Project Description - A sulfur-based, carbon-negative pathway for biomass for bioenergy production

1. Introduction

New energy technologies are required to address global climate change and new bioenergy technologies are particularly promising because bioenergy is one of the few renewable energy sources that can generate dispatchable, baseload electricity and can be used to create substitutes for transport fuels through thermal, chemical or biological conversion. However, bioenergy systems can only be considered sustainable if the biomass on which they are based can be sustainably sourced. All bioenergy sources are fundamentally limited by the availability of biomass feedstocks, and the growth of these feedstocks generally require arable land, fresh water, and solar energy, all of which are demanded by other users and this almost always reduces the overall sustainability of the system.

Furthermore, with the possible exception of algal production systems, biomass for bioenergy production systems use the same agricultural species and basic technologies that humans have used for centuries; however, biosystems engineering requirements for biomass for bioenergy production are significantly different from food production systems, and new approaches are needed. While there continues to be significant research investment on new thermal, chemical and biological methods for the conversion of biomass to higher-value energy products, research on new, transformational approaches to the biosystems engineering of growing biomass for bioenergy are also needed.

One novel method for the growth of biomass for bioenergy is the use of sulfur-oxidizing bacteria such as *Thiomicrospira crunogena*. *T. crunogena* has a number of advantages over conventional biomass-producing species:

- It grows in seawater and thus has no impact on freshwater supplies;
- In is chemolithotrophic and requires no sunlight or energy source beyond reduced sulfur;
- It can be grown at extremely high densities, eliminating the need for scarce land resources;
- It is a bacteria, has been genetically sequenced, and is thus more amenable to eventual genetic manipulation than eukaryotes (Scott et al. 2006).

However, sulfur-oxidizing bacteria have not been evaluated for their potential as bioenergy "crops". Here, we seek to analyze the biological and economic potential of the use of *T. crunogena* as a novel biomass for bioenergy production system. We envision a system in which *T. crunogena* is grown and the biomass pyrolyzed to create valuable energy products and biochar; the biochar is then stored in the soil to create a carbon negative process, and we evaluate the biologic and techno-economic feasibility of this concept.

2. Specific Aims

Specifically, we will:

1. Determine the growth rate of *T. crunogena* under varying concentrations of molecular sulfur, nitrogen and phosphorus, and the consumption of molecular sulfur and nutrients per unit of dry biomass produced;
2. Determine the growth rate and nutrient removal capability of *T. crunogena* using marine aquacultural wastewater as a growth medium.
3. Determine the maximum dry biomass production rate of *T. crunogena* in continuous culture under nutrient enriched and non-enriched conditions;
4. Analyze the energetic content of *T. crunogena* and the products of biomass pyrolysis;
5. Use the data collected in Specific Aims 1-4 to develop and parameterize a bio-economic model of a hypothetical large-scale system for the growth of *T. crunogena* to estimate the production costs per dry ton of biomass and the costs of an integrated biomass pyrolysis system.

3. Background

***Bioenergy sustainability*.** Bioenergy systems can be used to generate electricity as well as gas and liquid fuels and therefore have the potential to replace hydrocarbon sourced energy systems with relatively modest modifications to the energy logistical infrastructure and end-user technology. However, there are two major issues impeding the development of the bioeconomy. First, current approaches for the conversion of biomass feedstocks into higher-value fuels are expensive or inefficient, and better technologies are under development and a focus of NSF funding.

Secondly, and just as critically, biomass feedstocks typically require arable land which could otherwise be used for food production. The conversion of agricultural land from food production to biomass for bioenergy production has the potential to reduce food supplies and increase prices, a special concern in the developing world. One proposed solution is to use agriculturally marginal lands for bioenergy production, but these marginal agricultural lands may be important for biodiversity and their conversion to bioenergy ecosystems may negatively impact biodiversity. A sustainable bioeconomy requires economically efficient biomass conversion systems as well as ecologically sustainable biomass production systems, yet at present, a large scale, sustainable method for biomass for bioenergy production is not well established. The purpose of the present proposal is to develop a novel, sustainable, and scalable biomass production system.

Thiomicrospira crunogena. *T. crunogena* is a motile, rod-shaped, gram-negative bacterium isolated from deep-sea hydrothermal vents near the Galapagos rift in the early 1980s (Ruby and Jannasch 1982). In the lab, it is typically grown in an artificial seawater medium supplemented with thiosulfate, but can also grow on sulfide or molecular sulfur. It is mesophilic, growing well at temperatures of 25-28°C, and at these temperatures it can double in as little as 35 minutes. Its

rapid reproduction and growth at moderate temperatures makes it an excellent candidate for biomass for bioenergy utilization. In addition, *T. crunogena* has a carbon concentrating mechanism (Dobrinski et al. 2005) which allows it to grow in nearly anaerobic conditions.

Sulfur as a chemosynthetic substrate. Sulfur is the fifth most abundantly produced element in the world and the 13th most abundant element in the earth's crust. While elemental sulfur can be mined, it is more typically produced via the oxidation of the hydrogen sulfide that occurs in sour natural gas and oil. In 2013, approximately 70 million tons of molecular sulfur were produced worldwide. As the world's supplies of easily accessible sweet gas and oil decline, the importance of sour gas and oil will increase, and global production of elemental sulfur is also expected to increase. For example, the Athabasca oil sands and the sour gas fields in Qatar both have high quantities of sulfur which must be removed from the hydrocarbons before combustion. Thus, there is abundant molecular sulfur both now and in the future which could be used to support a sulfur-based bioenergy industry. Due to the large production of molecular sulfur, it is relatively inexpensive, selling for $125 per metric ton in 2013. A low price of sulfur is critical to the economic feasibility of the system and is a reason to use a sulfur oxidizing bacteria over other chemotrophs that grow on reduced iron or hydrogen or electrosynthetic microorganisms.

Biomass pyrolysis. Pyrolysis is the thermal decomposition of organic material and is accomplished by the heating of carbonaceous material in the absence of oxygen. Pyrolysis is endothermic but produces three energy rich products: biochar, bio-oil and syngas. Biochar is a fine biologically derived charcoal. When used as a soil additive, biochar can increase crop productivity (Lehmann 2007) by improving nutrient and water retention, increasing cation exchange capacity (Laird et al., 2009), and increasing mycorrhizal and microbial growth (Warnock et al., 2007; Laird et al., 2009). The half-life of biochar in the soil is not well known, but it is thought to be on the order of centuries to millennia. Biochar was intentionally applied to soils in the Amazon Basin (terra preta) over 1,000 years ago; these soils currently contain 2.5 times as much carbon as adjacent non-amended soils (Glaser et al., 2001). Given that the effects of climate change are on the timescale of decades, biochar added to the soil can be thought of as a relatively long-term carbon sink. Thus, the model system can create a CO_2 negative process in which the growth of *T. crunogena* removes CO_2 from the atmosphere and its pyrolysis creates a long-lived carbon sink.

Bio-oil often is considered the most economically promising product of the pyrolysis system. Bio-oil is a complex mixture of organic compounds. It is hydrophilic, immiscible in petroleum based oils and contains 15 to 30% water (Bridgewater 2002). It has an energy content that is approximately half of the energy content (LHV = 13 to 18 MJ/kg) of diesel fuel (40% by weight; 60% by volume). Bio-oil can be used directly without further processing; however, the low energetic value precludes its use as a transportation fuel and while raw bio-oil has been shown to be operational in diesel engines, there are concerns about the effects of the oil on the engine, the flow of the oil in cold temperatures, and other technical problems (Briens et al., 2008).

However, after processing, bio-oil has a number of potential uses. Processed bio-oil could be utilized as a transportation fuel, especially as a blend with diesel fuel, or may produce high value products such as liquid fuels, fertilizers, acetic acid, food flavorings, and adhesives (Briens et al., 2008).

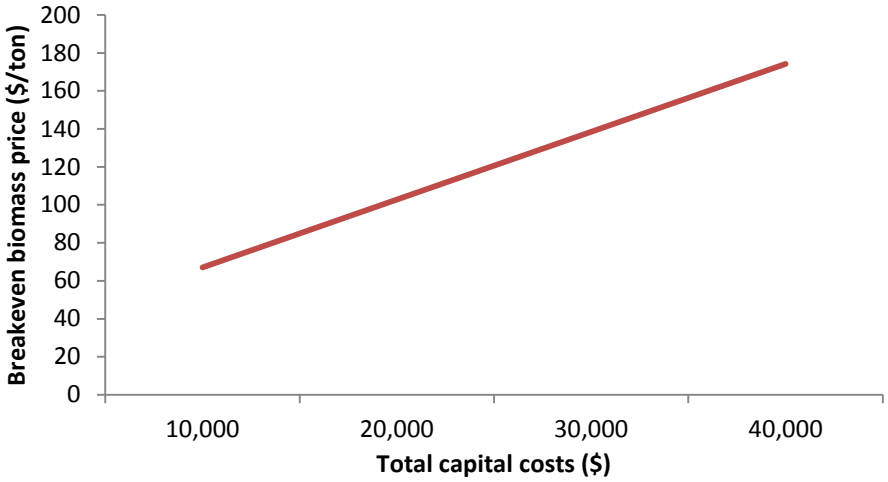
The syngas derived from pyrolysis consists primarily of H_2, CO, CO_2, and CH_4. Syngas can be used to power the pyrolysis reactor, may be combusted to produce electricity for sale, or may be further separated to produce feedstocks for industrial processes.

4. Prior Research

Electrofuels. The U.S. Department of Energy Advanced Research Projects Agency (ARPA-E) funded an "electrofuels" program based on the concept of using renewable electricity and/or hydrogen as an energy source for microbial growth and biofuel production via chemosynthesis or electrosynthesis (Hawkins et al. 2011; Lovely and Nevin 2013; Girguis and Holden 2012; Conrado et al. 2013). However, none of the funded electrofuels programs investigated the use of a sulfur-oxidizing bacteria and most were focused on the genetic engineering of model systems (e.g. engineering *E. coli* to take advantage of electrosynthesis). Thus, while the present proposal is not the first to attempt to decouple bioenergy production from photosynthesis, it is the first to focus on the use of sulfur-oxidizing, chemolithoautotroph for biomass for bioenergy (but see Syed et al. 2006 for the use of a sulfur-oxidizing bacteria for non-biomass for bioenergy purposes). Because of sulfur's availability and the lack of a need for genetic engineering, *T. crunogena* has important advantages over the proposed electrofuels systems. Further, all of the electrofuels projects focused on liquid transportation fuels and did not include a carbon negative component.

Preliminary Studies. In preliminary work, the PI has developed a simple bio-economic net present value model of the production costs of *T. crunogena* on a small scale and has found them to be relatively competitive, depending on the capital costs of the reactor. Figure 1 depicts the break-even costs of a 10,000 L system under a range of capital expenditures; break-even costs range from approximately $67 to $174 per ton of dry biomass. While these prices exceed prices for switchgrass or other common bioenergy crops (Mooney et al. 2009; Khanna et al. 2008) they are well within an order of magnitude and given the novelty of the system, are promising.

Figure 1. Breakeven costs of biomass under varying assumptions of the capital costs of a 10,000 L *T. crunogena* growth chamber.



However, many of the parameters used to generate the results depicted in Figure 1 are poorly understood. For example, to generate the data depicted in Figure 1, we assumed a sulfur consumption of 10 g/min based on known growth rates and sulfur concentrations (Wirsen et al. 1998; Jannasch et al. 1985), but the actual sulfur consumption rate is unknown and is a major factor in the costs of biomass production. While we consider the assumptions used to generate Figure 1 realistic, a major goal of the present proposal is to validate the growth rate, sulfur consumption rate and other parameters to better define economic relations of the type depicted in Figure 1.

Previous Metabolic Studies. There have been several prior studies on the metabolism and growth of *T. crunogena* (Wirsen et al. 1998; Jannasch et al. 1985; Ruby and Jannasch 1982; Javor et al. 1990). These studies were intended to describe the basic physiology of the organism, as a result, their data, while useful, are not adequate for the present purpose. Furthermore, all of these studies reported data primarily from growth on thiosulfate rather than molecular sulfur. Since thiosulfate is manufactured in small amounts, it is of limited utility for the present purpose. In contrast, molecular sulfur is readily available in industrial quantities, its production is expected to increase, and its price history is well known, all of which are critical for a useful bio-economic evaluation.

5. Methods

Culture. A continuous culture will be established and used as a source for all growth experiments. The culture will be grown at 27° C in an artificial seawater medium supplemented with 8 mM thiosulfate. As a preliminary experiment, 1 mL samples of the culture will be taken and the number of cells counted via dilution and direct cell counts after staining with acridine orange (Jannasch et al. 1985). Cell biomass will also be dried and the weight per cell estimated (Romanova and Sazhin 2010).

Specific Aim 1: *Determine the consumption of molecular sulfur, nitrogen, and phosphorus per unit of dry biomass and the growth rate of T. crunogena under varying concentrations of molecular sulfur, nitrogen, and phosphorus.*

T. crunogena will be grown in liquid batch culture under varying concentrations of sulfur, nitrogen and phosphorous for 12, 24 or 36 hours. Artificial sea water (ASW) cultures will be inoculated with a specified volume of the maintenance culture with a known (estimated) number of cells. Table 1 shows the initial concentrations of sulfur and nutrients to be used. The control treatment represents unsupplemented ASW media. The concentration of phosphorus and potassium will be identical since they are both supplied in the ASW media by KH_2PO_4. All cultures will be replicated five times for a total of 60 cultures.

Table 1. Treatments used to determine maximal growth rates of *T. crunogena*.

	Treatment				
Nutrient	Control	Low	Medium	High	Incubation period (hours)
Nitrogen (as NH_4)	16 mM	16 mM	32 mM	64 mM	12, 24, 36
Phosphorus (as PO_4)	4 mM	4 mM	8 mM	16 mM	12, 24, 36
Sulfur	0%	0.5%	1%	2%	12, 24, 36

Following the incubation period, the pH of the media will be measured using electronic probes while the nitrogen and phosphorus concentrations of the media will be measured using standard spectrophotometry techniques. To determine the sulfur remaining in the media, we will use the spectrophotometric method developed by Javor et al. (1990). Cells will be separated by centrifugation, diluted, stained with acrimide orange and counted.

Data and Results. Specific Aim 1 will provide data on the growth rate of *T. crunogena* under varying nutrient concentrations and the difference between the final and initial concentrations of sulfur and nutrients will inform the consumption rate per cell. Given a known weight per cell, the quantity of sulfur, nitrogen and phosphorus required per unit of biomass will be estimated.

Potential Pitfalls. The treatments are designed to capture the maximum growth rate of *T. crunogena*, however, the maximum growth rate may not be represented by any of the specified treatments. For example, we may observe a linear relationship between the treatments and growth rate, indicating that higher growth rates are possible with higher concentrations of sulfur and nutrients. If this occurs we will simply repeat the protocol with higher concentrations of sulfur, nitrogen and phosphorus. Similarly, a maximum growth rate may exist with low (control) levels of nitrogen and phosphorus and high levels of sulfur; therefore, once the maximum growth rate from the defined treatments is determined, we will alter the concentration of nutrients to ensure we capture the maximum growth rate. Because the experiment is direct and straightforward, we anticipate that this will be possible.

Specific Aim 2: *Determine the growth rate and nutrient removal capability of T. crunogena using marine aquacultural wastewater as a growth medium.*

Batch Culture: Marine aquacultural wastewater (MAW) will be collected from The University of Southern Mississippi's Gulf Coast Research Laboratory and sampled for concentrations of nitrogen and phosphorus. MAW will be autoclaved and supplemented with varying concentration of sulfur and used as a growth media for *T. crunogena* grown in batch culture for 12, 24 or 36 hours. Concentrations of nitrogen and phosphorus will not be manipulated. The sulfur concentrations employed will be similar to and informed by the results from Specific Aim 1. Following the growth period, assays for sulfur, nitrogen and phosphorus will be conducted and the cell biomass and quantity will be determined, as in Specific Aim 1.

Continuous Culture: T. crunogena will be grown in continuous culture in chemostats in MAW supplemented with molecular sulfur. Based on the results of the batch culture experiments described above, a concentration of sulfur will be selected that maximizes the growth rate of *T. crunogena*. MAW will be continuously added to the culture to maintain the nitrogen and phosphorus concentrations near their initial maxima, and *T. crunogena* will be continuously collected from the overflow of the chemostat. Once collected, the *T. crunogena* will be starved as additional sulfur will not be added and growth will eventually cease (note that this starvation system is required to use up available sulfur since the cells tend to form intracellular sulfur globules). The fact that growth will not end immediately upon removal from the chemostat is not a concern since we intend only to maximize biomass production given specified levels of nutrients and sulfur. A flocculent will be used to separate the bacteria from the media in the overflow chamber.

The continuous culture will be maintained for 15 days, and three replications will be performed. Following the 15 day growth period, all biomass will be collected, dried and weighed, and cell counts will be performed.

Data and Results: One potential application of the proposed system is to use *T. crunogena* growth to remove excess nutrients from marine aquacultural wastewater. For the batch experiment, the data will provide an estimate of the growth rate of *T. crunogena* under MAW conditions, and will describe the efficiency with which *T. crunogena* can remove excess nutrients from aquacultural wastewater. For the continuous culture experiment, the data will provide an estimate of the biomass production rate of *T. crunogena* under MAW conditions.

Potential Pitfalls: The continuous culture of *T. crunogena* has been well established by Dr. K.T. Scott (see letter of support) and no major problems are anticipated. We note that the concentration of nutrients in marine aquacultural wastewater will vary seasonally, however, for the present purpose, the MAW media will be collected from an indoor, climate controlled facility with little annual variation.

Specific Aim 3: *Determine the maximum dry biomass production rate of T. crunogena in continuous culture under nutrient enriched and non-enriched conditions.*

Using the results generated in Specific Aim 1, we will identify a combination of sulfur, nitrogen, and phosphorus that yields the highest growth rate of biomass and grow *T. crunogena* in continuous culture at this nutrient concentration. Continuous cultures will last 15 days, and as in Specific Aim 2, overflow from the chemostat will be routed into a starvation chamber where the cells may continue dividing and consume the remaining sulfur. Samples will be collected from the chemostat every 48 hours and tested for nutrient concentrations and cell counts. Following the 15 day growth period, all biomass will be collected, dried and weighed. An identical experiment will be performed with standard artificial seawater media, supplemented only with sulfur (but not nitrogen or phosphorus).

Data and Results: The results of Specific Aim 3 will determine the maximum growth rate of *T. crunogena* in continuous culture and can be compared to the results obtained in Specific Aim 2.

Pitfalls: The continuous culture of *T. crunogena* has been well established by Dr. K.T. Scott (see letter of support) and no major problems are anticipated.

Specific Aim 4: *Analyze the energetic content of T. crunogena and the products of biomass pyrolysis.*

Using the biomass generated via Specific Aim 2, we will determine the energetic content of dry *T. crunogena* using a bomb calorimeter. We will then contract with a third-part laboratory (Conversion And Resource Evaluation Ltd) to have samples of the biomass pyrolyized and these samples analyzed. Three experiments will be conducted corresponding to the three major types of pyrolysis:

- Slow pyrolysis in a moving bed reactor at 450°C with recovery of chars and liquids. During these runs, the pyrolysis gas will be analyzed using a mass spectrometer capable of measuring H_2, N_2, CO, CO_2, CH_4 and C_2 hydrocarbon gases.
- Fast pyrolysis in a fluidised bed reactor at 500°C with full recovery of the fine char and the pyrolysis liquids. Gases will be analyzed online by gas chromatography for H_2, CO, CO_2, CH_4, N_2, C_2H_4, C_2H_6, C_3H_6, C_3H_8. Liquids will be recovered for detailed chemical analysis by GC-MS and water determination by modified Karl-Fischer titration as required.
- Fast pyrolysis in a fluidised bed reactor at 800°C with full recovery of the fine char and the pyrolysis liquids. Gases will be analyzed online by gas chromatography for H_2, CO, CO_2, CH_4, N_2, C_2H_4, C_2H_6, C_3H_6, C_3H_8. Liquids will be recovered for detailed chemical analysis by GC-MS and water determination by modified Karl-Fischer titration as required.

Data and Results: The data will consist of the calorific value of *T. crunogena* and the quantity of the char, liquids, and gases produced by pyrolysis. The pyrolysis data will be used as input in the model described in Specific Aim 5.

Potential Pitfalls: While the pyrolysis of bacteria is well established at the lab scale for microorganism identification purposes (Schmidt et al. 2006), we are not aware of any studies that investigate the pyrolysis of bacteria for bioenergy purposes. Since the cell walls of bacteria and plants are quite different, the results of the pyrolysis reactions could differ significantly and these differences could prove positive or negative for the economics of the system. While the specific reactions and quantities of the end products may differ, the pyrolysis system is still highly likely to yield chars, liquids and gases. Interestingly, the biochar derived from the pyrolysis of bacteria may have different effects on plant growth than the biochar derived from plant matter (Steinbeiss et al. 2009) and these differences could be examined as part of an undergraduate research project (see broader impacts).

Specific Aim 5: *Develop and parameterize a bio-economic model of a hypothetical large-scale system for the growth of T. crunogena to estimate the production costs per dry ton and the costs of a biomass gasification with carbon capture and storage system.*

We will develop two separate models; one to estimate the biomass production costs and another that uses the biomass costs as input to determine the costs of a biomass pyrolysis system.

Biomass Production Costs Model

We will model a system composed of 100, 72,000 L bioreactors with a total volume of 7.2 million L. The bioreactors will be based on commercially available water containers and each reactor is expected to cost approximately $5,000. The bioreactors will be continuously supplied with marine aquacultural wastewater or seawater supplemented with molecular sulfur. The sulfur consumption will be determined based on the results of Specific Aims 1 and 2. *T. crunogena* will be continuously harvested from the bioreactor at a rate determined by the results of Specific Aims 2 and 3. Capital costs will consist of the costs of bioreactors and facility construction, the costs of equipment including pumps, and a biomass harvesting system. The purpose of this model is to determine the costs associated with production of the biomass compared to other common types of biomass such as switchgrass.

Pyrolysis Model

Following biomass production and drying, biomass is assumed to be pyrolyzed in a medium-scale reactor. The products of pyrolysis will be determined via the results of Specific Aim 4. The major capital cost will include the cost of the reactor. Input costs such as energy costs and labor costs associated with the pyrolysis process will also be included. This process yields three products: char, bio-oil and syngas at varying proportions based on the results of Specific Aim 4. Two of these commodities, specifically the bio-oil and syngas have significant potential

commercial value. The value of the char will also be studied both as a soil additive and as a means of carbon sequestration and the value of the treated water will be estimated using standard ecological economic techniques such as the replacement cost method (DeGroot et al. 2002).Using these costs of production, we will analyze the economic feasibility of producing these two commodities compared to and (a) crude oil prices (b) natural gas prices. This will inform us of two things. First, what price of crude oil and natural gas would be needed to make this technology economically feasible on a commercial scale. Second, given reasonable projections of future crude oil and natural gas prices, two secondary questions will also be answered. First, what level of subsidy, if any, would be needed to make this economically feasible. Second, the char could have significant value if sold for carbon credits. Given the currently low trading value of carbon credits (Snyder 2014), it is unlikely that this will significantly impact the economics of this project. But what is not known is the price of carbon needed in order to make production of these commodities economically feasible when compared to crude oil and natural gas prices.

As previously mentioned, the major factor that will impact the economic feasibility of production on a commercial scale will be the price of crude oil and natural gas. These globally traded commodities have seen large swings in prices in relatively short periods of time. With the recent developments in horizontal drilling combined with hydraulic fracturing, this has provided even more uncertainty about future prices. For example, during the months prior to writing this proposal, crude oil prices have seen a steep decline of more than 20 percent over the past five months. For this reason, any analysis of economic feasibility will be outdated as soon as it is published if it is not discussed in relation to different levels of future prices. For this reason, we will produce an (a) subsidy amount and (b) a carbon price required to make this new technology economically feasible when compared to a range of future crude oil and natural gas prices. This will provide policy makers with a realistic view of the economic feasibility under a number of different price scenarios that will not be outdated once the price of crude and natural gas change.

Potential Pitfalls: The PI and Co-PI (G. Upton) have extensive experience in techno-economic modeling, bio-economic models, and forecasting in the energy industry. The methods used to create these models are standard and straightforward and we do not expect to encounter methodological problems. It is possible that the models will suggest that extremely high oil and gas prices, carbon prices, or subsidies are required; if this is the case we will explore ways to reduce costs.

6. Broader Impacts

Broader impacts of the proposal stem from two main proposal goals: the research itself and education.

Research Broader Impacts. If the research demonstrates that sulfur-oxidizing bacteria can be a cost effective novel biomass for bioenergy source, the results could be potentially transformative.

If a non-photosynthetic method of biomass for bioenergy growth is developed, then biomass growth would not need to compete for land with food production and would be instead limited by sulfur production. While sulfur production is currently limited to 70 million tons per year, sulfur is widely abundant in the earth's crust and sulfur mining could fuel sulfur-based biomass production on a large scale. If this biomass production was coupled with pyrolysis-based carbon capture and storage, this could create a system in which molecular sulfur was used to pull CO_2 out of the atmosphere and store it in long-lived sinks while simultaneously creating a valuable energy product (bio-oil and syngas).

Education Broader Impacts. The proposed research is highly amenable to participation by non-specialists and education will be a major focus of the proposed research. We envision educational broader impacts to come from four activities:

1. A master's seeking graduate student will be employed to conduct the majority of the research, working under the close supervision of the PI and Co-PI Dr. Crystal Johnson.
2. The PI and Co-PIs will recruit undergraduates to work on projects related to, but separate from the specific aims identified in the proposal. Specific projects may include some of the experiments associated with the potential pitfalls in Specific Aim 1, studies of the impacts of derived chars on plant growth as in Specific Aim 4, or genetic engineering of *T. crunogena* to increase extracellular lipid production for biofuel applications (Bharti et al. 2014). We anticipate including at least one undergraduate in each semester (including summers) for a total of nine undergraduate researchers. While we anticipate that the majority of these undergraduates will work in the lab under the supervision of the PI and co-PI, Dr. Crystal Johnson, we will also recruit one or more undergraduate researchers to work on economic modeling with Dr. Greg Upton.
3. The PI and Co-PI, Dr. Crystal Johnson, will recruit biology teachers from local high schools and create a three (half) day summer research experience project based around a laboratory experiment they could perform with their students. This program will accommodate three to seven teachers per year and will run in two project summers, educating a total of six to 14 teachers. An example experiment may demonstrate the principle of chemosynthesis via growth in ASW media with and without sulfur and compare to photosynthetic and heterotrophic bacteria. All teachers will leave the program with a kit containing all of the materials needed to repeat the experiments in their classrooms.
4. This project will create the opportunity for community outreach to local high school students. Local high school students will compete for positions in a Summer Research Institute (SumRI) in which four students will be chosen based on grades, essays, and letters of recommendation; minorities and girls will be especially encouraged to apply. These students will spend six weeks in a laboratory at LSU where, under mentored supervision, students will be exposed to current scientific literature and allowed to develop their own hypotheses and design experiments to test them. At the end of the

Internship, students will write a 10-page scientific report of their findings and will present a summary of their research in a 30-minute campus seminar. Students will be encouraged to take their findings back to their high schools and present them as a poster in their school's science fair.

The Broader Impact component of this proposal takes advantage of the expertise of the Co-PI, Crystal N. Johnson, Ph.D., a former NSF GK-12 Program graduate fellow and a female minority, and will therefore leverage and the capacity-building experience of the Co-PI. This synergistic combination will be very beneficial to the design and implementation of these summer programs and will contribute to the nation's production of research scientists. In sum, we anticipate that the proposed research will directly contribute to the education of at least one graduate student, nine undergraduates, eight high school students, and six to 14 K-12 educators.

7. Results of Prior NSF Support

PI: B. Snyder. Not Applicable.

Co-PI: G. Upton. Not Applicable.

Co-PI: C. Johnson.