communities from healthy batches are present from the beginning.

The aerobic bacteria in the sludge digest the organic material around them to reproduce and grow, and change the chemical makeup of the sludge by oxidizing ammonia into nitrate and nitrite in a process called nitrification. The process follows a progression that will be familiar to anyone who has studied microbiology: there is a lag period where these bacteria initially begin to grow, followed by an exponential growth phase, a stationary phase and finally a senescent phase where starving bacteria begin to die off. In their bubbly, sludgy new home, these bacteria are doing most of the work for us, turning sludge into more bacterial cells.

Most of the role that we play in this process is trying our best to keep the microbes on track. This involves taking samples of the sludge to track its progress. Metrics, like the amount of dissolved oxygen and organic matter, the amount and types of bacteria, such as culturable indicator species (e.g., coliform bacteria), and other indicators, are used to identify various stages of the process. Waste management facilities also use biological oxygen demand (a measure of the amount of oxygen being consumed by microbes) to calculate the food-to-mass (or microbe) ratio. These values allow scientists to chart their course to the stationary phase when the sludge no longer needs to be aerated.



A floc of bacteria at 400X magnification removing phosphorus from medium in the lab. All bacteria were stained green, and Candidatus Accumulibacter Phosphatis, which accumulates phosphorus, were stained in blue. Courtesy of Connor Skennerton. Source: Wikimedia Commons.

When things are going well, it's easy to see. Clumps of bacteria, called flocs, form in the sludge as these microbes help us reclaim the water within. Similar to its homophone, flocculation is a process where these aerobic bacteria produce biofilms composed of extracellular polymeric substances that allow them to stick together. These biofilms help microbiologists monitor when a healthy consortium of bacteria are actively working to digest waste, while signs like excessive foam point to microbes that aren't team players.

If the wrong microbes show up or the process goes off track, then we intervene chemically or remove excess sludge. Filamentous bacteria can become the "wrong microbes" if they don't cooperate with the biofilm consortia of bacteria and produce excessive filaments. This "filament bulking" prevents sludge from settling. In particular, *Nocardia* species and *Microthrix parvicella* convert oil and grease into a brown foam by increasing hydrophobicity in the system, which stabilizes the foam.

By the end of the process, a mature food chain of diverse microorganisms capable of transforming the sludge biochemically emerges. Bacteria feed on sludge, amoebae and ciliates (such as peritrichs) feed on the bacteria and tardigrades (and the occasional nematode) comprise the apex predators. These higher-order members of the food web become more prominent during the exponential phase of bacterial growth, but if they become too common, it's a sign something has gone wrong (such as aerating the sludge for too long).

CLARIFICATION AND DISINFECTION

When it's time to stop bubbling, the activated sludge enters its next phase: clarification. As it says on the back of juice bottles, "settling is natural," and so we wait while the flocs and remaining sludge settle out of a now-watery solution. Once the water has clarified to the satisfaction of the facility, the activated sludge, which has concentrated at the bottom, is sent off for further processing.

Remaining sludge goes through a second bacterial digestion without oxygen. Anaerobic bacteria further break down the sludge and reduce nitrate and nitrite into nitrogen gas through a process called denitrification. Biogas (primarily methane and carbon dioxide) produced during this anaerobic digestion is burned off or further purified for sale to energy companies. Such anaerobic digestion can occur at various stages of the process.

The very last of the activated sludge that survives primary and secondary microbial digestion is then dried. This "waste-activated sludge" is ready to leave the facility as fertilizer or smoke, depending on its composition. Here, many weeks and microbial assists later, is the final destination of our modern sewage.

The supernatant (Latin for "great swimmers") on the top after clarification is disinfected with chemicals, like chlorine, or with ultraviolet (UV) radiation. This final step effectively kills any remaining organisms, pathogenic or otherwise, that have made it this far. If pathogens are a concern, checks are made throughout the process to ensure they are eliminated as the water is reclaimed. Finally, the water that may have once passed through your kidneys is ready to rejoin the ecosystem, often by way of local lakes and rivers.

For most of us, our contribution to this process is pressing the toilet handle. As is often the case, we can thank microbes for doing the hard work and making it look easy.

Interested in learning more about wastewater? Gain a deeper understanding of how wastewater spreads AMR, and learn how this knowledge can help microbiologists mitigate this global health threat.

Read the Article

Boosting Bee Health With Probiotics and Vaccines

BY VILHELMIINA HAAVISTO

This article, originally published in June 2023, was updated for inclusion in the Spring 2024 issue of Microcosm, "Microbes and the Bioeconomy: Greasing the Gears of Sustainability."

Honeybees, especially the Western honeybee *Apis mellifera*, are important pollinators in agricultural environments contributing to the success and sustainability of the bioeconomy. Worker bees carry out all colony functions except for laying eggs, and their health is critical for the wellbeing of the whole hive. If these workers disappear, destructive events known as colony collapses, can result. In a colony collapse, worker bees die or leave the hive, abandoning their queen and thus forfeiting the entire hive.

The driving forces of colony collapses are both complex and unclear, but they are crucial to understand if we want to protect honeybees and the ecosystem and economic services they provide. Thus, bee health is an important focus of both scientific research and conservation efforts around the world. Researchers are considering factors such as diseases, pesticides, changes in food sources and even stress as they study how these kinds of perturbations affect the survival, behavior and gut microbiota of honeybees. Some of these dangers have microbial origins, and some even have microbial solutions.

Honeybees live in dense and complex societies, whose ecosystem services are indispensable. Source: Flickr/Ivan Radic.

HONEYBEE GUT MICROBIOME OFFERS CLUES

One particularly microbe-dense habitat is the gut, which is just as important for insects, such as honeybees, as it is for mammals like humans. Though the gut microbiota of honeybees is relatively simple, harboring just 5 core members, it provides many benefits for their health. These include improved growth, digestion and protection from opportunistic pathogens.

What's more, the gut microbiota may play a role in what is known as the gut-brain axis, the 2-way communication between gastrointestinal tract and the central nervous system. Scientists have shown that the gut microbiota affects the social behavior of honeybees, as it appears to be important for mediating social interactions and parsing sensory information from their environment. Bees use such social cues to transmit information amongst themselves, helping them to navigate the world around them and underscoring the importance of the gut microbiota for a functional hive.

Head-to-head interactions among honeybees help them transmit information and keep the hive functioning. Source: Flickr/U.S. Department of Agriculture.

In honeybees, as in other animals, a healthy gut microbiota is crucial for a healthy host. However, much like ours, the honeybee gut microbiota is also vulnerable to disturbances that induce a disrupted state known as dysbiosis. Threats come from many sides: antibiotics, habitat loss, diet, pesticides and even the wide-reaching impacts of climate change. Dysbiosis in the gut can leave honeybees more vulnerable to pathogens and negatively impact their health.

PRO-BEE-OTICS

Given the importance of the gut microbiota, some bee health efforts start with microbiota-focused treatments. In an approach similar to interventions for human gut disorders, some researchers are looking to probiotic treatments to protect honeybees from gut dysbiosis and its negative effects. Although robust evidence for the efficacy of probiotics in honeybees is still mostly lacking, native bee strains seem to have more success at remaining in the gut after the probiotic treatment has stopped than commercial probiotic mixes that are not necessarily bee-derived.

Engineering Probiotics to Combat Deformed Wing Virus

Going a step further, efforts are also underway to produce "designer" probiotics for bees, which can help to protect them against parasites and pathogens. For example, the *Varroa* mite, a destructive honeybee pathogen, both parasitizes the bees and transmits a viral pathogen known as deformed wing virus (DWV). *Varroa* and DWV are unwelcome, but extremely common, hive inhabitants and can cause colony collapses.

However, researchers are now beginning to understand how protection against these pathogens can come from within. In one study, researchers genetically engineered *Snodgrassella alvi*, 1 of the 5 core honeybee gut microbes, to stimulate the bee's immune system and mount the RNA interference response. In this response, the immune system recognizes exogenous double stranded RNA (dsRNA) inside cells and degrades any matching dsRNA by chopping it up. This response can be harnessed to target pathogen-specific RNA, although directly injecting targeted dsRNA has had limited success in honeybees. However, engineering *S. alvi* to produce it inside the host was highly effective at protecting bees against both *Varroa* and DWV.

The *Varroa* mite is a destructive honeybee pathogen. Source: Flickr/BBC World Service.

Added Protection Against *Varroa* Mites

For DWV, *S. alvi* produced dsRNA matching sections of the viral genome, priming the bees to chop up matching RNA belonging to the virus, while the mechanism for fighting *Varroa* was slightly more complex. When *Varroa* mites parasitize bees, they eat dsRNA-containing fat bodies on the bee thorax and abdomen. When the mites ingested dsRNA matching 14 of their own essential genes, produced by *S. alvi* in the gut, the bees were protected from infection as the dsRNA triggers the mites' RNA interference response, leading them to chop up their own RNA. Although it is a promising step forward, whether this kind of technology can be scaled up to protect entire hives from different kinds of diseases is still an open question.

THE FIRST HONEYBEE VACCINE GENERATES BUZZ

In addition to these new insights in the field of probiotics, major strides in vaccine development are changing the way we look at American foulbrood, a widespread bacterial disease that causes colony collapses. In January 2023, the U.S. Department of

Agriculture (USDA) authorized the first oral vaccine to protect honeybees against American foulbrood. The vaccine technology rests on feeding inactivated *Paenibacillus larvae* (the causative agent of the disease) to the queen bee via the royal jelly, her special food source. Once the queen bee ingests the vaccine, her body produces antibodies that propagate into her eggs. This also renders her progeny, *P. larvae*'s target, immune. This vaccine technology paves the way for protection of honeybees, as well as other insects, against other microbial threats.

Wild bees, such as the carpenter bee, are much less studied than honeybees. Source: Flickr/Jim Nelson.

BEE-YOND THE HONEYBEE

Although significant progress has been made, some argue that the overwhelming research focus placed on honeybees is myopic, as they are by no means the only pollinators on the block. For example, wild bees are an incredibly diverse group comprising around 20,000 species.

While wild bees don't produce honey, they are extremely important members of ecosystems in their own right. We know very little about many of them, especially their gut microbiota, limiting the microbially minded steps we can take to protect them.

Saving the bees—both honey- and wild—is an ongoing global effort. Understanding bees from the inside out can help us to protect them from some of the threats they face, though we cannot lose sight of larger issues, like habitat loss and climate change, to which microbially based interventions are little more than a band-aid.

Interested in learning more about the factors that are threatening bee colony survival? Check out this next article, which explains microscopic and macroscopic stressors, as well as what can be done to help address the problem!

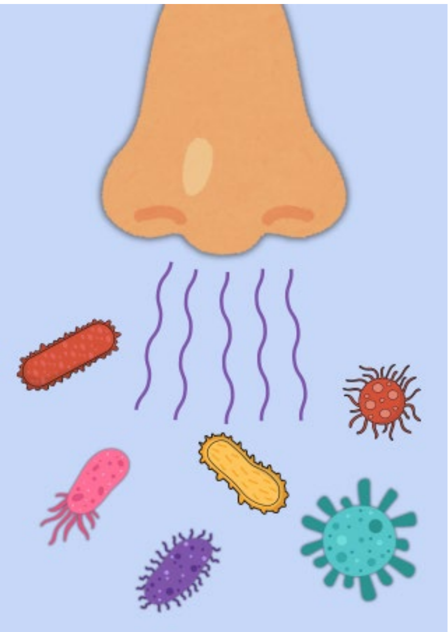
Read the Article

What’s That Smell? The Role of Microbes in the Scented World

BY ELISE PHILLIPS, PH.D.

This article, originally published in June 2023, was updated for inclusion in the Spring 2024 issue of Microcosm, *"Microbes and the Bioeconomy: Greasing the Gears of Sustainability."*

Humans subconsciously interact with a multitude of microorganisms through the scents they produce; the yeasty smell of fresh dough, geosmin after it rains, acidic ferments mediated by *Lactobacilli* and even stinky feet. The molecules that we recognize as scents play an important, yet poorly understood, role in microbial physiology and interactions with other microorganisms and larger eukaryotes. Some of these scents are unique and can act as microbial fingerprints, allowing us to identify colonizing organisms, which may offer a non-invasive glimpse into infectious diseases. Microbes are also adept at creating non-native flavors and scents that are used in the industrial production of scent and flavor compounds for food and cosmetic enhancement.



Source: American Society for Microbiology.

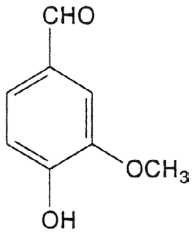
VOLATILE ORGANIC COMPOUNDS

What we understand as scents and flavors are typically small aromatic molecules, known as volatile organic compounds (VOCs). These molecules have high vapor pressures, essentially boiling and turning into gas at room temperature, which is why they are considered volatile. Because of their gaseous nature, VOCs can travel far distances, making them valuable tools for communication amongst diverse organisms.

MICROBIAL GREENING OF FLAVOR PRODUCTION

Many scents and flavors that are used in food, cosmetics and medicines are inspired by natural products, often plants. Unfortunately, some of these plants are rare because they are limited to very specific environmental niches, so demand regularly exceeds supply. To overcome this, many such compounds are produced artificially, frequently from petroleum-based starting materials. While this protects some natural resources and drives down costs, there are environmental impacts, and many people are wary of artificial additives.

A solution to this involves microbial factories, using microorganisms to make compounds of interest. These microbially produced versions of flavor compounds are referred to as "like-nature," and are not considered artificial by most legislative bodies. Flavor compounds can be produced either enzymatically or with whole cells. The compound can be produced by cells natively, or cells can be metabolically engineered to increase yield. Many compounds have a similar structure, so engineered metabolisms can be manipulated to become modular, requiring only the final reactions to be exchanged. For example, mint, citrus, patchouli and sandalwood scents are all terpenoids that can be produced through a single biosynthesis pathway expressed in yeast that diverges enzymatically at the final steps. The type of starting material, or feedstock, used in scent production is important for ecological and economic considerations. It is beneficial to use feedstocks that would otherwise go to waste, such as agricultural by-products, to add value to the waste product.



Vanillin

The chemical structure of vanillin. Source: Science Direct.

One particularly exciting waste feedstock is the plastic polyethylene (PET). This strategy couples enzymatic PET breakdown with engineered *E. coli* that can metabolize the monomers into vanillin. Vanillin is the primary molecule that we associate with the flavor of vanilla, which is one of the most expensive spices when derived from the plant. By converting PET into vanillin, producers can generate a valuable flavoring compound from trash.

SCENTS-ING DISEASE

Throughout history, infectious disease diagnosis has included the detection of characteristic scents known to be associated with certain diseases. Although, this fell out of practice for many years, it is starting to see a resurgence. For example, *Pseudomonas aeruginosa* colonization, which is associated with disease progression in cystic fibrosis patients, is known for its grape-like odor, which can be attributed to the VOC 2-aminoacetophenone (2-AA). Because this compound is known, investigation of a non-invasive diagnostic approach in which patient breath is collected then analyzed for 2-AA using Gas Chromatography-Mass Spectrometry (GC/MS) is underway.

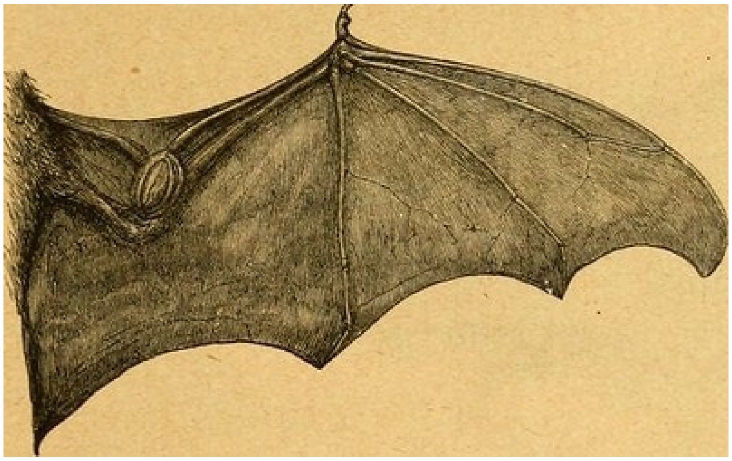
Some vector-borne diseases go so far as to rely on scent alteration of infected host species for their own survival. Mosquitos use smell to find their blood-meals, and improving the host's scent increases the likelihood of mosquito visitation and disease spread. Malaria and flaviviruses, including Zika and dengue, alter the scent profile of infected hosts by increasing host (or host microbiome) production of VOCs, including monoterpenes and acetophenone by malaria and flaviviruses, respectively. Flaviviruses increase acetophenone production by suppressing an antimicrobial protein (RELMα) that normally inhibits some of the acetophenone-producing skin commensal bacteria. By inducing RELMα with vitamin supplementation, the VOC production is reduced and consequently so is mosquito host-seeking. This suggests a potential strategy for reducing community transmission of mosquito-borne flaviviruses.

VOLATILE ORGANIC COMPOUNDS MEDIATE INTERKINGDOM COMMUNICATION

Humans are not the only organisms that sense and cultivate microbial scents. Microbially produced VOCs are important modes of interkingdom interactions, ranging from cooperative to antagonistic.

Animal Responses to VOCs

In the animal kingdom, microbes are a key component of "perfumes" used to attract a mate. For example, male greater sac-winged bats transfer secretions (and microbes) from their genitals and throats to their wing sacs in a daily ritual that maintains their individual scents. Female bats, who do not do this, have a different wing microbiome composition, so it is thought that males use their microbe-influenced scent as a form of courtship. However, no specific microbes have been tied to specific scents or mating success for male bats.



An illustration of a sac-winged bat wing. Source: Flickr.

Many other animal species demonstrate similar behaviors, in which mating rituals are mediated by microbial produced scents. For example, mouse commensal microbes contribute to biosynthesis of trimethylamine, which helps with species identification when choosing a mate.

Plant Responses to VOCs

Although not all VOCs are known to produce detectible odor, research shows that gas exchange resulting from microbial VOC production can have significant impacts on soil community composition, which can influence plant colonization, plant pathogenesis and dispersal. In the lab, stark defects in leaf size and survival are apparent when plants are grown under exposure to VOCs produced by *Chromobacterium violaceum* and *Burkholderia pyrrocinia*. This observed reduction in growth suggests a pathogenic effect for certain VOCs. Alternatively, evidence indicates that competitive inhibition generated by VOC production may also be used to control the spread of other plant pathogens. For example, *Aspergillus flavus* and *Ralstonia solanacearum*—2 pathogens that are capable of independently causing harm to plants—may use VOCs to act antagonistically against one another and effectively limit colonization of peanut plants by either microbe.

Insect Responses to VOCs

Microbial VOC production also encourages distribution of some sessile organisms by attracting pollinators. The microbial composition of nectar has a strong influence on its attractiveness to pollinators. Parasitoid wasps have been observed to preferentially visit nectar with native nectar-inhabiting yeast species (e.g., *Metschnikowia gruessi, Metschnikowia reukaufii*) compared to non-native yeasts (e.g., *Aureobasidium pullulans, Hanseniaspora uvarum, Sporobolomyces roseus, Saccharomyces cerevisiae*). Wasps that were attracted to isolated VOCs extracted from native nectar-inhabiting yeasts were also shown to live longer than those feeding on nectar fermented by non-native yeasts. This increased attraction likely helps the plant's pollen and the yeast disperse to new places, improving fitness for both organisms.

Protubera nipponica mushrooms. Source: Hideyuki Matsui/Flickr

A similar "pollination" principle is utilized in fungi. Fungi that are dispersed by hornets and fruit flies have a different scent profile than those distributed by flies and wasps that are attracted to carrion (dead, decaying flesh). The fungus *Protubera nipponica* produces a fruity scent and is frequently visited by the giant hornet, which passes viable spores after eating parts of the mushroom.

Microbially produced volatile organic compounds give us a glimpse of microbial lifestyles. Whether they are using scents to communicate, or prevent the growth of organisms, VOCs are an important, yet underexplored facet of microbial physiology that will continue to inform our understanding of the microbes around us.

Looking for related content and wondering what to read next?
Read the article below to learn more about how microbial VOCs can be used for disease diagnosis.

Metabolomics in Diagnostic Microbiology

Are Bacteria the Next Big Thing in Fashion?

BY MADELINE BARRON, PH.D.

This article, originally published in June 2023, was updated for inclusion in the Spring 2024 issue of Microcosm, "Microbes and the Bioeconomy: Greasing the Gears of Sustainability."

It costs to look good—and more than just money. The clothes people wear require a massive amount of energy and resources to produce; it's a lot to invest in garments that will ultimately be thrown away, bulking up already bulky landfills. When it comes to sustainability, the fashion industry is rather unfashionable. What can take it from environmentally drab to fab? Bacteria, or rather, bacterial cellulose (BC), a network of carbohydrate molecules excreted by some organisms, can be crafted into everything from handbags to jackets. BC can be generated using organic waste and is biodegradable, making it an attractive solution to an unattractive problem. But how can these bacterial fibers be transformed into fabulous "'fits?" And how soon can people expect to find microbial garments at their favorite clothing store?

THE UGLY SIDE OF FASHION

The underbelly of the fashion industry is not glamorous. The process of converting raw materials (e.g., cotton) to products that grace runways and store shelves requires hundreds of thousands of tons of insecticides and pesticides, and millions of tons of fertilizers, oil and chemicals. Textile manufacturing also contributes to climate change, generating ~1.2 billion tons of carbon dioxide every year, or roughly 10% of global greenhouse emissions.

Most pieces of clothing don't last long in the closets of consumers—in the U.S., the average person throws away an estimated 81 pounds of clothes each year, many after only being worn a few times. When these garments end up in landfills, they can leach chemicals and microplastics into the environment and waterways, which can negatively impact the health of people and the planet.

Producing clothing is a resource-intensive process.
Source: Unsplash/Markus Winkler.

This picture has propelled a push for practices that "green" up clothing production, including the use of raw materials that bolster sustainability without sacrificing the features that make standard natural and synthetic materials desirable (i.e., comfortability, permeability, flexibility, etc.). It's a big ask—but bacteria could be up to the task.

THE BEAUTY OF BACTERIAL CELLULOSE

Bacterial cellulose consists of a meshwork of glucose molecules excreted by various species of gram-negative bacteria. It's structurally like cellulose from plants that is already used for textile production (think cotton), just with tinier fibers; in its raw form, BC looks and feels a bit like wet leather. Manufacturers grow the bacteria in vats of culture media and harvest the resulting mats of cellulose to make products (the BC is generally washed and treated to remove bacteria and other impurities).

Unprocessed bacterial cellulose is a bit like wet leather. When dried, it can be made into products like handbags. Source: Choi M.S., et al./Polymers, 2022

After processing, the final texture is akin to (dry) leather, though it can vary depending on treatment. For example, Modern Synthesis, a U.K. based startup company, grows their bacteria on a framework of thread; the BC fills in the gaps to produce a unique, structured material that looks somewhat like nylon and feels dry and paper-like. Indeed, microbial cellulose is an incredibly flexible material that can be grown to various thicknesses and textures and dyed a slew of different colors. It also has a large surface area, high purity, permeability, dexterity and stellar stability. Moreover, by altering factors like nutrients and oxygen supply—or even the microbes themselves via genetic engineering—producers can modulate BC output and structure.

THE SUSTAINABILITY OF MICROBIAL CELLULOSE

And then there's the "green" component. BC is a rapidly renewable raw material and can be generated using waste and alternative energy sources. Polybion, a Mexican biomaterials company, uses fruit waste from local producers to feed the bacteria that make BC. This fruity waste would normally end up in landfills and give rise to methane, a potent greenhouse gas. Using the waste prevents the gas from contributing to climate change; it also minimizes the cost required for pricey culture media. Researchers have fed BC-excreting microbes everything from potato peels to wastewater.

There's also the matter of clothing as a waste product itself. People will throw clothes away—that's a given. But those made from BC are biodegradable, which shrinks the impact considerably. Malai Eco—a company in Southern India that generates BC using local waste coconut water to produce a leather-like material—notes that their products can be placed in a compost bin, where they will naturally break down. Manufacturing BC clothes using natural dyes (including those made from bacteria themselves) and fewer harmful chemicals than traditional manufacturing processes gives the sustainability factor an "oomph."

THE FUTURE OF FASHION

For all its intriguing qualities, BC-based clothing exists largely in the realm of prototype. Polybion debuted a BC jacket developed in partnership with the Danish fashion brand, Ganni, at the 2023 Global Fashion Summit; Modern Synthesis debuted a handbag at the same event. The jacket and handbag weren't for sale, but simply illustrated the potential of BC. This is common in the BC fashion space—though awareness of microbial cellulose as a raw material has existed for years, products made from the stuff have yet to hit the mainstream market.

Examples of pieces of clothing made from bacterial cellulose.
Source: Choi M.S., et al./Polymers, 2022.

Turning bacterial excretions into wearable clothing requires optimization, which takes time. For instance, BC absorbs loads of water (i.e., is highly hydrophilic), which can be undesirable for making clothes. As such, efforts to confer hydrophobicity to BC while maintaining its flexibility and durability, among other beneficial features, is an important step in development.

Even when a material is ready for consumer use, scaling up production is a lengthy, iterative process that can take upward of 15 years, depending on how ready the technology is and whether the supply chain can support it. This time frame is seemingly discordant with the rapidity with which the fashion industry moves (trends are in, out and in again in the blink of an eye). Because of this, it can feel as though innovations like, say, BC clothes, which generate a lot of buzz during their early development, will never make it to the average consumer.

This isn't to say it won't happen, though. Modern Synthesis is exploring how to use existing manufacturing infrastructure to scale up production of their material. Polybion built the world's first facility for industrial-scale production of BC, and, throughout 2024, is focusing on automating their processes for optimal efficiency. Malai already offers pre-made items and swaths of BC material for purchase.

While the timeline for BC clothing availability may be uncertain, the implications of advancing production are bigger than the potential to snag a cool jacket. The U.S. has made it a priority to bolster the bioeconomy (i.e., the share of economy based on products, services and processes derived from biological resources, like microbes) as climate change looms large. Harnessing the metabolic power of bacteria to literally put clothes on people's backs is a step in the right direction.

Besides making clothes, bacteria are being used for a variety of applications, including aiding in the detection of disease and environmental contaminants. Check out this next article to learn more.

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