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# Pilot-Scale Continuous-Flow Hydrothermal Liquefaction of Filamentous Fungi

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# Pilot-Scale Continuous-Flow Hydrothermal Liquefaction of Filamentous Fungi

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**ABSTRACT:** This study examined the potential of using the filamentous fungus *Rhizopus oligosporus* as a feedstock for hydrothermal liquefaction (HTL). The fungal biomass, cultivated in thin stillage from a corn ethanol plant, was processed at pilot-scale using a 1.5-L capacity continuous-flow HTL system. HTL operating conditions of 300−400 °C at 27 MPa for 12−30 min were tested. Biocrude yields ranging from 48.2 to 60.9% were obtained. At low reaction temperatures (300 °C), yields as high as 59.9% could still be achieved. Aside from the least severe reaction condition studied (300 °C, 12 min), neither the yield nor elemental composition of the biocrude was significantly impacted by residence time or temperature, as is typically seen with batch reactors. Similarities in the biochemical and elemental composition between *R. oligosporus* and microalgae resulted in biocrude yields that were comparable to those previously reported for continuous-flow systems using microalgae. These findings demonstrate the viability of using fungal biomass as a feedstock for the HTL process, and they show that lower temperatures can be used at pilot-scale while still achieving maximal yields.

## 1. INTRODUCTION

The U.S. corn ethanol industry has seen a considerable amount of growth over the past decade, resulting in the production of 15 billion gallons of ethanol per year from over 200 plants nationwide.[1] Biofuels provide a renewable source of energy and reduce dependency on fossil fuels. However, some remain skeptical of the sustainability of corn ethanol production,[2,3] and issues regarding energy efficiency and handling of byproducts remain. Specifically, the liquid centrate produced from centrifugation of suspended solids following distillation is a resource that can be better utilized to improve plant operations. This centrate, referred to as thin stillage, is currently being evaporated in an energy-intensive process to produce syrup containing 30% solids. The syrup is subsequently mixed with the dewatered suspended solids, and then dried to form an animal feed called dried distiller's dried grains with solubles (DDGS).[4] There is, however, limited demand for the syrup, and it is often sold at low prices. The added nutritional benefit of the syrup is also questionable.[5]

In order to avoid incurring high energy costs to produce a low-value coproduct, alternative uses of thin stillage are being considered. Because thin stillage has a high organic content (90 g/L COD) and an acidic pH that is in the ideal range (pH 4−5) for fungal growth, previous studies have been performed to study the cultivation of the filamentous fungi *Rhizopus oligosporus* and *Mucor circinelloides* in thin stillage.[5,6] Fungi were found to provide effective water treatment of the thin stillage by reducing COD, suspended solids, and organic acids. The reduction in suspended solids is reportedly due to solids attaching to the fungi, as well as through biological mineralization.[5] The reduction in COD, suspended solids, and organic acids could allow the ethanol plant to recycle much of the water back into the ethanol production process and potentially reduce water needs by 75%.[5,7] Additionally, the harvested fungi could be used as an animal feed due to their high protein and essential amino acid content.

Fungal biomass cultivated in thin stillage was also found to be a possible feedstock for biofuels, due to a short cultivation period of 2−3 days and an increased lipid content when compared to fungi cultivated in yeast malt broth.[6] Production of a renewable diesel fuel from filamentous fungi *Rhizopus oligosporus* cultivated on thin stillage could potentially add a high-value coproduct from the corn ethanol process, and improve energy return on investment. Hydrothermal liquefaction (HTL) was investigated in this study as a pathway for fungal biocrude production. This thermochemical process uses water at high temperature and pressure to break down the long-chain organics of the biomass and repolymerize them into short-chain hydrocarbons to form a liquid fuel referred to as biocrude. Unlike other thermochemical processes such as pyrolysis or gasification, no drying of the biomass is needed, since water is required for HTL. Thus, energy consumption due to drying is eliminated,[8] making HTL an attractive process for use with fungal biomass cultivated in thin stillage.

To date, the majority of studies using HTL as a biofuel production process have used algae or lignocellulosic biomass,[9−28] with research on algae being particularly prominent most recently. Limited research has been conducted using fungi as a feedstock for HTL, and is limited to the yeasts *Cryptococcus curvatus* and *Saccharomyces cerevisiae*.[29−32] Additionally, continuous-flow HTL processes have exclusively investigated the use of microalgae and lignocellulosics.[12,26,33,34]

However, no information on continuous-flow HTL with filamentous fungi has been reported. This study aimed to determine the feasibility of using fungal biomass as an HTL feedstock. Performance at pilot-scale in a continuous-flow reactor was studied, and optimization of reaction conditions was investigated. The results of this study were compared to

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results obtained by researchers using other feedstocks that have received more attention The feasibility and optimization of the HTL process on fungal biomass were evaluated based on the quantity and composition of the biocrude created at reaction conditions previously determined to be optimal for biocrude production.[10,18,28,35]

Assessing the biocrude quality included determining the oxygen, nitrogen, and sulfur contents of the biocrude. Low oxygen levels were desired to produce a biofuel comparable to fossil fuels. Similar composition would allow for the fungal biofuel to be more readily utilized in the existing petroleum-based infrastructure. The nitrogen content of the biocrude was also of concern. Increased nitrogen in the biocrude demonstrates improved protein conversion during the HTL process. However, excessive amounts would potentially increase $NO_x$ emissions when combusted.[8] Specifications for automotive fuels limit the sulfur concentration because of issues regarding environmental pollution, cylinder wear, and cylinder deposits.[36] Therefore, it was important to determine reaction conditions which produced high yields of biocrude with a composition similar to that of petroleum crude oil.

## 2. MATERIALS AND METHODS

**2.1. Feedstock Cultivation and Harvesting.** The culture of the fungus *R. oligosporus* was obtained from the American Type Culture Collection (ATCC# 22959, Rockville, MD). Cultivation, collection, and storage of spores were conducted according to methods used by Ozsoy et al.[37] The spores were aseptically cultured using HIMEDIA RM301 agar for 36−48 h at 30 °C. Spores were then collected using deionized dilution water containing 0.85% (w/v) sodium chloride and 0.05% Polysorbate 80. The collected spores and solution were passed through a 50 mL syringe containing glass wool to remove any mycelia. Subsequently, yeast mold (YM) broth (Difco Laboratories, Detroit, MI) was mixed with the filtrate at a 1:1 ratio, and glycerin was added to a final concentration of 20% (v/v). This spore stock solution was collected in sterile 2 mL cryo-vials and stored at −80 °C.

A fungal inoculum was prepared by transferring one 2 mL cryo-vial of spore suspension to 1 L of autoclaved YM broth. The inoculum flask was incubated at 37 °C in an orbital shaker at 200 rpm for 24 h prior to inoculating the thin stillage.[6] A total of 8 L of fungal inoculum was prepared for the pilot-scale bioreactor.

*R. oligosporus* biomass was cultivated in thin stillage obtained from Golden Grain Energy LLC (Mason City, IA), a dry-grind corn ethanol plant. 1400 L of thin stillage was collected for the pilot-scale bioreactor operations. Since the temperature of the thin stillage can reach 80 °C at the time of collection, it was allowed to cool to 35 °C over the course of 15 h before inoculation to prevent heat damage to the fungal inoculum. Prior to fungal cultivation, the average pH was 5.0.

The 1600-L pilot-scale bioreactor was cleaned and disinfected with a 3% (v/v) sodium hypochlorite solution, followed by a sodium hydroxide solution (pH ∼ 10.5) for 24 h prior to thin stillage transfer. Following the cooling period, thin stillage was transferred to the bioreactor, and 8 L of fungal inoculum was added to the thin stillage. Cultivation was allowed to proceed over the following 48 h.[6] During cultivation, the temperature in the bioreactor was maintained at 37 ± 3 °C,[6] and four ceramic diffusors provided air at a flow rate of 300 L/min. In order to control excessive foaming, 450 mL of a liquid antifoaming agent (Antifoam 204, Sigma-Aldrich, St. Louis, MO) was added to the bioreactor.

Upon completion of fermentation, the solids content of the output from the fungal reactor was 1%. Therefore, dewatering was required to increase the solids content to the desired level for the HTL work. The fungal biomass was harvested and dewatered by filtering with a fine mesh screen to remove excess thin stillage and achieve a solids content of approximately 25 wt %. The dewatered fungi served as a feedstock supply for the HTL tests discussed in this paper, as well as for pumping tests using slurries with 2−15% solids. The solids content of each slurry was confirmed by drying a known weight of wet fungal biomass (approximately 5−10 g) at 70 °C for 48 h. After harvesting, the fungi were stored at 10 °C until they were needed for testing. Although dewatering the fungi from the fungal reactor was required, an interesting test to perform in future work would be to dewater the fungi to just 4−5% solids (rather than 25% solids) and then use that fungi/stillage mixture as the feedstock for the HTL reactor, as opposed to performing more extensive dewatering and then adding water to make the desired solids content in the slurry.

Analysis of fungal biomass from the pilot-scale bioreactor to determine concentrations of C, H, N, O, and S and heating values was conducted by Keystone Materials Testing, Inc. (Newton, IA) using ASTM methods D5291 and E711, respectively. Exact Scientific Services, Inc. (Ferndale, WA) performed proximate analysis to determine the carbohydrate, lipid, and protein content of the biomass.

**2.2. Feedstock Slurry.** Prior to feedstock slurry preparation, the stored fungi were mixed until uniform consistency was achieved to ensure a homogeneous moisture content. Fungal slurries containing 4 wt % solids were prepared 1−7 days prior to each HTL test and stored at 10 °C until needed. The solids content of the slurry was limited by the supercritical reactor equipment due to difficulties in pumping the slurry and the risk of valves plugging when the fungal slurries contain greater than 5 wt % solids. All of the biomass used in the slurries came from one batch of fungi grown on the same thin stillage. The same batch of fungi was used in order to eliminate the potential risk of biochemical variations between batches, which could influence biocrude yields.[8]

Fungi and water were blended into a slurry in a two-step method. First, the biomass and water were mixed in a commercial-grade food blender. Approximately 3000 g of wet fungal biomass was blended with an equal amount of distilled water for about 1 min. Additional distilled water was subsequently added to the blend and mixed to a 4 wt % solids content in a high-shear in-line mixer until a uniform slurry was obtained.

A proximate analysis was performed to confirm a 4 wt % solids loading and determine the amount of ash present in each slurry sample. For determining moisture content, 50−75 g of fungal slurry was added to a ceramic crucible and dried in an oven at 105 °C for at least 24 h. Moisture content was calculated by the mass difference of the wet and dried fungal biomass. Ash determination was conducted in a similar manner by heating the dried biomass in a muffle furnace for a minimum of 3 h at 550 °C.[34] Analyses on the fungal slurries were performed in triplicate, and the average values from those analyses were reported.

**2.3. HTL Tests.** HTL was performed in a 1.5-L capacity pilot-scale supercritical flow reactor (SCFR) located at the Iowa Energy Center's Biomass Energy Conversion (BECON) Facility in Nevada, IA. A schematic diagram of the reactor system is shown in Figure 1. The SCFR was designed by Supercritical Fluid Technologies Inc. (Newark, DE), with maximum operating conditions of 450 °C and 69 MPa. A 10-L tank equipped with a paddle mixer was used to contain the prepared slurry. The paddle mixer provided continuous agitation in order to maintain a homogeneous slurry. A plunger pump was used to transport slurry from the tank through a preheater, consisting of stainless steel piping coiled around a cylindrical heating unit, before entering the SCFR. Effluent was discharged from the SCFR via a pneumatic valve when the pressure inside the reactor reached the set point. A liquid-to-liquid heat exchanger cooled the reactor effluent to approximately 25 °C.

HTL of fungi was studied at 12 different operating conditions. Four nominal residence times ranging from 12 to 30 min were investigated at temperatures of 300, 350, and 400 °C while using a constant pressure of 27 MPa for each test. Attempts to keep residence times constant across the temperature range proved to be difficult based on the SCFR setup. Since the effluent flow rate was dictated by the reactor reaching the set-point pressure, residence times would vary at different temperatures despite operating the pump at a constant speed. The average residence times were 12, 16, 19, and 30 min, while the actual residence times sometimes varied significantly from these "nominal" values. Table 1 lists the nominal and actual residence times


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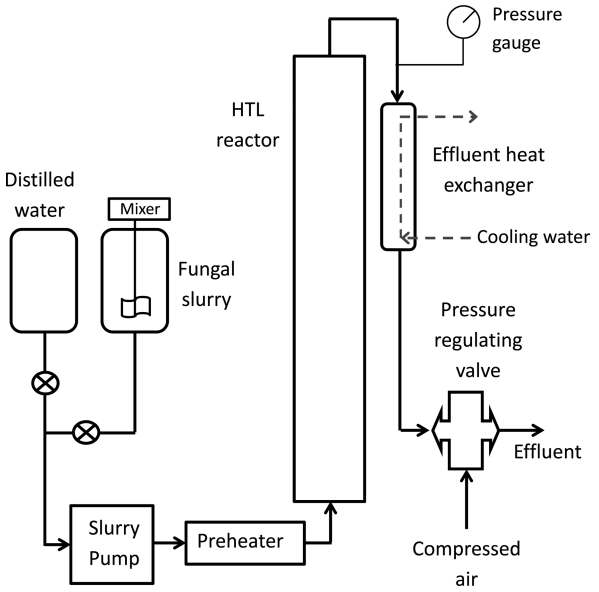


**Figure 1.** Schematic diagram of the HTL reactor system.

**Table 1. Nominal and Actual Residence Times for Each Reactor Temperature Tested**

| Temp (°C) | Nominal Residence Time (min) | Actual Residence Time (min) |
|---|---|---|
| 300 | 12 | 11.9 |
| 300 | 16 | 15.6 |
| 300 | 19 | 17.9 |
| 300 | 30 | 24.8 |
| 350 | 12 | 12.2 |
| 350 | 16 | 16.0 |
| 350 | 19 | 18.9 |
| 350 | 30 | 31.0 |
| 400 | 12 | 12.7 |
| 400 | 16 | 17.5 |
| 400 | 19 | 19.5 |
| 400 | 30 | 29.9 |

for each of the three reactor temperatures used. Although previous continuous-flow reactor studies have used shorter residence times,[26,33,34] these times were selected for our study based on the operating capacity of the slurry pump and the rate at which the heat exchanger could effectively cool the effluent.

Prior to each HTL test, distilled water was pumped through the SCFR for a minimum of 4 h to achieve the desired operating conditions. During this period, the residence time was gradually decreased as the reactor was heating up. By starting with a high residence time (i.e., a low feed rate through the reactor), heating the reactor to the desired temperature could be performed much more quickly, since starting with a high feed rate provides excessive cooling of the reactor. The system was switched over to the fungal slurry once a steady state of temperature and residence time had been reached. The preheater was set at 133 °C, as temperatures above this could potentially bake the slurry and cause plugging issues.[33] After switching to the slurry, the initial 2 L of collected effluent was discarded. This effluent was found to be predominately composed of the distilled water remaining in the SCFR prior to switching to the fungal slurry. Due to the aforementioned issues with residence time consistency, samples of aqueous effluent were collected on a volumetric basis as opposed to time increments. Effluent samples of 500 mL each were collected in polypropylene bottles throughout each HTL test and each of those samples was processed individually. In previous HTL tests (unpublished) using fungal slurries, biocrude yields were determined as a function of sample number at 300, 350, and 400 °C using retention times of 12−30 min. The biocrude yields initially increased with increasing sample number and then eventually leveled off. The results showed that the biocrude yields always leveled off by the time the 12th sample was collected, at which point it was assumed that steady state had been reached. Therefore, a minimum of 12 samples of effluent were collected for each HTL run discussed in our current study.

**2.4. Biocrude Yields.** Determining the biocrude yield consisted of separating the biocrude from solid and aqueous phase fractions. Although a variety of organic solvents can be used for this purpose,[19,23] dichloromethane (DCM) is the most commonly used. Therefore, DCM (Fisher Scientific, Waltham, MA) was selected as the solvent to be used for processing biocrude samples from the HTL process. Because of health considerations associated with using DCM, all of the work involving the use of open vessels containing DCM were performed in a fume hood, and laboratory personnel working with DCM always wore lab coats and safety glasses. In addition, North Silver Shield EVOH/PE Laminated Gloves were always worn and inspected prior to each use. Those gloves are available from numerous vendors, including Fisher Scientific.

Biocrude was extracted by adding 100−200 mL of DCM to each 500 mL sample of reactor effluent, followed by shaking at 30 rpm in a rotator for 60 min. Solids from the DCM and effluent mixture were subsequently vacuum-filtered in a fume hood and removed via glass-fiber filters with an effective pore size of 1.6 μm. After filtration was complete, the filters sat in the fume hood for 15−60 min, were subsequently placed in vented convection oven at 105 °C for 24 h, and then weighed to determine the mass of solids present in the aqueous sample. The filtrate was collected and transferred to a separatory funnel, where the denser DCM and oil separated from the lighter aqueous fraction. The oil and DCM fraction were transferred to a crystallizing dish, where the DCM was allowed to evaporate at room temperature in a fume hood for 3−5 days. The biocrude remaining after evaporating the DCM was weighed and recorded, and a dry, ash-free (daf) biocrude yield was determined using eq 1.

Analyses on the biocrude from each of the 12 HTL tests (four residence times at each of three operating temperatures) were conducted by Keystone Materials Testing, Inc. (Newton, IA) to determine heating values and elemental composition (C, H, N, O, and S) using ASTM methods D240−09 and D5291, respectively.

$$yield\ (daf\ \%) = \frac{Biocrude\ Mass}{(Sample\ Mass\ In)(Slurry\ \%\ solids)(1 - \%\ ash)} \times 100 \quad (1)$$

**2.5. Statistical Analysis.** A statistical analysis was performed to determine the significance of reactor operating conditions on biocrude and biochar yields. Software JMP (SAS Institute, Cary, NC) was used to perform a Tukey's honest significant difference (HSD) test. "P" values greater than 0.05 were determined to show significance.

## 3. RESULTS AND DISCUSSION

**3.1. Fungal Feedstock Analysis.** The fungal feedstock composition and higher heating value (HHV) of *R. oligosporus* cultivated on thin stillage are shown in Table 2. The biomass was predominately composed of carbohydrates (34.8%) and proteins (34.2%). The crude lipid content accounted for 22.4% of the biochemical composition. Fungi benefit from being cultivated in thin stillage due to their ability to accumulate 20% more lipids compared to the same fungi cultivated in YM broth.[6] Increased lipid content is desirable since biocrude production has been demonstrated to come primarily from lipids, followed by proteins, and then carbohydrates.[8,27,38] Compared to other fungal species used in HTL studies, *R. oligosporus* contained a significantly higher lipid content than yeast *Saccharomyces cerevisiae* (2.7%),[30] and less than the oleaginous yeast *Cryptococcus curvatus* (32.8%).[32] Relative to


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**Table 2. Composition and Heating Value of *R. oligosporus* Fungus**

| | |
|---|---|
| Proximate Analysis (wt %, as received) | |
| Moisture | 1.44 |
| Ash | 7.13 |
| Carbohydrates | 34.8 |
| Proteins | 34.2 |
| Lipids | 22.4 |
| Elemental Composition (wt %, daf) | |
| C | 63.2 |
| H | 9.3 |
| N | 5.9 |
| O (by difference) | 21.2 |
| S | 0.4 |
| Higher Heating Value (MJ/kg, daf) | 28.3 |

other feedstocks used in continuous-flow HTL systems, the lipid content of *R. oligosporus* was comparable to that of microalgae,[12] specifically, *Chlorella* (25%)[8] and *Nannochloropsis* (28%).[39] The elemental composition and HHV of the fungal biomass feedstock was also found to be similar to those of microalgae. The biochemical and elemental similarities between the fungus *R. oligosporus* and the microalgae suggests that the fungus should make a good feedstock material for HTL, since biocrude yields of up to 64% daf and oxygen contents as low as 5% have been reported for microalgae in continuous-flow HTL systems.[33]

**3.2. Reactor Performance.** Biocrude production as a function of the reactor conditions was determined in order to study the performance of the SCFR versus the sample collection number for each set of conditions tested. Biocrude yield results were reported on a dry, ash-free (% daf) basis for each 500 mL sample that was collected, with each sample number increasing sequentially as the reactor run progressed. Those results are shown in Figure 2. Despite being held at constant temperatures, biocrude production required a start-up period of 30−60 min before near steady state conditions were reached. This lag time suggests that the water that was pumped into the reactor during the initial start-up period had not yet been completely removed, despite the fact that the first 2 L of effluent were discarded. Water used during the start-up period apparently diluted the slurry, thereby reducing the actual solids content present at the time of reaction. In turn, this resulted in decreased biocrude production during the early portions of the HTL run. The up-flow design of the SCFR may have contributed to additional mixing due to settling by gravity of the water and the fungal slurry.

Data on biocrude yields are the most meaningful after steady state conditions have been reached. Because of the lag times noted in Figure 2, decisions had to be made regarding which data best represented biocrude samples collected under steady state or near steady state conditions. Those decisions were not always clear-cut in view of the lack of clearly defined trends in some cases, but the point was to exclude the early samples where yields were still clearly increasing with increasing sample number (indicating nonsteady state conditions). Although that approach involves some subjectivity, it still gives a much better representation of the biocrude yields obtained under each set of test conditions than would be possible by including results from all of the samples collected under each set of conditions. The

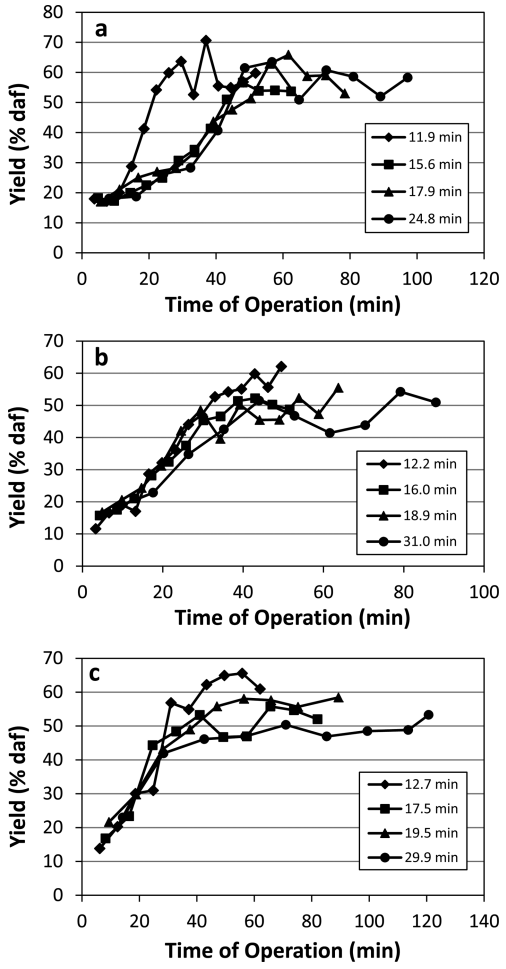


**Figure 2.** Biocrude production as a function of operating time, residence time in the reactor, and reactor temperatures of (a) 300 °C, (b) 350 °C, and (c) 400 °C.

sample number (and associated total operating time) which we took to represent the beginning of near steady state conditions for all of the residence times and reactor temperatures are tabulated in Table 3. Again, the intent of this approach was to exclude the samples collected during the "lag" phase where the

**Table 3. Operating Time and Sample Number Assigned to Represent the Beginning of Steady State Conditions**

| | | Assumed Beginning of Steady State Conditions | |
|---|---|---|---|
| Temp (°C) | Residence Time in Reactor (min) | Operating Time (min) | Sample No. |
| 300 | 11.9 | 29.6 | 8 |
| 300 | 15.6 | 48.0 | 10 |
| 300 | 17.9 | 56.0 | 10 |
| 300 | 24.8 | 48.6 | 6 |
| 350 | 12.2 | 42.9 | 13 |
| 350 | 16.0 | 38.7 | 9 |
| 350 | 18.9 | 29.4 | 6 |
| 350 | 31.0 | 44.0 | 5 |
| 400 | 12.7 | 31.0 | 10 |
| 400 | 17.5 | 41.0 | 10 |
| 400 | 19.5 | 47.0 | 10 |
| 400 | 29.9 | 71.0 | 10 |



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water introduced into the reactor during start-up was still being purged.

After approximately 3 L of effluent samples had been collected, the water had been sufficiently purged from the reactor and a near steady state production of biocrude was achieved. Residence time and temperature appeared to influence biocrude production trends at steady state. The more severe the reaction conditions were, the more stable the biocrude yields became. This is most evident during the 30 min residence time at 400 °C, where the yields at steady state varied by ±2.4% compared to ±5.8% seen at the same residence time at 300 °C. These variations occurred despite the fact that the same number of samples had been collected and the same amount of effluent slurry had been pumped through the reactor. Even greater variation was found to occur at the least severe condition (300 °C, 12 min), where steady state yields differed by as much as ±6.2%. Stability of the reaction conditions at higher temperatures and longer residence times at pilot-scale have been noted in other studies using continuous-flow systems.[34]

**3.3. Biocrude Yield.** In terms of the yields calculated as a percentage of the fungal biomass, the average biocrude yields (% daf) obtained after the reactor reached steady state conditions are shown in Figure 3 for all temperatures and residence times. As noted in Section 3.2, longer times of operation could have produced more well-defined trends, specifically at lower temperatures and residence times where more variation was noted to occur. However, 3−8 samples at steady state conditions were averaged for each condition tested, and are believed to provide representative biocrude yields for the test parameters. Repeatability of these results was determined by conducting a second HTL test for one of the given operating conditions. Only a 0.52% (absolute) difference in biocrude yield was seen between the duplicate tests.

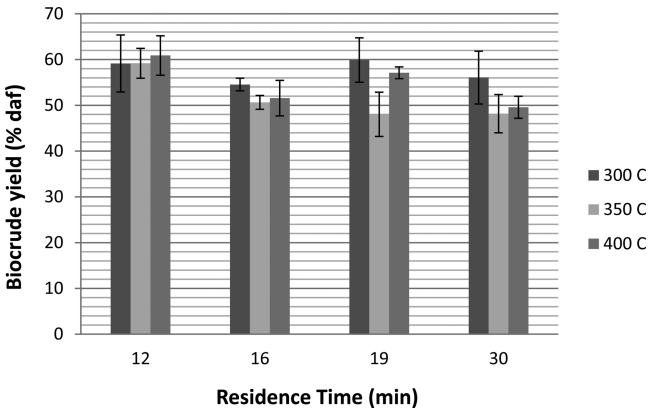


**Figure 3.** Biocrude yields (% daf) from HTL testing. Results are averages of at least three samples collected at steady state. Error bars represent one standard deviation.

The optimum temperature range for biocrude production during HTL, as opposed to biochar and biogas formation, is generally reported to be 300−350 °C.[11,35,40,41] However, more severe temperatures may increase biocrude formation from the protein fraction, since it has been reported that biocrude samples created at higher temperatures have a higher nitrogen content.[12] Therefore, the reaction temperature of 400 °C was also studied since fungal slurries contain a high level of proteins. From the HTL test data, it can be seen that the maximum yield (60.9% daf) was obtained at 400 °C for 12 min. This finding is consistent with studies both in batch reactors as well as continuous-flow systems, in which the highest biocrude production is observed at the most severe reaction temperature, which may be due to improved conversion of proteins and carbohydrates to biocrude.[10,12,34] However, in Figure 3 it must be noted that the highest temperature (400 °C) did not produce the highest biocrude yields for any residence time other than the 12 min residence time, suggesting that perhaps using a temperature of 400 °C did not actually lead to increased conversion of the protein. Also, in work reported by Teri et al. (2014), it was noted that there appears to be an interaction between the protein and carbohydrates that affects biocrude yields in the HTL process. Therefore, caution must be exercised when drawing any conclusions about biocrude yields and the protein content of the feedstock material.

It must also be noted that while the maximum yield was obtained at the most severe reaction temperature (400 °C), it was not significantly higher than the other temperatures tested ($P < 0.05$). A range of yields between 48.2−60.9% daf was found to be attainable using *R. oligosporus* as a HTL feedstock, but did not appear to be dependent on temperature or residence time. The fungal biomass used for this study produced higher yields than the oleaginous yeasts used in batch reactor tests performed by other researchers, where biocrude yields ranged from 40−49% daf.[29,30,32] When compared to biocrude yields obtained with other continuous-flow systems, the observed range of yields obtained with *R. oligosporus* is similar to those obtained when using microalgae as a feedstock, in which biocrude yields of 38−64% daf have been reported.[12,33,34] It should be noted that in a previous continuous-flow study conducted by Elliott et al., temperatures of 350 °C were required to produce yields near 60%.[33] Conversely, the HTL system used herein for these fungal biomass tests achieved yields as high as 59.9% daf even when the lowest reaction temperature of 300 °C was employed. Therefore, these observations suggest that pilot-scale continuous-flow processes are highly system specific, and the significance of temperature will vary depending on the equipment and parameters used.

For this system, acceptable biocrude yields were obtained when the least severe reaction temperature (300 °C) was used. This has implications for commercial-scale applications, where energy consumption of the HTL process could be reduced by lowering the reaction temperature to 300 °C. Improved energy return on investment can occur when lower HTL temperatures are employed with lipid-rich feedstocks.[41] As previous studies have shown that temperatures below 300 °C result in decreased yields,[10] this fungal HTL process should be further investigated using lower temperatures to determine when temperature starts to become a significant factor.

As determined by Anastasakis and Ross[42] and Jena et al.,[43] residence time significantly impacts biocrude yields in batch reactors. However, residence time did not play a large role in biocrude production during our pilot-scale continuous-flow HTL tests. This observation has been reported by Jazrawi et al. using microalgae as well.[34] Extending the residence time from 12 to 30 min did not improve yields in this present study. Maximal biocrude production (60.9%) was found to occur even at the shortest residence time of 12 min. Shorter residence times allow for higher biocrude output at commercial-scale, and can help improve the economic viability of the technology.



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In our HTL tests, biocrude yields of about 6 g/L of thin stillage were obtained when cultivating fungi in thin stillage, filtering the solids, and then adding water to the fungi to produce the 4% fungal slurries used as the feedstock material for the HTL reactor. A test performed in a prior year used only thin stillage containing about 8% suspended solids (mostly organic) as the feedstock for the HTL reactor. That test was performed using reactor conditions of 4000 psi, a temperature of 350 °C, and a residence time of 30 min. This produced a biocrude yield of 26 g/L of thin stillage. Part of the reason for the higher yields when using only thin stillage is because the solids content in the thin stillage was twice as high (8%) relative to that in the fungal slurries. If biocrude yields from the fungal slurries were calculated based on yields per liter of feedstock slurry (rather than per liter of thin stillage), and if the solids content was the same as in the raw thin stillage (i.e., 8%), then the biocrude yields from the fungal slurries would be expected to exceed the 26 g/L obtained when using just thin stillage as the feedstock material. This is based on the fact that biocrude conversion rates of 50−60% were observed for the fungal biomass in the tests discussed in this paper. Another consideration is that the amount of fungal biocrude produced per liter of thin stillage used to grow the fungi is inherently linked to the fungal yields obtained in the fermentation step, which is typically about 10−12 g/L at pilot-scale. However, laboratory tests have shown that fungal biomass yields in thin stillage can exceed 30 g/L. Although this has not yet been accomplished at pilot-scale, we believe it may be achievable with modifications in the fungal cultivation procedures.

**3.4. Solids Content Analysis of HTL Effluent.** Solids were collected from the effluent samples and were reported as % daf mass of sample. The results for each reaction condition are presented in Figure 4. Solids collected in the effluent were typically low, ranging from 0.13−0.71% when the reactor was determined to be at steady state. A greater amount of solids was expected to occur at lower temperatures due to this reaction condition favoring biochar formation via hydrothermal carbonization.[44] Overall, a trend of decreasing biochar formation with increasing temperature was observed at each residence time. Deviation from this trend occurred at the 12 min residence time, and may be an indication that this length of time is not sufficient for maximal biochar formation. Due to the relatively low solids content, biocrude yields were not impacted by solids formation. While the overall yield was not affected, solids do raise a concern with regards to further processing of the biocrude.

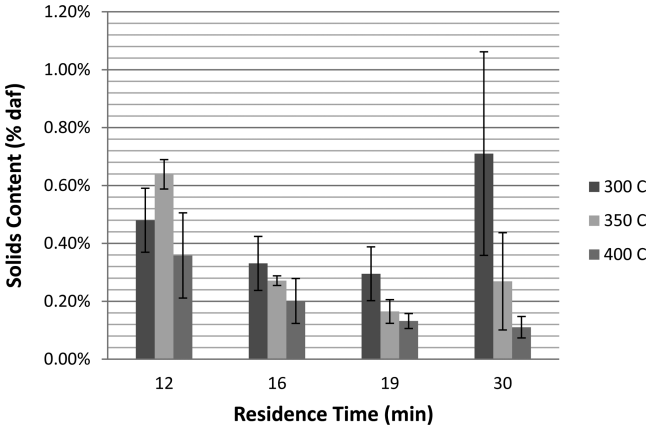


**Figure 4.** Solids content present in aqueous sample collected for each given reaction condition after reaching steady state conditions. Error bars represent one standard deviation.

**3.5. Elemental Composition and HHV of Biocrude.** While it was found that the reaction conditions did not significantly impact biocrude yields, the same cannot be said about the quality of the product. To help assess the quality of the biocrude product, concentrations of C, H, N, O, and S and the higher heating value (HHV) of the biocrude were determined. A summary of the composition and HHV of the biocrude at each reaction condition is presented in Table 4. HTL substantially lowered oxygen concentrations relative to that present in the fungal biomass. The biocrude samples contained an average of 14% oxygen, which is 30% (relative) lower than the oxygen content of the fungal feedstock. Continuous-flow reactor results reported by other researchers show a broad range of oxygen levels in the biocrude, with approximately 5−18% oxygen being reported when using microalgae.[33,34] Batch reactor studies previously performed by Miao et al.[31] and Jena et al.[32] using yeast have shown reductions in oxygen concentrations as high as 63% (relative) when compared to the starting feedstock. Differences in the biochemical composition of the feedstock materials and using a larger scale reactor in our study may have contributed to less efficient oxygen removal when using fungal biomass. With regards to operating conditions, the oxygen value in the biocrude produced in 12 min at 300 °C was most notable. A higher oxygen concentration (16.2%) was found in the biocrude at this condition compared to the rest of the samples. All other reaction conditions produced biocrude with lower oxygen contents, averaging 13.7%, and showed little deviation (±0.95% absolute) between each condition tested. Higher levels of oxygen suggest that the residence time was insufficient, resulting in decreased quality of biocrude.[8] At temperatures of 350 and 400 °C, the lowest oxygen concentrations in the biocrude were obtained at the longest residence times, which is consistent with previous work.[34]

The lowest nitrogen content was seen at the least severe condition tested (300 °C, 12 min), although nitrogen contents varied by no more than 0.7% (absolute) for all of the tests. The variability in nitrogen concentrations tended to decrease with increased temperature. Although it has been reported that the nitrogen concentration in biocrude is often the lowest when the reaction severity is minimal, thereby converting fewer proteins to biocrude,[10] our data overall do not provide strong evidence that more severe reaction conditions lead to higher nitrogen concentrations in the biocrude under the conditions tested in view of the minimal variability in the nitrogen results and lack of consistent trends. However, the fact that the least severe reaction condition (300 °C for 12 min) produced biocrude with the lowest carbon content, the lowest nitrogen content, and highest oxygen content does suggest that 12 min at 300 °C is not sufficient for complete reactions to occur. In cases where the severity of reaction conditions more clearly correlates with higher nitrogen levels in the biocrude, a balance between optimal conversion and low nitrogen levels needs to be met. Elevated levels of nitrogen can become a concern due to the potential formation of $NO_x$ compounds during combustion.[8] While more nitrogen present in biocrude is potentially detrimental to the environment, the nitrogen levels found in this biocrude were lower than what has typically been seen in biocrude from other continuous-flow systems (4−8%).[12] Further treatment of biocrude will be needed to remove


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Table 4. Elemental Composition (daf) and Higher Heating Value (daf) of Biocrude as a Function of Residence Time and Temperature

| Temp (°C) | Residence Time (min) | Carbon (%) | Hydrogen (%) | Nitrogen (%) | Oxygen (%) | Sulfur (%) | HHV (MJ/kg) |
|---|---|---|---|---|---|---|---|
| 300 | 11.9 | 70.5 | 10.3 | 2.51 | 16.2 | 0.47 | 35.8 |
| | 15.6 | 72.7 | 10.3 | 3.19 | 13.2 | 0.57 | 36.4 |
| | 17.9 | 72.1 | 10.3 | 3.05 | 14.0 | 0.56 | 36.2 |
| | 24.8 | 72.4 | 10.4 | 2.92 | 13.7 | 0.53 | 36.6 |
| 350 | 12.2 | 73.4 | 10.7 | 2.87 | 12.5 | 0.52 | 36.8 |
| | 16.0 | 71.5 | 10.3 | 3.05 | 14.6 | 0.55 | 36.3 |
| | 18.9 | 71.3 | 10.3 | 3.02 | 14.8 | 0.57 | 36.4 |
| | 31.0 | 73.6 | 10.8 | 2.81 | 12.2 | 0.56 | 37.0 |
| 400 | 12.7 | 72.3 | 10.4 | 2.85 | 13.9 | 0.52 | 36.9 |
| | 17.5 | 71.6 | 10.4 | 2.76 | 14.6 | 0.57 | 37.1 |
| | 19.5 | 71.5 | 10.3 | 2.83 | 14.7 | 0.64 | 36.5 |
| | 29.9 | 73.4 | 10.6 | 2.79 | 12.6 | 0.61 | 37.2 |

nitrogen to obtain petroleum-like quality (<2%) if $NO_x$ production is a concern.

Due to the majority of reaction conditions producing a biocrude of similar composition, the HHV varied only slightly (±0.7 MJ/kg) for the parameters tested, as shown in Table 4. However, the minor variations in oxygen and nitrogen content did provide an overall trend for the impact of temperature and residence time on HHV. Slightly higher HHVs were obtained at 400 °C (37.2 MJ/kg), while slightly lower values were obtained at 300 °C (35.8 MJ/kg). This trend resulted from slightly improved oxygen removal at higher temperatures and longer residence times. However, the improvement in biocrude quality seen at more severe reaction conditions was minimal, and it is unlikely that an energy return on investment analysis would show that increased temperature provides a significant benefit. These values are larger than what has been seen in other continuous-flow HTL systems using microalgae[33,34] as well as lignocellulosic biomass.[9] Additionally, the fungal biocrude HHVs greatly exceed that of pyrolysis bio-oil (16–19 MJ/kg) and reach values near those typically observed in petroleum fossil fuels (40–45 MJ/kg).[45] Additional upgrading would be needed to further remove oxygen and nitrogen and to produce liquid hydrocarbon transportation fuels.[15,28]

**3.6. HTL Catalysts and Solvents.** No catalysts or organic solvents were used in our HTL testing. However, interesting results have been reported regarding the use of catalysts and alternate solvents in the conversion of biomass to biocrude via HTL. Catalytic HTL has predominantly involved the use of homogeneous catalysts, most notably sodium and potassium hydroxides and carbonates. Use of such catalysts in some studies has led to substantial increases in biocrude yields and increased biocrude quality.[20,28] Organic solvents used in HTL generally involve the use of alcohols (generally ethanol), either alone or in aqueous mixtures,[13,16,21,25,32] although other solvents such at heptane and toluene have also been used when in situ fractionation of the biocrude was desired.[26] Using organic solvents, either in neat form or as cosolvents, has been shown to significantly increase biocrude yields.[13,21,25] However, one study reported that lower biocrude quality accompanied the higher yields.[21] When an alcohol is used as the sole solvent, it has the advantage that HTL can be performed under supercritical conditions at much lower temperatures and pressures compared to using water as a solvent. In future work, it would be interesting to explore the use of homogeneous catalysts and cosolvents as they relate to our application. The use of ethanol as a cosolvent would be particularly interesting in view of the fact that the fungi used as a feedstock material are grown in thin stillage from corn ethanol plants. Therefore, it may be very economical to use off-spec ethanol from the plant for this purpose.

## 4. CONCLUSIONS

Biocrude derived from the fungi *R. oligosporus* was successfully produced using a pilot-scale continuous-flow HTL reactor. At pilot-scale, higher temperatures did not prove to significantly enhance biocrude production, and 300 °C was found to generate yields equal to those seen at 400 °C. Reaction conditions had less of an impact in our study compared to what has been previously reported in batch reactors, and the reaction conditions only became a significant factor at the least severe condition of 300 °C for 12 min. Biocrude from fungi had similar yields and quality to that of microalgae at temperatures of 300 °C for residence times of 16 min or longer, which makes fungi an attractive feedstock for HTL.

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### Notes

The authors declare no competing financial interest.

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