# **Fungal Decay: Using Fungi as a Biological Pretreatment for Anaerobic** Digestion **Leslie Johnson 2012 BioEnergy and Sustainability School** August 7, 2012





# Outline

### Introduction

- Anaerobic Digestion
- Hydrolysis
- Pretreatment
- Objectives
- Hypothesis
- Methods
- Results
- Future Studies



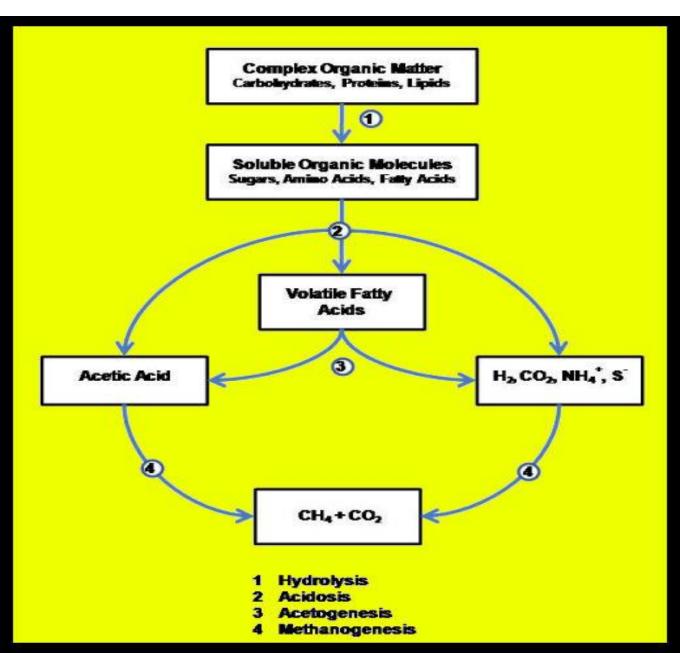


# Introduction

- Anaerobic digestion is defined as the microbial degradation of organic material under anaerobic conditions.
- It is a natural process that occurs in oxygen-free environments, such as swamps.
- Anaerobic digestion produces two products: biogas and biofertilizer



# Hydrolysis



## Pretreatment

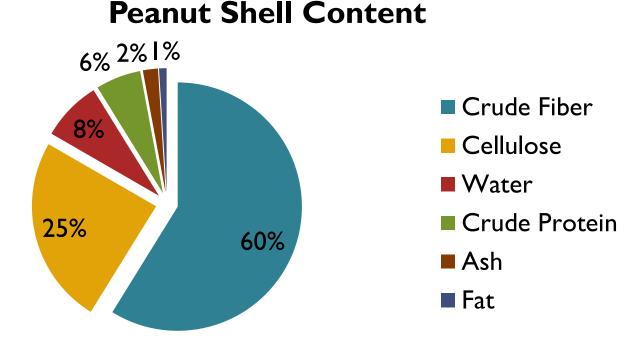
### Why is Pretreatment Necessary?

- Pretreatment can increase kinetics
- Physical pretreatment requires lots of heat and energy
- Chemical pretreatment requires the use of enzymes, such as cellulase
- Biological pretreatment can be an inexpensive alternative





• The feedstock used in this experiment were peanut shells and coffee grounds.



## Objectives

- Determine the baseline biological methane potential of peanut shells and coffee grounds
- Find and cultivate local fungi
- Test if fungi can increase hydrolysis step by solubilizing cellulosic materials

# Hypothesis

 Fungal pretreatment will show an increase in biogas production in coffee grounds and peanut shells as a result of the increase in the hydrolysis step.



# Methods

Total solids (dry matter content, at 105° C) and volatile solids (organic matter content 500° C) tests were conducted to characterize peanut shells (PS) and coffee grounds (CG).

## Methods



BMP bottles (12) were filled with 200 mL of dairy manure and 0.4 grams of untreated substrates.

Biochemical Methane Potential (BMP) tests were conducted using peanut shells and coffee grounds

An active microbial consortium (dairy manure) was added as an inoculum and also used as the blank. Cellulose was used as a control.

The BMP test determines actual potential methane production under specific conditions.

### Measurement of Biologically-produced Methane Gas





# Methods

- The three species of fungi were cultivated in petri dishes at 35° C.
- Fungal solutions containing fungi, cellulose broth, and deionized water were created in Erlenmeyer flasks.
- ImL of solution were placed on petri dishes. Solutions were diluted to a 1:10 and 1:100 ratio.



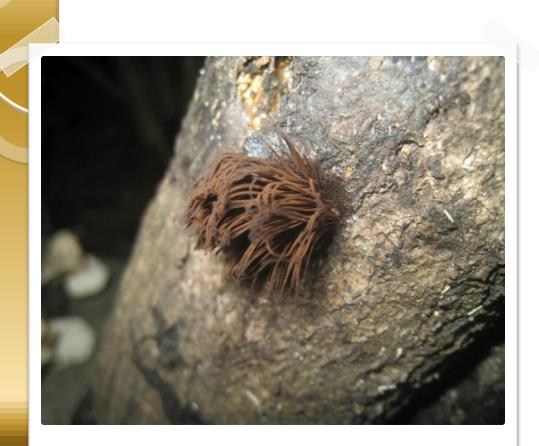
# Methods

- Total Chemical Oxygen Demand (COD) and Soluble COD were measured over time.
  - Total COD measures the total quantity of oxygen required for oxidation and gives a theoretical maximum methane production.
  - Soluble COD is a similar process that tells how much organic matter is soluble in water and available for biogas production.



# Fungal Prospects

- Collected from rotting wood
  - Already capable of using a high lignocellulose material
- Three organisms were investigated:
  - Stemonitis sp.
  - Tramates versicolor
  - Cyathus sp.



#### Fungal Prospects for Pretreatment

#### "Chocolate Tube Slime"

### Stemonitis sp.



### Tramates versicolor

#### Fungal Prospects for Pretreatment

### "Turkey-Tail Fungi"



### Cyathus sp.

#### Fungal Prospects for Pretreatment

#### "Bird's Nest Fungi"



# **Biological Incubation**

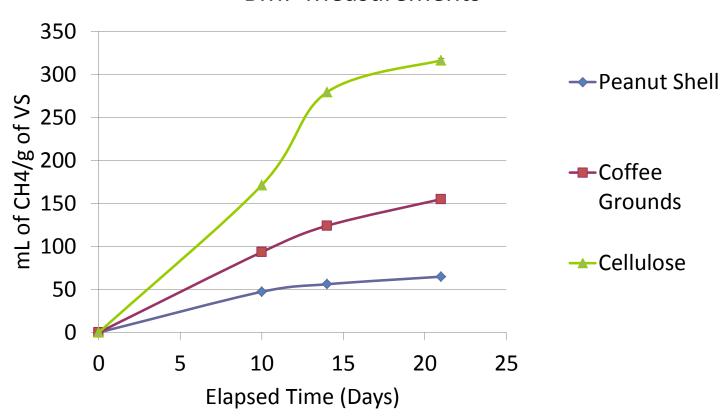


 Pretreatments were placed in 125mL flasks and shaken for 24 & 48 hours.
Measurements were recorded each day.

## Results

Feedstock	Total Solids	Volatile Solids
Average CG	28.43 ± 2.65	96.43 ± 0.31
Average PS	90.33 ± 0.07	96.68 ± 0.156

### Results



**BMP** Measurements

Pretreatment is necessary to increase maximum CH<sub>4</sub> production

#### \*BMP measurements are of untreated substrates

## **Results-Fungal Cultivation**

Tramates versicolor

After Day 1

(Kell/3)

After Day 5



After Day 1

After Day 5





Stemonitis sp.



### Tramates versicolor







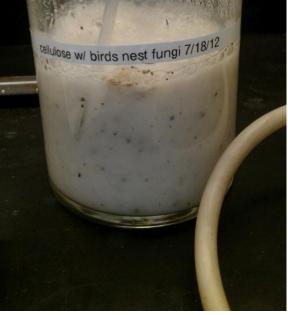


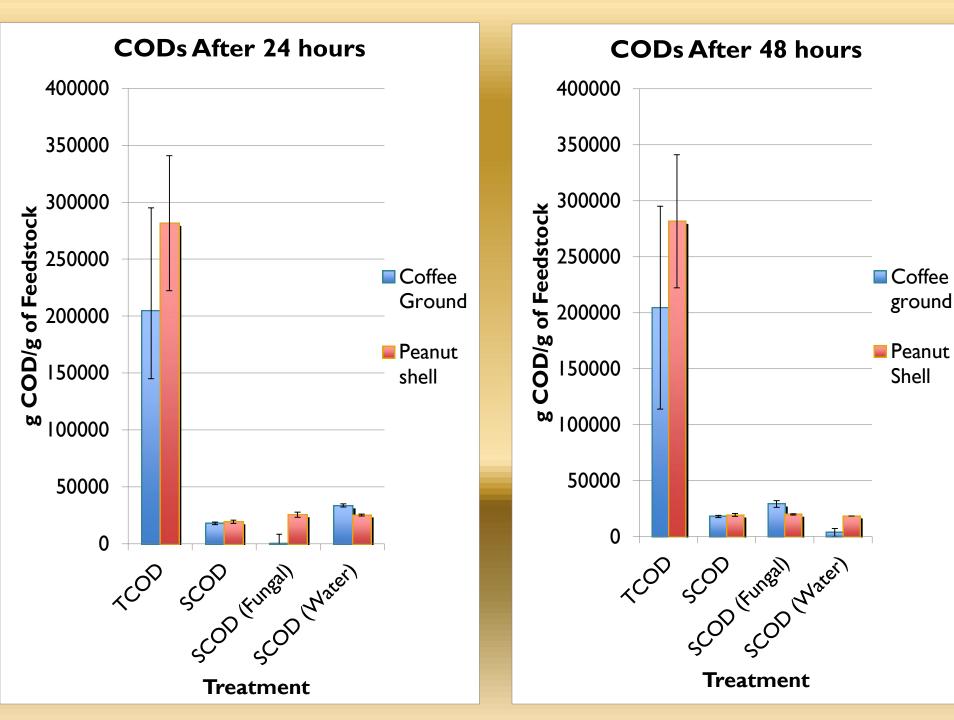
Day 1

cellulose w/ birds nest fungi 7/18/12

Day 12

cellulose w/ birds nest fungi 7/18/12







### Conclusion

- TS/VS tests indicate that both substrates consist of at least 96% of organic matter.
- Pretreatment is necessary for PS and CG.
- Biological incubations did not rapidly increase the soluble COD of either coffee grounds or peanut shells



## **Future Studies**

- Test SCODs for a longer period of time. (ex. 2-4 weeks)
- Cultivate the fungi under various temperatures to maximize the growth potential.
- Test the BMPs with the pretreated substrates.
- Testing more substrates to compare the SCODs and BMPs. (ex. wood chips, leaves, and filter paper)

# Questions or Comments?

