ENVIRONMENTAL COMMISSION MEETING
January 3, 2013 6 PM
7700 Southern Avenue (Public Services Building)

M I N U T E S

Present		**Staff**
Chairman Joe Skelley	Andy Foster	Bo Mills
Clyde Davidson	Linda Kaplan	Joe Nunes
Jimmy Davis	Paul Mosteller	Leslie Throneberry
Urania Erskine	Anne Wesberry	
Steve Fleischmann	Alderman John Drinnon	

Absent: David Thorpe

CALL TO ORDER:
Chairman Skelley called the meeting to order at 6:00 p.m.

ESTABLISHMENT OF QUORUM:
A quorum for tonight's Environmental Commission meeting was established.

INTRODUCTION OF MEMBERS:

Anne Wesberry: Mrs. Wesberry has been a Germantown resident for over 20 years. She grew up in Collierville. She serves as the Chairperson for SERVES, an organization for adults with disabilities. She has two grown children.

Jimmy Davis: Dr. Davis is a 25 year resident. He has been on the Commission approximately 5 years. He works for Union University and is a chemist by background.

Steve Fleischmann: Mr. Fleischmann moved to Germantown from Houston, Texas ten years ago. This is his second year on the Commission. He has one son that lives in Seattle.

Paul Mosteller: Mr. Mosteller has been a resident of Germantown for many years. This is his second year on the Commission. He is also on the Financial Advisory Commission and has been on the Athletic Club Commission. He recently became a grandfather to a baby girl.

Joe Skelley: Mr. Skelley is the Chairman of the Environmental Commission. He is a chemist and has been on the Commission for many years. He is a 20 year resident of Germantown and has two grown kids.

Bo Mills: Mr. Mills is the Director of Public Services and has been employed with the City of Germantown for 28 years.

Clyde Davidson: Mr. Davidson is a 20+ year resident of Germantown. He retired from the Purchasing Department of St. Jude Hospital in 1992.

Alderman Drinnon: Alderman Drinnon has been a Germantown resident since 1973. He has been appointed as the liason to the Environment Commission.

Urania Erskine: Mrs. Erskine has been a Germantown resident since 1987. Previously, she was on the Public Education Commission, Library Commission and Library Board. She has been on the Environmental Commission for 3 or 4 years.

Linda Kaplan: Mrs. Kaplan is a returning member of the Commission.

Joe Nunes: Mr. Nunes is the Neighborhood Services Manager and has been with the City of Germantown for over 8 years. Neighborhood Services is the department that oversees the contract for solid waste collection and recycling.

Leslie Throneberry: Mrs. Throneberry is the Administrative Assistant for Public Services and has worked for the City for just over 4 years.

Our first order of business is in consideration of an honorarium for Susan Threlkeld for her many years of service on the Commission. We would like to take up a collection to make a donation to the Library or the Farm Park

because she is a Master Gardener. At the February meeting, we will take up a collection and decide on the course of action for the honorarium. We will then invite Susan to the March meeting to present the award.

MINUTES APPROVAL:
The minutes of the December meeting were approved.

ELECTION OF OFFICERS:
The Chairman, Mr. Skelley, has been appointed by the Board of Mayor and Alderman. Andy Foster was elected to be our Vice Chairman again this year.

STAFF REPORTS:

Joe Nunes: Mr. Nunes had nothing to report because the recycling reports haven't been received yet this month.

Bo Mills: Mr. Mills shared which services are provided by our Public Services Division. We provide water and sewer services to the residents. We produce our own water from aquifers below ground. We bring it up, treat it and supply it. We collect the sewer water but we do not treat it. The City of Germantown has a long term agreement with the City of Memphis for our sewer treatment. Public Services also takes care of all of the paved public roads in the City other than the State routes (Germantown Rd and Poplar Ave). Storm water is also a part of the Public Services division. We are responsible for all of the storm water infrastructure maintenance including curbs and gutters. The Germantown Animal Shelter is another part of Public Services. It is located next door to the Public Services facility. Our Grounds maintenance division provides maintenance for the Public Services complex, City Hall, GPAC, GAC, Great Hall and the 27 City parks, including all grasses, flower beds and median strips. About half of the Grounds maintenance is contracted out. We contract all of the grass cutting, pavement maintenance and street sweeping duties. 79 employees work out of the Public Services department. The average tenure for our division is 18 years of service. We have employees with 38 years of service still employed. This is a great City and a great place to work. Our Board of Mayor and Alderman is very supportive of our department. Please contact our department if you ever have issues with any of these services.

Mr. Mills circulated a December 13, 2012 press release concerning leaf disposal (see Attachment A). The City tries to give instructions to our residents on how to process their leaves. Our solid waste contractor does collect bagged leaves. We encourage residents to shred their leaves and use it for mulch. We have had several storm inlets stop up due to leaves being washed into the inlets.

Mr. Mills also shared a chart from USGS (U.S. Geological Survey) on the water levels in our Southern Avenue well field. (See Attachment B) The news has reported that the average summer temperatures were the highest they have ever been and the drought impact was high as well. Mr. Mills' concern was what this did to our aquifer. Overall, our water consumption numbers stayed very reasonable to be in a drought year. According to the chart, the water levels declined between June and October. The water levels began recharging in November and December to near normal levels.

COMMITTEE REPORTS:

Paul Mosteller: Mr. Mosteller has been in favor of rain barrels. He had a discussion with a friend who was not in favor of them because he felt the Yellow Fever epidemic was attributed to mosquito breeding due to the rain barrels. After researching this information, Mr. Mosteller found that it wasn't just rain barrels that affected the epidemic. It was also the lack of proper sewers and sanitation and a variety of other things. He brings this up to state that the people that go to the time and expense of installing rain barrels these days, would also be concerned about sanitation and mosquito breeding.

During his research on rain barrels, Mr. Mosteller found some information from the Philadelphia Water Department about storm water runoff, rain barrels, water conservation and other information. He passed around the Table of Contents for "A Homeowner's Guide to Stormwater Management" (see Attachment C). If you would like to read the document, please go to http://www.delawareestuary.org/pdf/HomeownersGuideSWMgmnt.pdf.

Steve Fleischmann: Mr. Fleischmann asked Joe Nunes when we will be able to single stream plastics 1 through 6 for recycling. Mr. Nunes has a document being developed to reinforce the recycling effort and to announce that we are now accepting plastics 1 through 7. Plastic shopping bags are not recyclable. Bottle caps can now be left on the bottles.

Jimmy Davis: Dr. Davis asked Mr. Nunes if someone puts what they consider to be a "clean" pizza box into the recycling container, whether it will be removed or not? Mr. Nunes felt that it would probably not be removed.

Dr. Davis circulated an article from Chemical Engineering News Magazine on Microbiome Mining (see Attachment D). Food stock, like corn and soybeans, is being converted to ethanol and biodiesel. Waste cellulose is difficult to break down chemically into useful feed stock to make fuel. Cows, kangaroos and pandas are able to do this. Mississippi State graduate students are studying the pandas at the Memphis Zoo to see which microorganisms in them might break it down. There is hope that a microbe will be found that can be manipulated so that it can break down the waste in corn and soybeans that we currently throw away.

Linda Kaplan: Mrs. Kaplan reminded us that the Eco Expo at Temple Israel is scheduled for 1/27/13 from 11:00 a.m. to 3:00 p.m. 60-70 exhibitors and approximately 600-800 people will be attending. There is an hybrid and electrical car parade, food vendors, etc.

Andy Foster: Mr. Foster has noticed people using Segways to tie flyers to mailboxes.

Urania Erskine: Mrs. Erskine shared some information on rain gardens. Los Angeles is offering to put in eco-friendly landscaping to cut down on the water consumption in their area. They landscape it with native plants.

Alderman Drinnon: Alderman Drinnon thanked everyone for their service. He also stated that he is here to support and provide any assistance to the Commission.

Clyde Davidson: Nothing to report.

Mr. Skelley asked the commissioners to consider which subcommittee functions they would like to oversee. Next month, everyone will be given the opportunity to choose.

There are several programs that the Environmental Commission assists with. The following is the 2013 schedule:

Amnesty Dumpster Days are scheduled for April 20 and Sept 21.
Mulch Days are scheduled for March 16 and October 19.
Senior Expo is scheduled for November 7.
Arbor Day is scheduled for March 1.

ADJOURNMENT:
There being no further business, the meeting was adjourned at 7:13 p.m.

Attachment A

CITY OF
GERMANTOWN
TENNESSEE
1930 South Germantown Road • Germantown, Tennessee 38138-2815
Phone (901) 757-7200 Fax (901) 757-7292 www.germantown-tn.gov

Contact: Stacey Ewell, Assistant to the City Administrator at 901-237-6521 or sewell@germantown-tn.gov

FOR IMMEDIATE RELEASE: **December 13, 2012**

Going Green with Leaf Disposal

For proper collection by the City of Germantown, leaves must be placed in a yard debris container or clear plastic bags for weekly pickup by the yard waste contractor. Leaves must never be blown or swept into the street, curb or storm drain. Bagged leaves must not be placed in the gutter or street but on the curb away from light poles, mail boxes or any other obstruction.

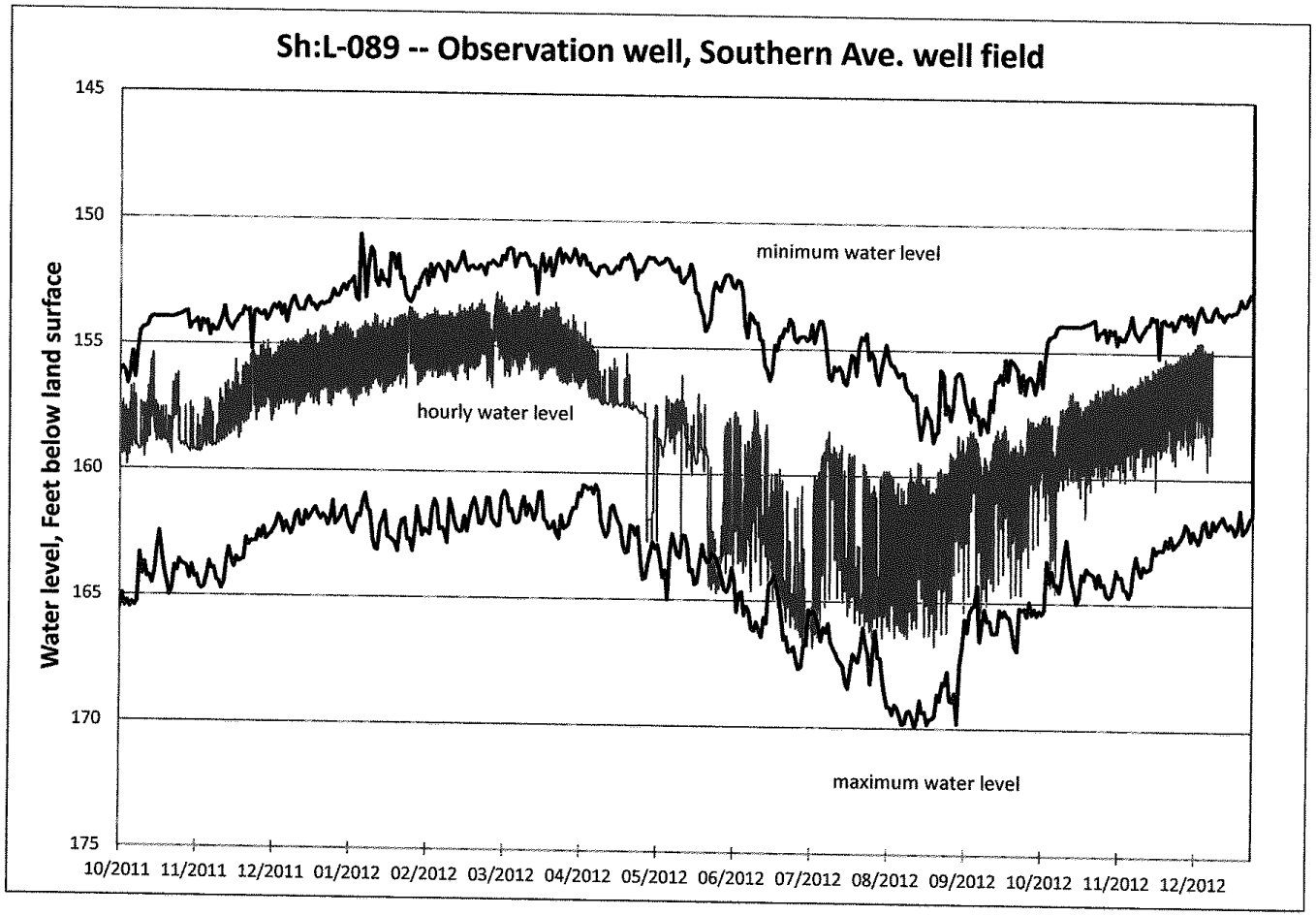
"During the intense rainfall last weekend, loose leaves left in the street were swept by the runoff water to storm drain inlets, putting the inlets at risk for blockage or potentially clogging the underground storm system," said Mayor Sharon Goldsworthy. "The force of the water can be substantial, even pushing bags of leaves to or into the inlets. It doesn't take much for those to jam the underground storm sewers and cause backup at inlets and flooding."

Home owners are responsible for dealing with all leaves falling on their property. Residents may see Public Service crews vacuuming leaves that have fallen naturally into the street. That effort is only to clear inlets and prevent flooding. It should not be confused with citywide street sweeping which occurs over a period of several months, starting in late fall.

Fall leaves can easily be turned into valuable soil-enhancing organic matter. Dry leaves can be mulched and left in place on lawns, tilled into the vegetable or annual flower beds or placed in a compost bin. Shredding the leaves will speed their decomposition.

-end-

Attachment B

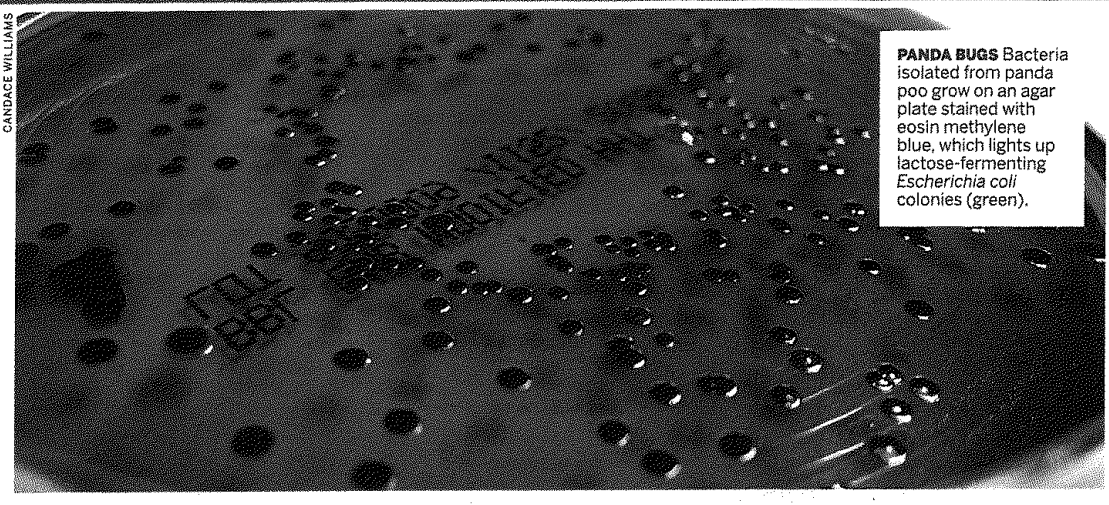


Attachment C

A Homeowner's Guide to Stormwater Management

Table of Contents

Attachment D



CANDACE WILLIAMS

PANDA BUGS Bacteria isolated from panda poo grow on an agar plate stained with eosin methylene blue, which lights up lactose-fermenting *Escherichia coli* colonies (green).

MICROBIOME MINING

Microbes within pandas and other creatures may hold keys to cost-competitive **CELLULOSE-BASED BIOFUELS**

STEPHEN K. RITTER, C&EN WASHINGTON

YA YA AND LE LE are a couple of giant pandas at the Memphis Zoo who don't realize they are participating in an important science experiment. Once in a while a person will come into their space at the zoo, grab their poo, and scurry away. Ya Ya and Le Le shrug off the intrusions and go back to what they do best: eating bamboo.

The results coming from their backsides could benefit the pandas. Scientists studying the microbial community in the animal's gastrointestinal tract are gaining a better understanding of panda nutrition, which could help improve the health and reproduction of the lovable and endangered creatures.

But people have still another motive for these panda poo investigations. They want to figure out which symbiotic microbes living in the panda gut are deconstructing the tough, fibrous lignocellulose that makes up the plant cell walls of bamboo. By analyzing DNA they glean from the stool samples, scientists are working backward to identify the microbes and pinpoint genes that code for their cellulose-busting enzymes.

And researchers aren't stopping at pandas: They are searching for such enzymes in a variety of vegetarian animals. Ultimately, they want to cut and paste the genetic blueprints for such enzymes into other microorganisms, giving the engineered microbes the ability to break apart biomass into soluble sugars that can be fermented into biofuels.

It remains to be seen whether such technologies will eventually lead to an economically viable cellulosic biofuel market. But those challenges aren't stopping biochemist Ashli Brown and members of her Mississippi State University research group. They watch Ya Ya and Le Le eat and wait for them to poo. When the time comes, they alert Memphis zookeepers to move in for a search and recover operation.

And it's not such a dirty job. "Panda pellets are fairly desiccated and fibrous," Brown says. "They are like mini hay bales. For anyone with experience working with animal fecal material, I can assure you panda poo has a fairly pleasant smell and is probably the nicest to work with."

To find useful enzymes in the pellets, Candace L. Williams in Brown's group takes them, grinds them up, performs extractions, and then uses traditional anaerobic bacterial culture methods to grow and identify bacterial colonies. The microbes found clinging to incompletely digested bamboo in panda poo can be difficult to cultivate in a lab, like any microbe when it's out of its element. Brown's group inevitably misses a few of them.

To circumvent that problem, Brown's team has added another tool to its arsenal: metagenomics. This relatively new DNA sequencing approach can reveal the diverse array of microbes that live in a given environment, such as the panda gut. With metagenomics, scientists can take a census of all of an environment's microbes—collectively known as a microbiome—without having to culture them.

The researchers chemically dice up the DNA extracted from the panda poo into pieces of about 100 to 600 base pairs and sequence each piece, Williams explains. They then take the thousands of short sequences generated from the microbiome, align them to find overlapping regions, and piece them together to isolate the 16S ribo-

"DNA is a common currency no matter where it comes from."

somal RNA genes. This gene codes for a component of the ribosome, the machinery used to build all the proteins in a cell. It has a relatively short sequence—about 1,500 base pairs long—and each microbial species has a unique one.

The team then turns to computational algorithms to sort through the billions of base pairs sequenced to match the 16S rRNA and all the other genes in the microbiome to the individual microbes. They also use publicly available bioinformatics database tools to compare microbial diversity between multiple microbiomes to determine what all the different microbes in the panda gut do and which enzymes they produce.

Creature: Panda
Eats: Bamboo
Microbial enzymes allow pandas, like Le Le at the Memphis Zoo, to rapidly digest large amounts of bamboo.

SYMBIOTIC ZOO In search of cellulose-degrading enzymes to make biofuels, scientists mine the gut microbial communities of a menagerie of creatures. They use a combination of metagenomics, whole-genome sequencing, enzyme activity assays, and sugar and fatty acid composition analyses.

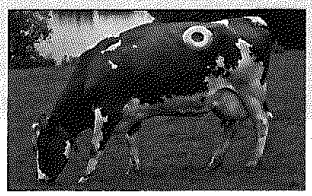
In the end, because the sequencing process and data analysis are not 100% accurate, the Mississippi State team is validating its metagenomics data by using the polymerase chain reaction to amplify the microbial cellulase genes they discover in panda poo.

THE MISSISSIPPI STATE team is not alone in its quest. By using a combination of metagenomics, whole-genome sequencing, enzyme activity assays, and sugar and fatty acid composition analyses, scientists have been profiling the gut microbiomes of a variety of animals to begin to understand how they degrade plant biomass, says biophysicist Susannah G. Tringe, head of metagenomics at the Department of Energy's Joint Genome Institute (JGI).

"We now have a pretty standard approach to metagenomics projects," Tringe says. "DNA is a common currency no matter where it comes from, allowing us to study microbiomes in soil, water, plants, or animals, including humans."

Animal metagenomics studies like that of the panda serve as a foundation for biofuel development, Tringe says. "The Human Genome Project, for example, hasn't actually cured any diseases itself. But it has given scientists a much faster way to understand the genetics of individual diseases and potential treatments. Now that we are getting cellulosic enzyme metagenomics data, it's creating opportunities for a variety of downstream studies."

Like pandas, cows are unwitting participants in these gut metagenomics projects. Enzymes in the cow's rumen, the primary grass-digesting chamber in the cow's multistomach GI tract, allow the ruminants to process massive amounts of grass. In 2011, Tringe was part of a multi-institution team that conducted a metagenomics study of the cow rumen (*Science,* DOI: 10.1126/science.1200387). The researchers took 268 billion bases they sequenced and whittled them down to 27,755 genes that encode for carbohydrate-active enzymes. They ultimately identified 51 enzymes with cellulose-degrading activity and are now testing them for their catalytic abilities.



Creature: Cow
Eats: Grasses and grains
This cow, fitted with a surgical port, participated in a study to identify enzymes in its rumen that break down switchgrass to oligosaccharides.

Another animal with potentially useful cellulase enzymes is the hoatzin, a chicken-sized, reddish-brown bird with a spiky crest that hails from South America. The hoatzin is unusual among birds in that it primarily eats leaves. Like other birds it has a crop, a built-in storage bin that is part of the digestive system—a kind of prestomach. But in the hoatzin, the crop functions as a biomass fermenter, similar to the cow's rumen. In fact, the hoatzin is nicknamed the stink bird because its droppings smell like cow manure.

Studies on the hoatzin microbiome, led by Filipa Godoy-Vitorino and María G. Domínguez-Bello of the University of Puerto Rico, Río Piedras, detected hundreds of microbes. The researchers also carried out a comparative analysis of the hoatzin and cow microbiomes.

"Despite being phylogenetically distant, adult hoatzins and pasturing cows share specific digestive microbes," says Godoy-Vitorino, who is now at JGI. After classifying the microbial species, the researchers found seven that were unique to the hoatzin, she says. They are now classifying the hoatzin cellulose-degrading enzymes.

Creature: Hoatzin
Eats: Leaves
The South American hoatzin has a digestive tract similar to a cow's, loaded with cellulose-degrading microbes that aid in digesting its leafy diet.

And then there are pandas. "If you can use a microbe that efficiently breaks down biomass naturally and as efficiently as pandas do, and convert them into sugars that could be converted to oils and other chemicals, production costs for alternative fuels would be cut tremendously," Mississippi State's Brown says. "That would be fantastic."

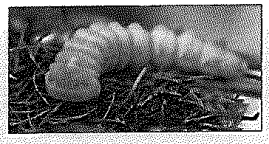
Pandas have to be efficient at breaking down biomass—they eat as much as 30 lb of bamboo each day, and not much else, Brown notes. Bamboo makes up a whopping 99% of the giant panda's diet in the wild. The creatures have unusual adaptations to accommodate, she says. For one, pandas have a pseudothumb that allows them to grab bamboo and strip away leaves and stems. They also have well-developed teeth, jaws, and chewing muscles, designed

"You can't design enzymes or biosynthetic pathways like a computer chip and have them work."

more for masticating like a cow than ripping meat like a carnivore.

But the panda's digestive system isn't built like a cow's, Brown continues. Whereas cows and other ruminants typically have a long GI tract with a multichambered stomach system designed to digest grasses, she says, the panda sports a short GI tract and a single-chambered stomach. The combination of bamboo gluttony and short GI tract means the panda processes bamboo quickly, in only about four hours from front to rear; in cows, it's a one- to three-day process.

Brown's research has been guided by the panda genome, which was sequenced and reported in 2009 by an international consortium led by scientists in China (*Nature*, DOI: 10.1038/nature08696). The Chinese team assembled the genome of Jingjing, a panda at the Beijing Zoo named after one of the mascots of the 2008 Olympic Games.



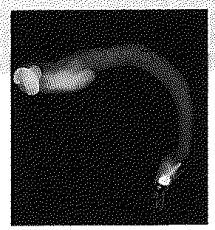
Creature: Asian longhorned beetle
Eats: Wood
Beetle larvae bore in and feed on wood, using enzymes produced by a fungus living in their guts to break down lignin to aid digestion.

The study revealed a genetic basis for the panda's love of bamboo. It turns out pandas have mutations resulting in loss of function of the gene that codes for umami taste receptors, which recognize glutamate, Brown notes. These mutations appear to have robbed pandas of the ability to savor the taste of meat and other high-protein foods, she explains, pushing them toward their diet of readily available bamboo.

REGARDLESS OF the panda's food preferences, it is genetically speaking a carnivore, Brown says. Bamboo-loving pandas and dogs, the only other carnivore sequenced to date, have 99% of their genes in common.

"What we took away from the study of the panda genome and our study of the panda's digestive microbiome is that the enzymes for bamboo digestion must all come from symbiotic microorganisms living in the gastrointestinal tract," Brown says. "And these bacteria have to be highly specialized to efficiently break down bamboo so quickly. Pandas really are marvels in their ability to have adapted to the highly fibrous bamboo diet. It seems that the panda recruited the microorganisms based on a functional need, rather than an evolutionary process."

As for biofuel prospects, among the hundreds of microbe species inside pandas, the Mississippi State researchers discovered 17 cellulose-degrading microbes. They are still trying to identify the exact species of them all, but some of them have also been found in other biomass-digesting animals. Brown's team has done preliminary work culturing the newfound microbes, feeding them cellobiose, the primary disaccharide in cellulose. They are finding that some of the microbes convert the sugar to fatty acid methyl esters for energy storage.



Creature: Shipworm
Eats: Submerged wood
Known as termites of the sea, shipworms are a type of saltwater clam that relies on bacterial enzymes in the digestive tract to break down wood.

"We see considerable cellobiose consumption and quite a mix of different triglycerides and free fatty acids forming," she says. Those that yield primarily oils or alcohols are likely to be more amenable to producing biofuels, she adds. Her group is collaborating with Mississippi State chemical engineer Darrell Sparks Jr. and colleagues to explore the possibility of transplanting genes unearthed from the panda gut into the yeast *Rhodotorula glutinis* to enhance its oil-producing ability. For example, they are hoping to engineer yeast strains that can feed off waste lignin in paper mills or residual organic carbon in wastewater at treatment plants.

"There is now a ton of sequence data that's been generated hinting at putative cellulolytic enzymes," JGI's Tringe says. "I am not sure how many more animal microbiomes we need to explore. The real need now is to understand enzyme functions better to move the data downstream to make them useful for industry."

To that end, chemical engineer Trent R. Northen, director of array-based assays at the Joint BioEnergy Institute (JBEI), is leading work on methods for rapid analysis of microbiome enzymes to determine which types of bonds the enzymes are forging or breaking. JBEI is one of three DOE bioenergy centers focused on biofuel research.

Chemists traditionally use high-performance liquid chromatography often coupled to mass spectrometry for this task, Northen says. But the throughput is limited by the chromatography. JBEI has designed assays that eliminate this time-consuming step.

Creature: Tammar wallaby
Eats: Leaves, seeds, grasses
This marsupial, the size of a large rabbit, has a digestive system comparable to a cow's. Beyond mining its gut microbes for enzymes to make biofuels, scientists are hoping to pinpoint the enzymes that allow wallabies to produce much less methane than cows.

"We are trying to address the mismatch between the rate we can sequence genomes and the rate we can characterize gene functions," Northen says. For this function-based screening, Northen and his team use nanostructure-initiator mass spectrometry (NIMS). Northen helped develop this technique, originally designed with biomedical research and drug discovery in mind, when he was in physical chemist Gary Siuzdak's group at Scripps Research Institute.

In NIMS, a laser beam vaporizes initiator molecules, typically fluorinated siloxanes, which are trapped in nanometer-sized pores on a silicon surface, Northen explains. As the initiator molecules are ablated, they carry away sample molecules that have been deposited on top of the pores, desorbing the molecules for mass analysis.

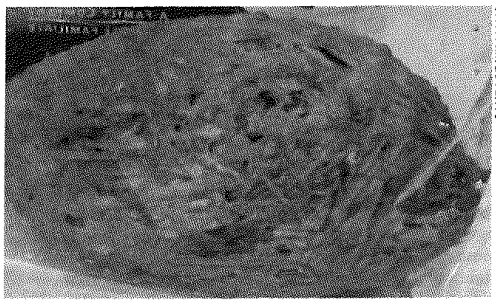
Northen and his colleagues have done more than 50,000 assays on 200 glycoside hydrolases—a family of enzymes that includes cellulases—originating from JGI's cow rumen, insect, and other metagenomics projects. The assays pit oligosaccharides found in biomass against different enzymes under different temperature, pH, and salt concentration conditions. The analysis

MEMPHIS ZOO (PANDA), JONAS LØVAAS GJERSTAD (COW), FABIAN MICHELANGELI (HOATZIN), JOSHUA PETER KAFFER/PENN STATE (BEETLE), DAMON TIGHE/JGI (SHIPWORM), MEHGAN MURPHY/SMITHSONIAN'S NATIONAL ZOO (WALLABY)

determines how the enzymatic reactions unfold by identifying the products formed when oligosaccharide bonds are modified.

Accumulating this type of functional information in public databases will be a resource for synthetic biologists and others, Northen relates. It will enable them to look through databases, find a specific reaction catalyzed by a specific enzyme under specific conditions, download a sequence, synthesize the gene, and clone it into a microorganism to make the biofuel ingredients or other products they want, he says.

"There are tremendously exciting possibilities with this approach," Northen continues. "We don't yet have a predictive understanding of biology. You can't design enzymes or biosynthetic pathways like a computer chip and have them work. We need ways of characterizing them—the equivalent of an oscilloscope or a voltmeter. That's where these high-throughput screening tools to characterize the libraries of genes and enzymes fit in."



CANDACE WILLIAMS

PANDA PELLET Panda poo, sampled at the Memphis Zoo, is kept under anaerobic conditions while it is being mined for cellulose-degrading enzymes.

But even as hundreds of researchers and millions of research dollars have been dedicated to enzyme bioprospecting, there is one thing still missing: a viable market for cellulosic biofuels. If scientists like Brown come up with improved technologies for processing the biomass, a viable market will require more, including a practical and economical source for the biomass, companies to produce biofuels, and consumer demand.

Biomass supplies are ample, according to DOE estimates. It projects that by 2030 the U.S. could produce some 1.1 billion dry tons of bioenergy crops or agricultural residues annually without upsetting food, animal feed, and fiber production—enough to replace about 30% of the country's petroleum consumption.

& VIDEO ONLINE **See how Ya Ya and Le Le's poo may reveal the secret to better biofuels at http://cenm.ag/panda.**

With that in mind, enzyme producers, chemical and oil companies, and biofuel start-ups are forming partnerships as they pick and choose technology platforms to make cellulosic biofuels. But no companies have yet to achieve commercial-scale production (C&EN, Oct. 8, page 22).

ANY NEW TECHNOLOGY that emerges from animal microbiome mining would need to improve upon the proprietary enzyme systems already in companies' arsenals. For example, enzyme company Novozymes is already marketing cellulase enzymes from *Trichoderma reesei*, a fungus originally discovered because it was degrading cotton military uniforms and canvas tents in the South Pacific during World War II. The company, which sequenced *T. reesei* with JGI, has slipped some of the fungal genes into other microorganisms to produce enzymes it is selling to a handful of cellulosic biofuel companies around the globe.

But biofuel producers are still looking for enzyme systems that can reduce or eliminate the need for harsh chemical pretreatments—acids, ammonia, or ionic liquids—currently required in cellulosic biofuel production processes. Pretreatment serves to open up the lignocellulose matrix to give enzymes more room to work. Ultimately, cocktails of 30 to 40 enzymes, tailored to accommodate different types of biomass, will be needed by biofuel producers to efficiently convert untreated biomass into fermentable sugars and on into biodiesel or biogasoline.

Scientists anticipate that the mounds of data coming out of the animal metagenomics studies will provide replacement or supplemental genes needed to improve current biofuel production systems to make them cost-competitive. But so far, neither metagenomics researchers nor enzyme producers have reached out to each other to collaborate on the gut microbiome results.

"There is potentially great synergy between what the Joint Genome Institute and academic institutes are doing to discover genes that code for cellulase enzymes and what enzyme companies are doing with expression technology to take new enzyme technology to the marketplace," says Mark A. Emalfarb, president and chief executive officer of enzyme company Dyadic International. "But we need to do a better job helping each other bring these discoveries to market."

Dyadic has spent 20 years developing a robust enzyme expression system based on a genetically modified soil fungus. The company has licensed its technology to other companies to make biofuels, and it's developing its own line of biofuel cellulases.

"This industry is not for the faint of heart," Emalfarb says. "Expression technology takes years and millions of dollars to develop. No company today produces enzymes that are cost-effective enough for economically viable production of cellulosic biofuels."

Beyond the science and process engineering challenges, policy decisions out of the control of scientists impact consumer demand for biofuels, Emalfarb adds.

The biofuel industry faces a formidable foe in fossil fuels that are cheaper and will remain readily available for years to come. Increased federal fuel-efficiency standards will put biofuels at a further disadvantage. And although the federal Renewable Fuel Standard, which gradually increases the amount of cellulosic biofuels required to be produced each year, continues to create market demand for new enzymes, the federal government has declined to mandate that carmakers produce vehicles that can accept a range of biofuel/fossil-fuel blends.

Emalfarb suggests the cellulosic biofuel industry needs to become more engaged in the outcome of the metagenomics studies to prevent the research on pandas, cows, hoatzins, and other creatures from sitting on a shelf collecting dust.

In Memphis, oblivious to these dilemmas, Ya Ya and Le Le keep chowing down on bamboo. Mississippi State's Brown and her team, probing pellets for more enzymatic clues, are more aware of what's at stake.

"Our work suggests that metagenomics is useful for enzyme bioprospecting," Brown states. "Hopefully we are helping standardize DNA sequencing technology and high-throughput screening technology to make this approach more credible. And hopefully in doing that we can make a difference for the pandas and for biofuels." ■