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Abstract

This project is aimed at the development of the biofuels industry in Kentucky by establishing a laboratory to develop improved processes for biomass utilization. The facility is based at the University of Kentucky Center for Applied Energy Research and the Department of Biosystems and Agricultural Engineering, and constitutes an “open” laboratory, i.e., its equipment is available to other Kentucky researchers working in the area. The development of this biofuels facility represents a significant expansion of research infrastructure, and will provide a lasting resource for biobased research endeavors at the University of Kentucky. In order to enhance the laboratory’s capabilities and contribute to on-going biofuels research at the University of Kentucky, initial research at the laboratory has focused on the following technical areas: (i) the identification of algae strains suitable for oil production, utilizing flue gas from coal-fired power plants as a source of CO_2; (ii) the conversion of algae to biofuels; and (iii) thermochemical methods for the deconstruction of lignin. Highlights from these activities include a detailed study of bio-oil production from the fast pyrolysis of microalgae (*Scenedesmus* sp.) and the application of pyrolytic gas chromatography coupled with mass spectrometry (Py-GC-MS) to the characterization of high lignin biomass feedstocks.

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Executive Summary

This project is aimed at the development of the biofuels industry in Kentucky by establishing a laboratory to develop improved processes for biomass utilization. Kentucky is well positioned to become a leading player in biofuels production: Kentucky's abundance of natural resources, including forestry wastes and agricultural residues, in addition to crops such as corn and soybeans, offers a huge resource of available biomass. The facility is based at the University of Kentucky Center for Applied Energy Research (UK CAER) and Department of Biosystems and Agricultural Engineering (BAE). The laboratory has been conceived as an open access facility, such that its equipment is available to researchers in other Kentucky institutions working in the field of biofuels. The development of a biofuels facility represents a significant expansion of research infrastructure, and will provide a lasting resource for biobased research endeavors at the University of Kentucky.

In order to continue to build research capacity, several major items of equipment were acquired for the Laboratory, including a FT-IR spectrometer, an anaerobic digester (for conversion of algae biomass to methane), a high pressure/high temperature pressure reactor for thermochemical conversion experiments with algae, an accelerated solvent extractor for use in the extraction of lipids from algae, an HPLC, required for studies pertaining to lignin utilization for the production of fuels and chemicals, and a pyrorobe pyrolyzer unit. Initial research at the laboratory has focused on three technical areas: (i) the identification of algae strains suitable for oil production, utilizing flue gas from coal-fired power plants as a source of CO_2; (ii) the conversion of algae to biofuels; and (iii) thermochemical methods for the deconstruction of lignin.

To facilitate research aimed at the identification of algae strains suitable for oil production, utilizing flue gas from coal-fired power plants as the source of CO_2, four different culture systems were assembled. These permit the cultivation of algae in a controlled environment, utilizing synthetic flue gas. Studies were begun to investigate the effect of SOx (represented by sulfuric acid) on *algae* strains of interest in terms of growth rate and general health. Compared to *Chlorella vulgaris*, *Scenedesmus* adapted to and thrived under acidic conditons with a higher growth rate. Microalgae were also tested for their tolerance to ashes produced from nearby Kentucky coal-firing plants (Dale, Spurlock, Gillbert, and Tyrone). Exposure to ash at a concentration of up to 1 g ash per 400 mL culture volume produced no algal growth inhibition for either algal strain.

Research on the conversion of algae to biofuels was focused on the production of bio-oil from the fast pyrolysis of a dried microalgae feedstock (*Scenedesmus* sp.*)*. Product analysis showed the various fractions of bio-oil produced were, in certain respects, comparable to pyrolysis products from wood and other microalgae species. Indeed, the oxygen and moisture contents of the products were fairly typical of pyrolysis oil produced from lignocellulosic feedstock. However, the average total acid number of the oil was lower than for bio-oil produced from wood pyrolysis. Furthermore, the products possessed a relatively high nitrogen content due to the high protein content in the algae feedstock. Overall these results indicate that microalgae can be pyrolyzed to produce bio-oil and a biochar rich in ash, although the bio-oil would require upgrading (hydrotreating) prior to use for most fuel applications.

In separate work, the use of decarboxylation as a route for the conversion of triglycerides (typically the major component of algal lipids) to hydrocarbons was studied. Initial work focused on uncatalyzed reactions in order to ascertain the contribution of thermal reactions to

subsequent catalyzed experiments. Cracking experiments were performed in a steel reactor over the temperature range 350-500 ºC, using soybean oil as a surrogate for algae oil. The yield of the diesel-like fraction (i.e., total liquid yield x % diesel-like fraction in liquid) initially increased with the cracking temperature, approaching *ca*. 30 wt% at 500 °C under atmospheric pressure. Under 300 psig N_2 the yield of the diesel-like fraction was slightly higher, reaching 37 wt% at 450 °C, but then decreased to 34 wt% at 500 °C due to a decrease in the total amount of liquid product, this being a result of increased gas formation (light hydrocarbons). Overall, these results show that the production of diesel-like product is favored by high reaction pressures and by temperatures in the range 450-500ºC; however, yields are fairly modest. Consequently, future efforts will focus on catalytic decarboxylation, using semi-batch and fixed bed reactors.

The deconstruction of lignin represents the third area under study. In order to identify and examine feedstocks that possess naturally high lignin content, pyrolytic gas chromatography coupled with mass spectrometry (Py-GC-MS) was employed. The resulting pyrograms showed that walnut shells, coconut shells, olive pits and switchgrass vary in their lignin and hollocellulose content, causing a variation in pyrolysis product distribution. Switchgrass contained the least amount of lignin and coconut shells the greatest; the latter also showed a much larger increase in the production of phenol when compared to pyrolysis of the biomass types. Additionally, pyrolysis products from whole biomass contained large amounts of acetic acid and furfural whereas these compounds are produced only in small amounts from the corresponding lignin fractions.

Finally, in keeping with the open laboratory objective, experiments and sample analyses were completed at the request of a number of other institutions, as well as other research groups within the University of Kentucky. These requests included GC analysis of biodiesel samples, bomb calorimetry of biomass samples, the evaluation and steam activation of carbon derived from fast pyrolysis, the thermochemical processing of algae, and GC analysis of gas and liquid bio-oil samples produced from the fast pyrolysis of biomass.

Introduction

The principle objective of this project is the establishment of a laboratory dedicated to researching improved processes for biomass utilization, with the goal of supporting the development of the biofuels industry in Kentucky. Kentucky is well positioned to become a leading player in biofuels production: Kentucky's abundance of natural resources, including forestry wastes and agricultural residues, in addition to crops such as corn and soybeans, offers a huge resource of available biomass. The facility is based at the University of Kentucky Center for Applied Energy Research (UK CAER) and Department of Biosystems and Agricultural Engineering (BAE), and constitutes an "open" laboratory, i.e., its equipment is available to other Kentucky researchers working in the area. The development of a biofuels facility represents a significant expansion of research infrastructure, and will provide a lasting resource for biobased research endeavors at the University of Kentucky. Emphasis is placed on working with local biofuel producers in order that research findings can be readily implemented at the industrial scale.

Phase 2 of this project focused on building research capability at the laboratory with the aim of supporting research initiatives in the following areas:

- The identification of algae strains suitable for oil production, utilizing flue gas from coal-fired power plants as a source of CO_2
- The conversion of algae to biofuels
- (Thermo)chemical methods for the deconstruction of lignin

Towards these goals, the necessary laboratory instrumentation was acquired and installed. As detailed below, these tasks were duly completed, after which activities were focused on the application of these facilities to these research areas, in addition to community outreach.

Upon completion of the Phase 2 tasks in mid-2011, Phase 3 of the project was commenced. Phase 3 goals likewise focus on the cultivation of algae strains suitable for biofuels production, together with the study of thermochemical methods for the conversion of algae biomass to fuels. This will also include a techno-economic feasibility study of the available options.

Results and Discussion

Phase 2 Tasks
Task number: 1.0 (Acquisition of laboratory instrumentation)

Major items of equipment that were acquired for the Laboratory consisted of the following:

- An anaerobic digester (Ankom Gas Production System) for conversion of algae biomass to methane. This will be a key item of equipment for studies in Phase 3 of this project.
- A high pressure/high temperature pressure reactor (Parr Instrument Company) for thermochemical conversion experiments with algae (sub-task 2.2).
- A Dionex ASE 350 Accelerated Solvent Extractor with 100 mL extraction cells, for use in the extraction of lipids from algae (for use in sub-task 2.2).
- An Agilent HPLC, required for studies pertaining to lignin utilization for the production of fuels and chemicals (sub-task 2.3).

- A CDS Pyrorobe pyrolyzer unit, similarly acquired to assist in studies pertaining to lignin utilization (sub-task 2.3)

Task number: 2.0 (Installation and commissioning)

The installation and commissioning of equipment for this phase of the project was completed. Work then transitioned to the analysis of algae-derived oils, as well as the development of analytical protocols for the analysis of lignin and its depolymerization products.

Sub-task 2.1: Assembly of algal growth system
Four different culture systems were assembled and made operational. One is located in a walk-in hood with 500 mL flasks (21 in total) on a shaking table (see Fig.1 below). This set up is intended for tests with SOx and simulated flue gas. Another set up includes a temperature controlled platform, for use with 500 mL flasks, which is used to test the temperature dependence of algae growth for various strains with varying media. Another set up is located in a controlled environment chamber with four racks, each capable of housing 27 flasks. This set up is intended to be large enough to run up to four experiments simultaneously. These experiments will focus on media optimization, waste water treatment, and effects of the material used for photobioreactor construction on algae growth. The final system is a pilot-scale reactor with six photobioreactors, each of 10 L capacity. This system is intended for continuous, larger scale experiments. The pilot-scale reactor with four photobioreactors for a total volume of 60 L has been modified to ensure equal flowrates in each of the photobioreactors and to ensure adequate mixing and easy sampling.

Figure 1. Algae culture system for use with simulated flue gas.

Phase 2 of this sub-task was completed in March 2011 and studies were initiated in order to transition to Phase 3 of the project. Specifically, studies were begun to investigate the effect of SOx (represented by sulfuric acid) on *algae* strains of interest in terms of growth rate and general health. The influence of a buffer in order to mitigate the pH swing when flue gas is added is also being examined. Initial results are presented below as part of the Phase 3 activities.

In separate work, the anaerobic digester system was used for sorghum fermentation, in order to test the system. A future goal in Phase 3 of the project is to run the digester with algae, with the particular goal of analyzing the digestate and ascertaining whether it can be recycled for use in the preparation of algal growth media (given that it should be rich in P, N, K, etc.). Paperwork and clearance forms have been completed in order to obtain activated sludge samples from a local waste water treatment plant. The experimental design and procedures have been developed for initial studies. Algae cultures are being grown up in order to have enough biomass for the initial studies.

Sub-task 2.2: Analysis of algae-derived oils

This activity has focused on an examination of different routes for the upgrading of microalgae to liquid fuels, the ultimate goal being to arrive at a techno-economic assessment of the different options. In order ascertain the viability of producing liquid fuels from whole algae (i.e., avoiding the necessity of extracting the algal lipids), the pyrolysis of algae (*Scenedesmus*) was conducted in a bench-scale fluidized bed fast pyrolysis reactor, operating at a biomass feed rate of 5 lb/h. During operation, the biomass was fed into the reactor where it contacted a hot bed material (sand) and underwent pyrolysis. The effluent exiting from the reactor passed through a hot gas cyclone and a ceramic filter (at 450-500 °C) to remove solids (mainly char), and then entered a series of quenching coolers for recovering condensable oil and water. Pyrolysis was performed at 480 °C, 1 bar and *ca.* 2 s residence time using *ca*. 20 lb of dried algae (3% moisture content) which had been ground to a powder (< 2 mm).

Fractions collected from the quenching coolers of the fast pyrolysis unit are defined such that C1 corresponds to the heaviest oil fraction collected and C4 corresponds to the lightest oil fraction, where C2 and C3 are intermediate fractions. Each of these fractions was obtained as a mobile, brown liquid. The total oil yield was estimated at approximately 55 wt.%, based on the yield of bio-oil fractions collected and the approximate amount of oil remaining on the reactor walls and piping. Note that this figure is based on the weight of feedstock, excluding its ash content.

Considering first the char product, the ratio of crude oil:char obtained was 3.76 by weight for the oil fractions collected. The char had low calorific content (1970 Btu/lb) and contained 13.3 wt.% volatile matter, while ultimate analysis showed it to contain 15.9 wt. % carbon, together with small amounts of nitrogen (2.3 wt.%), sulfur (0.8 wt.%) and hydrogen (0.8 wt.%). 75 wt.% of the biochar mass was attributed to the presence of ash. SEM images (not shown) indicated that a significant portion of the ash content resulted from the presence of frustules derived from *Navicula* diatoms that were present in the algae feedstock as a contaminant. The presence of these organisms also explains the high ash content (35.2 wt.%) in the original feedstock. The ash obtained from the biochar consisted of 49.5 wt.% SiO_2, 4.1 wt.% Fe_2O_3, and 11.0 wt.% Al_2O_3 which is consistent with the presence of the silicate frustules [1]. The biochar ash also contained 10.7 wt.% CaO, 1.6 wt.% Na_2O, 5.9 wt.% K_2O, 9.7 wt.% P_2O_5, and 3.1 wt.% SO_3 which were mainly associated with the *Scenedesmus* sp. (originating from the nutrients supplied to the algae feedstock).

Turning to the oil fractions, the filter oil constituted the largest percent of recovered oil product by mass (33.8% of the total), followed by the C3 oil fraction (28.5% of the total, see Table 1). The average density of the oil was 1.1 g/ml, which is slightly lower than that of wood pyrolysis oil [2] but similar to values reported for pyrolysis oil derived from autotrophically grown algae [3,4]. The average total acid number for the oil products was 68 mg KOH/g, which is somewhat lower than typical bio-oil produced from wood pyrolysis [2]. Elemental analysis showed the oil products to contain an average of 27.6 wt.% oxygen, 51.9 wt.% carbon, 9.0 wt.% hydrogen and 8.6 wt.% nitrogen (dry basis), the relatively high nitrogen content being a consequence of the high protein content of the algae. Figure 2 displays the results from ultimate and proximate analyses for each fraction obtained. The lighter oil fractions contained more water than the heavy fractions (up to 72 wt.%), although the overall average oxygen and moisture content (~25 wt.%) were typical of lignocellulosic-derived pyrolysis oil.

Table 1. Product distributions for select oil fractions based on GC-MS analysis.

Compounds (Class of Compounds)	C2 (Area %)	C3 (Area %)	C4 (Area %)	Filter (Area %)
Alkanes	0.0	2.0	0.0	2.6
Alkenes	1.5	8.9	0.0	9.4
Fatty Oxygenates	21.0	12.1	0.0	32.3
Steroids	2.8	0.0	12.9	3.1
Aromatics	0.0	0.0	0.0	1.8
N-containing Compounds	18.7	70.4	86.2	21.7
Unidentified	56.0	6.7	1.0	29.1
Yield of oil fraction (% of total oil recovered)	3.1	28.5	11.4	33.8

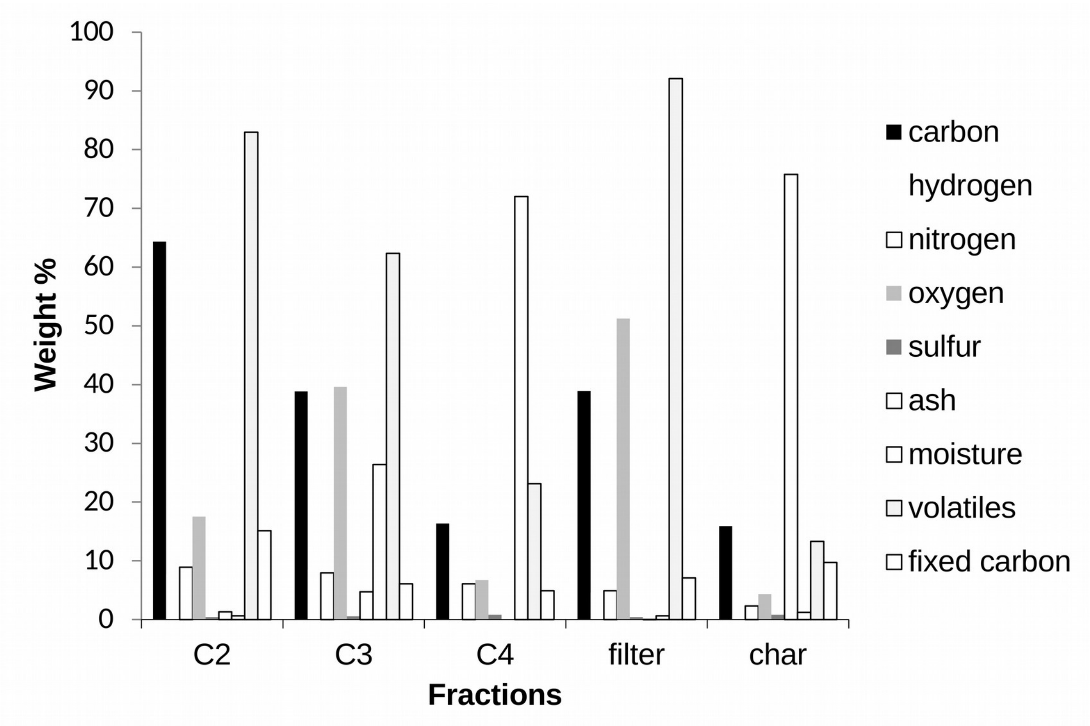


Figure 2. Ultimate and proximate analysis of select fractions obtained from *Scenedesmus* sp.

pyrolysis in the fluidized bed reactor.

The results of bomb calorimetry showed the average calorific content of the oil to be approximately 18.4 MJ/kg. This is comparable to bio-oil produced from the fast pyrolysis of wood [2] but is lower than the value of 30 MJ/kg reported by Miao and Wu [3] for pyrolysis oil obtained from fast pyrolysis of *Chlorella protothecoides* cultured autotrophically. This difference can be attributed to the lower oxygen content (19.43%) of the oil obtained by Miao and Wu (and correspondingly higher carbon and hydrogen contents) as compared to the oil produced in the current study. Additionally, there may also be differences in the water content of the bio-oils (the water content is not reported in references 3 or 4). The reason for these differences in bio-oil properties is not apparent, although we note that Babich *et al*. [5] reported an intermediate heating value of ~26 MJ/kg for bio-oil obtained from pyrolysis of *Chlorella* sp. at 450 ºC. The lighter fractions in the current work (C3 and C4) had lower heating values than the heavy fractions due to their higher water content. Simulated distillation GC results, shown in Figure 3, indicated that each fraction contained a high proportion of components boiling in the range for heavy gas oil (343°C-524°C). The lighter fractions also show a significant proportion of products that boil in the range typical of kerosene (204°C-288°C).

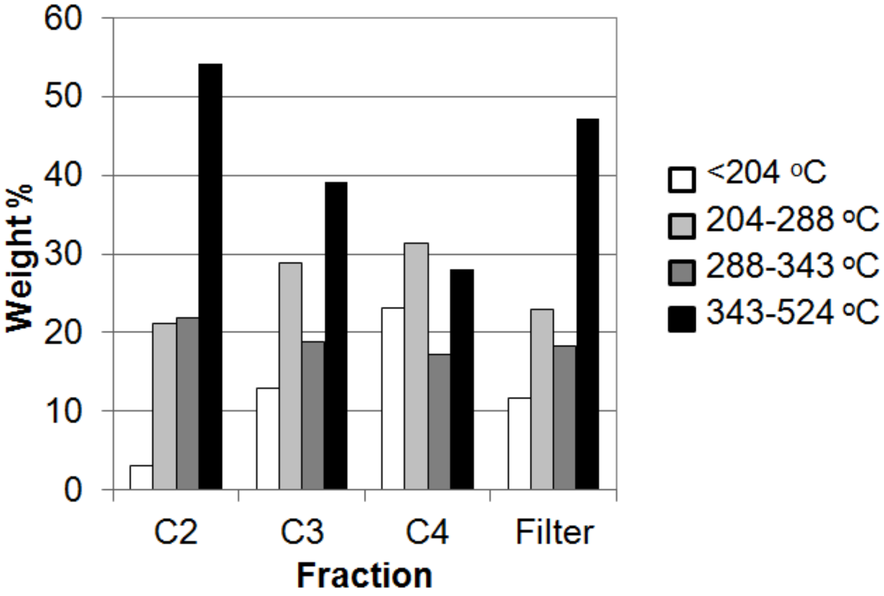


Figure 3. Simulated distillation GC results for select oil fractions.

GC-MS analysis indicated the presence of nitrogenous and oxygenated compounds in the oil fractions such as amides and fatty acids, as well as a variety of hydrocarbons. Many of the compounds were branched or unsaturated as indicated by the C:H ratios and GC-MS results. The area % for the various types of compounds identified for each oil fraction using GC-MS is summarized in Table 1. Nitrogenous compounds identified include amines, amides, pyridines, pyrroles, pyrazoles, pyrazines, nitriles, imidazoles and indoles, although the majority of these compounds were amides. The amides varied in chain length ranging from acetamide to stearamide and also included cyclic amides such as 2-pyrrolidone. Cyclic amides may be formed from protein and amino acid intramolecular cyclization [6-11] whereas linear amides may be formed from primary protein decomposition or from amines in amino acids that reacted with carboxylic acids to produce amides and water (Figure 4). The presence of pyrroles can be attributed to the decomposition of glutamine amino acids present in proteins [10], as well as decomposed chlorophyll that was present in the algae feedstock [12]. Pyrazines, pyridines, piperidines and pyrazoles are also likely formed from protein decomposition and/or intramolecular cyclization. Imidazoles may be formed from the decomposition of histidine amino acids present in proteins [9] and indoles may be produced from decomposed tryptophan amino acids [7,10]. Each of these compounds may be the result of primary or secondary reactions that occurred during pyrolysis or in the condensed oil phase. Decomposition mechanisms that lead to these products include, but are not limited to: homolytic cleavage, heterolytic cleavage, decarboxylation, decarbonylation, dehydration, deamination, dehydrogenation, condensation and cyclization [11]. While the amount of nitrogenous compounds formed seems high, the results agree with elemental analysis. For example, if the average nitrogenous compound is compositionally similar to octanamide, then based on its empirical formula, a nitrogen content of 10 wt. % would be expected. Since N-containing species constituted less than 100% of the various oil fractions, a nitrogen content of less than 10 wt. % is to be expected.

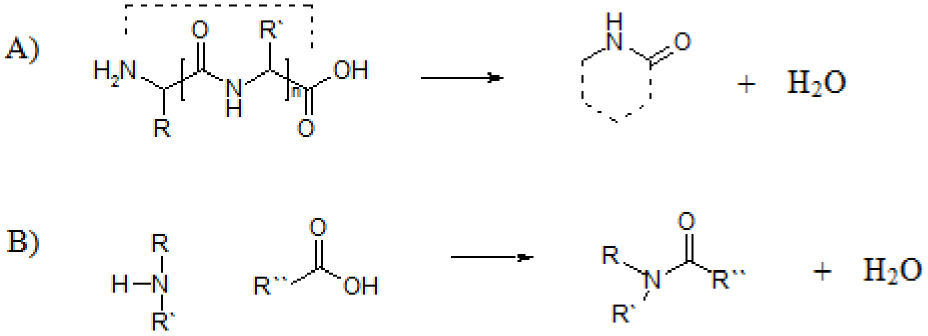


Figure 4. A) Intramolecular cyclization of proteins resulting in pyrrolidone structures. B) Carboxylic acids react with amines to produce linear amides.

Fatty oxygenates identified include aldehydes, ketones, acids, and alcohols with long carbon chains, including saturated and unsaturated, branched and linear isomers. Alkanes and alkenes in the products were identified in accordance with retention time calibrations and mass spectra analysis based on a NIST library. The majority of these hydrocarbon compounds were formed primarily from the pyrolysis of the lipid fraction (triglycerides and fatty acids) of the algae feedstock. Lipid pyrolysis mechanisms are complex and have been thoroughly investigated [13-19]. Steroids and aromatic compounds such as phenols, naphthalene and toluene were also observed in the oil products, particularly in the filter oil.

Overall these results indicate that microalgae can be pyrolyzed to produce bio-oil and a biochar rich in ash, although the bio-oil would require upgrading (hydrotreating) prior to use for most fuel applications

Sub-task 2.3: Method development for the analysis of lignin deconstruction products

(i) Pyrolytic gas chromatography

This task has focused on the deconstruction of lignin, to guide the development of improved processes for the efficient processing of lignin into fuels, as well as valuable chemicals.
While cellulose has received much attention for deconstruction via biochemical [20,21] or thermochemical means [22,23], lignin, as the second most abundantly renewed biopolymer on

the planet, is often looked upon as a waste product, due to its complex structure and recalcitrance. However, lignin contains structural units that could serve as a source of fuels and high-value chemicals [24], if means can be found to free those structural units from the polymer.

In order to identify and examine feedstocks that possess naturally high lignin content, we have employed pyrolytic gas chromatography coupled with mass spectrometry (Py-GC-MS). The use Py-GC-MS can provide valuable insight into the structure of lignin through the analysis of the products obtained when the latter is pyrolyzed. Parenthetically, this analysis can also provide valuable information regarding the potential of a particular lignin sample as a fuel source. Given that Py-GC-MS allows for lignin to be pyrolyzed in the presence of a catalyst, this technique can also be used to screen potential catalysts for the pyrolytic depolymerization of lignin. With this in mind, a Pyroprobe 5200 pyrolyzer with thermal desorption and reactant gas operation capabilities was purchased from DCS Analytical, Inc. and interfaced with an existing GC-MS. Subsequently, the GC-MS was calibrated using appropriate standards to allow quantification of the yields of the main products expected (phenol, m-cresol, p-cresol, guaiacol, syringol, etc.) and used to identify the differences in lignin composition and structure in wild-type switchgrass, walnut shells, coconut shells and olive pits. In addition to analyzing the whole biomass, lignin was also extracted from each of these sources (using a literature procedure) and analyzed separately using Py-GC-MS. The composition and the relative amounts of the pyrolysis products are used to distinguish different lignin types and to determine whether a given biomass source is capable of producing specific pyrolysis products.

Pyrolysis experiments were conducted at 650°C (1000°C/s heating rate) for 20 s under He. Whole biomass pyrolysis yielded products associated with hemicellulosic, cellulosic and lignin fractions within the biomass. Holocellulosic pyrolysis products included hydroxyacetaldehyde, furan derivatives, furfural, acetic acid and other short chain oxygenated compounds. These compounds appear in pyrograms at retention times of up to 8.8 minutes. Lignin pyrolysis products included methoxyphenols and other aromatic compounds derived from the monomeric units coumaryl, coniferyl, and sinapyl alcohol within the lignin structure. These compounds have retention times mostly in excess of 8.8 minutes. Table 2 shows selected marker compounds for both lignin and holocellulosic fractions with their retention times and sources as they appear in the pyrograms.

Whole biomass pyrolysis products for each sample are compared in Table 3 and lignin pyrolysis products are compared in Table 4. As expected, switchgrass shows the least amount of total lignin pyrolysis products and a higher amount of holocellulose based pyrolysis products when compared to walnut shells, coconut shells, and olive pits. Olive pits appear to contain less lignin than walnut and coconut shells which function as protective barriers for the seeds of the walnut and coconut trees. Coconut shell pyrolysis produces much higher amounts of phenol and less 2-methoxyphenol than the other samples as indicated by both whole biomass pyrolysis and pyrolysis of lignin extracted from the samples. Figure 5 shows a pyrogram of walnut shell pyrolysis at 650°C. 2-methoxyphenol and 2-methoxy-4-methylphenol are the primary products resulting from lignin pyrolysis where acetic acid, acetone, and 1-hydroxy-2-propanone are produced primarily from the hollocellulosic fraction. Pyrolysis of coconut shell lignin is shown in Figure 6 and shows that pyrolysis of the lignin fraction also creates a small amount of acetic acid and furfural. This could also be due to leftover hemicellulose in the lignin fraction. It also shows a large amount of phenol production, whereas the amount of 2-methoxy-4-methylphenol is less than that produced from olive pits or walnut shells. Coconut shell lignin pyrolysis also produced more 2,6-dimethoxyphenol than the other biomass samples. Given that 2,6-dimethoxyphenol is considered to be a marker for sinapyl alcohol, the result suggests that coconut shells may contain higher amounts of the sinapyl alcohol monomer in the lignin structure [24].

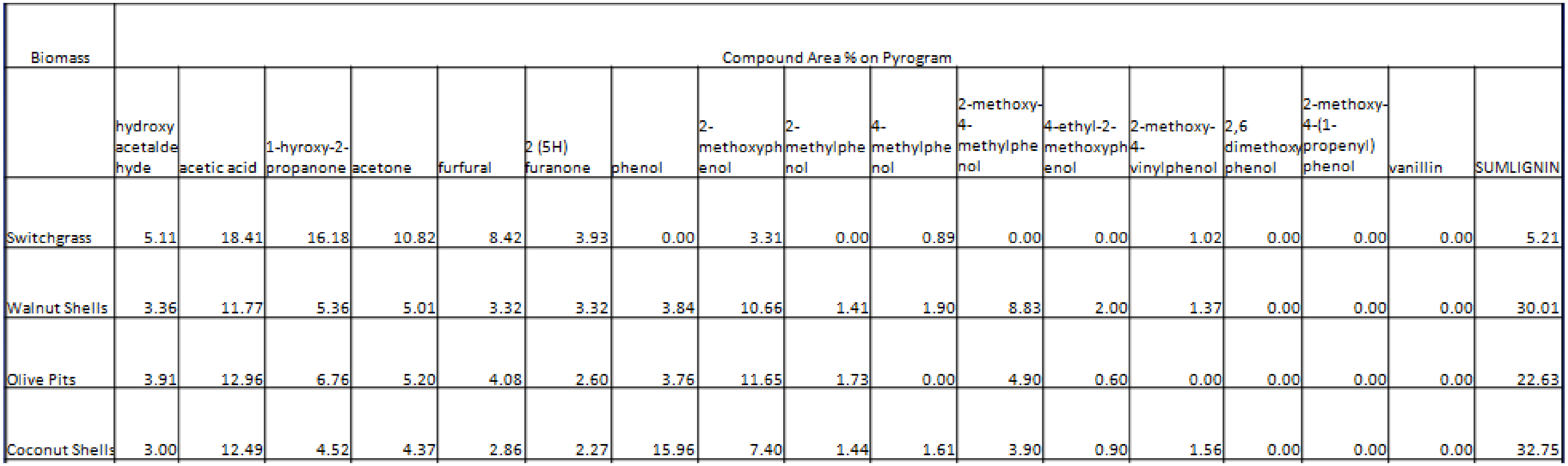
Table 2. Select compounds identified in pyrograms obtained from biomass and lignin pyrolysis.

Compound	Retention Time (min)	Source
benzene	2.6	Lignin
hydroxyacetaldehyde	2.8	Lignin + holocellulose
acetic acid	3.0	Lignin + holocellulose
toluene	3.4-3.5	Lignin
1-hydroxy-2-propanone	3.5	Lignin + holocellulose
acetone	5.2	Lignin + holocellulose
furfural	5.4-5.9	Lignin + holocellulose
2 (5H) furanone	7.9	Lignin + holocellulose
phenol	8.9-9.1	Lignin
2-methoxyphenol	9.1-9.2	Lignin
2-methylphenol	9.4-9.5	Lignin
2,6-dimethylphenol	9.7-9.8	Lignin
4 methyl phenol	10.1-10.5	Lignin
2-methoxy-4-methylphenol	10.7-11.2	Lignin
4-ethyl-2-methoxyphenol	11.8-12.1	Lignin
2-methoxy-4-vinylphenol	12.4-13.1	Lignin
2,6 dimethoxyphenol	13.2-13.6	Lignin
2-methoxy-4-(1-propenyl) phenol	14.2	Lignin
2-Methoxy-4-(2-propenyl) phenol	12.6	Lignin
vanillin	14.4	Lignin

The distribution of products obtained from pyrolysis of walnut shell lignin and olive pit lignin is similar, except 4-methylphenol constitutes a higher area % for the pyrolysis of walnut shell lignin. Also, walnut shell pyrolysis appears to produce a higher amount of 2-methoxy-4-vinyl phenol and lower amount of 2-methoxy-4-(2-propenyl) phenol although these two compounds are very close in retention times and the sum of their areas is similar to the sum of the areas for these two compounds for olive pit pyrolysis. The production of these two compounds is the result of pyrolysis of the same monomeric unit within the lignin structure, coniferyl alcohol.
It should also be noted that different amounts and types of lignin pyrolysis products are seen when the lignin is pyrolyzed separately from the hollocellulose fraction for each biomass sample. This is not surprising and indicates that the pyrolysis process is altered depending on the coexistence and variation in linkages between the individual biopolymers.

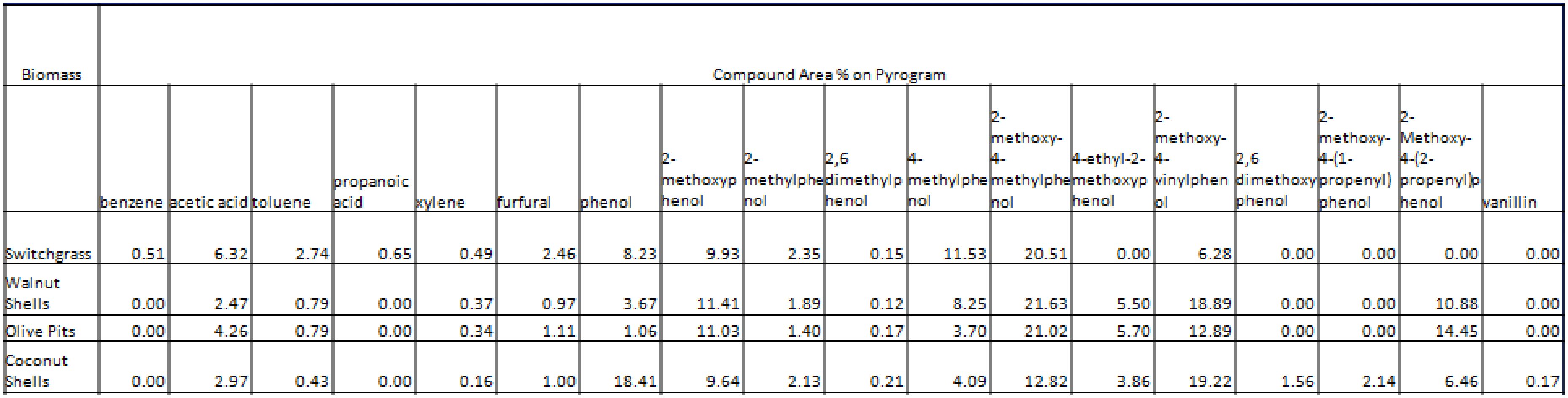
In conclusion, pyrograms show that walnut shells, coconut shells, olive pits, switchgrass vary in their lignin and hollocellulose content, causing a variation in pyrolysis product distribution. Switchgrass contained the least amount of lignin and coconut shells show a much larger increase in the production of phenol when compared to pyrolysis of the other samples. Pyrolysis products from whole biomass contained large amounts of acetic acid and furfural whereas these compounds are produced only in small amounts from the corresponding lignin fractions. Overall, these results serve to illustrate the potential of high-lignin endocarp feedstocks such as coconut and walnut shells, which are readily available as agricultural waste from horticultural crops, to generate valuable chemicals by thermochemical deconstruction.

Table 3. Comparison of compounds produced from whole biomass pyrolysis at 650 ºC



Biomass	Compound Area % on Pyrogram															
	hydroxy acetaldehyde	acetic acid	1-hyroxy-2-propanone	acetone	furfural	2 (5H) furanone	phenol	2-methoxyphenol	2-methylphenol	4-methylphenol	2-methoxy-4-methylphenol	4-ethyl-2-methoxyphenol	2-methoxy-4-vinylphenol	2,6 dimethoxyphenol	2-methoxy-4-(1-propenyl) phenol	vanillin	SUMLIGNIN
Switchgrass	5.11	18.41	16.18	10.82	8.42	3.93	0.00	3.31	0.00	0.89	0.00	0.00	1.02	0.00	0.00	0.00	5.21
Walnut Shells	3.36	11.77	5.36	5.01	3.32	3.32	3.84	10.66	1.41	1.90	8.83	2.00	1.37	0.00	0.00	0.00	30.01
Olive Pits	3.91	12.96	6.76	5.20	4.08	2.60	3.76	11.65	1.73	0.00	4.90	0.60	0.00	0.00	0.00	0.00	22.63
Coconut Shells	3.00	12.49	4.52	4.37	2.86	2.27	15.96	7.40	1.44	1.61	3.90	0.90	1.56	0.00	0.00	0.00	32.75

Table 4. Comparison of compounds produced from lignin pyrolysis at 650 ºC



Biomass	Compound Area % on Pyrogram																
	benzene	acetic acid	toluene	propanoic acid	xylene	furfural	phenol	2-methoxyphenol	2-methylphenol	2,6 dimethylphenol	4-methylphenol	2-methoxy-4-methylphenol	4-ethyl-2-methoxyphenol	2-methoxy-4-vinylphenol	2,6 dimethoxyphenol	2-methoxy-4-(1-propenyl) phenol	2-Methoxy-4-(2-propenyl)phenol	vanillin
Switchgrass	0.51	6.32	2.74	0.65	0.49	2.46	8.23	9.93	2.35	0.15	11.53	20.51	0.00	6.28	0.00	0.00	0.00	0.00
Walnut Shells	0.00	2.47	0.79	0.00	0.37	0.97	3.67	11.41	1.89	0.12	8.25	21.63	5.50	18.89	0.00	0.00	10.88	0.00
Olive Pits	0.00	4.26	0.79	0.00	0.34	1.11	1.06	11.03	1.40	0.17	3.70	21.02	5.70	12.89	0.00	0.00	14.45	0.00
Coconut Shells	0.00	2.97	0.43	0.00	0.16	1.00	18.41	9.64	2.13	0.21	4.09	12.82	3.86	19.22	1.56	2.14	6.46	0.17

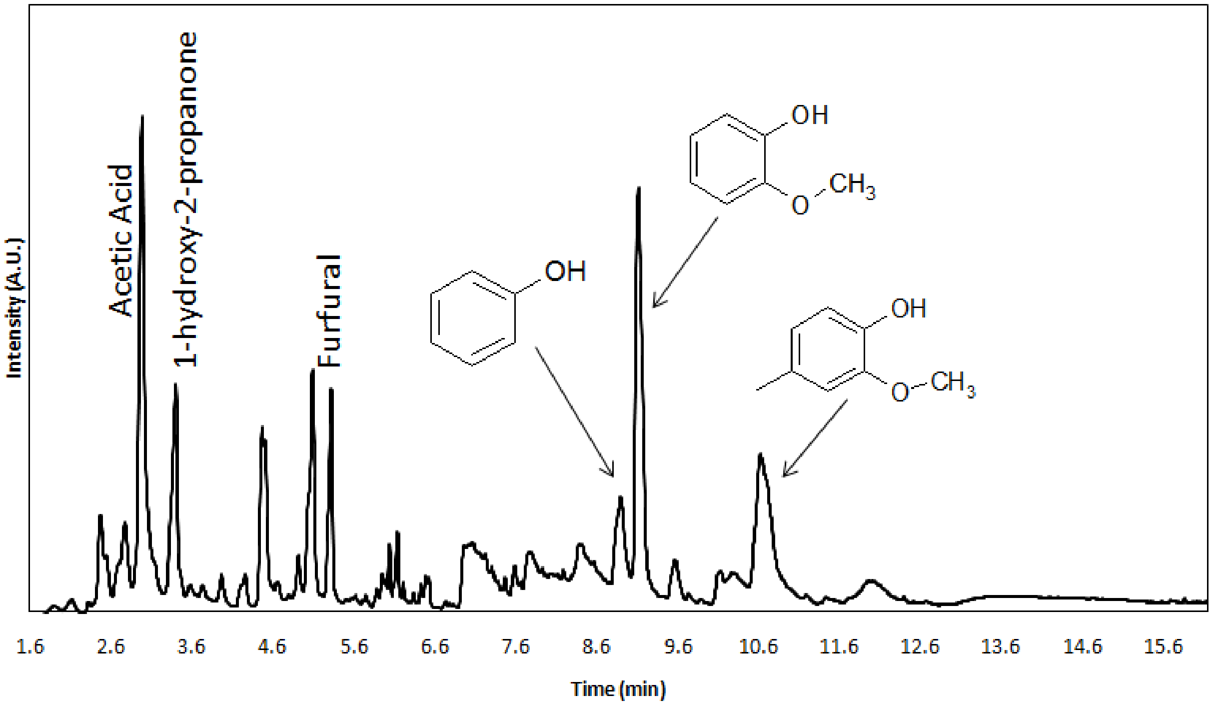


Figure 5.Pyrogram resulting from walnut shell pyrolysis at 650 °C.

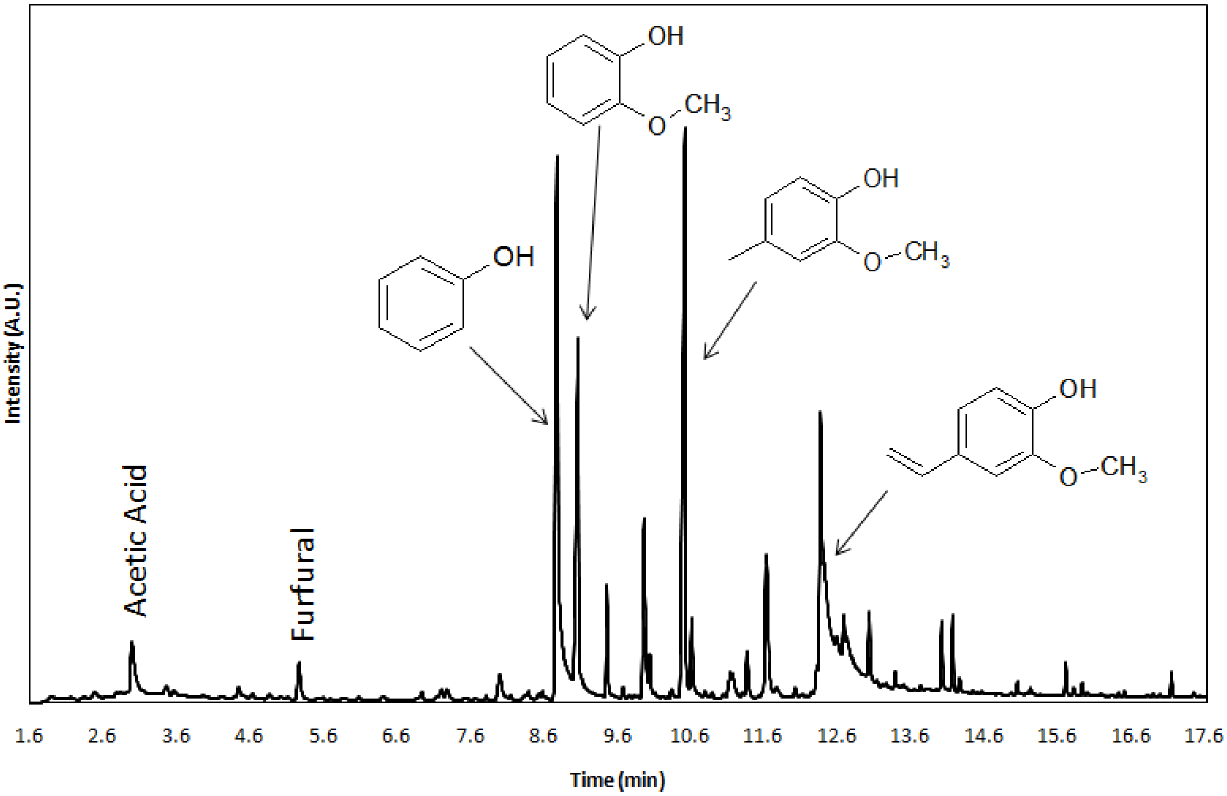


Figure 6. Pyrogram resulting from coconut shell lignin pyrolysis at 650 °C.

(ii) HPLC analysis of lignin and its deconstruction products
The development of a robust HPLC protocol for the analysis of lignin and its deconstruction products has constituted a second important goal in this task. Without such an analytical method, experiments aimed at the catalytic deconstruction of lignin would be severely hampered. After examining numerous literature procedures, as well as investigating new approaches, a new protocol was developed which affords results which we consider to be both repeatable and reliable. A mobile phase of 50/50 dimethylsulfoxide (DMSO)/water was chosen on the basis that it allows for complete dissolution of lignin, which is a critical pre-requisite for size-exclusion analysis. A flow rate of 0.5mL/min for 50 min was found to optimal for the analysis, while 100% HPLC grade DMSO was used as the solvent. Under these conditions, satisfactory chromatograms were obtained for switchgrass lignin using both a Corona Charged Aerosol Detector (CAD) and a UV/VIS diode array detector in the wavelength window of 260-320 nm. Applying this analytical protocol, preliminary lignin deconstruction experiments are currently being performed under the auspices of a NSF-sponsored project focusing on lignin utilization.

Phase 3 Tasks:

Task number: 1.0 Acquisition and Installation of Laboratory Instrumentation

Task 1.0 focused on the acquisition of the instrumentation necessary to support the Phase 3 initiatives of the Biofuels Laboratory. To this end, a Fourier transform-infrared (FT-IR) spectrometer was acquired, together with various smaller equipment items, including an ATR-IR cell.

Task 2.0 Screening of Algal Strains

Task 2.0 concerns the cultivation of algal strains at various temperatures, salinities, pH values, media concentrations and light levels with the ultimate goal of identifying algae strains that are tolerant of conditions associated with coal-derived flue gas. *Scenedesmus* and *Chlorella vulgaris* were first cultured in a media study to select a medium that would yield the best growth rate. Results showed that a urea medium (utilizing industrial fertilizer urea as the nitrogen source) is the best suited for cultivation of the aforementioned microalgae. Next, both algal strains were cultured in a buffered urea medium under various acidic conditions (0-120 ppm H_2SO_4) and 3% CO_2 for four days. The buffered medium (1 g/L $NaHCO_3$) stabilized and maintained pH at 6.7-6.9 (the ideal pH range for *Scenedesmus* and *Chlorella vulgaris*). Compared to *Chlorella vulgaris*, *Scenedesmus* adapted to and thrived in the acidic environment with a higher growth rate.

Microalgae were also tested for their tolerance to ashes produced from nearby Kentucky coal-firing plants: Dale, Spurlock, Gillbert, and Tyrone. In the first study, *Scenedesmus* and *Chlorella vulgaris* were tested for ash toxicity. They were each cultured in a 500 mL Erlenmeyer flask containing 400 mL urea media and 2 g ash. Both *Scenedesmus and Chlorella vulgaris* adapted to and thrived (with a short lag phase) in media containing Tyrone ash. Tyrone ash is the least treated of the ashes tested. The extra particulates and minerals in the ash acted as nutrients for algal absorption. For Dale, Spurlock, and Gilbert, algae slowly adapted to the ashes and recovered towards the end of a four day experiment. Next, algae were exposed to smaller amount of ashes (0-1 g ash per 400 mL culture volume) to determine the level of growth inhibition. Results showed no algal growth inhibition at lower levels of ash for either algal strain.

In our continuing effort to identify viable algae strains for flue gas utilization (i.e., for CO_2 capture and utilization), *Chlorella vulgaris*, *Scenedesmus* sp., and *Neospongiococcum bisorpum* were cultured in a comparison study at 3% CO_2, a pH of 5.5, a temperature of 35°C and 35% RH in either urea (*Chlorella* and *Scenedesmus*) or Waris (*Neospongiococcum bisorpum*) media. *Neospongiococcum bisorpum* had the highest growth rate of 0.0029 g dry weight/L/h, while *Chlorella* and *Scenedesmus* both had a similar growth rate of 0.0027 g dry weight/L/h. This result indicates that *Neospongiococcum bisorpum* would be a viable strain for cultivation in the elevated temperatures during experiences during the summer months in Kentucky. However, based on results from the media, ash toxicity/tolerance, and temperature studies, *Scenedesmus* is currently the preferred algae strain for cultivation using flue gas.

Task 3.0 Anaerobic Digestion of Algae Biomass

Anaerobic digestion of algae (before and after oil extraction) is of interest in order to evaluate biogas production as a cost effective means of utilizing algae biomass for the production of energy. An active sludge (contains groups of active bacteria, hereby referred to as the inoculum) was collected from a local wastewater treatment facility (West Hickman Treatment Plant) located in Nicholasville, Kentucky. The total solids of sludge were determined by drying triplicate samples at 105°C for 24 hours. The dried samples were then cooked in the furnace at 550°C for 1 hour to determine the volatile solids content. The total solids content was measured at 14.59% while the volatile solids value was measured at 11.55%. The initial algae slurries intended for testing consists of 3% dry weight and 97% water. In future work the COD (chemical oxygen demand) of the algae will be first measured. Based on the COD value, the amount of inoculum required for anaerobic digestion will be determined on a ratio basis (COD/volatile solids of inoculum). An initial experiment will be conducted to determine an appropriate ratio to use for maximum methane gas production.

Task 4.0 Thermochemical Conversion of Algae Biomass to Liquid Fuels

Work in FY2011 focused both on the pyrolysis of whole algae (as described under the Phase 2 activities), as well as the conversion of triglycerides to hydrocarbons via decarboxylation. Initial studies focused on uncatalyzed reactions in order to ascertain the contribution of thermal reactions to subsequent catalyzed experiments. In addition, a brief study was performed with the aim of assessing the potential of glycerol carbonate synthesis as an outlet for glycerol utilization. This is of relevance in a Kentucky context due to the significant amount of biodiesel (fatty acid methyl esters), and hence glycerol, produced in the state. Currently, this biodiesel is produced almost exclusively from soybean oil, although in the future algae oil might conceivably serve as a feedstock.

(i) **Thermal decarboxylation of triglycerides:**
Experiments were conducted at 300 psig in a fixed-bed reactor (9 mm x 100 mm, d x l). The reactor bed was packed with glass beads of 1 mm average diameter. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min (STP). Soybean oil (as a surrogate for algae oil) was fed to the reactor at 10.64 g/h, giving a nominal LHSV of 1 h^{-1}. Tables 5 and 6 show the conversion of SBO and the yields of the main fractions in the liquid product for experiments conducted at different temperatures under both atmospheric pressure and 300 psig pressure. The conversion

was estimated based on the total yield of the product obtained, i.e., conversion = 100 x (weight of liquid product + gas + coke)/weight of feed, where weight of liquid product = product fraction (BP<375^{o}C) x total weight of liquid recovered. Note that for the purposes of this calculation, all of the liquid components boiling above 375 ^{o}C are considered to be starting material (SBO), while everything below this cut point is considered product. The diesel-like fraction is defined to be the components from C10 to C18 with boiling point from 177^{o}C to 325^{o}C, while C17 and C18 are defined to be the fractions with boiling points ranging from 295^{o}C - 314^{o}C and 314^{o}C - 325^{o}C, respectively. Likewise, palmitic acid is assumed to be the fraction with boiling point from 330^{o}C to 341^{o}C and stearic acid to be the fraction with boiling point from 352^{o}C to 368^{o}C.

Table 5. Conversion of soybean oil (SBO) and product yields from thermal cracking under atmospheric pressure

Temp, °C	Yield (% of liquid product)					
	Conversion (%)[1]	**Diesel-like fraction**	**C17**	**C18**	**Palmitic acid**	**Stearic acid**
350	15	7.3	0.6	0.4	2.3	4
375	33	10.4	1.4	0.5	4.6	15
400	51	15.1	3.0	1.0	5.9	15.5
450	65	29	5.4	2.0	8	17
500	67	33.4	5.4	2.0	7.1	16

[1] Conversion% = 100 x (weight of liquid product + gas + coke)/weight of feed, where weight of liquid product = product fraction (BP<375^{o}C) x total weight of liquid recovered.

Table 6. Conversion of SBO and product yields from thermal cracking under 300 psig pressure

Temp, °C	Conversion	Yield (% of liquid product)				
	(%)	**Diesel-like fraction**	**C17**	**C18**	**Palmitic acid**	**Stearic acid**
350	19	7.3	0.9	0.4	2.8	4
375	29	8.4	1.4	0.5	4.6	9
400	61	20.9	4.3	1.3	7.4	23
450	73	46.6	9.0	2.8	5.6	5
500	74	54.1	7.0	2.6	3.3	3

As shown in Figure 7, the conversion of SBO increased from 16 wt% to 67 wt% when the cracking temperature was increased from 350^{o}C to 500^{o}C at atmospheric pressure. Correspondingly, the SBO conversion can reach more than 80 wt% at 500^{o}C under 300 psig pressure. Indeed, the conversion of SBO is higher under 300 psig pressure than under atmospheric pressure at all cracking temperatures except at 375^{o}C. Interestingly, the yields of a number of the components (C17, C18, palmitic acid and stearic acid) increase with cracking temperature initially and then decrease when the cracking temperature is above 400°C or 450°C. In the case of stearic and palmitic acid, these data suggest that the acids undergo decarboxylation at high temperatures to afford hydrocarbons (C17 and C15, respectively).

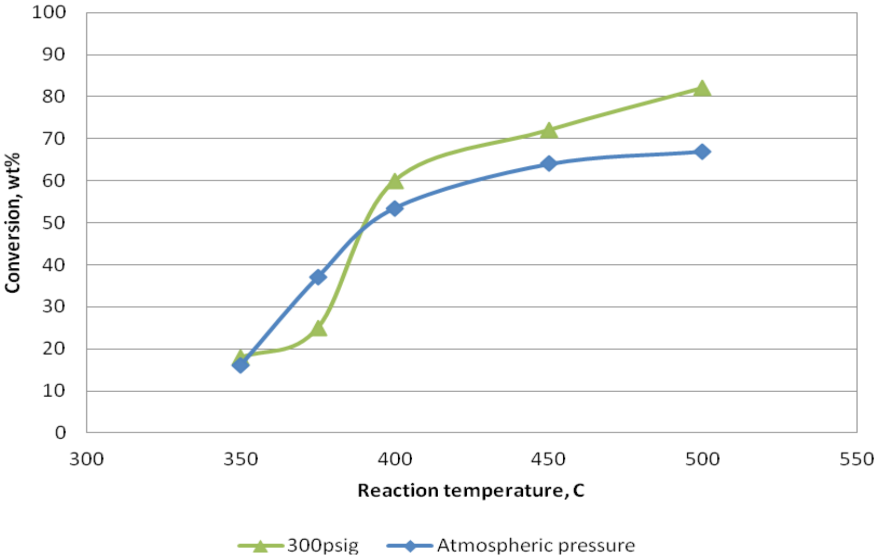


Figure 7. Effect of reaction pressure on the conversion of SBO at the different cracking temperatures

Figure 8 and 9 show the yields of the total liquid product and the diesel-like fraction as a function of temperature for the thermal cracking of SBO at atmospheric pressure and 300 psig, respectively. In both cases, the yield of total liquid product decreases with increasing cracking temperature. Figure 8 shows that the yield of the diesel-like fraction (i.e., total liquid yield x % diesel-like fraction in liquid) initially increases with the cracking temperature, until it approaches *ca*. 30 wt% at 500°C under atmospheric pressure. Under 300 psig the yield of the diesel-like fraction reaches 37 wt% at 450°C, but then decreases to 34 wt% at 500°C because the amount of liquid product drops significantly. A comparison of Figures 8 and 9 shows that the yields of the diesel-like fraction obtained at 300 psig are higher than those obtained at atmosperic pressure. Overall, these results show that the production of diesel-like product is favored by high reaction pressures and by temperatures in the range 450-500ºC; however, yields are fairly modest. Consequently, future efforts will focus on catalytic decarboxylation, using semi-batch and fixed bed reactors.

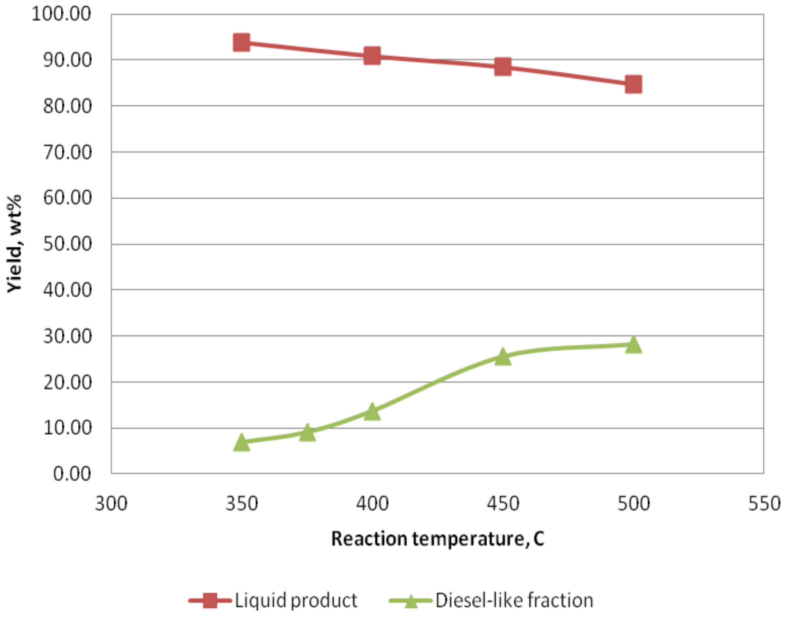


Figure 8. Yields of liquid product and diesel-like fraction obtained from thermal cracking of SBO at atmospheric pressure

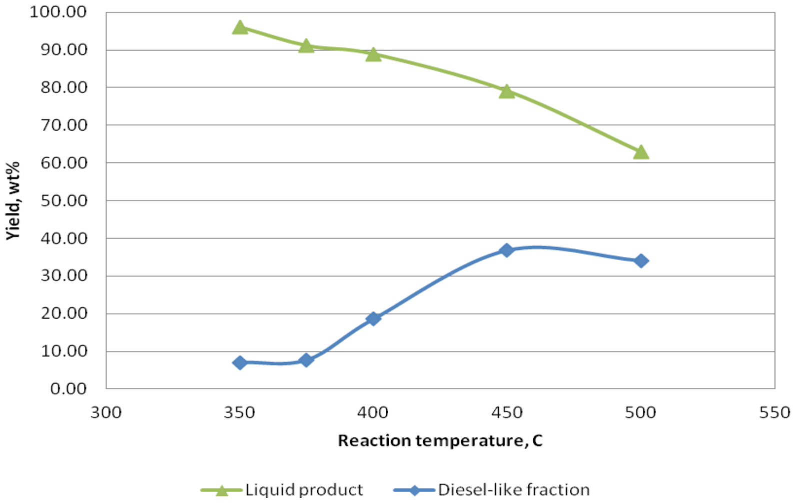


Figure 9. Yields of liquid product and diesel-like fraction obtained from thermal cracking of SBO at 300 psig

(ii) A catalytic approach to the synthesis glycerol carbonate from glycerol and urea: Glycerol is a co-product of biodiesel production from the transesterification of oils with methanol. As biodiesel production has increased in recent years, and supply of glycerol has begun to outstrip demand, the market price of glycerol has fallen sharply. Consequently, the development of new applications for glycerol represents a means of improving the overall economics of biodiesel production. One potential application which appears particularly promising is the synthesis of glycerol carbonate from glycerol. Glycerol carbonate is used in numerous and diverse applications due to its unique properties. For instance, glycerol carbonate is an ideal bio-lubricant and solvent due to high adhesiveness and chemical stability, as well as its low vapor pressure. Glycerol carbonate can also be a promising starting material for polymer production. While several methods have been reported for the synthesis of glycerol carbonate, we have recently focused on the synthesis of glycerol carbonate from glycerol and urea. This pathway was selected based on the low cost of the urea reactant and the relatively mild reaction conditions:

In a typical run, 27.6 g of glycerol and 2.76 g of TEG was first added to a round-bottom flask with volume of either 100ml or 250 ml equipped with a condenser and purged in flowing N_2 for 10 min. Next, 27.0 g of urea and 0.552 g of catalyst were added to the reactor to start the reaction. Samples were taken during the run by syringe, diluted with methanol and quenched in iced water. After removing the catalyst using a PTFE micro-filter, the filtrate was analyzed by GC. Tetraethylene glycol (TEG) was selected as an internal standard for sample analysis due to its high boiling point (~330 ^{o}C) and thermal stability. Calibration was accomplished by analyzing a series of standard mixtures, which were prepared from glycerol, glycerol carbonate and TEG dissolved in methanol.

Following literature leads suggesting that the reaction of glycerol with urea is catalyzed by Lewis acids, a Zn-Al layer doubled layer hydroxide (Zn/Al= 75:25) was tested as a catalyst for this reaction. The catalyst was calcined at 450 ºC in air for 2 h prior to use in order to convert it to the mixed oxide. The glycerol conversion and glycerol carbonate (GC) selectivity from the different runs are shown in Table 7.

Table 7. Results of glycerol carbonate experiments

Entry	**Temp (^{o}C)**	**Time (h)**	**Glycerol conv. (%)**	**GC yield (%)**	**GC sel. (%)**
1	120	3	31	16	51
2	140	3	65	32	49
3	140	4	53	46	86
4	160	1	54	38	70
5	160	2	59	18	30
6	160	3	67	2	3

N_2 flow rate = 60 ml/min, urea/glycerol molar ratio = 1.5, pressure = ambient

Evidently, temperature is a critical factor in this reaction. Increase of the temperature from 120 to 140 ºC is beneficial for GC production. However, although a very reasonable glycerol carbonate conversion and GC yield was obtained during the first hour of the run at 160 ºC, the GC yield showed a dramatic drop with time. This suggests that the GC formed may undergo side reactions. Future work will aim at identifying the other products formed and closing the mass balance for the glycerol + urea reaction.

This preliminary work demonstrates that a catalytic approach can be used to synthesize glycerol carbonate from glycerol. Additional work will focus on further improvement of the glycerol conversion and glycerol carbonate yield by optimizing relevant parameters, evaluating alternative catalysts and also understanding the kinetics of glycerol carbonate formation.

Task 5.0 Techno-economic Feasibility Study of Biofuels Production from Algae

In the coming year a techno-economic analysis will be conducted to evaluate the available options for biofuels production from algae. Work on this task will commence once sufficient data have been collected under Task 4.

Conclusions

The University of Kentucky Biofuels Laboratory is equipped and functioning. Research in the past year has focused on the cultivation of algae strains suitable for biofuels production, together with the study of thermochemical methods for the conversion of algae biomass to fuels. A third focus area has concerned the development of analytical protocols for the analysis of lignin and its depolymerization products, to support an on-going NSF-sponsored research project aimed at the development of (thermo)chemical methods for the deconstruction of lignin to fuels and chemicals. Based on results from media, ash toxicity/tolerance, and temperature studies, *Scenedesmus* sp. has been identified as the preferred algae strain for cultivation using flue gas derived from coal-fired power plants (containing significant concentrations of SO_2 and fly ash). Pyrolysis studies performed on *Scenedesmus* sp. indicate that microalgae can be pyrolyzed to produce bio-oil and a biochar rich in ash, although the bio-oil would require upgrading (hydrotreating) prior to use for most fuel applications. Thermal cracking studies have also been performed on soybean oil as a model for algae oil. Results show that the production of diesel-like product is favored by high reaction pressures and by temperatures in the range 450-500ºC; however, yields are fairly modest. Consequently, on-going efforts in this task are focused on catalytic approaches to triglyceride decarboxylation. Finally, Py-GC-MS and HPLC protocols have been developed for the analysis of lignin. Differences in product yield and molecular species distribution among feedstocks evaluated using Py-GC-MS have illustrated the potential of high-lignin endocarp feedstocks (readily available as agricultural waste from horticultural crops) to generate valuable chemicals by thermochemical deconstruction.

Patents

None.

Publications / Presentations

1. J. Mobley, L. Ware, E. Santillian-Jimenez, A. Placido, R. Andrews, M. Crocker, S. DeBolt, M. Meier, S.A. Morton III, “Dissolution and Analysis of Lignin-Rich Biomass Feedstocks”, 2011 Kentucky Renewable Energy & Energy Efficiency Workshop, Louisville, KY, March 14, 2011.

2. S. Mohapatra, T. Morgan, E. Santillan-Jimenez, M. Crocker, “Conversion of Triglycerides and Fatty Acids to Hydrocarbons over Supported Nickel Catalysts”, poster presentation (P-Tu-53) at the 22nd North American Catalysis Society Meeting, June 5-10, 2011, Detroit, MI.

3. L. Ware, T. Morgan, M. Wilson, S. Mohapatra, M. Crocker, J. Zhang, K. Liu, “Fast pyrolysis of *Scenedesmus* algae”, poster presentation at the 242nd ACS National Meeting, Denver, CO, August 28-September 1, 2011, FUEL-152; Preprints of Symposia - American Chemical Society, Division of Fuel Chemistry, 2011, 56(2), 533-534.

4. V. Mendu, A.E. Harman-Ware, M. Crocker, J. Jae, J. Stork, S. Morton III, A. Placido, G. Huber, S. DeBolt, “ Identification and thermochemical analysis of high-lignin feedstocks for biofuel and biochemical production”, Biotechnol. Biofuels, 4 (2011) 43.

5. A.E. Ware, T. Morgan, M. Wilson, M. Crocker, J. Zhang, K. Liu, J. Stork, S. DeBolt, “Microalgae as a renewable fuel source: fast pyrolysis of *Scenedesdmus* sp.”, submitted to Biomass Bioenergy.

6. T. Morgan, E. Santillan-Jimenez, A.E. Harman Ware, Y. Ji, D. Grubb, M. Crocker, “Catalytic deoxygenation of trigylcerides to hydrocarbons over supported nickel catalysts”, submitted to *Chem. Eng. J*.

Outreach Activities

In keeping with the open laboratory objective, experiments and sample analysis have been completed at the request of other institutions, as well as other research groups within the University of Kentucky. These requests have included the following:

American Science & Technology, Inc.: GC analysis of biodiesel samples, bomb calorimetry of biomass samples.

Eclipse Renewables: Evaluation and steam activation of carbon derived from fast pyrolysis.

Sapphire Energy: Thermochemical processing of algae.

University of Kentucky Center for Applied Energy Research (UK CAER): members of the Power Generation and Utility Fuels Group submitted samples for gas chromatographic analysis (consisting of gas and liquid bio-oil samples produced from the fast pyrolysis of biomass).

Department of Biosystems and Agricultural Engineering, University of Kentucky: GC analysis of gas sampled from biomass storage silos.

To further publicize the fact that the UK Biofuels Laboratory is available to external institutions, the CAER public relations department has created a website, http://www.caer.uky.edu/renewablefuels/lab.shtml, outlining the laboratory’s analytical capabilities and providing appropriate contact details.

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