V(A). Planned Program (Summary)

Program # 3

1. Name of the Planned Program

Sustainable Energy

☑ Reporting on this Program

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
101	Appraisal of Soil Resources		0%		10%
102	Soil, Plant, Water, Nutrient Relationships		0%		10%
123	Management and Sustainability of Forest Resources		0%		10%
201	Plant Genome, Genetics, and Genetic Mechanisms		0%		20%
203	Plant Biological Efficiency and Abiotic Stresses Affecting Plants		0%		10%
403	Waste Disposal, Recycling, and Reuse		0%		20%
511	New and Improved Non-Food Products and Processes		0%		20%
	Total		0%		100%

V(C). Planned Program (Inputs)

1. Actual amount of FTE/SYs expended this Program

Year: 2014	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.5	0.0	7.3
Actual Paid	0.0	0.0	0.0	4.5
Actual Volunteer	0.0	0.0	0.0	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c	**1890 Extension**	**Hatch**	**Evans-Allen**
0	0	0	244000
1862 Matching	**1890 Matching**	**1862 Matching**	**1890 Matching**
0	0	0	249347
1862 All Other	**1890 All Other**	**1862 All Other**	**1890 All Other**
0	0	0	33480

V(D). Planned Program (Activity)

1. Brief description of the Activity

WVSU Agricultural and Environmental Research Station research efforts are focused on improving thermophilic digestion of poultry waste, developing anaerobic microbial energy conversion, and increasing awareness of soil remediation technology among mining operators and agencies. We are expanding into studying the functional role of additional oil biosynthesis genes in Arabidopsis and translate the proven examples from the model plant to a dedicated bioenergy crop for the production of bioenergy.

2. Brief description of the target audience

Digester manufacturers and users, poultry farmers, other agricultural waste producers, environmentally concerned citizens, undergraduate and graduate students, engineers and scientists who study bioreactors and anaerobic microbial processes. Mine operators, mine reclamation contractors, land owners

3. How was eXtension used?

eXtension was not used in this program

V(E). Planned Program (Outputs)

1. Standard output measures

2014	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Actual	0	0	0	0

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year: 2014

Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2014	Extension	Research	Total
Actual	0	1	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

- Train students in digester operation, biochemical analysis, microbial analysis.

Year	Actual
2014	0

Output #2

Output Measure

- Improve the operation of thermophilic poultry waste digesters.

Year	Actual
2014	0

Output #3

Output Measure

- Add an extension component to the digester program
  Not reporting on this Output for this Annual Report

Output #4

Output Measure

- Evaluate the feasibility of adopting anaerobic digestion on poultry farms in WV through extension outreach to farmers.
  Not reporting on this Output for this Annual Report

Output #5

Output Measure

- Develop soil management practices for the use of biochar and anaerobic digester products as soil amendments for regional soils.

Year	Actual
2014	0

V(G). State Defined Outcomes

V. State Defined Outcomes Table of Content

O. No.	OUTCOME NAME
1	Improve the operational parameters that have been used to control thermophilic poultry waste digesters.
2	New knowledge concerning how microbial diversity gives rise to anaerobic microbial energy conversion and anaerobic digestion.
3	Increase awareness of beneficial use of waste and byproducts to improve soil productivity and ecological services.

Outcome #1

1. Outcome Measures

Improve the operational parameters that have been used to control thermophilic poultry waste digesters.

2. Associated Institution Types

- 1890 Research

3a. Outcome Type:

Change in Action Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	1

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Anaerobic digestion (AD) is a well-established process for converting organic wastes into bioenergy (methane). The economics of AD can be improved when different types of wastes are combined (co-digested). WVSU has a long-term research program that has investigated the efficiency, stability and microbiology of a thermophilic, pilot-scale, digester that treats poultry farm waste. We are also interested in whether this digester and its microbiome can successfully utilize other types of organic wastes. Our current research is testing whether glycerol, which is the major waste produced by biodiesel manufacturing, can be co-digested with poultry litter. If these wastes could be processed together in this digester, that would improve the economics of thermophilic digestion which is somewhat more costly than mesophilic digestion. We are also interested in improving the economics of thermophilic digestion by creating smaller scale, less expensive digesters. To this end, we have also investigated the feasibility of a thermophilic plugflow digester design.

What has been done
To investigate the potential of digesting glycerol in a thermophilic digester adapted to poultry litter, we began a co-digestion experiment using three replicate model 10-liter digesters. These digesters were started with inoculum from the WVSU pilot plant thermophilic digester. We also completed an experiment that tested the feasibility of developing a thermophilic digester with a plug flow design for poultry litter using microorganisms from the WVSU CSTR digester. The new plugflow digester was pilot-scale and contained 1 cubic meter of liquid. The start-up of the digester was done during the previous year and the experiment was completed during 2014. The performance of the digester and associated microbial ecology were evaluated.

Results
The digesters used for the glycerol experiment were stabilized at a 15-day steady state hydraulic retention time with poultry litter substrate. The effect of 3% glycerol co-substrate was evaluated in terms of reactor performance and microbial diversity dynamics. This level of glycerol was found to be inhibitory to methane production and caused a large shift in the primary bacterial diversity. The evaluation of the new pilot-scale thermophilic plugflow digester was completed; the entire experiment lasted nearly 600 days. We found that biogas was produced and COD was reduced in the new digester indicating that the design and process did work. The microbial diversity of the digester was tested using pyrosequencing to target 16S rRNA genes. During a period of lower temperature lasting several weeks, biogas production decreased and the diversity of the microbial community shifted to dominance by Proteobacteria. However, when the temperature was restored, digester performance and community diversity began to recover. Two WVSU graduate students contributed to this research, as well as two technicians, one visiting graduate student (Mexico), and two undergraduate students. Dr. Teodoro Espinosa-Solares, Department of Agroindustrial Engineering, Universidad Autonoma Chapingo (Mexico) was a collaborator. In addition, analysis and modeling of data from a previous fatty acid kinetics experiment was continued and a paper from this work is in progress.

4. Associated Knowledge Areas

KA Code	Knowledge Area
403	Waste Disposal, Recycling, and Reuse

Outcome #2

1. Outcome Measures

New knowledge concerning how microbial diversity gives rise to anaerobic microbial energy conversion and anaerobic digestion.

2. Associated Institution Types

- 1890 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Transitioning from a fossil fuel-dependent economy to one based on sustainable energy

production is a major national objective. One of the most promising types of sustainable methods for energy generation is the microbial conversion of biomass to energy. This can be accomplished most effectively with mixed microbial consortia because no single species possesses the required suite of metabolic processes. However, mixed microbial consortia are quite complicated and the ability to engineer their properties has been limited. Future advances in environmental biotechnology will require systems-level analysis of these microbial processes in order to reveal and engineer underlying mechanisms. These goals require new approaches to studying bioenergy communities and new educational curricula to train the next generation of students. This project was supported by a USDA CBG Research/Teaching Integration grant (Huber, PI) that investigated the systems-level properties of methanogenic microbial consortia, and created a systems biology and metagenomics component for the biotechnology curriculum at WVSU. The research conducted with this grant will be useful for applications in agriculture and industry where bioenergy produced from organic wastes can be used to offset the energy costs of manufacturing and farming. The educational component of the grant provided training in advanced DNA sequencing technologies, bioinformatics, and microbial systems biology for students at WVSU and visiting international students and scientists.

What has been done

This CBG had both research and education objectives. The research objectives were to understand how microbial communities perform biomass-to-bioenergy conversion using a systems analysis of methanogenic reactors. Three long-term bioreactor experiments were continued during the past year. The first experiment sought to elucidate the relationship between the stability and efficiency of bioenergy production in terms of microbial community properties. Six replicate five-liter reactors were operated for more than three years of continuous operation to measure metabolic performance, stability and resilience. A second long-term experiment was conducted which measured the resilience of methanogenic reactor communities toward environmental perturbations. Another set of replicate reactors was used to provide a detailed time series profile of performance during steady state operation prior to environmental disturbances and during the recovery period. Using these system parameters, we measured the properties of resistance, resilience and stability following pulse disturbances caused by increased organic loading. In addition, metagenomics analysis was done to compare the microbiomes of thermophilic and mesophilic digesters that treat poultry litter and plant biomass. Another major objective of the grant was to build the educational capacity of the biotechnology program at WVSU with regard to incorporating microbial systems biology.

Results

For the first experiment, systems analysis of the reactors consisted of time series measurements of primary metabolites and microbial community dynamics. Continuous measurements of biogas composition, COD removal, fatty acid production, and other chemical variables were made during this experiment. Fine-scale microbial population diversity was sampled using pyrosequencing. We found that food web structure and population interactions changed in response to feeding level. To our knowledge, network analysis of methanogenic ecosystems have never been performed at this level. A second long-term experiment was conducted which measured the resilience of methanogenic reactor communities toward environmental perturbations. A set of five replicate 10-liter reactors was used to provide a detailed time series profile of performance during steady state operation prior to environmental disturbances and during the recovery period. We found that the history of the reactors (previous substrates) affected their resilience to the pulse disturbances, and that resilience varied for individual fatty acids. In another experiment, we demonstrated that replicate microbiomes could occur in alternative stable states. Time series analysis showed that replicate reactors originating from the same stable source community could

stabilize in alternative states with different energy conversion efficiencies. Metagenomics analysis showed that the thermophilic digester (WVSU) and the mesophilic digester shared primary functions based on SEED subsystem classification. However, important differences, including carbohydrate utilization pathways, were also found. In addition, modeling was done of fatty acid kinetics in this hydrogenotrophic microbiome. For the educational component of the grant, training was provided in environmental biotechnology, microbial systems biology, and genomics to graduate and undergraduate students at WVSU and visiting international graduate students. The technology provided by the grant also supported other research at WVSU (freshwater ecosystems) and research with collaborators at Universidad Autonoma de Coahuila (Mexico) and Universidad Autonoma Chapingo (Mexico). A ?Microbial Metagenomics Workshop? was taught at WVSU. The Workshop was attended by thirteen participants, including graduate students, undergraduates, and visiting international scientists.

4. Associated Knowledge Areas

KA Code	Knowledge Area
403	Waste Disposal, Recycling, and Reuse

Outcome #3

1. Outcome Measures

Increase awareness of beneficial use of waste and byproducts to improve soil productivity and ecological services.

2. Associated Institution Types

- 1890 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Current remediation and reclamation practices of disturbed lands fall short of reviving a natural system?s ability to sustain ecosystem services, such as clean water and good fertile, non-eroding soils. Best management practices (BMP) for reclamation must be demonstrated, implemented and adopted in order to improve post-mining land use and reclaimed soil fertility and productivity.

What has been done

With support of the Appalachian Regional Commission, we initiated a public-private partnership with a regional land-holding company and a mining operator to identify a location and to implement BMPs on recently reclaimed coal mine, which included deep contour ripping and reforestation. In addition, a field experiment was conducted to evaluate and compare establishment and growth of different bioenergy crops (switchgrass, miscanthus, big blue stem and broom sage) under different fertilization regimes (none and optimal).

Results
Over 30,000 black locust trees were planted and a genetic trial for selection of suitable hybrid poplar genetic line (60 lines tested) was established on nearly 40 acres of reclaimed land. Outreach activity was conducted to provide university staff and students, as well as members of the local community, with the opportunity to visit a reclaimed mine site and to have a hands-on experience in planting trees and establishing pollinator?s garden. More than 45 people participated and more than 800 trees were planted. Field trial of bioenergy grasses failed and had to be reseeded.

4. Associated Knowledge Areas

KA Code	Knowledge Area
101	Appraisal of Soil Resources
123	Management and Sustainability of Forest Resources
403	Waste Disposal, Recycling, and Reuse

V(H). Planned Program (External Factors)

External factors which affected outcomes

- Natural Disasters (drought, weather extremes, etc.)
- Economy
- Appropriations changes
- Public Policy changes
- Government Regulations

Brief Explanation

The anaerobic digestion research program has been impacted by recent State regulations concerning above ground storage tanks. Mine site access can be a complicating factor in addition to weather. All research programs have been impacted by the delay in receiving federal funds and a decrease in State matching.

V(I). Planned Program (Evaluation Studies)

Evaluation Results

The Sustainable Energy program is an area of emphasis at WVSU as a new position was created: Assistant Professor of Bioenergy and Environmental Biotechnology. The Anaerobic Digestion program has been hampered by a lack of funding and new State regulations on above ground storage tanks. McIntire Stennis funding has contributed significantly to this area of research.

Key Items of Evaluation

The Sustainable Energy program is an area of emphasis at WVSU as a new position was created: Assistant Professor of Bioenergy and Environmental Biotechnology. The Anaerobic Digestion program has been hampered by a lack of funding and new State regulations on above ground storage tanks. McIntire Stennis funding has contributed significantly to this area of research.