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#### ASSESSING THE ENERGY PRODUCTION POTENTIAL OF MISSISSIPPI CROPS AND CROP RESIDUE USING ADIABATIC BOMB CALORIMETRY

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#### ABSTRACT

A variety of crops and crop components are being considered for their potential as alternative fuel sources for synthesis of biofuels. Development and continued improvements in conversion technologies have increased potential sources of alternative fuels. More importantly, recycling of crop residues formerly discarded as waste may enhance the sustainability of agricultural production. We are interested in exploring the total potential energy stored in crops and crop residue. Caloric values of all portions of several agricultural crops commonly grown in Mississippi were measured by adiabatic bomb calorimetry to evaluate their potential as an energy source. Corn, cotton, rice, soybeans, sweet potatoes and winter wheat were included in this study. Crops were grown and harvested under traditional production practices. Total crop biomass was harvested, and separated into individual plant components. Total energy content of each plant component was determined using standard procedures for adiabatic bomb calorimetry. Additional parameters evaluated were moisture content, density, and ash content. The experimental results show that corn cobs, cotton seeds and stalks, soybean seeds and leaves, sweet potatoes, and wheat have sufficient energy to make them feasible to use as alternative fuel sources. Efficiency of conversion of biomass will reduce the net potential energy production of this biomass material.

*Keywords*: crop energy content; alternative uses of crops; crop waste; adiabatic bomb calorimetry; biofuel production; alternative fuel production.

#### INTRODUCTION

Crop producers are facing unique challenges to maintain economically feasible production systems. The dominant large-scale industrial model of agricultural production in the US is dependent on inputs of increasingly expensive externalities to maintain the high level of production (Sassenrath et al., 2008). Demands for agricultural products have grown substantially with the linkage of crop production and energy through the biofuels industry (Naylor et al., 2007). As the consumption and expense of fuel rises faster than that for food, crops traditionally produced as feed stocks have been converted for use as fuels, exacerbating lack of food resources for food-insecure people globally (Naylor et al., 2007). Moreover, producers are relying on expensive inputs to the production system for fertilizer, fuel, pest control, and water, and are compromising the conservation of natural resources (Sassenrath et al., 2011).

Creating sustainable agricultural production systems requires balancing productive output harvested from the system with inputs required for that production, and the economic and environmental goals of the larger society that benefit from that production (Tilman et al., 2009). Interest has arisen in improving the sustainability of the agricultural production system. One key principle of sustainable systems is the reduction of waste (Hanson et al., 2008), partly attainable by reducing inefficiencies in the production system, and primarily by increasing recycling, reusing and repurposing excess produced that is currently labeled as waste. Recycling products or crop components that were formerly considered trash also adds value to crop production systems, improving the sustainability and economic return to farmers. To this end, biomass produced in cropping systems may serve as a fuel source, expanding the saleable products harvested from crops without diverting food products to bioenergy production.

Alternative crops and crop uses offer potential new revenue streams that can enhance the return on investment for farmers. In addition to generating revenues for the farmer, local production of biofuels can potentially contribute to energy independence (Lal, 2005; Wyman, 2007). An estimated 446 M dry tons of crop residues are produced per year in the US (Perlack et al., 2005). It has been shown that more than 8 M acres of land in Mississippi would be suitable for conversion to production of bioenergy crops (Lemus et al., 2011).

Mississippi has approximately 30M acres of land, of which 23% is used for crop production, 15% for pasture or rangeland, and nearly 60% is forested. The cropland is dominated by traditional row crop production of soybeans, corn, cotton, winter wheat and rice typical of the Southeast US (Sassenrath et al., 2010). These cropping systems are concentrated in the Mississippi River Valley alluvial flood plain in the Northwest portion of the state, a region locally known as the Delta. Other minor acreage is planted to sorghum, sweet potatoes, vegetables, fruits and nuts, and other crops. The acreage planted each year varies as farmers respond to adjustments in crop prices (Sassenrath et al., 2013). Crop production alone generates nearly 2B\$ in food, feed, and fiber revenues in the state (NASS, 2013). Animal production, primarily poultry but also swine and cattle, contribute an additional 3B\$ to the agricultural production budget of the state. The agricultural production of Mississippi is the tenth highest contributor to the US agricultural GDP (EconPost, 2010). Bioenergy is an additional potential source of revenue for the state.

Biofuel production relied on conversion of plant sugars to energy. However, it is anticipated that ethanol production from plant sugars will not provide sufficient energy to address the growing needs of society (Hahn-Hagerdal et al., 2006). Use of cellulosic biomass feed stocks introduces problems due to the lignin content of plant materials. Significant recent research efforts have gone into developing methods of converting lignocellulosic feed stocks from grains and other plant materials into a suitable biofuel energy source such as ethanol (Hahn-Hagerdal et al., 2006). Successful development of these methods would open up the potential of using crop residues for biofuel synthesis.

Use of crop residues for fuel production has several key advantages over the use of edible or feed components, such as corn or other grains, but the conversion efficiency must improve before it is feasible to rely primarily on residues. Pre-treatments and new procedures have enhanced the efficiency of conversion of biomass into energy (Zhang and Shahbazi, 2011; Pierre et al., 2011), making use of cellulosic materials more amenable as feed stocks for biofuel production. Additionally, concerns have been raised of the consequences to soil quality from removal of crop residues (Lal, 2009). However, farmers in the Delta traditionally remove the residue by burning, especially for corn stubble, and rice and wheat straw (Korontzi et al., 2012; Larson, 2011). Moreover, much of the crop residue is removed from the field at harvest, and discarded during the processing of the harvested material, such as during ginning of the cotton lint and processing of rice for grain. Although cotton stalks are shredded in the field, the stalks do not contribute substantially to the soil organic matter because of the slow degradation of the woody stalk material. In current production practices in Mississippi, very little crop residue remains in the fields (Larson, 2011).

In the research reported here, we explore the potential role of crop and residue biomass and its management on improving the sustainability of cropping systems in Mississippi. We determine the energy content stored in a variety of crop products and byproducts for potential use as biofuel stocks. Identifying potential biomass components of the agricultural production cycle that were traditionally disposed of as trash can greatly benefit producers and consumers alike. As a first step in exploring the potential biofuel production capacity of MS, we determined potential biomass output from traditional row crop production. To determine potential energy production, we compared the caloric energy content of six commonly grown crops: corn, cotton, rice, soybeans, sweet potatoes, and winter wheat. The relative importance of crops and crop residues and their potential contribution to biofuel production is discussed.

#### MATERIAL AND METHODS

#### i. Plant Growth and Sampling

Plants were grown at the Delta Research and Extension Center Experiment Station farm in Stoneville, MS. The soils of the Delta region are geographically young alluvial soils ranging from rapidly draining sandy loams to slowly draining clays and silty clays. Row crop production was conducted on the soil type best suited to each crop. Corn (Zea maize, L.), cotton (Gossypium hirsutum, L.), rice (Oryza sativa, L.), soybeans (Glycine max, L.), and sweet potatoes (Ipornoea batatas, L.) were planted in the spring and harvested in the fall of each growing season. Wheat (Triticum aestivum, L.) was planted in the fall, and harvested the following spring. Standard agricultural practices were used for planting, weed and insect control, fertilization and irrigation. All crops were grown under optimal conditions, as described according to state recommendations (MSUCares, 2012).

All above-ground plant parts were harvested at maturity, with the exception of sweet potatoes. Because sweet potatoes are harvested by digging, no above-ground

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plant parts remain. For consistency in harvesting, a metal grid 1 m x 1 m was placed on the ground. All plant materials within the grid were harvested using pruning shears, scissors or hack saws, depending on the size of the plant materials. Plant materials were then bagged, and transferred to the lab for processing.

#### ii. Sample preparation

The plant materials were weighed, dissected into plant parts (leaves, stems, reproductive organs), and dried in an oven at 15 C for several days. The plant material was then reweighed to determine dry weight. Percent moisture was calculated as the mass difference between wet (initial) and dry weight.

The dried plants were ground several times to a fine powder. The samples were chopped or cut into small pieces, and then ground using a BHG food processor and a GE coffee grinder. Some samples, such as the corn cobs, required initial grinding in a large mill prior to grinding in the food processor. The sweet potatoes were sliced into thin slices with a knife, and then ground with the food processor. The samples were stored in sealed glass containers until analysis. Following the determination of moisture content, three one-gram aliquots of the sample were pelletized using a Parr pellet press (Parr Instrument Company, Moline, IL) in preparation for calorimetric determination. Cotton lint was too light to form into a pellet, and so was not assayed calorimetrically. An approximation of the ash content was obtained by weighing the inorganic residue remaining after the total consumption the organic matter during the calorimetric of measurements. A more accurate determination would be attained with use of an ashing oven (Sluiter et al., 2008), which was not available. Ash represents the inorganic fraction that is not available for energy production, and can reduce the quality of the biofuel produced (Monti et al., 2008). The ash content was determined for all samples except wheat.

#### iii. Calorimetry

A 1341 Parr Bomb Calorimeter (Parr Instrument Company, Moline, IL) was used to perform the energy content measurement for each component of the crop samples (Núñez-Regueira et al., 2001). In adiabatic bomb calorimetry, samples are combusted in an oxygen rich atmosphere under constant volume conditions. The samples are isolated inside a bomb at approximate 30 atm of oxygen and an electrical impulse is passed through a very small fuse, which generates heat and initiates the combustion process. The exothermic combustion releases heat into a two liter water jacket that surrounds the bomb. The temperature of the water is monitored for change.

The operation of the adiabatic bomb calorimeter entailed the following steps. First, 2000 ml of distilled water is added to the oval calorimeter bucket and a 10 cm of Ni-Cr fuse wire was measured and attached to the electrodes. The pellet was placed in the ignition cup ensuring that the electrode made a good connection with the pellet but did not touch the ignition cup to prevent grounding. The bomb head was then placed into the steel bucket and sealed, and the bomb was purged of nitrogen to prevent the formation of nitrous oxides by adding about 15 atm of oxygen, releasing it, and finally pressurizing the bomb to 30 atm. The steel bucket containing the 2000 ml of water was placed into the calorimetry jacket with cover and the lid of the calorimeter was sealed, stirring motor engaged, and the timing device started. The temperature of the water in the reservoir was measured at one minute intervals and recorded; thermal equilibrium was allowed to be established in the first six minutes of the timing. This was instituted to form a plateau by which the change in temperature could be determined. At the 6 minute mark the bomb was fired. The remaining inorganic material was weighed to obtain an estimate of ash content. The entire apparatus was cleaned, and reset prior to the next measurement. A minimum of three calorimetric measurements were made for each plant sample.

#### iv. Energetic Calculations

In adiabatic bomb calorimetry, the molar volumes of the reactants and products do not change drastically due to the constant volume conditions (Equation 1); therefore, the  $p\Delta V$  term of Equation 1 can be neglected, revealing Equation 2.

$\Delta \mathbf{H} = \Delta \mathbf{U} + p \Delta \mathbf{V}$	Equation 1

#### $\Delta H \sim \Delta U$ Equation 2

The change in enthalpy of the reaction ( $\Delta$ H) can be assumed to be approximately the change in internal energy ( $\Delta$ U) (Atkins and de Paula, 2006). Since the system was under constant volume conditions, no expansion work could occur, and the assumption that the total change in internal energy was heat can be made (Equation 3 and 4).

$\Delta \mathbf{U} = q + w$	Equation 3
$\Delta \mathrm{U} = q$	Equation 4

To establish the heat capacity (c) of the bomb, a standardized sample (benzoic acid) with a known enthalpy of combustion is run. With heat as a function of temperature, the energy released during combustion is simply the heat capacity of the calorimeter multiplied by the change in temperature in degrees Celsius (Equation 5). Therefore, the energy released by the biomass can be

determined by subtracting the heat released by the wire (Equation 6).

$q = c \Delta T$	Equation 5
$\Delta U = c\Delta T - c_w l$	Equation 6

To calculate the amount of energy released per gram of biomass combusted, Equation 7 was utilized. The heat capacity of the bomb calorimeter is "c" (in calories/°C), while  $\Delta T$  (in °C) is the change in temperature during the combustion. The heat capacity of the wire in calories/cm is "c<sub>w</sub>", *l* is the length of the wire in centimeters, and "m" is the mass of the sample in grams. The units of  $\Delta U$  are in calories/g.

$$\frac{c\Delta T - c_w l}{\Delta U = m}$$
 Equation 7

Biomass is primarily composed of cellulose, a polymer of D-glucose units  $(C_6H_{10}O_5)_n$ . The combustion of cellulose would involve the reaction of these units with oxygen,

 $C_6H_{10}O_5 + 6 O_2(g) - 6 CO_2(g) + 5 H_2O.$ 

#### v. Total potential energy content

Total potential energy content of crop biomass was calculated based on published records of average harvested hectarage for each county for the past three years (2009 -2011) (NASS, 2013). This will reduce the variability in crop area harvested in response to anticipated economic return. Reported crop yield (NASS, 2013) was averaged from the prior 10 years to reduce variability in crop productivity due to changes in yearly growing environment. The change in crop productivity due to cultivar improvement would be less in a ten-year time period, but enough to capture the year-to-year variability in yield from growth environment. Total crop biomass produced in the state was calculated using the reported acres planted for three years, the reported per-acre harvest, and the measured ratio of crop components to harvested component.

#### **RESULTS AND DISCUSSION**

#### i. Crop yield and biomass

Annual planting patterns in US agricultural production systems vary in response to anticipated return on investment (Halloran et al., 2011). In Mississippi, average hectarage planted to crop production for the past three years has been dominated by traditional row crop production, primarily of corn (21%), cotton (11.5%), and

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soybeans (54%) (Figure 1; NASS, 2013). Rice (6.2%) and winter wheat (5.3%) were planted on less area. Other crops, such as small grains (sorghum, oats, rye, and millet), peanuts, sweet potatoes, and vegetables, sunflowers, fruits and nuts, were planted on a very small portion (1.7%) of the total crop production area.

Harvested yield depends on a variety of edaphic, biotic, and abiotic factors, many of which are outside of the control of the producer and contribute to annual variation in yield. All row crops produced in Mississippi showed variability in harvested yield (Figure 2; NASS, 2013) over the past ten years. Corn showed the greatest range in harvested yield over the past ten years, varying more than 3000 kg/ha statewide as seen by the upper and lower percentiles (Figure 2). Soybeans, rice and winter wheat had the most consistent production over the 10 year period. Production of sweet potatoes and cotton were both fairly consistent across years, with sweet potatoes experiencing some years of low yield. Sassenrath et al. (2013) concluded the observed steady increase in crop production from 1998 - 2008 for rice, corn and soybeans resulted from improved cultivars and management practices. Corn was particularly sensitive to growth environment, however, which most likely accounts for the large range of harvested yield observed here.

Of the crops analyzed in this study, sweet potatoes produce the greatest biomass of the harvested component per acre harvested (Figure 2). Corn and rice also produce substantial harvested biomass. Cotton, soybeans and winter wheat have the lowest harvested biomass of the principle yield component. Note that harvested yield for seed cotton is reported; yield for cotton lint would be approximately one-third of seed cotton weight.

The potential biomass produced from crop production in Mississippi was calculated from harvested yield (NASS, 2013), average annual area planted to individual crops (NASS, 2013), and measured crop biomass of total plant parts harvested (Table 1). Seed residue includes shucks and cob for corn, bracts for cotton, and pods for soybeans. Rice seed includes grain and husks. Sweet potato culls, cuts and bites were used for "stalks". Cotton is defoliated prior to harvest, so very few leaves remained at harvest. Any remaining leaves were included with bracts for analysis.

With the higher per-area biomass production and large area planted to corn, corn had the greatest annual biomass production potential, followed by soybeans, cotton and rice, based on current planting patterns. While sweet potatoes account for only a very small portion of total hectarage planted (Figure 1), their significantly greater harvested yield led to a greater contribution to potential biomass production (Table 1).



Figure 1. Crop Production in Mississippi. Average land area planted to each crop from 2009 - 2011, percent of total planted area (NASS, 2012). Miscellaneous crops include vegetables, fruits and nuts, and sunflowers. Small grains include sorghum, millet, oats and rye.



Figure 2. Average harvested crop yield for principle crops in Mississippi, 2001 – 2010, kg/ha (NASS, 2012). Cotton yield reported is harvested weight of seed cotton. Note break and change in scale in the vertical axis. The median is the solid line within the box, while the upper and lower boundaries of the box indicate the 25th and 75th percentiles, respectively, and error bars above and below the box give the 90th and 10th percentiles, respectively.

Potential Annual Biomass, m ton/yr				
Crop	Crop Primary Harvested Component Crop Residue			
Corn	1,763,472	1,720,273	3,483,745	
Cotton	1,237,486	1,794,825	3,032,312	
Rice	701,923	503,095	1,205,018	
Soybeans	1,535,440	1,894,179	3,429,619	
Sweet potatoes	125,887	62,944	188,831	
Winter Wheat	308,744	425,515	734,259	
Total, all crops	5,672,952	6,400,830	12,073,783	

Table 1. Potential annual biomass production from crops in metric tons, for current cropping patterns and yields in Mississippi.

#### ii. Energy content

Total energy of plant components was determined calorimetrically from total harvested plant material (Table 2). Corn, cotton, rice and soybeans were dissected into principle plant components, and energy content determined on each component separately. Sweet potatoes are harvested by digging, so no above-ground plant parts remained. Calorimetric analysis was performed on the entire ground wheat samples and not the individual components. Cotton and soybean seeds had the highest energy content per gram of plant material, most likely due to the oil content in the seed. All of the other plant components were very similar in energy content for all crops, ranging from a low of 14.7 kJ/g for corn leaves to a high of 18.0 kJ/g for soybean leaves.

The amount of plant materials produced by each plant will determine the net energy per plant. By far the highest energy per plant came from sweet potatoes (Table 2), followed by corn and cotton. Rice, soybeans, and winter wheat produced much less energy per plant. For corn, rice and soybeans, most of the per-plant energy came from the primary cash component (kernels, seeds or grain). For cotton, the stalk and seed contributed similar per plant energy levels.

Plant Component	Energy Content (kJ/g)	Average Mass (g/plant)	Average Energy per plant (kJ/plant)
	С	orn	
Kernels	$15.8\pm0.60$	164.30	2595.9
Leaves	$14.7\pm0.10$	15.77	231.8
Stalks	$15.6\pm0.50$	103.22	1610.2
Cobs	$16.2\pm0.34$	29.31	474.8
Shucks	$15.6\pm0.46$	11.95	186.4
Total		324.55	5099.23

Table 2. Energy content, biomass, and energy per plant for crops and crop components.

Cotton			
	1		
Seeds	20.7 ± 1.90	28.08	581.3
Lint	NA	12.00	NA
Bracts & Leaves	$15.8 \pm 1.00$	21.06	332.7
Stalks	$17.4 \pm 1.90$	37.09	645.4
Total		98.23	1559.37
	ŀ	Rice	
Grain	$15.4\pm0.20$	3.92	60.4
Leaves	$15.1\pm0.50$	0.91	13.7
Stalks	$14.1\pm0.06$	1.90	26.8
Total		6.73	100.90
	Soy	beans	
Seeds	$21.3\pm0.20$	7.40	157.6
Pods	$15.2\pm0.03$	3.76	57.2
Leaves	$18.0\pm1.00$	0.92	16.5
Stalks	$16.7\pm0.30$	4.45	74.3
Total		16.53	305.63
	Sweet	Potatoes	
Potato	$16.6\pm0.29$	963.79	15998.9
Winter Wheat			
Grain		1.47	
Residue		2.03	
Total Plant	16.2	3.50	56.7

The plant materials were harvested at full crop maturity, so most plant components contained very low levels of moisture (Table 3). The exception was the sweet potatoes, which contained substantial moisture. The ash content represents that fraction of plant material that will remain after total digestion, is thus unusable for energy production, and can reduce the quality of the biofuel (Monti et al., 2008). Sweet potatoes had the highest ash content. All rice components had high ash contents as well.

Energy content for plant components was determined on an area basis (Table 4). Seed residue includes shucks and cob for corn, bracts for cotton, and pods for soybeans. Rice seed includes grain and husks. Sweet potato culls, cuts and bites were used for "stalks". These are damaged sweet potatoes usually sold at a discount. Cotton is defoliated prior to harvest, so very few leaves remained. Any remaining leaves were included with bracts for analysis. The cotton yield component measured seed energy content only. The lint was too fine to make a pellet for analysis. Winter wheat was not dissected, so the energy content includes all plant parts.

Sweet potatoes had the highest energy content per hectare (Figure 3). Corn and rice also produced substantial energy per hectare. Corn, cotton and sweet potatoes had the crop residues with the highest energy content per hectare. Seed residue from corn (cobs and shucks) and soybeans (pods) remain in the field after the harvest operation. Seed residue from cotton and rice is removed from the field during the harvest operation. Seed cotton is then ginned to separate the seed and lint. Gin trash has been used for fertilizer and soil amendment, cattle feed, and biofuel (Stancil, 2006; Wilde et al., 2010). Cotton seed is

processed after ginning for oil and feed production. Rice is milled after harvest to separate the grains from the hulls. Inclusion of the hulls in the crop residue would increase the total energy content of the residue.

Table	3.	Moisture	content	and	percent	ash	of	plant
compo	ne	nts						

Plant Component	Moisture Content (%)	Ash Content (%)		
	Corn			
Kernels	$12.69\pm0.57$			
Leaves	$4.05 \pm 0.15$			
Cobs	$9.24\pm0.25$			
Shucks	4.51 ± 1.20			
	Cotton			
Seeds	$9.90 \pm 0.10$	0.03		
Bracts & Leaves	$10.4 \pm 0.20$	3.33		
Stalks	$7.1 \pm 0.30$			
	Rice			
Grain	9.4	14.4		
Leaves	7.5	9.8		
Stalks	6.8	6.8		
	Soybeans			
Seeds	$5.40 \pm 0.20$	$4.08\pm0.18$		
Pods	$12.5 \pm 0.70$	$3.80 \pm 0.75$		
Leaves	$9.40\pm0.50$	6.72		
Stalks	$8.60\pm0.70$	$1.43\pm0.58$		
Sweet Potatoes				
Potato	$77.0\pm0.85$	$20.22 \pm .01$		
	Winter Wheat			
Grain	6.0 <u>+</u> 0.1			
Residue	6.1 <u>+</u> 1.2			
Total Plant	$6.2 \pm 0.8$			

Table 4.Potential annual energy production fromcrops and crop residues, based on current plantingpatterns and yields for Mississippi, from energy contentmeasured calorimetrically.

Potential Annual Energy, GJ/yr				
Crop	Primary Harvested Component	Crop Residue	Total	
Corn	27,865,023	26,870,543	54,735,567	
Cotton <sup>1</sup>	13,341,605	22,450,712	35,792,317	
Rice	10,808,992	7,257,144	18,066,136	
Soybeans	32,704,733	22,194,208	54,898,941	
Sweet potatoes	2,089,724	1,044,862	3,134,586	
Winter Wheat <sup>2</sup>	5,001,647	6,893,341	11,894,989	
Total, all crops	91,811,725	86,710,811	178,522,536	

<sup>1</sup> Cotton harvested component is seed cotton, including both seed and lint.

<sup>2</sup> Energy content of winter wheat grain is estimated from rice grain.

## iii. Distribution of crops and potential crop components

The total crop biomass and crop residue biomass produced in each county was determined from reported yield and planted area (NASS, 2013). For wheat, the total biomass production was estimated using ten years' average yield, three years planting area, and measured harvest index. Note that winter wheat yield ranges from approximately 3 - 4 m ton/ha in MS (Sassenrath et al., 2013), which is about half the average yield reported elsewhere (White and Wilson, 2006).

The primary crop production area of Mississippi is the Delta, in the northwest portion of the state (NASS, 2010). This area also had the greatest production of plant biomass (Figure 4). The Delta region was identified as a significant contributor of agricultural net primary production (NPP) based on the agronomic production in the region (Lobell et al., 2002). Although it has been suggested that it may be best to keep land that is optimal for food-crop production in production of food and feed (Lemus et al., 2011), these lands also produce substantial levels of residue that would be amenable for biofuel energy.



Figure 3. Potential energy produced per hectare of principle row crops of Mississippi based on measured energy content of crop components, and state average annual yield for previous 10 years. Winter wheat was not dissected prior to calorimetric analysis. Reported values for wheat are for the entire plant.



Figure 4. Spatial distribution of crop biomass production in MS, million metric tons per year. County-level maps with A) total biomass for all crops produced with current cropping patterns and yields, and B) crop residue biomass from all plant materials given current cropping patterns and yields.

#### CONCLUSIONS

Agricultural production in Mississippi generates substantial reserves of potential biofuel feed stocks that are currently underutilized. Even with current planting patterns, additional biomass in excess of six million metric tons is generated in crop residues alone. Modification of the planting patterns could increase those potential biofuel feed stocks without greatly impacting food production.

In developing sustainable cropping systems, we need to balance the economic and environmental costs of importing externalities into the agricultural system (fertilizer, water, and so on) with the potential return on investment. The harvesting of all plant components for biofuel synthesis may negatively impact the environment, particularly soil quality, by reducing organic matter that improves soil tilth (Larson, 2011). However, current production practices in Mississippi remove much of the crop residue. Harvesting crop residue for use in biofuel synthesis instead of the current practice of burning crop residue in the field will improve air quality (Korontzi et al., 2012), contribute a second income source, and contribute to the energy self-sufficiency of Mississippi.

The potential of crops and crop residues to contribute to the energy budget of the state depends on the conversion efficiency of the process and the particular biochemical makeup of the biomass. Improvements in energy sustainability are possible but must be tempered by planning the overall focus of agriculture to balance production of crops for food, feed, fuel and fiber. Developing cropping systems (rotations, crop sequences, and new crops and crop uses) can be established with a focus on sustainability. Most notably, development of crop residue processing should be coordinated with location to reduce transportation expenses of residues (Hill, 2007). Moreover, the majority of production expenses in current cropping systems occur during the harvest and post-harvest processing (Martin and Cooke, 2002). Additional expenses associated with harvesting crop biomass and processing crop material to separate components will add additional expense. The additional revenues from biofuel production will need to cover these additional expenses to make production feasible. Given the biomass production potential of the Northwest Delta region of the state, this would be an ideal location for development of biofuels. Moreover, the establishment of processing facilities could add to the rural communities (Fannin et al., 2008), contributing to an additional component of sustainable agriculture.

Alternatively, crop residues could be used on-farm for energy production. Particularly clever methods are being developed such as plasma-thermochemical gasification (Van Oost et al., 2009), or hybrid technologies incorporating both thermochemical and biochemical approaches to production of syngas (Advanced Biofuels USA, 2011). The potential to convert agricultural residues into energy on-farm is particularly attractive for their opportunity to directly reduce costs of production by lowering energy costs on-farm (Boateng et al., 2007). Initial setup of biomass processing facilities may be expensive, but use of agricultural residues on-farm would reduce the costs associated with establishing the infrastructure to transport residues to a central processing facility (Steiner and Banowetz, 2004). With the rising costs and demands for energy, development of methods to reduce on-farm production costs while reducing waste will improve the sustainable production of crops in Mississippi.

In this study, we limited the potential biofuel feed stocks to crops and crop residues. Additional inputs to biofuel synthesis are possible from abundant hay production in the hills of Mississippi, as well as forestry and poultry litter. These alternative fuel sources could provide additional energy for Mississippi, contributing to the energy sustainability.

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#### DISCLAIMER

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## EFFECTS OF VARIOUS MICROORGANISMS AND PRETREATMENTS ON MICROBIAL DEGRADATION OF LIGNOCELLULOSIC MATERIALS

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#### ABSTRACT

This study was conducted to analyze the effects various pretreatments on the ability of *Trichoderma reesei*, *Pycnoporus cinnabarinus*, *Fusarium solani*, an unknown bacterial isolate, and an unknown fungal isolate in the degradation of lignocellulosic biomass of Southern pine sawdust under aerobic and anaerobic conditions. Samples of Southern pine sawdust were either autoclaved or treated with 25% H<sub>2</sub>SO<sub>4</sub>. Liquid medium was prepared and added to each of 48 Erlenmeyer flasks. Various microorganisms were inoculated into each flask and left to incubate for 0, 6, 9, 13, 16, and 22 days under aerobic and anaerobic conditions. At specific time intervals samples were analyzed to determine the concentration of glucose present using the Dinitrosalicylic Acid (DNS) method. Our results revealed that *Fusarium solani* produced more reducing sugar followed by *Trichoderma reesei*, across all days as compared to other isolates. Additionally, we found that more reducing sugar was produced under aerobic conditions as compared to anaerobic conditions. We concluded that pretreatment methods may play significant roles in the degradation of lignocellulosic biomass of Southern pine sawdust and that the rate of degradation may depend on aerobic conditions.

#### INTRODUCTION

Increased concern for the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions into the atmosphere, and rising prices of crude oil due to increasing fuel demands, has put pressure on society to find renewable alternative sources of bioenergy [1, 2]. Currently, research is being done to enhance the digestibility of lignocellulosic biomass mainly for the efficient conversion of indigestible materials such as lignin, hemicelluloses, and cellulose to reducing sugar, ethanol, methane, and hydrogen. Fortunately, biologically mediated processes seem promising for energy conversion, in particular for the conversion of lignocellulosic biomass into fuels [3].

Biological, enzymatic, or microbial hydrolysis commonly involves four biologically-mediated transformations: the production of saccharolytic enzymes (cellulases and hemicellulases); the hydrolysis of carbohydrate components present in pretreated biomass to sugars; the fermentation of hexose sugars (glucose mannose, and galactose); and the fermentation of pentose sugars, xylose and arabinose [3, 4]. However, it is unclear which characteristics of the lignocellulosic biomass are important to determine a successful pretreatment method [3].

Lignocellulosic biomass refers to plant biomass that is composed of cellulose and hemicellulose tightly bound to the lignin by hydrogen and covalent bonds, and is the most abundant organic material on earth, and is therefore a promising raw material for bioenergy production [5,6]. Cellulose is a polymer of glucose while hemicellulose ispredominantly composed of xylans which, after hydrolysis, yield the pentose sugar and xylose [7].

The four categories of biomass resources in the world include: (1) wood residues which are by far the largest current source of biomass for energy production, (2) municipal solid waste, the next largest, (3) agriculture residues and, (4) dedicated energy crops [8].

Generally, the use of celluloses and hemicelluloses (cellulosic biomass) is recommended instead of traditional feed stocks. Ideally, cellulosic biomass could be used as an inexpensive and abundantly available source of sugar for fermentation into the sustainable transportation fuel, ethanol [9, 10].

To initiate the production of industrially important products from cellulosic biomass, bioconversion of the cellulosic components into fermentable sugars is necessary [2]. The two main obstacles in the degradation of lignocellulosic materials are the resistance of lignin, and the crystal-like structure of cellulose fibrils [7]. It would therefore be of great benefit if microorganisms were

developed that could utilize cellulose and other fermentable compounds available from pretreated biomass and produce desired product at high yield and titer [4].

In filamentous fungi, production of the cellulose- and hemicelluloses-degrading enzyme, cellulases and hemicellulases, are controlled at the transcriptional level by the available carbon source [11].

The filamentous fungus Trichoderma reesei is well known as an efficient producer of cellulases [12, 13]. This fungus is the main industrial source of cellulases and hemicellulases used to depolymerize biomass to simple sugars that are converted to chemical intermediates and biofuels, such as ethanol [14]. It has been proposed by other researchers [9, 15] that T. reesei produces a family of different cellulolytic enzymes, including endoglucanases, exocellobiohydrolases, and  $\beta$ -glucosidases; a view that is different than the one proposed by Kovacs and his colleagues [16] who proposed that  $\beta$ -glucosidases is practically not secreted by Trichoderma reesei. Still, others claim that the cellulolytic enzyme system of T. reesei can efficiently degrade crystalline cellulose to glucose [17]. Studying solid-state fermentation with Trichoderma reesei for cellulase production, Chahal [12] concluded that the cellulase potential of various mutants of T. reesei ranges between 160 and 250 IU/g of pure cellulose in liquid-state fermentation.

Higher fungi (white rot fungi) which cause white rot in wood are believed to be the most effective lignin-degrading microbes in nature [18, 19, ] and are the most promising microorganisms used for biological pretreatment because of their abilities to selectively degrade. **Pycnoporus** cinnabarinus is the basidiomycete commonly known for its ability to efficiently degrade lignin by an unusual production of ligninolytic enzymes, ligninolytic phenol oxidases [20]. Pycnoporus cinnabarinus has a simple ligninolytic system. Neither lignin peroxidase nor manganese peroxidase activity has been detected, but laccase is produced [21. Pycnoporus cinnabarinus laccase appears to occur in only one single acidic form, a usual feature among fungal laccases [22]. Overall. P. cinnabarinus has proven to be an interesting model organism for studying new mechanisms of lignocelluloses degradation by white rot fungi [20, 22].

*Fusarium solani*, a pathogenic plant fungus causes root rots, which results in considerably economical losses in many important crops [23], has a beneficial importance in degrading cellulosic materials in biomass. Among organisms isolated from the soil, *F. solani* was the most vigorous microbial degrader of a synthetic lignin, such as a dehydrogenation polymer of coniferyl alcohol. The findings of other researchers with different microbes strongly indicate that aeration of *F. solani* cultures with

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oxygen could dramatically increase the rate and quality of lignin degradation under most, if not all, otherwise suitable incubation conditions.

This study was therefore carried out to examine the effects of five microorganisms: *Trichoderma reese*i, *Pycnoporus cinnabarinus*, *Fusarium solani*, an unknown bacterial isolate, and an unknown fungal isolate on the degradation of cellulosic materials that had been subjected to pretreatments of heat and sulfuric acid, and incubated under aerobic and anaerobic conditions.

#### MATERIALS AND METHODS

#### Preparation of Lignocellulosic and Cellulose Materials

Samples of Southern pine sawdust (obtained from a sawmill in Taylorsville, Mississippi) were prepared according to treatments 1 thru 8, (Table 1). For Treatments 1 and 2, thirty mL of distilled water were added to 12 Erlenmeyer flasks that contained 10 g of the sawdust. The flasks were then covered and autoclaved (121°C, 15 psi) for three consecutive days for one hour each day. For treatments 3 and 4, ten g of sawdust were placed into 5 flasks each and 30 mL of 25% H<sub>2</sub>SO<sub>4 were</sub> added and mixed with the sawdust. The flasks were covered and incubated for three days at room temperature after which the sample was filtered four times using 20 mL of distilled water and saving the filtrate each time. Thirty mL of the filtrate were poured into each of 12 previously prepared flasks and covered. For treatments 5 and 6, ten g of original sawdust that had not been autoclaved or treated with 25% H<sub>2</sub>SO<sub>4</sub> was placed into 12 Erlenmeyer flasks. and covered. For treatments 7 and 8, five g of pure cellulose were added to 12 Erlenmeyer flasks and covered.

#### Liquid Media Preparation

Liquid medium was prepared according to Miller [24]. Briefly, in each 1000 mL of distilled water, the following reagents were added; 2 g potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>), 1.4 g ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.3 g calcium chloride (CaCl<sub>2</sub>.2H<sub>2</sub>O), 0.3 g magnesium sulfate (MgSO<sub>4</sub>.7H<sub>2</sub>O), 0.6 g urea, 10 mg ferrous sulfate (FeSO<sub>4</sub>.7H<sub>2</sub>O), 2.8 g zinc sulfate , ZnSO<sub>4</sub>.7H<sub>2</sub>O), 3.2 g cobalt chloride (CoCl<sub>2</sub>.6H<sub>2</sub>O), 1.6 g manganese sulfate (MnSO<sub>4</sub>.H<sub>2</sub>O), 0.1 % peptone, and 0.1% tween 80 (Polyoxyethylene (20) Sorbitan mono-oleate)/L, The resulting solution was then boiled with frequent agitations using a magnetic stirrer for ten minutes. One hundred fifty mL of the medium was added to each of the 48 previously prepared and described flasks containing a carbon source. The flasks were autoclaved at 121°C at 15 psi for 15 minutes.

#### Microorganisms

Fungal cultures including *Trichoderma reesei* (ATCC # 26921), *Pycnoporus cinnabarinus* (ATCC # 48748), and *Fusarium solani* (ATCC # 52176) were purchased from the American Type Culture Collection (ATCC) Manassas, VA. Unknown bacterial and fungal isolates from rotting wood samples were also used.

#### Isolation of Bacteria and Fungi from Rotting Wood

Ten grams of rotting-wood samples were dispensed into a 100 mL dilution bottle containing 90 mL of sterile distilled water. The bottle was vortexed for five minutes to ensure thorough mixing of the samples and the diluents. From the initial suspension (10<sup>-1</sup>), serial dilutions were made using sterile distilled water as the diluent [25]. Aliquots (0.1 mL) of the chosen dilutions (i.e., 10<sup>-3</sup> to 10<sup>-7</sup>) were dispensed onto nutrient agar for bacteria, and potato dextrose agar for fungi. Solidified petri dishes and spread plates were prepared and incubated at 24° C for five days. Colonies that developed on the plates were subcultured for subsequent use in this experiment.

#### **Media and Culture Conditions**

Potato dextrose agar (PDA) was used to grow *Trichoderma reesei* culture at 24 °C. A known culture of *Pycnoporus cinnabarinus* was grown in ATCC medium 200 Yeast Mold Agar (YM agar, BD 271210) on petri dishes and slants, and then incubated at a room temperature (24 °C). A culture of *Fusarium solani* was grown on potato dextrose agar (PDA) plates and slants at 24 °C. All cultures were grown for 72 hours.

A bacterial isolate from a rotting wood sample was inoculated into nutrient agar plates and incubated for three days. Fungal isolate with the highest growth was selected and inoculated into potato dextrose agar plates and incubated for two days. After the designated growth period, both cultures of bacterium and fungi were harvested and maintained at 4°C for use in this experiment.

#### **Microbial Inoculation of Liquid Media**

Ten mL of each culture were inoculated into each of the twenty 250 mL Erlenmeyer flasks containing 150 mL of liquid medium plus a carbon source as specified in Table 1. Four flasks containing a carbon source were not inoculated with microorganisms and represented the control. Foam plugs were inserted into each of the flasks and the flasks were left undisturbed on the laboratory table at room temperature. This was considered as an anaerobic condition. For aerobic treatments, another set of 24 flasks were prepared as described above, covered with parafilm, and placed into a shaker and shaken at a speed of 60 rpm. Thus, a total of forty-eight flasks were used in this experiment.

Table 1. Organisms used in this study were: *Trichoderma reesei*, *Pycnoporus cinabarinus*, *Fusarium solani*, an Unknown Bacterial Isolate and an Unknown Fungal Isolate. Pretreatments are: Heat (Autoclaved), Acid (Sulfuric Acid), No Pretreatment (Untreated Sawdust) and Pure Cellulose.

	Pretreatments	Aerobic	Anaerobic
1.	Autoclaved (Sawdust + Water + Liquid Medium (Control)	1	1
2.	Autoclaved (Sawdust +		
	Water) + Liquid Medium + 1 of 5 Organism per flask	5	5
3.	Sawdust + H <sub>2</sub> SO <sub>4</sub> + Liquid Medium (Control)	1	1
4.	Sawdust + H <sub>2</sub> SO <sub>4</sub> + Liquid Medium + 1 of 5 Organism	_	_
5	per flask Sawdust original + Liquid	5	5
5.	Medium (Control)	1	1
6.	Sawdust original + Liquid Medium + 1 of 5 Organism per flask	5	5
7.	Pure cellulose + Liquid Medium (Control)	1	1
8.	Pure cellulose + Liquid Medium + 1 of 5 Organism per flask	5	5

#### **Determination of Reducing Sugar (Glucose)**

At specified time intervals (day 0, 6, 9, 13, 16, 22, and 28), samples were analyzed to determine the concentration of glucose present in each using the dinitrosalicylic acid (DNS) method of Miller [24]. Five mL of each sample were pipetted into centrifuge tubes. The samples were then centrifuged for two minutes, and one mL of the supernatant from each sample was pipetted into 25 mL test tubes and replicated 3 times. One mL of DNS was added to each test tube and the test tubes were allowed to boil for ten minutes. The color of the solution changed to red. The tubes were then transferred into a cold water bath. After cooling, the solution was diluted to 25 mL with distilled water. The absorbance readings of the samples were determined with a Cole Palmer SQ-2800 UV/Visible Spectrophotometer at 520 nm.

#### **Glucose Standard Preparation**

A stock solution of 1.0 mg/mL of glucose was first prepared in a test tube. Using this stock solution, the following standards were then prepared: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/mL. One mL of dinitrosalicylic (DNS) acid reagent was added to each tube containing the glucose standard solutions. The test tubes were then boiled for ten minutes (the color of the solution changed to red) and were transferred into a water bath with a temperature of 4 °C. After cooling, the solution in each tube was diluted to 25 mL with distilled water and the absorbance reading was taken at a wavelength of 520 nm using a SQ-2800 UV/Visible Spectrophotometer (Cole Palmer).

#### **Statistical Analysis**

Statistical analyses were performed using SAS Computer Software Program. Comparisons of data between incubation period, and among pretreatment methods were made by the analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test. Pretreatments were defined as: heat (autoclaved), acid (sulfuric acid), no pretreatment (untreated sawdust) and pure cellulose. Statistical differences were assessed at p < 0.05 (95% confidence).

#### **RESULTS AND DISCUSSION**

Our study further revealed that samples that were treated with Trichoderma reesei produced higher amounts of suga under the conditions of heat and acid, especially Overall, results of this study suggest that pretreatment of Southern pine sawdust with heat and sulfuric acid increased the degradability of lignin, hemicelluloses and a cellulose to produce reducing sugars under both aerobic (p = 0.0047) and anaerobic (p = 0.0101) conditions, respectively (Table 2).

Our study further revealed that samples that were treated with *Trichoderma reesei* produced higher amounts of sugar under the conditions of heat and acid, especially on day 13 under aerobic (Figure 1) and anaerobic (Figure 2) conditions. *Trichoderma reesei* is well known as an efficient producer of cellulases [12, 13] and is the main industrial source of cellulases and hemicellulases used to depolymerize biomass to simple sugars that are converted to chemical intermediates and biofuels such as ethanol [14]. Sugar production in sawdust with no pre-treatment and treatments of pure cellulose were the lowest (Figures 1 and 2). Both conditions, aerobic (Figure 1) and anaerobic (Figure 2) showed variation in reducing sugar production

with peaks on days 13 and 16 followed by a gradual decline on day 22.

Samples that were pretreated with acid and *P. cinnabarinus* under aerobic conditions (Figure 3) produced almost the same amount of reducing sugar (0.837 mg/L) as samples under anaerobic conditions (Figure 4) (0.810 mg/L) on days 9 and 22, respectively.

Table 2. Microbial effects of pretreated pine sawdust on reducing sugar production (mg/L). Data are means  $\pm$  of standard deviations of three replications.

Pretreatment	Aerobic	Anaerobic
Trichoderma reesei		
Heat	0.52 <u>+</u> 0.17	0.54 <u>+</u> 0.24
Acid	0.58 <u>+</u> 0.20	0.59 <u>+</u> 0.23
No pretreatment	0.49 <u>+</u> 0.19	0.41 <u>+</u> 0.22
Pure cellulose	0.39 <u>+</u> 0.13	0.40 <u>+</u> 0.17
Pycnoporus cinnabarinus		
Heat	0.52 + 0.11	0.59 + 0.06
Acid	0.63 + 0.13	0.67 + 0.10
No pretreatment	0.43 + 0.08	0.41 + 0.11
Pure cellulose	0.50 + 0.06	0.55 + 0.07
		_
Fusarium solani		
Heat	0.51 <u>+</u> 0.17	0.61 <u>+</u> 0.21
Acid	0.49 <u>+</u> 0.11	0.75 <u>+</u> 0.29
No pretreatment	0.15 <u>+</u> 0.04	0.15 <u>+</u> 0.05
Pure cellulose	0.37 <u>+</u> 0.14	0.33 <u>+</u> 0.05
Bacterial Isolate		
Heat	0.27 <u>+</u> 0.13	0.29 <u>+</u> 0.17
Acid	0.27 <u>+</u> 0.15	$0.30 \pm 0.20$
No pretreatment	$0.11 \pm 0.02$	$0.13 \pm 0.03$
Pure cellulose	$0.20 \pm 0.11$	$0.28 \pm 0.09$
		_
Fungal isolate		
Heat	0.35 <u>+</u> 0.09	0.35 <u>+</u> 0.09
Acid	$0.37 \pm 0.11$	0.39 <u>+</u> 0.13
No pretreatment	0.13 <u>+</u> 0.02	0.15 <u>+</u> 0.04
Pure cellulose	$0.44 \pm 0.20$	$0.32 \pm 0.08$



Figure 1. Effects of incubation periods on sugar production by *Trichoderma reesei* under aerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD (p < 0.05).



Figure 2. Effects of incubation periods on sugar production by *Trichoderma reesei* under anaerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).



Figure 3. Effects of incubation periods on sugar production by *Pycnoporus cinnabarinus* under aerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

*Fusarium solani* showed a different pattern of variation in reducing sugar production under aerobic (Figure 5) and anaerobic (Figure 6) conditions. Maximum amount of reducing sugar was produced on day 22 under anaerobic condition (1.159 mg/L) with acid pretreated samples followed by heat pretreated sample under aerobic (0.732 mg/L) condition. The mean amount of reducing sugar produced by acid pretreated samples (0.49 mg/L) was lower compared to those of heat-pretreated samples (0.51



Figure 4. Effects of incubation periods on sugar production by *Pycnoporus cinnabarinus* under anaerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

mg/L) under aerobic conditions (Table 2). Under anaerobic conditions acid pretreated samples produced more sugar (0.75 mg/L) as compared to heat-pretreated samples (0.61 mg/L). In all cases, untreated samples produced the least amount of sugars under both aerobic and anaerobic conditions (0.15 mg/L and 0.15 mg/L, respectively) (Table 2). Generally, *F. solani* is one of the most vigorous microbial degraders of synthetic lignin, and it has been indicated that aeration of *F. solani* cultures with oxygen

could dramatically increase the rate of lignin degradation under most incubation conditions [23]. The difference between our findings and the findings of other investigators are unexplained at this time.

In nature, cellulosic materials are degraded with the cooperation of many microorganisms. It has been reported that the utilization of microbial community composed of one cellulolytic bacterium and another non-cellulolytic bacterium or comprising of aerobic and anaerobic bacteria could degrade cellulosic materials effectively [27]. In this



Figure 5. Effects of incubation periods on sugar production by *Fusarium solani* under aerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).



Figure 7. Effects of incubation periods on sugar production by an unknown bacterial isolate under aerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

study, a bacterial isolate was incubated under aerobic (Figure 7) and anaerobic conditions (Figure 8). The highest sugar production was observed on day 6 under aerobic conditions and day 13 under anaerobic conditions, respectively. However, less sugar was produced by the unknown bacterial isolate as compared to *T. reesei*, *F. solani, and P cinnabarinus* (Table 2). Overall, samples that were pretreated with acid or heat showed significant differences in the amount of sugar production when compared to untreated samples (p = 0.0069).



Figure 6. Effects of incubation periods on sugar production by *Fusarium solani* under anaerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).



Figure 8. Effects of incubation periods on sugar production by an unknown bacterial isolate under anaerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

Fungal isolates produced the highest amount of reducing sugar on day 16 where we observed 0.48 mg/L and 0.40 mg/L under aerobic (Figure 9) and anaerobic conditions (Figure 10), respectively. There were high amounts of sugar produced after 16 and 22 days of incubation (Table 3). Generally, under both aerobic and anaerobic conditions samples that were not pretreated



Figure 9. Effects of incubation periods on sugar production by an unknown fungal isolate under aerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

either by heat or sulfuric acid produced less sugar compared to pretreated samples. Our results support the position that pre-treatment: (1) improve the formation of sugars or the ability to subsequently form sugars; (2) avoid the degradation or loss of carbohydrate; and (3) avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes [28].



Figure 10. Effects of incubation periods on sugar production by an unknown fungal isolate under anaerobic conditions. For each incubation period, means with similar letters are not significantly different according to Tukey's HSD test (p < 0.05).

		1						
Organism	Condition	Day 0	Day 6	Day 9	Day 16	Day 22	Day 22	Day 28
Trichoderma	Aerobic	$0.36\pm0.05$	$0.42\pm0.07$	$0.39\pm0.07$	$0.77\pm0.14$	$0.65 \pm 0.11$	$0.38\pm0.08$	
reesei								
	Anaerobic	$0.34 \pm 0.03$	$0.43 \pm 0.07$	$0.30 \pm 0.06$	$0.87 \pm 0.12$	$0.55 \pm 0.19$	$0.40 \pm 0.16$	
Pycnoporus cinnabarinus	Aerobic	$0.54 \pm 0.49$	0.44 ± 0.57	$0.55 \pm 0.60$	$0.45 \pm 0.47$	$0.42 \pm 0.46$	0.59 ± 0.59	0.53 ± .046
	Anaerobic	$0.61 \pm 0.58$	$0.44 \pm 0.53$	$0.55 \pm 0.62$	$0.54 \pm 0.53$	$0.53 \pm 0.51$	$0.65 \pm 0.67$	$0.54 \pm 0.45$
Fusarium solani	Aerobic	$0.22\pm0.08$	$0.41\pm0.26$	$0.36\pm0.17$	$0.38\pm0.19$	$0.40 \pm 0.21$	$0.50\pm0.18$	$0.40 \pm 0.20$
	Anaerobic	$0.21\pm0.08$	$0.49 \pm 0.34$	$0.40 \pm 0.21$	$0.51 \pm 0.31$	$0.44 \pm 0.21$	$0.66 \pm .046$	$0.51\pm0.35$
Bacterial isolate	Aerobic	$0.21\pm0.07$	$0.35 \pm 0.16$	$0.20 \pm 0.06$	$0.25 \pm 0.09$	$0.08 \pm 0.04$		
	Anaerobic	$0.26 \pm 0.12$	$0.24 \pm 0.07$	$0.19 \pm 0.06$	0.43 ± .021	$0.13 \pm 0.06$		
Fungal isolate	Aerobic	$0.23 \pm 0.07$	$0.27 \pm 0.10$	$0.27 \pm 0.17$	$0.39 \pm 0.17$	$0.48 \pm 0.26$	$0.31 \pm .020$	
	Anaerobic	$0.26 \pm 0.03$	0.29 ± 0.11	$0.26 \pm 01.0$	$0.32 \pm 0.13$	$0.40 \pm 0.16$	0.29 ± 0.18	

Table 3. Amount of reducing sugar (mg/L) produced at various incubation periods. Data are means  $\pm$  standard deviation of three replications.

#### CONCLUSIONS

There is a significant amount of low-value or waste lignocellulosic materials that are currently burned or wasted. These materials are particularly well suited for reducing sugar, ethanol and for energy applications because of their large scale availability, low cost and environmentally benign production [4].

Combined pretreatment methods can be recommended to enhance the effectiveness of conversions of lignocellulosic materials from Southern pine sawdust. The study demonstrated that the combined pretreatments of acid plus microorganisms and heat plus microorganisms, were effective for sugar production. However, in order to realize the full potential of these methods, microorganisms must be developed that utilize cellulose and other fermentable compounds available from pretreated biomass with high rate and high conversion, and which produce a desired product at yield and titer [4].

The biodegradability of lignocellulosic biomass is limited by several factors like crystallinity of cellulose, available surface area, and lignin content. Pretreatment methods have an effect on one or more of the operating conditions. Acid-pretreated samples and heat-pretreated samples produced differently with different microorganisms and under aerobic and anaerobic conditions.

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#### LICE INFESTATION OF GOATS FROM SOUTHWESTERN MISSISSIPPI, USA

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#### ABSTRACT

There is increasing interest in goat production in the State of Mississippi and in the use of goat milk by the people. So goats are growing in economic importance. The present study was conducted to determine the distribution of goat lice in southwestern Mississippi and the extent to which they are a problem in the region. During the period of March 1997 to January 1998 lice were collected from the bodies of goats from 14 southwestern counties of Mississippi by brushing, searching and picking them with forceps. They were put in vials containing 70% alcohol, mounted in CMC medium and identified. Of 1445 goats examined 997 (69.0%) were infested. Nine-hundred and seventy-six (69.0%) of 1408 does and 21 (57.0%) of 37 bucks were infested. The prevalence was highest in Warren (100% of 106) and Franklin (100% of 24) counties and least in Lawrence (38.0% of 64) county. The lice species identified were *Linognathus stenopsis* (Anoplura: Linognathidae) and *Demalinia crassipes* (Mallophaga: Ishnocera). Lice appear to be of common occurrence on goats in Mississippi (they were found in all of the 14 counties surveyed), and are therefore a management and sanitation problem. The prevalence is more in goats (69.0% of 1445) than in pigs (31.7% of 407). This is the first significant report on lice infestation of goats covering the southwestern region of Mississippi.

#### INTRODUCTION

Goat industry is gaining prominence in the state of Mississippi. Lice are considered to be the most common ectoparasites of goats and one of the most serious menaces faced in the goat industry in the U.S. Lice infestation causes goats to bite, and pull their hair and scratch themselves with their horns and hooves. This results in hair loss, damaged and broken hair and skin. Severe lice infestation may cause anemia. In a similar study on lice infestation of swine in southwestern Mississippi, Acholonu and Epps (2009) reported 31.7 % (129) infested hogs out of a total of 407 sampled from 14 counties. The present study was conducted to determine the prevalence and distribution of lice in goats in the same area in Mississippi, the extent to which they pose a problem in the region and to compare the prevalence with that of swine.

#### MATERIALS AND METHODS

During the period of March 1997 to March 1998, goats were selected from farms in 14 counties located in southwestern Mississippi (Fig. 1). An average of five farms per county were selected. The number of goats in each farm ranged from 46 to 250. All goats in each farm were examined. Lice were collected from goats' bodies by brushing, searching, and picking them with the forceps following the procedure of Acholonu and Epps (2009). Lice collections were secured in vials containing 70% alcohol and transported to the laboratory in Alcorn State University. The lice specimens were mounted in CMC medium and identified using the key developed by Kim et al. (1986) and Bowman (1995), and confirmed by Dr. Tahir Rashid of the Department of Agriculture, Alcorn State University.

#### RESULTS

Of the 1445 goats examined, 997 (69.0%) were infested. In terms of sex, 976 (69.0%) of 1408 does and 21 of the 37 bucks (57%) were infested. Goat populations in Warren and Franklin Counties had the highest percentage of lice infestation 106/106 goats (100%) and 24 of 24 goats (100%), respectively. Wilkinson and Lincoln Counties showed greater than 75% lice infestation (76.1% of 67 and 76.0% of 96 goats, respectively). The least infested goats were found in Lawrence County (38.0%) (See Table 1 and Fig. 2). The lice species identified were the goat blood sucking lice, Linognathus stenopsis (Burmeister) (Anoplura: Linognathidae) and the chewing or biting lice, (Holokartikos) crassipes Damalinia (Mallophaga: Ischnocera), with chewing mouthparts (they do not actually bite) (See Figs. 3 and 4). The former was more prevalent (84.6%) than the latter (15.4%). Two (goats) (8.0%) had mixed or double infection. The does (69% of 1408) were more infested than the bucks (57.0% of 37). The two lice species have been deposited in the National Museum of Natural History in the Smithsonian Institute, Washington, D.C. (Accession Number: NMNH #2052431).

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Figure 1. Map of Mississippi showing Southwestern counties surveyed.

Table 1. PREVALENCE OF LICE ON GOATS BY SEX FROM 14 COUNTIES IN SOUTHWESTERN MISSISSIPPI

Counties	Goats Examined By County By Sex			Goats I	nfested And	Total Infested (%)
					(%)	
	<u>n</u>	DOE	BUCK	DOE	BUCK	
ADAMS	124	121	3	48 (40.0)	1 (33.3)	49 (40.0)
AMITE	46	44	2	26 (59.0)	2 (100)	28 (61.0)
CLAIBORNE	87	85	2	57 (6.0)	0 (0)	57 (66.0)
СОРІАН	152	148	4	110 (74.3)	1 (25)	111 (73.0)
FRANKLIN*	24	23	1	23 (100)	1 (100)	24 (100)
HINDS	250	245	5	175 (71.4)	3 (60)	178 (71.2)
JEFFERSON	73	71	2	51 (72.0)	2 (200)	53 (73.0)
LAWRENCE*	64	62	2	24 (39.0)	0 (0)	24 (38.0)
LINCOLN	96	93	3	73 (79.0)	0 (0)	73 (76.0)
PIKE	52	50	2	25 (50)	2 (100)	27 (52.0)
SIMPSON	200	196	4	145 (74.0)	3 (75)	148 (74.0)
WALTHALL	104	101	3	66 (65.3)	2 (66.6)	68 (65.4)
WARREN*	106	104	2	104	2 (100)	106 (100)
WILKINSON	67	65	2	49 (75.4)	2 (100)	51 (76.1)
TOTAL	1,445	1,408	37	976 (69.0)	21 (57.0)	997 (69.0)



Figure 2 Percentage of Goats infected in each Mississippi County



Figure. 3: Blood Sucking Louse (*Linognathus stenopsis*). Shows the characteristic narrow head.



Figure. 4: Chewing/Biting Louse (*Damalinia crassipe*). Shows the characteristic large or broad head and three-jointed antennae.

#### DISCUSSSION

High infestations of goats can be attributed to raising them on ranch (fencing and pasture) farms where they can roam and browse freely. Lice appear to be of common occurrence on goats in Mississippi and are therefore a management problem. The 69.0% infestation is relatively high and more than that recorded in swine (31.7%) in the same area (Acholonu and Epps, 2009). It was also high compared with the prevalence of the two genera of lice reported in Nigeria where goat meat is relatively used more than in the U.S. Linognathus occurred in 30% (18) of 60 goats and Damalinia (Bovicola) in 0.17% (1) of 60 goats (Idris and Ulmar, 2007). But in terms of numbers of different species of lice found in the goats, it was less than reported in Egypt where two species of biting lice (Bovicole caprae and B. ovis) and two species of sucking lice (Linognathus africanus and L. stenopsis) were reported in goats (el-Baky, 2001). Finding lice in all of the 14 counties surveyed shows that lice infestation is widespread and may be found in other counties not included in this study where goat production exists. It is thus recommended that this kind of study be extended to other counties to verify this surmise and that control measures be stepped up in the State of Mississippi especially the areas surveyed.

Goats' productivity depends on exemplary management style and more. Lice can be a problem for goat producers, especially during winter months. According to Medley and Drummond (1963) lice populations fluctuate during the year, with populations lowest in the summer and highest during late winter and early spring. Acholonu and Epps (2009) also observed that lice infestation of swine was highest during the winter when it was cold and early spring. Lice infestation of goats can cause intense pruritus, restlessness, scratching, and rubbing. The effect depends on the number of lice present. Blood-feeding lice cause more severe symptoms. Excessive feeding causes scabby and bleeding areas that may lead to bacterial infection (Medley and Drummond, 1963). Sucking lice feed almost constantly resulting in oozing blood, which clots on the surface of the goats' skin. This can cause a secondary bacterial skin infection as well as attract flies (http://www.goatbiology.com/lice.html). Chewing lice may cause their hosts considerable irritation when present in large numbers and especially in situations in which it is difficult for the animals to groom themselves. (Bowman, 1995.)

With the increasing interest in goat production in Mississippi and the waxing popularity in the use of goat milk by the populace, the problem posed by lice infestation of goats should not be treated with levity or overlooked. It is recommended that goats be treated without delay if any signs and symptoms of lice infestation are observed. Even without symptoms, goat rearers should periodically check the goats for lice infection. This will eliminate the chances of its becoming a serious economic problem. They can cause a reduction in meat and milk production (Medley and Drummond, 1963).

To our knowledge, this is the first significant report on lice infestation of goats which covers the southwestern region of Mississippi, thus constituting a geographic record.

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#### FRESHWATER STATUS IN MISSISSIPPI DELTA, USA.

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#### ABSTRACT

Environmental pollutants impact freshwaters by reducing their usability for economic and recreational purposes, as well as decreasing the number and variety of their resident organisms. The first goal of this research was to determine the presence or absence of 10 freshwater parameters (ammonium-nitrogen, chlorine, chromium, copper, cyanide, iron, nitrate, phosphate, silica, and sulfide) for Mississippi Valley State University (MVSU) north-campus pond, MVSU south-campus pond, Itta Bena Lake, Greenwood Blue Lake, and the Big Sand Creek in Mississippi Delta. The second goal was to document another set of freshwater parameters; temperature, pH, dissolve oxygen, total dissolve solids, and salinity, and then determine if any pollution and potential health hazards exist that could impact the use of the freshwaters for food and recreational purposes by humans, birds and wildlife. The magnitude of all the above parameters along with those of coliform bacteria loads, determine if water resources are pollution-free or polluted. Samples of water from the water resources were collected using the LaMotte Water Sampler<sup>™</sup> at depths of 1-5 m. The 10 freshwater parameters were tested using the Lab Aid<sup>®</sup> qualitative water pollution kit. Two of the five freshwater resources tested positive for nitrate or ammonium-nitrogen and chlorine was found in only one of the five. Iron was found in three of the five freshwaters tested, but phosphorous, silica, and sulfide were found in all five. Total dissolved solids, dissolved oxygen, pH, temperature, and salinity were determined using the HI 9828 Multiparameter meter and probes. Total dissolved solids concentration ranged from 24 to 121 ppm, while the dissolved oxygen ranged from 0.04 - 7.02 ppm. Measurements for salinity, pH and temperature were 0.02 - 0.11 ppm, 6.56 - 8.76, and 23.90- 31.42°C, respectively. Copper, chromium, and cyanide were not detected in the study and hence present no pollution issues. Presence of coliform bacteria was tested using the LaMotte™ Coliform Bacteria test kit. All the five freshwater resources tested positive for coliform bacteria, suggesting pollution and potential health risks; hence a more regular monitoring is recommended to document seasonal profile for each water resource.

#### **INTRODUCTION**

Water is an essential and integral chemical component of all living organisms. It is present at different amounts in all ecological habitats where it provides basis for all biological activities. For most organisms, water makes up as much as 70 to 90 percent of the body weight (Mader, 2008 and Mader and Windlespecht, 2014) and impacts both the population density and the quality of existence (Brooker et al., 2014 and Mader and Windlespecht, 2014). The ubiquity and uniqueness of water not only enables it to serve as a dynamic resource for food, drinking water, recreational activities but also as a hallmark of all successful human communities. In 2008, World Health Organization reiterated the importance of preserving freshwaters for drinking water supply, food production, and recreational activities; a task that is often compounded by moderate rainfalls. Prolonged and frequent thunderstorms and rainfalls as occur in Mississippi Delta (MD), adversely impact bodies of freshwater, including many ephemeral and intermittent water resources. A variety of wastes and pollutants often find their way into bodies of freshwater (Neal et al., 2002; Campbell et at., 2011 and <u>www.epa.gov/safewater/contaminations</u> <u>/ecoli.html</u>) through surface run-offs, septic tanks over-flow and ground water run-offs. In agricultural regions, such as the MD, where the use of pesticides and fertilizers by farmers are common, the potential for freshwater pollution through surface run-offs and ground water run-offs continues to draw questions and stir up profound fears of unsafe freshwaters that compromise humans' and wildlife health.

It is not uncommon in the MD to find local residents fishing for food in flooded ephemeral and intermittent water resource sites; such as by roadways, ditches, bogs and ponds. Knowledge of freshwater parameters and coliform bacteria loads are important in determining if waters are environmentally contaminated with pollutants. The first set of parameters tested in this study (ammonium-nitrogen, chlorine, chromium, copper, iron, nitrate, phosphate, silica, and sulfide) except cyanide, all serve as normal nutrients for freshwater organisms. Water resources however, become environmentally polluted when the concentration of one or more nutrients, coliform bacterial, or total dissolved solids, dissolved oxygen, pH, or salinity occur outside the safe range established by the Environmental Protection Agency (EPA) and or Mississippi Department of Environmental Control (MDEC). The ephemeral and intermittent water resources although not considered a major freshwater resource are used by birds and wildlife as sources of food and drink. Studies have shown that many animals that live in polluted waters tend to accumulate in their tissues the same pollutants that are present in such waters. Pollutant laden organisms are susceptible to parasitic infections. Ikenga and Wagner (2005) documented the presence of both parasitic and fungal diseases in many fish species from a watershed pond in Carroll Co. Mississippi. This research investigated the quality of five freshwater resources in the MD.

#### **OBJECTIVES**

The objectives of the study were to first document qualitatively 10 freshwater quality parameters for five freshwater resources in the MD. Freshwater parameters are measurable chemical and physical properties that are collectively used to determine the overall quality of health of freshwaters. The second objective was to determine quantitatively another set of freshwater quality parameters (Temperature, pH, Dissolve oxygen, Total dissolved solids, and Salinity) and third was to determine if any pollution and potential health hazards exist that could impact the use of freshwater resources for recreational purposes and for sources of food, by humans, birds and wildlife.

#### MATERIALS AND METHODS

The freshwater resources studied in this research are the Mississippi Valley State University (MVSU) north-campus pond (VNP) in Itta Bena, MS; the MVSU south-campus pond (VSP) in Itta Bena, MS; Itta Bena Lake (IBL) in Itta Bena MS; the Big Sand Creek (BSC) in Greenwood, MS; and the Greenwood Blue-lake (GBL) in Greenwood, MS. The IBL, GBL, and BSC are relatively lotic freshwaters. The VSP has a centrally installed deep aerator that circulates the water while the VNP has no circulation of water beyond that afforded naturally by wind. The five freshwaters studied were chosen because of their location in the region, accessibility, and prodivity for use by local residents. The project began in July 2008 and was completed in August 2008. At VNP, VSP, IBL, GBL, and BSC water samples were taken from a safe, none flow-obstructed location, using a wader and a LaMotte<sup>™</sup> Water Sampler at depths of 1.2 5 m, 1.21 m, 4.6 m, 2.9 m, and 0.91m, respectively. Depth was determined using the LaMotte<sup>™</sup> Water Sampler

calibrated rope attached to the sampler. At each freshwater location, five, clean, glass-bottles were carefully overfilled onsite with water sample from a LaMotte<sup>™</sup> Water Sampler and then tightly capped. Each glass bottle was labeled, dated and stored in a cooler-chest for transportation to the Environmental laboratory at MVSU. Water samples were kept cold in a refrigerator and tested within three days of collection. Chemical tests for the 10 water quality parameters were conducted using the Lab-Aid® Qualitative Water Pollution Kit per manufacturers' guidelines. Test kits were commercially supplied by the Carolina Biological Supply Company. On site, the HI 9828 Multiparameter Meter and probes were used to measure quantitatively the second set of parameters; temperature, pH, dissolve oxygen (DO), total dissolve solids (TDS), and salinity.

Prior to onsite water testing, the HI 9828 was fitted with the appropriate probes and calibrated with a Quick Calibration solution. Testing for coliform bacteria was conducted using the LaMotte<sup>™</sup> Coliform Bacteria Test Kit per manufacture's guidelines. The coliform bacterial test kit was commercially packaged and supplied by the Carolina Biological Supply Company. All data generated were tabulated and compared to the MDEC standards, to determine any anomaly that may suggest water pollution and possible health risks to humans, birds, and wildlife.

#### RESULTS

This research qualitatively found phosphorous, silica, and sulfide in the water samples from all five locations tested (Table 1). Iron was detected in water samples from the VNP, GBL, and BSC. Chlorine was detected in water samples from the GBL and ammonium nitrogen from the BSC. A trace of nitrate was found in BSC, but no chromium, copper, or cyanide was detected in any other water resources studied. Table 2 shows the quantified data for dissolved oxygen (DO), total dissolved solids (TDS), temperature, salinity and pH. The DO measured ranged from 0.04 ppm in water samples from GBL to 7.02 ppm in water samples from VNP (Table 2). Both IBL and VSP recorded respectively, 0.53 ppm and ppm for DO. Total dissolved solids found 4.94 ranged from 24 ppm in water samples from VNP to 121 ppm in water samples from VSP (Table 2). Salinity measurements were 0.02 ppm, 0.04 ppm, 0.05 ppm, 0.05 ppm, and 0.11 ppm in water samples from VNP, BSC, GBL, IBL, and VSP, respectively. The total dissolved solids or salinity found does not suggest any pollution. The pH measured ranged from 6.56 to 8.76.

Water Quality	Valley	Valley	Itta Bena	Greenwood	Big Sand
Parameters	South Pond	North Pond	Lake	Blue Lake	Creek
(NH <sub>4</sub> ) Nitrogen	-	-	+	_	-
Chlorine	-	-	-	+	-
Chromium	-	-	-	-	-
Copper	-	-	-	-	-
Cyanide	-	-	-	-	-
Iron	-	+	-	+	+
Nitrate	-	-	-	-	Trace
Phosphorous	+	+	+	Trace	Trace
Silica	+	+	+	+	+
Sulfide	+	+	+	+	+
Coliform Test	+	+	+	+	+

 Table 1. Qualitative Water Parameters from Five Locations in the Mississippi Delta.

Table 2. Quantified Water Quality	Parameters from Five Locations in the Mississippi Delta.
(~, comparative data not available.)	

Water Quality	VSP	VNP	IBL	GBL	BSC	MDEC Std.
Parameters						
Dissolved Oxygen (ppm)	4.94	7.02	0.53	0.04	4.64	≥4.0
рН	8.76	8.20	6.60	6.56	7.64	9.0
Temperature (° C)	31.42	28.62	29.97	23.9	26.62	30° C
Total Dissolved Solid	121	24	54	54	43	<400
(ppm)						
Salinity	0.11	0.02	0.05	0.05	0.04	~

The BSC (Figure 1) appeared the clearest of all the five freshwaters. Several minnows were observed at this location. At the time of this study, the BSC had not received much heavy rain falls and was not at its full capacity. The GBL appeared bluish but contained much decaying organic matter, algae, and aquatic weeds (Figure 2). No minnows were seen in GBL, but small fish were seen flipping out and diving back into the water. IBL (Figure 3) is a slow flowing lake and had a light green appearance with abundance of water hyacinth, especially at shorelines. The VNP had a little mud-brown coloration but with no observable algal growth (Figure 4). The surface of VSP was almost completely covered with algal growths (Figure 5). Coliform bacteria were found in water samples from all the five water resources tested.



Figure 1. The Big Sand Creek in northeast Greenwood, Mississippi, exhibiting a semidrought-like state.



Figure 2. The Greenwood Blue Lake in Greenwood, Mississippi, showing algal growth and floating aquatic plants.



Figure 3. The Itta Bena Lake in Itta Bena, Mississippi, showing abundant aquatic plants near shoreline.



Figure 4. The Mississippi Valley State University Northcampus Pond in Itta Bena, Mississippi, exhibiting a mudbrown appearance

#### DISCUSSION

Many bodies of water are negatively impacted by frequent thunderstorms and prolonged rainfalls through surface run-offs, including septic tanks over-flow and ground water run-offs. Both types of run-off usually enable dumping of a variety of wastes and pollutants into bodies of water (Neal et al., 2002; Campbell et at., 2011 and <u>www. epa.gov/ safewater/contaminations/ecoli.html</u>).

In agricultural regions, such as the Mississippi Delta, where the use of pesticides and fertilizers by farmers are common, water run-off into public bodies of water often elicit a profound sense of threat to human health, and collaterally to birds' and wildlife health. As in any environmental studies, sampling locations are important.



Figure 5. The Mississippi Valley State University Southcampus Pond in Itta Bena, Mississippi, showing a thick cover of algal growth.

During this study, efforts were made to collect water samples from each water resource from a reasonably suitable and safe location. It is suggested that equidistant water sampling sites across each water resource be undertaken to determine any possible differences. The IBL, GBL, and BSC are lotic freshwaters within the Yazoo River Basin. Water flow and mixing are enabled by wind and a centrally located deep aerator at the VSP and by wind only at the VNP. The deep aerator at VSP may be simulating some kind of eutrophication by upwelling bottom nutrients to the surface and hence, help maintain the algal bloom seen in Figure 5. Such abundance of algal growth is suggestive of water pollution. Data reported in Tables 1 and 2 of this study may be sensitive to season, and hence higher numbers may be recordable immediately following thunderstorms. It is therefore recommended that a regular seasonal water-sampling be implemented to confirm and elucidate the magnitude of seasonal changes.

Phosphorous, silica, and sulfide were found in water samples from all the five locations tested (Table 1). Both and sulfides are essential phosphorous plant macronutrients. These nutrients contribute to freshwater pollution at high concentrations. Aquatic vertebrates such as fish are common freshwater animals that require phosphorous and sulfides for building and replenishing of cellular proteins and nucleic acids (Mader and Windlespecht, 2013). Silica that was found in all the five locations tested may be responsible for some mineralization of the waters. Iron, an important mineral for binding and transport of oxygen in vertebrates and a plant micronutrient, was found in water samples from the VNP, GBL, and BSC. The concentration of iron in the above sites is not yet known, as only qualitative studies were done at this time. Nitrate that was found in trace amounts in the water samples from BSC and ammonium-nitrogen from IBL are essential plant macronutrients required for syntheses of proteins, nucleic acids, chlorophyll, coenzymes, and alkaloids (Brooker et al., 2014 and Mader and Windlespcht, 2013).

Some of the algae observed in the VSP and the GBL could be cayanobacterial, which may present with microcystin, a known hepatotoxin (Anderson, 2007; Dash et al., 2013; Falconer and Humpage, 2005; and Metcalf and Codd, 2004). The presence of ammonium nitrogen in IBL is perhaps why aquatic weeds, especially water hyacinth, were abundant in the IBL. Chlorine, a micronutrient of both plants and animals that was detected in GBL is used by plants in their photosystem cells and in the ion balance for photolytic activities, while animals need chlorine for acid-base and osmotic balances, as well as conduction of impulses (Brooker et al., 2014 and Mader and Windlespcht 2013). The presence of chlorine in these freshwaters is documented by this study but the concentration is unknown. However, the potential health risks from copper, chromium, and cyanide is at abeyance, since none was detected in the study. A quantitative analyses of all nutrients found would be needed to help determine presence or absence of pollution. A regular and seasonal water sampling is therefore recommended to help document any seasonal pollutants.

Both the IBL and the GBL were extremely anoxic and out of compliance of the minimum MDEC Standards. Such low DO levels in water indicate a high biological oxygen demand (BOD); suggesting some form of organic pollution (Okorie and Acholonu, 2008 and Okorie et al., 2013). Low DO levels negatively impact both variety and number of aerobic, aquatic organisms and possibly accounts for why no minnows were observed in the two water resources. It is possible that the fishes that periodically lipped out of the GBL and then dove back in were coming up for air. Most freshwater fishes breathe water. But a metabolic need for more oxygen in the environment that has less than optimum may be a driving force for the fishes observed leaping out of the water to adapt to aerial breathing.

The VNP recorded the highest DO concentration, suggesting the presence of a low BOD, hence a TDS of 24 ppm. Neither the total dissolved solids (TDS) found in this study (24 ppm to 121 ppm) nor the salinity (0.02 ppm to 0.11 ppm) represent any pollution concerns. The pH measured at the VNP, IBL, GBL, and BSC was not in disparity with the MDEC Standard. The VSP at 31.42° C was the only water resource tested that exceed the MDEC standard of 30° C. The high temperature may limit zooplankton and may also be one of the extrinsic factors promoting the dense algal bloom seen in the VSP (Figure 1).

This study documents the presences of coliform bacteria in all the test waters; a notably troublesome finding. Coliforms are microorganisms that represent a group of enteric bacteria. The latter along with other diseasecausing bacteria, viruses, and protozoans are associated with the fecal matter of warm-blooded animals, including humans. The coliforms in general, serve as indicators of possible sewage or fecal contamination with water resources (APHA, 1992). The detection of coliforms in this study suggests that a potential health risk exists for humans, birds or wildlife that use these water resources. A more sensitive but expensive bacterial assay is recommended to elucidate specific coliform species and load. IBL and GBL, in particular, are used often by local residents for fishing and or recreational purposes. Local youngsters sometimes swim in the IBL, raising concerns about recreational water illness. The latter are caused by microorganisms spread by coming in contact with, or breathing in mists, aerosols of, or swallowing in contaminated waters (http://www.cdc.gov/ healthywater /swimming/rwi).

#### CONCLUSIONS

This study documents both the freshwater parameters and the status of coliform bacteria for all the five MD freshwaters studied. Freshwater parameters are measurable chemical and physical properties that are collectively used to determine the overall quality of health of water resources. It is important to note that freshwaters can be considered polluted by the presence of one or more of the parameters tested in this study, if the concentrations exceed safe EPA and or MDEC standards. The finding of phosphorous, chlorine, sulfide, nitrate, iron, and ammonium-nitrogen in the test waters does not suggest presence or absence of pollution at this point. This qualitative study merely documents the kinds of nutrients present in the freshwaters. A follow-up quantitative analysis of each nutrient must be conducted to determine presence or absence of pollution by nutrients. Temperature, pH, DO, TDS, and salinity identify unhealthy waters quickly and help suggest possible state of pollution. Plant and animal tissues accumulate pollutants that are present in their environments. A regular seasonal study is therefore recommended for the five freshwaters to avert potential biological magnification. The latter occurs when plants and animals live and reproduce in polluted environments. The finding of coliform bacteria in all the test waters is notably troublesome. A more specific test for coli Е. (www. epa.gov/safewater/contaminations/ecoli.html and www.epa.gov/owow/monitoring/volunteer/stream/vms51

<u>1.html</u>) is recommended on a regular seasonal basis. Such a test would help determine if the *E. coli* is the strain that is highly pathogenic to humans, birds and wildlife.

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