Northeast SARE Farmer/Grower Grant

FINAL REPORT

To: Dale I. M. Riggs, SARE Northeast, Farmer Grant Specialist

From: John Morelli, Project Leader

Date: February 26, 2009

Project Title: ***Evaluating Spent Shiitake and Oyster Mushroom Substrate as Feedstocks for Ethanol Fuel Production***

Project Leader: John Morelli

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E-mail address: jmorelli@rochester.rr.com SARE grant amount: $10,000

Final Report to Northeast SARE

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Project Leader: John Morelli

North East Sustainable Agriculture Research and Education Program
Farmer Grant Final Report

1. Project name and contact information

Evaluating Spent Shiitake and Oyster Mushroom Substrate as Feedstocks for Ethanol Fuel Production

Project Leader: John Morelli
Flower City Mushrooms LLC
535 Five Points Road
Rush, NY 14543

E-mail: jmorelli@rochester.rr.com
Telephone: (585) 314-5009

2. Goal

The goal of this project was to determine whether spent cellulosic organic mushroom substrates are technically and economically feasible feedstocks for fuel ethanol production.

3. Farm profile

Flower City Mushrooms LLC (FCM) grows *Lentinula edodes* (shiitake) and a variety of *Pleurotus* (oyster) mushrooms, all certified organic by the Northeast Organic Farming Association of NY. These species are degraders of cellulose and lignin and consequently FCM seeks to use locally available organic products and by-products rich in these ingredients. FCM has established relationships with a local producer of organic corn seed to provide organic corn cobs to use in substrate preparation for oyster mushroom production, and with a local primary log mill to provide hardwood sawdust for shiitake.

Flower City Mushrooms has been operating as a small farm since 2002 and has be certified organic since 2003. It operates full-time all year around. During the first two years of operation, the lab and growing facility were constructed and research was conducted to identify local, potentially available substrate materials, secure samples of each, and develop substrate formulations to correspond to a variety of shiitake and oyster mushroom strains. The FCM lab and grow rooms are housed in a barn occupying approximately 2,500 square feet. FCM recently installed a ground source heat pump and ground loop to utilize the Earth's capacity to store energy in the form of heat, and therefore supplement the operation's heating and cooling needs. Operating at full capacity, FCM is capable of producing approximately 500 lb. of mushrooms per week.

4. Participants

The technical advisor to this project was:

Robert N. King, Ph.D., Director
Address Agriculture and Life Sciences Institute.
Monroe Community College
1000 E Henrietta Road, Rochester, NY 14623

Dr. King reviewed the original project proposal before it was submitted to SARE.

5. Project activities

Flower City Mushrooms LLC (FCM) has conducted a pilot scale project to examine the technical and economic feasibility of using spent cellulosic organic mushroom substrate to produce ethanol as an energy product for use on the farm.

This work examined the result of mushroom growth on three types of substrate: sawdust, wheat straw, and corncobs. Shiitake mushrooms (Lentinula edodes) were grown on the sawdust substrate and oyster mushrooms (sajor caju) were grown on the wheat straw and corncob substrates. FMC prepared mushroom substrate formulations and collected spent substrate samples at four stages: pre-colonization; post-colonization and pre-fruiting; post-fruiting; and post hydrolysis.

Two strategies were employed to evaluate changes in fiber content and water soluble carbohydrate concentrations as a precursor to ethanol in a fermentation process. The first strategy was to analyze the samples to compare fiber content and water soluble carbohydrates before inoculation, after mycelial growth, and after fruiting. This methodology would provide evidence of fiber hydrolization during the vegetative growth and fruiting stages. The second strategy involved microscopic inspection of the substrate at various stages for evidence of lignin destruction and weighing of dried substrate solids before and after enzymatic hydrolysis to determine the percentage of solids that were hydrolyzed.

Results of these inspections and analyses were compiled, compared and evaluated.

6. Results

The results of this research reveal that the mycelial growth of white rot fungi did in fact degrade the lignin by approximately 50% in the case of Lentinula edodes on hardwood sawdust substrate and by approximately 25% in the case of Pleurotus sajor caju on wheat straw. Concurrent with lignin reduction, fiber content was reduced by an average of 28% on the sawdust substrate and by 41% on the wheat straw substrate. These reductions in lignin and fiber coincide with a 500%

increase in simple sugars from the sawdust substrate and 400% increase in simple sugars from the wheat straw substrate.

While these increases in sugar content may appear significant, they are not significant enough to result in favorable economic production of ethanol. In the case of the sawdust substrate, the 500% increase in simple sugar content represented an increase from only 0.8% (the original sugar content percentage) to approximately 4.8% of the total substrate matter. At this sugar production rate and assuming a 100% efficient fermentation process, only about 41 gallons of fuel ethanol could be produced. In the case of the wheat substrate, the 400% increase in simple sugar content represented an increase from only 1.5% (the original sugar content percentage) to approximately 7.5% of the total substrate matter. At this sugar production rate and assuming a 100% efficient fermentation process, only about 64 gallons of fuel ethanol could be produced.

This process and analysis was also performed for *Pleurotus sajor caju* on a corncob substrate. This experiment resulted in the least promising result and the corncob substrate investigation was terminated at this point.

To improve the economic feasibility of fuel ethanol production on spent sawdust and wheat straw substrates, both substrates were subject to enzymatic hydrolysis using two proprietary enzymes made available by Genecor for this research. The hydrolysis process increased simple sugar content by approximately 150% for both substrates when using the Genecor Cellulose Enzyme CP. While addition of an enzymatic hydrolysis step again increased the simple sugar content, the results regarding ethanol production still appear to be economically infeasible. This finding is further supported when the cost of the cellulase enzyme and the process energy costs are considered. The results of this work indicate that conversion of 10,000 pounds of spent mushroom substrate would produce only 51 gallons of fuel ethanol using the shiitake/sawdust substrate formulation, and only 82 gallons of fuel ethanol using the oyster/wheat straw substrate formulation.

7. Conditions

Prepared samples were to be analyzed without cost by the National Renewable Energy Laboratory (NREL) using NREL LAP 009, Enzymatic Saccharification of Lignocellulosic Biomass (http://devafdc.nrel.gov/pdfs/9578.pdf**)** to determine the extent to which fermentation was possible (essentially an assay of simple and complex sugars). However, the NREL staff person who was originally contacted at the NREL regarding this work during the proposal development phase was no longer employed there when it came time to perform the analyses and the current director informed the project team that he would have to charge $3,000 per sample for this service. Since there were at least nine sample that needed to be analyzed, this cost was well beyond the project's budgetary capabilities. The loss of these analytical services limited comprehensive understanding of this regarding the breakdown products during the various steps

in the conversion process. However, alternative procedures were developed and carefully followed and these findings are presented with confidence.

8. Economics

The conclusion is that fuel ethanol production from spent mushroom shiitake/sawdust and oyster/wheat straw substrates is economically infeasible.

9. Assessment

Additional analytical resources could lead to a better understanding of the process by which cellulosic fiber is reduced to simple sugars that are then amenable to fermentation into fuel ethanol. What became apparent during this work was the lack of understanding regarding the intermediate breakdown products between cellulose/hemicellulose fibers and simple sugars. A reasonable next step would be to conduct such analyses.

10. Adoption

FCM will not continue to use the practice investigated here because it was determined to be economically infeasible.

11. Outreach

A copy of the full report was mailed (on 2/23/09) for consideration of publication to the:

Mushroom Grower's Newsletter

P.O. Box 5065

Klamath Falls, OR 97601 USA

12. Report Summary

Flower City Mushrooms LLC (FCM) has conducted a pilot scale project to examine the technical and economic feasibility of using spent sawdust, wheat straw, and corncob organic mushroom substrates to produce fuel ethanol.

Two strategies were employed to evaluate changes in fiber content and water soluble carbohydrate concentrations as a precursor to ethanol in a fermentation process. The first strategy was to analyze the samples to compare fiber content and water soluble carbohydrates before inoculation, after mycelial growth, and after fruiting. The second strategy involved microscopic inspection of the substrate at various stages for evidence of lignin destruction and weighing of

dried substrate solids before and after enzymatic hydrolysis to determine the percentage of solids that were hydrolyzed.

The results of this research reveal that the mycelial growth of white rot fungi did in fact degrade the lignin by approximately 50% in the case of Lentinula edodes on hardwood sawdust substrate and by approximately 25% in the case of Pleurotus sajor caju on wheat straw. Concurrent with lignin reduction, fiber content was reduced by an average of 28% on the sawdust substrate and by 41% on the wheat straw substrate and simple sugar concentrations increased by 500% on the sawdust substrate and 400% on the wheat straw substrate. Enzymatic hydrolysis of these substrates using the Genecor Cellulose Enzyme CP further increased simple sugar content by approximately 150%. The results of this work indicate that conversion of 10,000 pounds of spent mushroom substrate would produce only 51 gallons of fuel ethanol using the shiitake/sawdust substrate formulation, and only 82 gallons of fuel ethanol using the oyster/wheat straw substrate formulation, in both cases making it an economically infeasible pursuit.

Respectfully submitted,

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John Morelli Date