Researching the invisible world of microbes with computer tools: insights into improving wastewater treatment and producing bioenergy from agricultural waste.

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As a Research Associate at the Noguera Research Lab, which is part of the Great Lakes Bioenergy Research Centre (GLBRC) at the Wisconsin Energy Institute, University of Wisconsin-Madison, I am delighted to contribute this article to the program "Sharing UW-Madison Postdoctoral Scholarly Research with Non-Science Audiences," sponsored by the Wisconsin Initiative on Science Literacy (WISL). This program is made possible by the dedication of the WISL staff led by Professor Emeritus Bassam Z. Shakhashiri, the editor, Elizabeth Reynolds, and Cayce J. Osborne, the senior WISL outreach coordinator. It is instrumental in fostering connections between scientific exploration and a wider audience. My thanks go to Prof. Tim Donohue, the director of the GLBRC, my PI of the Noguera Lab, Prof Daniel Noguera, as well as all Staff, postdocs, and students of the GLBRC. I also thank the Wisconsin Energy Institute Communications team, especially Matt Wisniewski for providing illustrations used in this write-up.

In my role as a postdoctoral fellow, I see my path extending beyond the boundaries of research in the lab. It's not just about uncovering the secrets of science; it's a collective mission to share its marvels with people from all walks of life.

Tiny Invisible Architects of Life and A journey from Hygiene Class to Scientific Discovery

Microbes, short for microorganisms, are tiny, invisible living creatures that have had a massive impact on our world. They were discovered in the late 17th century when a Dutch scientist named Antonie van Leeuwenhoek peered through his homemade microscope and marveled at the teeming world of microbes he saw in a drop of water.

Today we know these minuscule beings are everywhere, from the soil beneath our feet to the air we breathe, and they come in various forms like bacteria, viruses, and fungi. Although they are often associated with disease, microbes also play essential roles in our daily lives. They help us digest our food, break down waste, and even produce essential products like bread and cheese. In recent years, scientists have also explored how microbes can be harnessed to address environmental issues and develop new medicines, shining a light on their incredible versatility and importance in the natural world.

Thanks to research, we now understand that microbes are not just tiny troublemakers, they are fundamental players in the grand scheme of life on Earth. They are like the hidden architects of our ecosystems, and their study has led to important breakthroughs in medicine, agriculture, and even biotechnology. This incredible world of tiny creatures continues to captivate scientists and holds the promise of even more discoveries that could change the way we live and care for our planet. So, the next time you take a sip of yogurt, tend to your garden or wash your hands, remember that you're interacting with a vast, invisible realm of microbes that have been shaping our world for billions of years.

Ahh yogurt! Early on in my childhood, I loved yogurt, and I thought all microbes were germs. I couldn’t have imagined both had so much in common. Germs came up only during hygiene class and our teacher warned us repeatedly about these invisible things, and how they could make us sick and die. Later, my interest in biology led me to study microbiology as a major at the undergraduate level. Only then did I realize microbes were literally behind all the delicious, fermented foods and drinks that I consumed every day. Even more, they produced drugs that saved millions of lives. This was confusing and intriguing at the same time, and I wanted to be a part of whatever it was that could turn foe into friend; My journey continued to a Master’s in Molecular Parasitology and Vector Biology, at the University of Buea, in Cameroon, PhD in Biotechnology and Biosafety at Eskisehir Osmangazi University in Türkiye, and now as a Postdoctoral Researcher at the Noguera Lab which is part of the Great Lakes Bioenergy Research Centre at the Wisconsin Energy Institute, University of Wisconsin-Madison, United States. The Noguera Lab is headed by Prof Daniel Noguera who is a Wisconsin Distinguished Professor at the Civil and Environmental Engineering Department at the University of Wisconsin Madison. He is also affiliated with the Wisconsin Energy Institute and the Great Lakes Bioenergy Research Center. Here I am part of a community of hundreds of scientists spanning diverse disciplines, collaboratively channeling our efforts towards harnessing the potential of microbes as green energy producers. Our shared goal is to contribute to a sustainable future, ultimately enhancing the health of our planet. I will explain further how the work I do contributes to this goal.

How We Study the Metabolism of Microbes and their Behavior.

Understanding the metabolism and behavior of microbes is essential to developing strategies that can turn them into partners for achieving our goals. For this, scientists can use several techniques to study microbes. When samples are obtained from the environment, one way is to immediately observe them for microbes using microscopy. Typically, we can see most microbes under microscopes and sometimes we use different staining techniques to help identify different types of microbes as in the example in Figure 1A below. After observation, microbes can also be grown in the lab on special foods called culture media on Petri dishes. These microbes manifest as a creamy substance on the petri dish, forming colonies comprising hundreds to thousands of individual microorganisms (see Figure 1B). Each colony typically represents a singular type of microbe, providing a basis for more in-depth microbial analysis. Next, the various ways in which they consume food and use it for energy, production of chemicals, growth, or even how they are related to other microbes can be studied. This is done by analyzing their genes, proteins, and metabolic pathways. This is the fun part where I come in.

Figure 1: On the left (A) Microscopy view of *Novosphingobium aromaticivorans* and (B) *Novosphingobium aromaticivorans* growing on a petri dish.

Do you remember DNA, the helical coil thing that holds all the information about a living organism? It is also present in microbes, albeit on a smaller scale, and holds all the information about the microbe's appearance and metabolism. Just like human DNA holds the instructions for our bodies, microbial DNA contains the information that makes these microorganisms tick. This DNA is made up of special molecules called nucleotides, which are like the alphabet of life. There are four types of nucleotides in DNA: Adenine (A), Cytosine (C), Guanine (G), and Thymine (T) (figure 2). To represent a DNA sequence, these letters are organized in a specific order. For example, figure 2 below shows a DNA sequence that represents a gene in the popular microbe *Escherichia coli* called 16S ribosomal RNA. The 16S ribosomal RNA gene is like a molecular ID card that we use to identify and classify bacteria. Just as we have unique fingerprints, bacteria have unique sequences in their 16S rRNA genes. By studying these genetic fingerprints, we can create a family tree of bacteria, showing how different types are related to each other.

Microbes have evolved and changed over the billions of years they have been around, and studying their DNA sequence can reveal the secrets of this evolution. It's like reading a history book written in a unique language, allowing us to understand how these microorganisms adapt, survive, and even impact our world with their metabolism.

One of the most remarkable tools in this genomic detective work is DNA sequencing technology. Imagine it as a super-powered magnifying glass that can decode the entire genetic language of microbes and tell us the exact sequence of AGCT nucleotides. This technology has helped revolutionize our understanding of the microbial world and revealed the incredible diversity and adaptability of these tiny beings. By exploring the secrets hidden within microbial DNA, we are uncovering the mysteries of life on a microscopic scale, and the implications are nothing short of groundbreaking for our world. We saw this recently with the development of CRISPR Cas technology, which is modified from microbial defense systems. It is currently transforming drug and vaccine development, and many other fields. The CRISPR Cas technology can make clean cuts in DNA at specific points and repair these cuts in a very precise way. Scientists can easily customize the technology to apply to humans. Thanks to this, soon it will be possible to cure health conditions acquired from birth, like anemia, sickle cell disease, and heart problems that were previously impossible to cure.

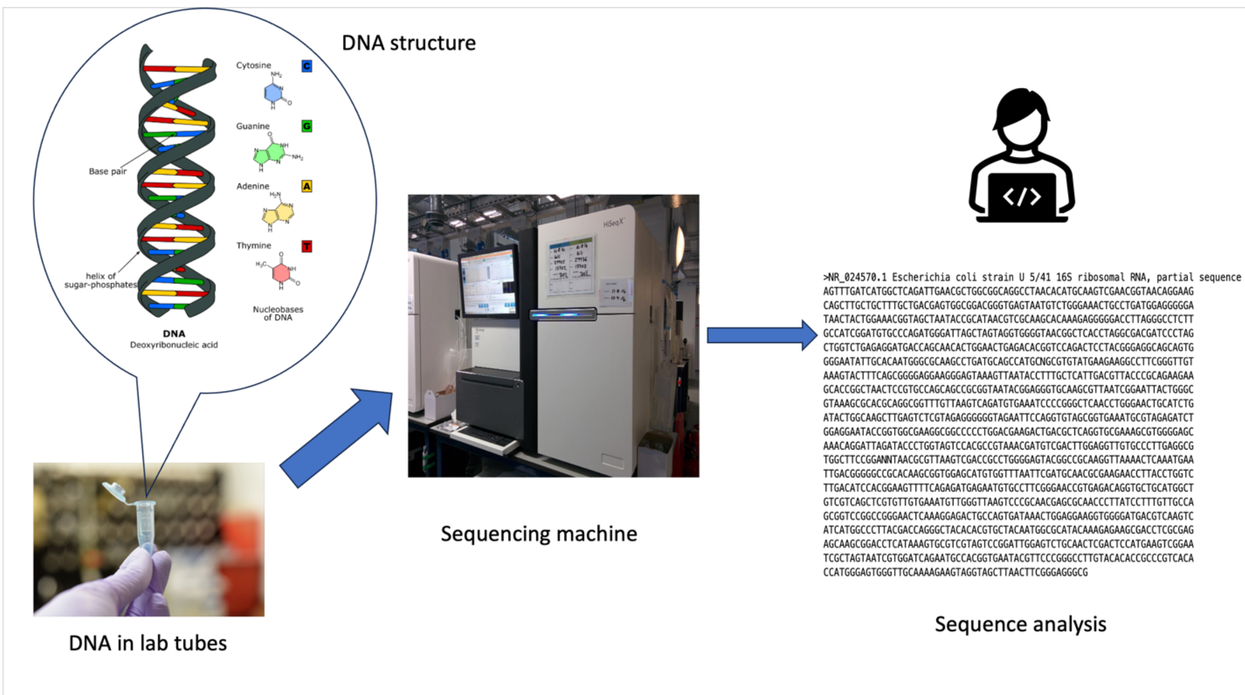

Figure 2: DNA in a lab test tube and an ultra-structure of DNA showing the base pairs between the nucleotides. The DNA is sent for sequencing and obtained as a file with the nucleotides arranged as a sequence of letters and other information. This file is analyzed using bioinformatics

tools to obtain the information encoded by the DNA. The example file shown is the DNA sequence for *Escherichia coli* 16S ribosomal RNA obtained from the NCBI RefSeq database.

Understanding DNA at a Large Scale: Hello Bioinformatics

When the DNA of a microbe is sequenced, the result is a long list of combinations of millions of AGCT letters. Understanding this is like trying to make sense of an enormously long and complex book with millions of letters. To decipher this colossal book, we turn to a special field called bioinformatics, which uses powerful computers and software to help scientists read, analyze, and make sense of this genetic code. This is crucial because DNA research isn't just about understanding this book; it's also about understanding an entire library of similar books. There are special online databases around the world with huge amounts of such microbial sequence data for scientists to use.

Think of bioinformatics as the librarian and translator for this massive genetic library. It can organize the data, identify patterns, and provide insights into how DNA works and how it can be applied in medicine, genetics, and many other fields. Without bioinformatics, we'd be lost in the genetic data maze, but with it, we can unlock the secrets of life and use that knowledge to improve our health, understand microbial ancestry, and tackle important challenges like disease and environmental conservation. So, in the fascinating world of genetics, bioinformatics plays a vital role in making DNA's enormous story accessible and useful for the benefit of all of us.

However, scientists didn’t stop there. As researchers began to look at the genome sequences, they saw that a lot was going on in living organisms that could not be explained. A couple of decades ago, some scientists came up with the crazy idea that they could piece together all the research done on individual microorganisms to reconstruct their entire metabolic system, much like bringing together pieces of a puzzle to get a view of the whole. Such an approach would enable us to better appreciate our gaps in knowledge and to research ways to fill these gaps. More help from math, algorithms, machine learning, and artificial intelligence will be needed. Several decades later, did these efforts yield fruits? We will also discuss how today, technologies resulting from this approach have evolved and are shaping the biotechnology field as a whole.

Unlike microbes, my interest in bioinformatics only came later in my academic path. Since my high school days, computers have always fascinated me. My parents didn't have the means to afford one for me, so I would always take advantage of computer lab sessions to peek around, and I would spend my allowance money on cybercafé subscriptions. I never thought biology and computers could blend. Not until during my postgraduate studies, when I finally got a laptop, was I able to do more research and learn what the range of options was. That was when I found out about bioinformatics, the R programming language, their applications in biological research, and the exciting discoveries they were helping to uncover. I knew immediately it was on that path I wanted to continue. I knew of no university around me offering training that could help me achieve my goals, so I sought opportunities abroad. My search eventually led me to the Ph.D. in Biotechnology and Biosafety program in Türkiye, where I began research work applying bioinformatics and computational biology tools to study the behaviors of microbes that live in extreme environments and how we could use them to benefit humans. In my research, we found microbes that survive in extremely salty environments can produce bioplastics, biosurfactants, ectoine, and potential antibiotics. These chemicals are useful in the food, pharmaceutical, cosmetic, and agricultural industries, etc. After my PhD, I moved to the GLBRC as a research associate where I am doing the research I will explain next.

Secret Heroes in Wastewater Cleanup

In one of our projects at the GLBRC, we are interested in studying the DNA of microbes that affect the way our wastewater is treated in wastewater treatment plants (figure 3). Did you know microbes are unsung heroes in the world of wastewater treatment plants? When we flush our toilets or send dirty water down the drain, it all ends up in the tanks of these facilities. What happens there is like a natural recycling process, thanks to hardworking microbes in the biological treatment stage. These tiny organisms feed on the impurities and contaminants in the water, breaking them down into harmless byproducts. It's like a microbial feast that cleans our water and makes it safe to release back into the environment. Understanding how these microbes work and their specific roles in this process is like having the recipe for a magic potion that can improve wastewater treatment. By fine-tuning the conditions to support these microbial workers, we can help treatment plants become more efficient, cost-effective, and environmentally friendly. This means cleaner water for us and a healthier planet. Let's see how my research contributes to bringing about these benefits.

Figure 3: The Madison Metropolitan wastewater treatment facility showing different tanks used in the biological treatment stage.

Identifying and Empowering Heroes for Better Wastewater Cleanup

Wastewater may seem dirty, but it's teeming with an entire ecosystem of microorganisms. Imagine it as a microscopic city with a population of billions.

In my research, I explore the world of wastewater by examining tiny samples and delving into the DNA hidden within them. Think of these DNA samples as treasure chests holding the secret codes of the microscopic beings living in this community. To unravel this genetic mystery, I use special computer tools from the field of bioinformatics. These tools help me figure out who these microbes are and understand the important roles they play in breaking down pollutants in wastewater. Picture it as solving a complex jigsaw puzzle where each piece represents fragments of DNA sequences, similar to the 16S ribosomal RNA I mentioned before. These bioinformatics tools not only identify these genetic pieces but also help me understand the functions of other genes and the microbes they belong to. This knowledge, together with knowledge in the scientific literature, allows me to recognize which microbes are present and how they work together to treat wastewater.

Why does this matter? Well, armed with this information, operators at wastewater treatment plants can tweak conditions in treatment tanks to encourage the growth of more microbes that excel at specific tasks. It's like having a strategy guide to optimize the processes and make wastewater treatment more efficient. In this project, I am providing data to help a wastewater treatment operator reduce costs associated with using high amounts of oxygen in their treatment processes. This improved microbial management process makes wastewater treatment eco-friendlier and more cost-effective.

Partnering with Microbes in Crafting a Greener Future with Bioproducts

Another term you may be familiar with is “biodegradable,” which is often used to refer to materials that do not stay long in the environment without complete transformation. Most products produced from fossil fuels, like plastics, are not biodegradable and, as such, are hurting the environment. But guess what? Microbes can help us with that too.

Bioproducts, often made by some interesting microbes, are like the eco-friendly alternatives of the manufacturing world. Microbes have a talent: they can transform simple and complex materials into valuable and sustainable materials in a process called fermentation (Figures 4 and 5). Most of the waste stuff you see around you - including plastics - can be broken down by microbes into something more useful to us.

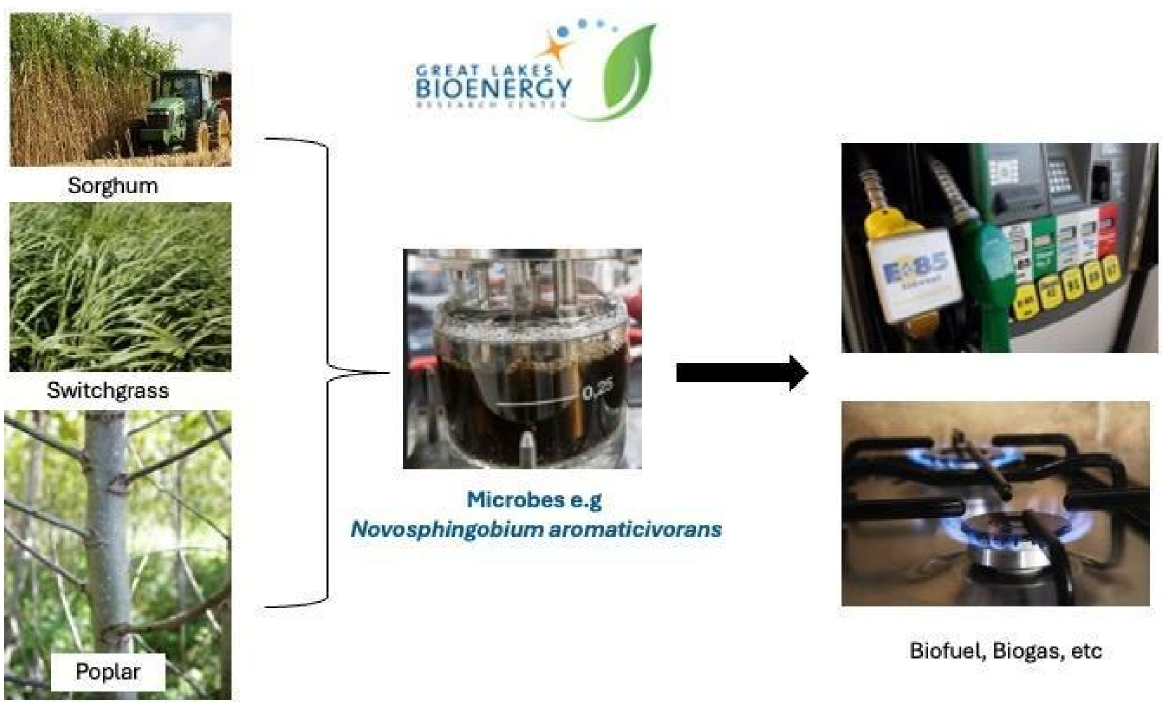

Figure 4: Processing plant biomass into biofuel and renewable cooking gas. The plant is processed into a liquid mixture and a microorganism called *Novosphingobium aromaticivorans* is added to the mixture for fermentation in bioreactors. Biofuels, butanol, ethanol, bioplastic, cooking gas, etc. can be obtained as end products depending on experimental conditions.

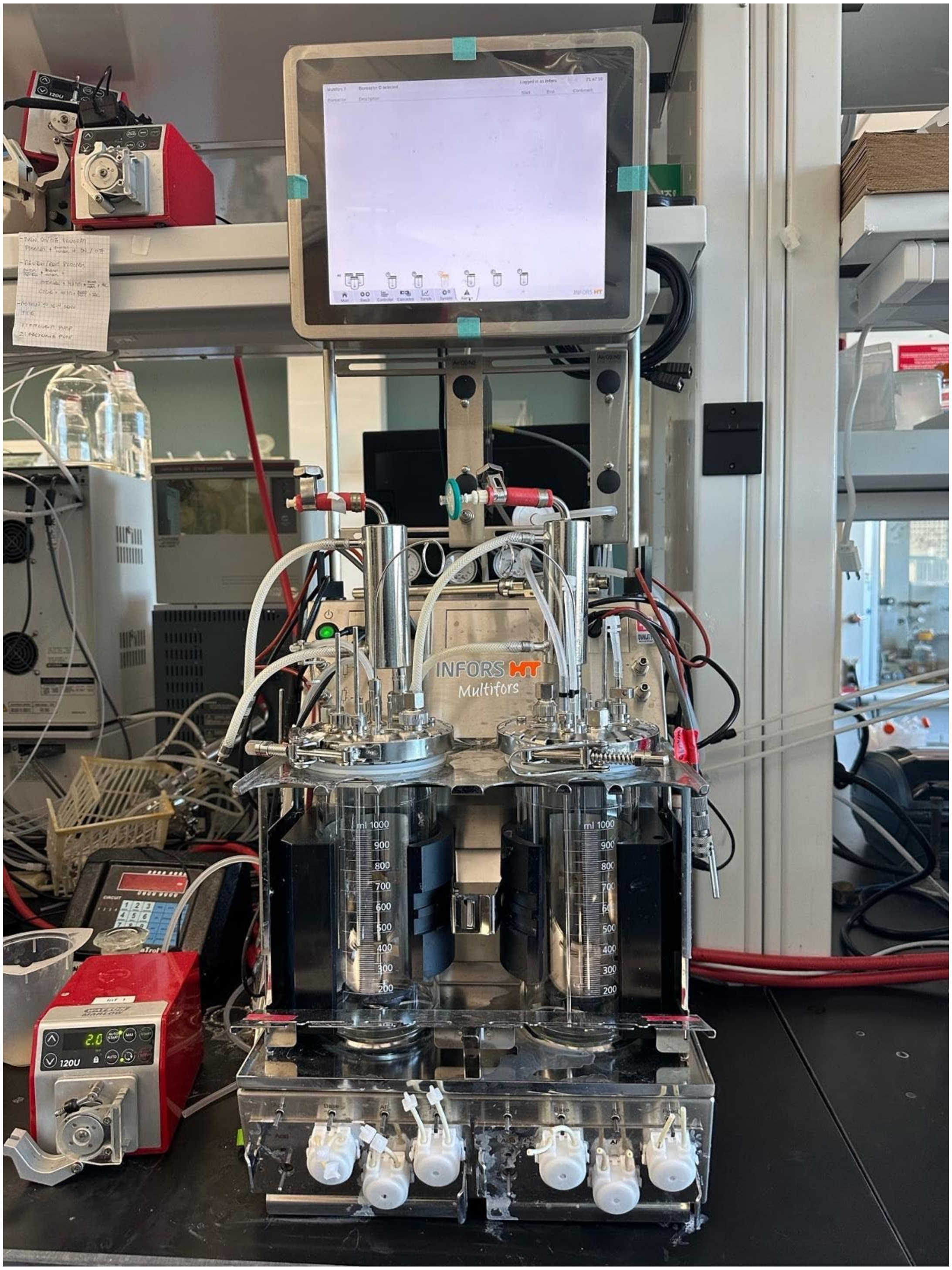

Figure 5: Example of laboratory-scale bioreactor equipment used to carry out fermentation, evaluate conditions, and beneficial end products. Production conditions are evaluated in this setup before proceeding to scale-up.

One of the most exciting things about bioproducts is that they offer an alternative to many of the products we use today that rely on fossil fuels like fuel, plastics, detergents, etc. Using bioinformatics and experimentation, microbes can be engineered to produce bioplastics, biofuels, and even medicines, offering us a cleaner and greener way to meet our needs. These bioproducts help reduce our carbon footprint and decrease our dependence on limited resources. They're like a breath of fresh air for the environment.

Furthermore, the industrial production of bioproducts using microbes can be done with fewer chemicals and less waste compared to traditional manufacturing methods. This means less pollution, less energy consumption, and a reduction in harmful byproducts. These benefits go beyond being environmentally friendly – they can also lead to cost savings, making bioproducts an attractive and sustainable choice for producers and consumers.

The US bioproduct market is a budding field brimming with potential, driven by the growing demand for sustainable alternatives to traditional materials and fuels. Bioplastics and biofuels derived from agricultural waste and other innovative products offer a glimpse into a greener future. The US government is also playing a crucial role in nurturing the bioproduct industry. President Biden's ambitious goals aim to replace 90% of fossil fuel-based plastics with bio-based alternatives over the next two decades and meet 30% of US chemicals demand from biomanufacturing by 2040. These goals are supported by initiatives like the BioPreferred Program, which gives purchasing preference to biobased products, and the Department of Energy's Bioenergy Technologies Office, which invests in research and development.

In this compelling landscape, my research project adds a unique dimension by delving into microbial technologies. Imagine a world where waste is not just disposed of but transformed into valuable bioproducts, enriching our lives in the process. In the next section, I will unravel the fascinating journey of how I contribute to this vision, illustrating the intersection of cutting-edge science and the promise of a cleaner, more sustainable future.

Turning Waste Wood into Green Gold with *Novosphingobium aromaticivorans* and Computational Biology

Yes, I know *Novosphingobium aromaticivorans* is a mouthful to pronounce. Let's keep it simple by calling it Novo. Producing beneficial chemicals using Novo is also one of our research interests at the GLBRC (Figure 6).

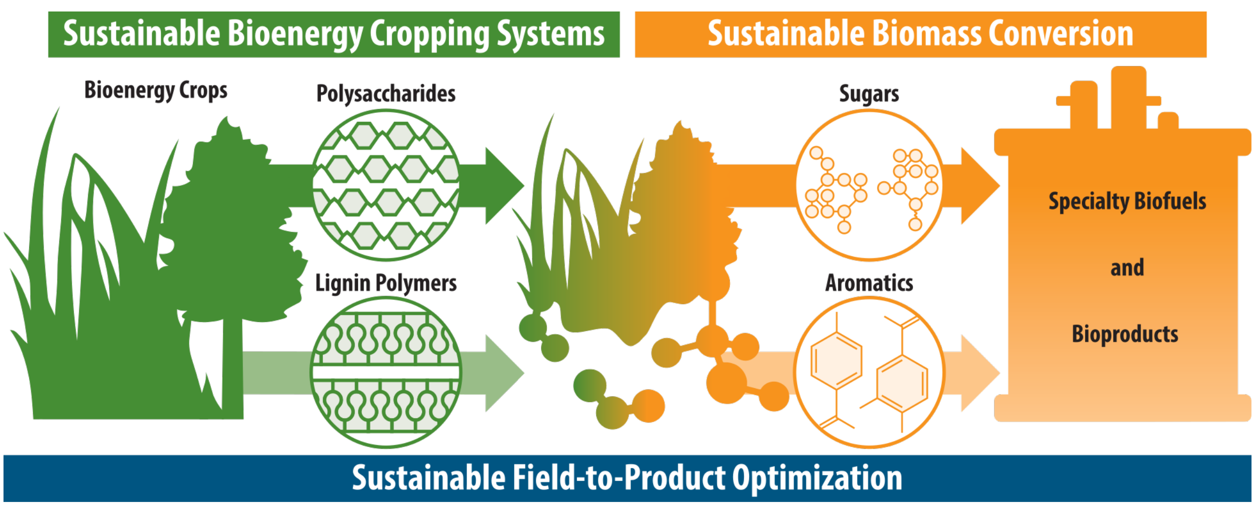

Figure 6: Summary of the main research areas at the Great Lakes Bioenergy Research Center (GLBRC). Research areas span research on sustainable cropping systems to produce polysaccharides and lignin polymers used as biomass inputs, and sustainable biomass conversion to specialty biofuels and bioproducts.

Earlier in this article, I mentioned how researchers intended to puzzle together the research done on individual microorganisms over the years to reconstruct their entire metabolic system. Using this data along with mathematical modeling techniques, algorithms, machine learning, and artificial intelligence, researchers are now able to reconstruct and simulate microbial cells in computers. These reconstructions are known as genome-scale metabolic network models and are used in computational biology. Genome-scale metabolic network modeling is like creating a detailed map of all the interconnected pathways and processes within a microbial cell (figure 7). This information can be obtained by exploring the genome of the microorganism. It helps scientists understand how different parts of the genome (the blueprint) influence the production of various substances in each cell. I teamed up this cutting-edge computational biology modeling technology with the tiny microbial superhero Novo to help us understand how to turn tough, wood-like lignin material in plants into valuable biochemicals. Lignin is like the protective armor of plants, and it's incredibly challenging to break down. But Novo is one of nature's experts at this task. Using this technology, I can predict how Novo's genes and enzymes work together to transform lignin into useful building blocks for making things like biofuels and plastics.

This modeling approach considers all the tiny molecular reactions happening inside cells, much like tracking all the roads and traffic between different parts of a city. By doing this, I can predict how changes in a cell's environment might affect the overall "traffic" of molecules within the cell. This information is crucial for understanding how cells function and can be useful in fields like medicine, bioengineering, and agriculture, where tweaking cellular processes can significantly impact health, technology, and food production. Essentially, it's a powerful tool that helps researchers explore and optimize the inner workings of cellular factories. This technology has helped produce high-value products like D-phenyllactic acid and L-valine. These are useful in supplements and drugs.

In my research, I build these genome-scale metabolic network models by sourcing information from the bioinformatics databases I mentioned previously. Then I use this as a computer model of the real live Novo and attempt to predict how it will behave in different concentrations of chemicals it typically needs for growth. So, using this Novo model we can predict the type of bioproduct that will be produced when Novo is provided with different kinds of “food”. These predictions are used to run further experiments in the lab and to engineer Novo species that produce the desired bioproducts, in this case, biofuels or bioplastics. These predictions from computational biology aren't just scientific fun and games; they hold the key to a greener future. If we can perfect harnessing Novo’s lignin-converting powers, we can reduce our reliance on fossil fuels and cut down on pollution, all while creating sustainable materials. So, this research is not just about understanding microbes and molecules; it's about unlocking a cleaner and more eco-friendly way to produce the things we use daily.

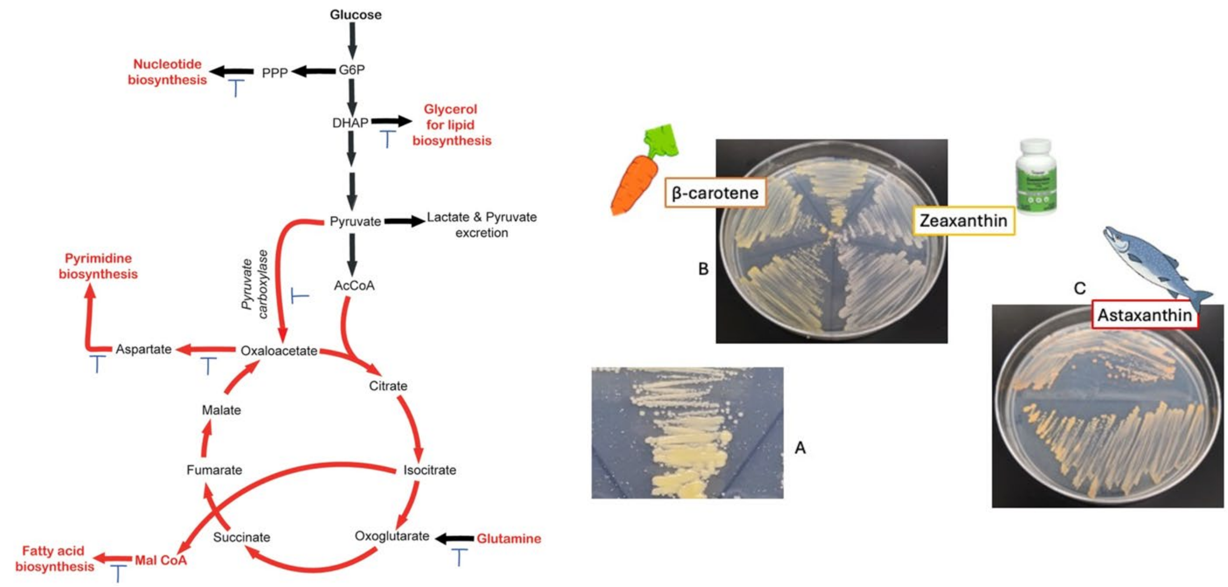

Figure 7: A small section of the microbial metabolic pathway showing how glucose is converted to other useful products. The area with dark lines marks the section known as glycolysis, while the red lines mark the section known as the TriCarboxylic Acid (TCA) cycle. Novo can be made to produce different useful pigments in Petri dishes when their metabolic pathways are genetically modified. In A, B, and C novo was made to produce different types of carotenoids beta carotene, zeaxanthin, and astaxanthin which are commonly found in food sources. This can be easily seen with the naked eye as the colors of their colonies change.

Conclusion

It has been a long and exciting journey so far from hygiene class to the cutting-edge science I do at the GLBRC. Technology is quickly improving and ushering in many novel ways we can approach and solve critical global problems. I am delighted to be on this path, and I look forward to the many exciting discoveries we will put out there for the world. There is hope for sustainable solutions to the global challenges we currently face.