Protocol to develop component additivity models that predict oil yield from hydrothermal liquefaction

Here, we describe steps for performing hydrothermal liquefaction (HTL) experiments and developing component additivity models that predict oil yields from HTL of mixtures with biomass and plastics. Such models could be developed for predicting outcomes from any thermochemical valorization process (e.g., pyrolysis) for any feedstock. The HTL protocol explains experiments with both a single component and mixture. The model is constrained to the specific plastic feedstocks and solvents for product recovery used in the experiments.

Publisher’s note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.
Protocol

Protocol to develop component additivity models that predict oil yield from hydrothermal liquefaction

Mahadevan Subramanya Seshasayee1,2,* and Phillip E. Savage1,3,*

1Chemical Engineering Department, Pennsylvania State University, 121D CBE Building, University Park, PA 16802, USA
2Technical contact
3Lead contact
*Correspondence: seshasay95@gmail.com (M.S.S.), pes15@psu.edu (P.E.S.)

SUMMARY

Here, we describe steps for performing hydrothermal liquefaction (HTL) experiments and developing component additivity models that predict oil yields from HTL of mixtures with biomass and plastics. Such models could be developed for predicting outcomes from any thermochemical valorization process (e.g., pyrolysis) for any feedstock. The HTL protocol explains experiments with both a single component and mixture. The model is constrained to the specific plastic feedstocks and solvents for product recovery used in the experiments. For complete details on the use and execution of this protocol, please refer to Seshasayee et al. (2021).

BEFORE YOU BEGIN

1. About hydrothermal liquefaction:

This protocol details the experimental and statistical methods used to develop component additivity models that predict oil yield from hydrothermal liquefaction (HTL) of mixtures containing both biomass and plastic components. HTL converts carbonaceous feedstocks to an energy-dense oil that could serve as a petroleum crude oil substitute in hot pressurized water (275°C–450°C, 6–40 MPa) (Gollakota et al., 2018). Carbonaceous feedstocks valorized using thermochemical methods such as HTL can be of synthetic origin, i.e., plastics, or natural origin, i.e., biomass. The main biochemical components of biomass are celluloses, non-cellulosic carbohydrates, proteins, lignin, and lipids. The HTL process outcomes (e.g., oil yield) for biomass directly depend on its biochemical composition (Yang et al., 2019). Component additivity model uses the biochemical composition of the biomass, the identities of the constituent plastic types in the feedstock, and the HTL process temperature as inputs to predict the oil yield from HTL.

2. Interactions among feedstock constituents:

Literature indicates the existence of biomass – biomass, biomass – plastic, and plastic – plastic interactions that influence the oil yield from HTL (Seshasayee and Savage, 2021b). For example, if processing cellulose alone, protein alone, and an equimass cellulose – protein mixture via HTL gives oil yields of A, B, and C, respectively, at a given set of conditions, then C > (A + B)/2. We term these positive interactions as synergistic. Similarly, interactions can also lead to decreases in oil yield, i.e., C < (A+B)/2. In such cases, the interactions are termed antagonistic.

Component additivity models (see Equation 1) contain unary factors (\(a_i\)) and binary interaction factors (\(a_{ij}\)). The unary factors give the oil yield expected from processing the component alone (\(X_i = 1\))
at a fixed process temperature. For example, in the illustration above, \( a_{\text{cellulose}} \) would be A and \( a_{\text{protein}} \) would be B. The interaction factors are derived from experiments with binary mixtures. For example, in the illustration above for cellulose – protein mixtures, \( a_{ij} = \frac{|C_i - (A_i + B_i)/2|}{A_i + B_i} \). The \( a_i \) and \( a_{ij} \) values can be temperature dependent.

\[
Y_{oi} = \sum_i a_i X_i + \sum_i \sum_j a_{ij} X_i X_j \quad \text{(Equation 1)}
\]

3. Scope of the model:

The component additivity model (see Equation 1) we developed includes cellulose, non-cellulosic carbohydrates, proteins, lipid, and lignin as potential biomass components along with four commonly used consumer plastics (polypropylene, polystyrene, polyethylene terephthalate, and polycarbonate). Hence, the model works well for predicting oil yield from HTL of most biomass, biomass + plastic, and plastic + plastic mixtures with the aforementioned plastics. The model is also constrained by specific process conditions (30 min, 300, 350, and 425°C). The mini-batch reactors heat up to the set point temperatures in less than 3 min (Gollakota and Savage, 2019). Thus, the process is isothermal for most of the reaction time. The model would not predict well the oil yields from shorter time experiments, the “fast HTL” region (which is non-isothermal and uses rapid heating), or temperatures below 275°C or above 450°C.

This is the first protocol detailing both hydrothermal liquefaction and developing component additivity models. Previous component additivity models were developed for HTL of biomass alone. Hence, the model discussed in this protocol is the first of its type to predict oil yield from HTL of plastic – biomass mixtures. The same was recently published in iScience with discussions based on the experiments (Seshasayee et al., 2021). We dive deeper into the methodology adopted in this manuscript.

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| Cellulose (microcrystalline) | TCI America | A17730-36 |
| Potato starch | TCI America | A117961-36 |
| Alkaline lignin | TCI America | L0082 |
| Soy Protein | MP Biomedicals, LLC | 9010-10-0 |
| Stearic acid | Sigma-Aldrich | 175366 |
| Polypropylene | Sigma-Aldrich | 427888 |
| Polystyrene | Sigma-Aldrich | 331651 |
| Polycarbonate | Sigma-Aldrich | 181625 |
| Polyethylene Terephthalate | Sigma-Aldrich | 429252 |
| Dichloromethane | WWR International | 75-09-2 |
| Hexane | WWR International | 110-54-3 |
| Acetonitrile | WWR International | 75-05-8 |

Software and algorithms

For t-test https://www.medcalc.org/calc/comparison_of_means.php

---

STAR Protocols 3, 101536, September 16, 2022
Table 1. Benchmarking component-additivity models in literature for HTL of biomass by comparing predictions for biocrude yields

| Model used to obtain parameter values | (Déniel et al., 2017) | (Yang et al., 2018) | (Lu et al., 2018) | (Yang et al., 2019) |
|-------------------------------------|------------------------|---------------------|------------------|---------------------|
| | $R^2_{avg}$ | 16.74 | 14.24 | 11.05 | 13.87 |
| % predicted within 10 wt% | 31 | 34 | 53 | 36 |

See Table S1 for dataset and values of the predictions.

**STEP-BY-STEP METHOD DETAILS**

The protocols used in this method are numbered in the following order:

1. **Identifying known interactions between components.**
   1. **Hydrothermal Liquefaction Reactions.**
   2. **Recovery, separation, and quantification of reaction products.**
   3. **Component additivity model development and benchmarking.**

**Identifying known interactions between components**

© Timing: Variable – 1 week for this study

The protocol identifies the known interactions among the different components in the component additivity model. It uses methodologies to review literature articles and determine interactions that influence oil yields. It further identifies experiments to be performed to develop component additivity models.

1. Review literature articles that present component additivity models that predict oil yields (for hydrothermal liquefaction in this case) for all feedstocks of interest.
   a. Record the equation, interactions among components, and coefficients in each model.
   b. Record the process conditions (temperature, time, extraction solvent) used in the experiments that provided the model parameters.
2. Record literature data for oil yields (or any modeled response) from hydrothermal liquefaction of feedstocks of interest. During the exercise, make sure the literature data are from conditions consistent with those to be used to develop your model. In our case these conditions were:
   a. The time of processing is greater than 15 min (to avoid non-isothermal HTL operation).
   b. The HTL reaction temperature is between 250°C–450°C.
   c. The solvent for recovering the oil phase is dichloromethane.
3. Use the literature component additivity models (identified in step 1) to predict the published oil yields collected in step 2.
4. Benchmark the performance of each model using two different metrics.
   a. Average absolute residual: Calculate the average value of absolute difference between predicted and experimental oil yields, i.e., $|\text{oil yield}_{\text{predicted}} - \text{oil yield}_{\text{experimental}}|$
   b. Percentage of data points predicted within 10 wt.% deviation.
      i. Count the number of data points with absolute residual less than 10 wt.%, i.e., $|\text{oil yield}_{\text{predicted}} - \text{oil yield}_{\text{experimental}}| < 10$.
      ii. Calculate the percentage of data points by dividing the value from step 4bi by the total number of data points in the dataset.

**Note:** Absolute residuals are considered here to benchmark the performance of the model because of their simplicity and physical significance.
5. Identify the models that work best in terms of each of the metrics in step 4 (see Table 1). All interactions considered in these models will then be included in the “to be developed” component additivity model.

△ CRITICAL: If for any of the feedstocks of interest in the “to be developed” component additivity model, there are no component additivity models available in the literature, skip steps 1–5 and follow step 6 onward. Otherwise, stop at step 5 and then proceed to step 8.

6. Look for clues in the literature regarding interactions that do/ do not have an effect on oil yields. In our case, we identified literature ([Seshasayee and Savage, 2021b](#)) indicating lignocelluloses interacted with all four plastics considered in the model.

7. If there are no literature clues available, then consider all possible interactions within the system. We had no information regarding plastic – plastic interactions in HTL and hence considered all possible interactions between these components.

8. Combine all of the information from steps 1–7 to determine all of the interactions that will be included in the “to be developed” model. The developed model has both unary or one component and binary or two component interaction terms.

Hydrothermal liquefaction reactions

© Timing: 1 day (4 work hours) per set

This protocol details methodology to assemble mini-batch reactors and perform HTL reactions for one component alone and two-component mixtures at a particular set point temperature and desired pressure.

9. Prior to every reaction, set the Techne fluidized sand bath at the desired setpoint temperature (300, 350, 425°C in our studies) with air flow rate as specified by the manual (depends on temperature). Wait two hours for the sandbath to heat to the specified temperature. Use a thermocouple to measure the temperature within the center of the bath.

10. Assemble 12 mini-batch reactors (~ 10 mL) from 3/4 inch Swagelok tube fittings – a port connector and two caps for each.
   a. Add 4.98 mL of water to each reactor, seal them, and process them at 350°C for 1 h in the sand bath.
   b. Empty the contents of the reactor with 10 mL of DI water and 10 mL of dichloromethane (DCM). This step seasons and cleans the newly assembled mini-batch reactors.
   c. Dry the seasoned and cleaned mini-batch reactors in an oven at 60°C overnight.

Note: Step 10 is only carried out with new mini-batch reactors. As long as the mini-batch reactors do not leak in an experimental run and there is no visible damage, one can use the mini-batch reactors for many experiments. Hence, step 10 can be skipped after the first reaction.

11. Perform one-component and two-component experiments with biomass and plastics. For two-component experiments, Table 2 gives the relative ratios among the biomass – biomass, plastic – biomass, and plastic – plastic mixtures.

Note: We derive these values from the relative composition of different components in municipal solid waste ([United States Environmental Protection Agency, 2018](#)).

12. Next, mass the empty mini-batch reactors. We aim to load 0.3892 g of total feedstock into the mini-batch reactors. The methodology followed for loading of feedstock differs for plastic or biomass. The masses added are detailed in Table 2. Keep massing the reactor after each
The difference between reactor mass measurements gives the mass of feedstock added in each run. We use this value for all calculations.

**a.** We use cellulose, starch, soy protein, stearic acid, and alkaline lignin as biomass feedstocks. We aim to add different amounts of the biomass in each run according to the target amounts in Table 2. We aim to maintain a mass of \( G_0.0020 \) g from target in each run. All of the materials mentioned above are obtained in dry powder form from the supplier.

**b.** We use polypropylene (PP), polystyrene (PS), polycarbonate (PC), and polyethylene terephthalate (PET) as plastic feedstocks. The target masses for each run are given in Table 2. All the plastics obtained are in pellet form. A single pellet mass ranges from \( 0.0200 – 0.0400 \) g. We hence aimed to maintain a mass of \( G_0.0050 \) g from the target in each run.

We add 6.15, 4.96, or 1.10 mL of DI water to the reactor using pipettes for HTL experiments at 300, 350, or 425°C, respectively. These amounts ensure the reactor is at saturation pressure for HTL at 300°C and 350°C and at 25 MPa for HTL at 425°C. This value is calculated using the density of water at the set point temperature and desired pressure (see Equation 2). We use a safety factor of 0.95 to avoid over pressurizing the reactors.

**a.** Mass the reactors again to ensure the proper amount of water was added.

Table 2. Feedstock compositions for all mixtures tested using HTL at 300°C, 350°C, and 425°C for 30 min to develop component additivity model

| Reaction number | Component A | Component B | Mass of A (g) | Mass of B (g) | wt% A | wt% B |
|-----------------|-------------|-------------|---------------|---------------|-------|-------|
| 1               | Cellulose   | Lignin      | 0.3252        | 0.0640        | 83.6  | 16.4  |
| 2               | Cellulose   | Soy Protein | 0.3466        | 0.0426        | 89.0  | 11.0  |
| 3               | Lignin      | Starch      | 0.2224        | 0.1668        | 57.1  | 42.9  |
| 4               | Lignin      | Stearic Acid| 0.2831        | 0.1061        | 72.7  | 27.3  |
| 5               | Starch      | Soy Protein | 0.2123        | 0.1769        | 54.6  | 45.4  |
| 6               | PP          | Cellulose   | 0.3036        | 0.0812        | 78    | 22    |
| 7               | PC          | Cellulose   | 0.3036        | 0.0812        | 78    | 22    |
| 8               | PS          | Cellulose   | 0.3036        | 0.0812        | 78    | 22    |
| 9               | PET         | Cellulose   | 0.3036        | 0.0812        | 78    | 22    |
| 10              | PP          | Starch      | 0.3036        | 0.0812        | 78    | 22    |
| 11              | PC          | Starch      | 0.3036        | 0.0812        | 78    | 22    |
| 12              | PS          | Starch      | 0.3036        | 0.0812        | 78    | 22    |
| 13              | PET         | Starch      | 0.3036        | 0.0812        | 78    | 22    |
| 14              | PP          | Lignin      | 0.3036        | 0.0812        | 78    | 22    |
| 15              | PC          | Lignin      | 0.3036        | 0.0812        | 78    | 22    |
| 16              | PS          | Lignin      | 0.3036        | 0.0812        | 78    | 22    |
| 17              | PET         | Lignin      | 0.3036        | 0.0812        | 78    | 22    |
| 18              | PP          | PC          | 0.1946        | 0.1946        | 50    | 50    |
| 19              | PP          | PS          | 0.1946        | 0.1946        | 50    | 50    |
| 20              | PP          | PET         | 0.1946        | 0.1946        | 50    | 50    |
| 21              | PC          | PS          | 0.1946        | 0.1946        | 50    | 50    |
| 22              | PC          | PET         | 0.1946        | 0.1946        | 50    | 50    |
| 23              | PS          | PET         | 0.1946        | 0.1946        | 50    | 50    |
| 24              | Cellulose   | –           | 0.3892        | –             | 100   | –     |
| 25              | Starch      | –           | 0.3892        | –             | 100   | –     |
| 26              | Soy Protein | –           | 0.3892        | –             | 100   | –     |
| 27              | Lipid       | –           | 0.3892        | –             | 100   | –     |
| 28              | Lignin      | –           | 0.3892        | –             | 100   | –     |
| 29              | PP          | –           | 0.3892        | –             | 100   | –     |
| 30              | PS          | –           | 0.3892        | –             | 100   | –     |
| 31              | PC          | –           | 0.3892        | –             | 100   | –     |
| 32              | PET         | –           | 0.3892        | –             | 100   | –     |
Mass of water added = Safety factor × Density of water (HTL temperature, desired pressure) × volume of reactor (mL)

(Equation 2)

14. Place the cap on the reactor to close and seal it. Follow the recommendations from Swagelok regarding how much to tighten the cap to form a leak-free seal.

△ CRITICAL: Step 14 is a critical step. If the reactors are not closed properly, they might leak or pose a safety hazard. Additionally, it is important to ensure that the proper amount of water is added to each reactor. Adding too much water increases the pressure within the reactor at the set point temperature. For the 10 mL reactors, the Swagelok parts have a safety limit of 30 MPa. This would correspond to adding 18.4, 9.0, and 2.1 mL of DI water for processing the reactors at 300, 350, and 425 °C, respectively. Thus, particularly for HTL at higher temperatures, more care must be taken. More information regarding reactors leaking is given in the troubleshooting section.

15. Place three reactors (at most) in a metal basket and process them simultaneously in a preheated sandbath. Since three independent runs are made for each desired data point, three mini-batch reactors with identical loadings are run simultaneously. The mini-batch reactors stay within the sand bath for 30 min. The timer starts the moment the batch reactors enter the sand bath.
   a. Place the reactors in the middle of the metal basket and place the metal basket in the middle of the sand bath. This placement ensures an even distribution of heat across all three reactors.
   b. Before introducing the reactors into the sand bath, use a thermocouple to measure the temperature of the sand bath. Say for example that one is running six reactions (two batches of three each) consecutively in the same sand bath. After the end of the first batch of three reactions, we allow the sand bath to stabilize for 10 min and measure the temperature before the next batch of reactors is placed within.

16. Once the stipulated time is complete, remove the reactors from the sandbath and immediately immerse them in water (room temperature) for 5 min to quench the reaction. The reactors are then left on a lab bench to equilibrate overnight at room temperature before the product recovery process starts. The room temperatures in these experiments were 20°C–25°C.
   a. If not possible to wait overnight, we recommend waiting for a minimum of two hours between quenching the reaction and beginning product recovery. One should use the same waiting time across all experiments in a study.

17. We use the following workflow for this study:
   a. Mass and clean the reactors after each run. Perform 12 reactions on day 1 using two sand baths. Run two batches of three reactors each on each of the two sand baths.
   b. Recover products from the 12 reactors on day 2. Let the reactors dry overnight.

**Recovery, separation, and quantification of reaction products**

**Timing:** 1 day (3 h of work) per set

This protocol details methodology for recovery, separation, and quantification of oil, aqueous, and solid products from the HTL reaction.

18. Mass the reactors after step 16 on day 2. The mass of the reactor should exceed the mass recorded in step 13. The reactor accumulates some moisture on its outer surface after the quenching is done in step 16. If the mass is lower, it indicates potential reactor leakage, i.e., the experiment is void.
   a. Mass a syringe filter (Tisch Scientific), syringe (Fisher Scientific), and glass pipette (Fisher Scientific).
19. Open the reactor and remove the contents using 10 mL (total) of DCM and 10 mL (total) of DI water. Add small aliquots (approximately 2 mL) containing both DCM and DI water. Aspirate and dispense each aliquot 10 times in the reactor and then pass it through a syringe filter into a centrifuge tube.
   a. The setup used for the product recovery process is visualized in Figure 1. The syringe filters are attached to the syringes and placed on top of the centrifuge tube. Pipet the aliquots removed from the reactor into the syringe and pass the dissolved products through the filter and into the centrifuge tube.
   b. Note that 10 mL of DCM and DI water each are added for 10 mL mini-batch reactors. Larger reactors would require larger (but still equal) amounts of solvents for extraction.
   c. Maintaining the same degree of contact between the solvents and the reaction products in the reactors for all runs is vital for the replicability of runs. Two of the plastics used in this study (polystyrene and polycarbonate), partially dissolve in DCM. Control experiments with no heating were carried out (steps 14–16 are skipped) to observe 29 and 25 wt.% of the polystyrene and polycarbonate pellets, respectively, dissolved in DCM during product recovery. This dissolution is a result of the product recovery process alone. We discuss this matter in greater detail in the limitations section.

Optional: Mass the reactor before and after opening. The difference gives the mass of gas produced during HTL. Follow Equation 3 to calculate the percentage yield of gas.

20. Centrifuge the contents of the centrifuge tube at 3000 RPM for 7 min. The liquids in the tube form two layers (see Figure 1). The DCM phase is denser and is the bottom layer. Ensure that the contents in the centrifuge tube post centrifugation have no solids. If they do, then pass all contents of the tube through the filter again until all solid contents are removed.

Note: Centrifugation increases the oil recovery (Jiang and Savage, 2019). Organic compounds otherwise trapped in the aqueous phase can coalesce and migrate to the oil phase upon centrifugation. Hence, using a centrifuge is optional but preferred.

21. Pipette out the DCM phase from the centrifuge tube and discharge the contents of the pipette into a pre-massed glass test tube. Then transfer the aqueous phase to a new pre-massed glass test tube.

---

**Figure 1. Schematic of experimental setup for recovery of products from HTL**

---

STAR Protocols 3, 101536, September 16, 2022 7
22. Dry the DCM phase in a RapidVap Vertex Evaporator at 40°C for 6 h. Continue the drying process for more time if the contents are not visibly dry. The mass of oil produced is the difference between the masses of the empty glass tube and the glass tube with a completely dried oil-phase product.

**Note:** To verify the DCM phase is completely dried, one can mass the test tube, dry it for 2 more hours, and mass the test tube again. If the mass loss between subsequent measurements is less than 0.0010 g, the drying is sufficiently complete. This dryness check is followed for the aqueous and solid phase products as well.

23. Repeat step 22 for the water phase but use an oven at 40°C for 30 days. For the component additivity model, we are primarily interested in the oil phase product. However, the yields of aqueous-phase products could give clues regarding the process. Hence, it is advised to complete the measurement process for this phase as well.

**Note:** If the water phase is of importance to the user, the drying time may be reduced significantly by using a container with a large surface area and less depth to evaporate the contents. Freeze drying is another option.

24. Dry the reactor, syringe, syringe filter, and pipettes in the oven at 60°C overnight. All products in these items did not dissolve in DCM or water and are considered as solids products. The mass of solid in each component is measured by the difference between the empty component and the component with a completely dried solid phase product.

25. The gravimetric yield for each product fraction is then calculated as the mass of each product fraction divided by the mass of total feedstock loaded into the reactor (see Equation 3).

\[
\text{Yield}_{\text{phase}}(\%) = \frac{\text{Mass of product from phase in run}}{\text{Mass of total feedstock added in run}} \times 100 \text{ (Equation 3)}
\]

26. Calculate the mean and standard deviation of yields for each phase of product – oil, solid, aqueous using the results from the replicated experiments.

**Pause point:** If the standard deviation of the oil phase yield is greater than either 5 wt% absolute or 10% of the mean value, repeat the experiments to reduce the uncertainty. The standard deviations, particularly for mixtures with PS or PC, are discussed in more detail in the troubleshooting section.

**Component additivity model development and benchmarking**

**Timing:** 4 h

This protocol details the method to develop a component additivity model from the HTL experiments detailed in Table 2 at 300, 350, and 425°C for 30 min. We further benchmark the performance of the component additivity model for predicting oil yields and compare it with other component additivity models in the literature.

27. The component additivity model here is developed for biomass (using five biomass components) and four plastics (polypropylene, polystyrene, polyethylene terephthalate, and polycarbonate). The following HTL experiments were conducted at 300, 350, and 425°C and 30 min using the methodology in step one and step two:

   a. Each component alone – cellulose, starch, lignin, soy protein, lipid, PP, PS, PET, PC.
b. For biomass – biomass interactions: cellulose – protein, cellulose – lignin, starch – protein, starch – lignin, and stearic acid – lignin binary mixtures.

c. For plastic – plastic interactions: All permutations of PP, PS, PC, and PET binary mixtures.

d. For plastic – biomass interactions: All permutations of cellulose, starch, or lignin with PP, PS, PC, or PET.

28. $a_i$ is equal to the mean oil yield (wt %) divided by 100. $a_{ij}$ is equal to $\frac{\text{oil yield}_{ij} - (\text{oil yield}_i + \text{oil yield}_j)/2}{\% \text{ in mixture} \times (100 - \% \text{ in mixture})}$.

29. Calculate $a_i$, $a_{ij}$ for each of the three HTL temperatures – 300, 350, and 425°C. These conditions sample sub-, near- and super-critical temperatures.

30. Use Equation 1 to predict the oil yields for more complex mixtures. The $a_i$, $a_{ij}$ values in Equation 1 are chosen based on the HTL temperature.

a. If the HTL temperature is greater than 275°C and less than 325°C, use coefficients from HTL experiments at 300°C.

b. If the HTL temperature is greater than 325°C and less than 400°C, use the coefficients from HTL experiments at 350°C.

c. If HTL temperature is greater than 400°C, use coefficients from HTL experiments at 425°C.

31. Supplement literature data with new experimental data for oil yields that includes HTL of all feedstocks in the component additivity model. For example, the component additivity model developed in this protocol contains both biomass and plastics. However, the previous dataset collected contained only biomass feedstocks. Hence, we supplement the dataset with more experimental data points for HTL of plastic – biomass and plastic – plastic mixtures.

32. Repeat step 4 with the new developed data set in step 30. Note that the component additivity model here also varies with set point temperature of HTL. Compare predictions from this model with other component additivity models in literature if comparable models exist. The model developed here however is the first of its type (i.e., it includes plastics, plastic – plastic, and plastic – biopolymer interactions). Hence, we come to conclusions based on the absolute values of average residual and percentage data points.

Note: Interactions between components are not limited to HTL and have also been observed in other thermochemical processes (Williams and Williams, 1998; Lin et al., 2020). However, no component additivity models have yet been developed in these fields. We provide this protocol as a resource for others to develop component additivity models for other thermochemical valorization methods such as pyrolysis or solvolysis. Component additivity models can also be used to determine different metrics associated with the quality of the oil produced from the process (Yang et al., 2019). The same would require the extension of this protocol to predict the heating value or elemental composition of the oil produced. This extension is achieved by replacing the response variable in this manuscript (oil yield) with the corresponding quantities of interest (e.g., heating value, heteroatom content).

EXPECTED OUTCOMES

All results presented in the "expected outcomes" section were presented and discussed in detail in our article (Seshasayee et al., 2021). Here, we simply provide a summary and highlight key observations and conclusions.

Product yields from HTL reactions
The mean product yields from HTL of cellulose at different temperatures (30 min) are presented in Figure 2. The rest of the mass (100 – solid – aqueous – oil (in wt. %)) is considered to be gas and volatile products that are not gravimetrically analyzed as a part of protocol 2. Note that through protocol 3, there are several drying steps. Volatile products can be lost to evaporation during these steps. We focus on the yields of products that have been measured gravimetrically. Standard deviations for the yields of each
phase at every process condition are also given in Figure 2. We fix the time to 30 min to attain approximately isothermal operation. The reactors take 3 min (Gollakota and Savage, 2020) to reach the set point temperature. Hence, for at least 90% of the reaction time, the contents of the reactor are at the set point temperature. We set 90% as the lower limit to consider an HTL experiment to be isothermal.

To determine whether two product yields differ to a statistically significant extent (with p value < 0.025), we perform a two way t-test with null hypothesis that the difference between the values is zero. We also stress the importance of evaluating whether similar observations have been made in prior work.

For HTL of binary mixtures, we determine the mass-averaged oil yield or calculated oil yield based on the yields from HTL of each component alone (see Equation 4). This value is the oil yield expected if there are no interactions among the components in the mixture (i.e., $a_{ij} = 0$). The standard deviation for the calculated yield is calculated by extrapolating errors in Equation 4.

$$\text{Calculated oil yield} = \sum_{i} \left( \text{wt. frac. of } i \text{ in mixture} \times \text{oil yield}_i \right)$$  \hspace{2cm} \text{(Equation 4)}

where $\text{oil yield}_i$ is the yield obtained for HTL of component (or pseudo-component) $i$ alone at the same conditions used for HTL of the mixture. We compare the calculated oil yield with the experimental oil yield by performing a t-test with a null hypothesis that the difference between them is zero. Interaction is present if $p < 0.025$. This comparison can be made for other product fractions as well, as desired.

**Component additivity model**

Parity plots showing the experimental and predicted oil yields (such as in Figure 3) visualize the predictive ability of the model. Figure 3 was previously published in iScience (Seshasayee et al., 2021). The closer the data points are to the diagonal dotted black line, the better the predictions. Additional markers for 5 wt. % and 10 wt. % deviations are added to give the reader a gauge of the deviations in predictions. We advise looking closely at any data points that have deviations over 10 wt. %. The following approaches were considered:

Consider the feedstock composition and process conditions used in the literature study that generated that data point. Check whether there are any significant departures from the conditions used for the model. These might be due to aspects in interactions overlooked for model development. For example, Seshasayee and Savage (Seshasayee and Savage, 2021a) reported non-linear interaction effects between cellulose and protein in their component additivity model. The $a_{\text{cellulose-protein}}$ factor
was modified to be a function of the percentage cellulose and percentage protein in the feedstock. This gave a better predictive ability for the model.

Attempt to see if any prior component additivity model predicts those data points better. Then further comparing the coefficients in the model could lead to clues behind why the models work better.

In this model, we found the few literature oil yields from HTL at 400°C to be better predicted using the model parameters determined at 350°C than the model parameters determined at 425°C. For HTL at temperatures near the midpoint of a given temperature interval, it may be useful to test the predictive ability of the model using both sets of parameters.

QUANTIFICATION AND STATISTICAL ANALYSIS

In this section, we show a sample data point wherein the reactors leaked and further follow through with the methodology used to calculate oil yields. We first performed triplicate runs for HTL of cellulose at 350°C and 30 min using protocol 2. Let us call this run 1. Table 3 shows the oil yields obtained from run 1. We observe reactor 103 giving a significantly (p-value < 0.025) lower oil yield (0.12%) when compared to the average oil yield from reactors 101 and 102. The water in the reactor also leaked during the reaction (observed in step 18). Hence, in run 1, we say reactor 103 leaked and then perform two more reactions – one with reactor 103 and one with a new reactor. Let us call this run 2. In run 2, reactor 103 leaks again and is discarded. We use the three good runs (A, B, E) to calculate the mean and standard deviation.

For performing t-tests and obtaining p values, we use medcalc (Medcalc, 3/23/2022). The link cited requires the user to input the mean, the standard deviation, and the number of experimental runs. The number of experimental runs corresponds to runs used to calculate the mean and standard deviation. For example, in the run described in Table 3, the number of experiments is three. Also, for calculated values (see Equation 4), we always assume that the number of experimental runs is 3 for simplicity. Note that standard deviations for calculated values are extrapolated from two separate experimental runs. These might have a different number of experiments performed in each.

LIMITATIONS

Constrained by the number of different plastic feedstocks considered:
The present component additivity model considers four plastics. The EPA (United States Environmental Protection Agency, 2018) indicates there are twelve common plastics used in the market. One would need to perform experiments with each of the twelve plastics, study interaction effects, and include coefficients in the model. Hence, it is important to extend the model to include coefficients for more plastics. We estimate extending the model to twelve plastics would require 92 experiments (60 plastic – plastic interaction + 24 plastic – biomass interaction + 8 plastic alone) at each HTL temperature. Expanding component additivity models to a large number of components (say 17 here) is very experiment intensive.

Effect of solvent on oil yield:

We refer to the DCM-soluble compounds as oil. Dichloromethane has a dielectric constant of 8.93, which enables it to dissolve both non-polar organic molecules and slightly polar organic molecules. Previous work in HTL of microalgae indicates DCM recovers more organic phase products from a given microalga than other solvents tested (Jiang and Savage, 2019).

For plastic, there is an issue with using DCM as the solvent to recover oil. It can dissolve plastics such as polystyrene and polycarbonate (Gündüz and Dinçer, 1980; Scheuer et al., 1989). We performed HTL control experiments (zero-time in the sandbath) for PP, PET, PS, and PC pellets and obtained 0.4, 0.8, 29, and 25 wt% oil yields. Hence, the coefficients for PS and PC will likely overpredict oil yields at HTL conditions where the depolymerization reaction is not yet complete.

We tested the effect of using solvents other than DCM to recover oil from HTL of plastics. We performed HTL experiments at 425°C for 30 min. At this temperature, both PS and PC completely liquefy, and no solid plastic is left in either case. We tested hexane, acetonitrile, and DCM as solvents using protocol 3. The oil yields are given in Figure 4. Across all four plastics, the oil yields are greater for DCM than for acetonitrile or hexane. Although DCM partially dissolves certain plastics even if they have not reacted, it also dissolves a larger fraction of the products at complete conversion. It is also the preferred solvent for biomass HTL lab-scale experiments. Hence, we used DCM to recover the oil for these HTL experiments. Alternative methods using GPC-MS could also be employed to evaluate the fraction of oil products that are actually decomposed vs. the fraction of unreacted plastic products.

Probing further into modeling binary interactions:

We model all binary interactions as \( a_{ij}X_iX_j \), where \( a_{ij} \) is constant within a given temperature range, and \( X_i \), \( X_j \) are wt.% of \( i \), \( j \) in the feedstock. This mathematical form implicitly assumes, for simplicity, that oil yield changes due to interactions are linear in both components. However, the literature (Seshasayee and Savage, 2021a) includes at least one report showing that interactions (for cellulose and protein) can be non-linear and composition dependent. The same might be true for other interactions. Hence, further experimentation is required for important interactions.

| Table 3. Example of raw data measured on for oil yield from HTL of cellulose |
|-----------------------------|-----------------------------|-----------------------------|
| Run ID | Run number | Reactor number | Feedstock added (g) | Oil product massed (g) | Oil yield (wt. %) | Valid data point (Yes/No) |
| A | 1 | 101 | 0.3688 | 0.0322 | 8.74 | Yes |
| B | 1 | 102 | 0.3695 | 0.0327 | 8.84 | Yes |
| C | 1 | 103 | 0.3699 | 0.0004 | 0.12 | No |
| D | 2 | 103 | 0.3676 | 0.0013 | 0.35 | No |
| E | 2 | 101 | 0.3701 | 0.0342 | 9.25 | Yes |

The present component additivity model considers four plastics. The EPA (United States Environmental Protection Agency, 2018) indicates there are twelve common plastics used in the market. One would need to perform experiments with each of the twelve plastics, study interaction effects, and include coefficients in the model. Hence, it is important to extend the model to include coefficients for more plastics. We estimate extending the model to twelve plastics would require 92 experiments (60 plastic – plastic interaction + 24 plastic – biomass interaction + 8 plastic alone) at each HTL temperature. Expanding component additivity models to a large number of components (say 17 here) is very experiment intensive.

Effect of solvent on oil yield:

We refer to the DCM-soluble compounds as oil. Dichloromethane has a dielectric constant of 8.93, which enables it to dissolve both non-polar organic molecules and slightly polar organic molecules. Previous work in HTL of microalgae indicates DCM recovers more organic phase products from a given microalga than other solvents tested (Jiang and Savage, 2019).

For plastic, there is an issue with using DCM as the solvent to recover oil. It can dissolve plastics such as polystyrene and polycarbonate (Gündüz and Dinçer, 1980; Scheuer et al., 1989). We performed HTL control experiments (zero-time in the sandbath) for PP, PET, PS, and PC pellets and obtained 0.4, 0.8, 29, and 25 wt% oil yields. Hence, the coefficients for PS and PC will likely overpredict oil yields at HTL conditions where the depolymerization reaction is not yet complete.

We tested the effect of using solvents other than DCM to recover oil from HTL of plastics. We performed HTL experiments at 425°C for 30 min. At this temperature, both PS and PC completely liquefy, and no solid plastic is left in either case. We tested hexane, acetonitrile, and DCM as solvents using protocol 3. The oil yields are given in Figure 4. Across all four plastics, the oil yields are greater for DCM than for acetonitrile or hexane. Although DCM partially dissolves certain plastics even if they have not reacted, it also dissolves a larger fraction of the products at complete conversion. It is also the preferred solvent for biomass HTL lab-scale experiments. Hence, we used DCM to recover the oil for these HTL experiments. Alternative methods using GPC-MS could also be employed to evaluate the fraction of oil products that are actually decomposed vs. the fraction of unreacted plastic products.

Probing further into modeling binary interactions:

We model all binary interactions as \( a_{ij}X_iX_j \), where \( a_{ij} \) is constant within a given temperature range, and \( X_i \), \( X_j \) are wt.% of \( i \), \( j \) in the feedstock. This mathematical form implicitly assumes, for simplicity, that oil yield changes due to interactions are linear in both components. However, the literature (Seshasayee and Savage, 2021a) includes at least one report showing that interactions (for cellulose and protein) can be non-linear and composition dependent. The same might be true for other interactions. Hence, further experimentation is required for important interactions.
TROUBLESHOOTING

Problem 1
Reactor leaking:

We could typically do 15–30 runs in a given reactor before discarding it. When a reactor leaks, the mass of the reactor post reaction will be less than the mass before the reaction started (see step 18). Upon opening the reactor, one may also notice the absence of water within. The oil yields from this process will also be low.

Potential solution

- Inspect the reactors and look for damage. This typically appears in the threads that allow the cap to seal tightly. Figure 5 shows an example of damaged threads on the cap. If this is the case, discard the reactor.
- If there is no evidence of damage, do the same reaction again in the same reactor. Ensure the reactor is properly closed, following the Swagelok guidelines for a leak-free seal. If it leaks a second time, discard it.

Problem 2
Large standard deviations for oil yields mixtures with PS, PC:

One may notice larger standard deviations in the oil yields from HTL of mixtures, particularly those with PS or PC. Both plastics can dissolve in DCM at room temperature (20°C–25°C). It is hence important to maintain the same degree of contact between solvent and plastic during the extraction process to minimize overall deviations. We control this by controlling the number of times each aliquot is aspirated and dispensed (refer to step 19).

Potential solution

- The number of times each aliquot is aspirated and dispensed must be the same for all product recovery processes in each study. A good practice would be to repeat the control experiments multiple times and lower the standard deviation caused during the extraction process there. Then repeat the experiment with the same precision performed in the control experiments for the product recovery step.
For future experiments, try testing other solvents to see whether one exists that can dissolve a large fraction of oil products but not dissolve the plastics of interest.

**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Seshasayee Mahadevan (sxm1778@psu.edu).

**Materials availability**
Cellulose (microcrystalline), potato starch, alkaline lignin, de-alkaline lignin, polypropylene (PP), polycarbonate (PC), polystyrene (PS), and polyethylene terephthalate (PET) were all obtained commercially and used as received. Reagent-grade dichloromethane (DCM), hexane, and acetonitrile were procured from VWR International, while deionized water was produced in-house. We constructed 10 mL (internal volume) 316 stainless steel mini-batch reactors from 3/4 in. Swagelok parts (a port connector and two caps).

**Data and code availability**
This paper uses commercially available chemicals. The source and unique identifier for each chemical is listed in the key resources table.

- All data used in the manuscript are made available in the Tables S1 and S2.
- All raw data reported in this paper will be shared by the lead contact upon request.
- The dataset used to benchmark the developed model is available publicly. The DOIs of sources used to make the dataset are given in the reference.
- No code is utilized in this paper.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**SUPPLEMENTAL INFORMATION**
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101536.

**ACKNOWLEDGMENTS**
This work was supported with funds from the Walter L. Robb Family Chair in Chemical Engineering at Penn State.
AUTHOR CONTRIBUTIONS
Conceptualization, S.M.S. and P.E.S.; methodology, S.M.S. and P.E.S.; investigation, S.M.S.; validation, S.M.S.; formal analysis, S.M.S.; visualization, S.M.S.; writing – original draft, S.M.S.; writing – review & editing, P.E.S.; funding acquisition, P.E.S.; resources, P.E.S.; supervision, P.E.S.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
Deniel, M., Haarlemmer, G., Roubaud, A., Weiss-Hortala, E., and Fages, J. (2017). Modelling and predictive study of hydrothermal liquefaction: application to food processing residues. Waste Biomass Valorization 8, 2087–2107. https://doi.org/10.1007/s12649-016-9726-7.

Gollakota, A., and Savage, P.E. (2019). Biocrude production from fast and isothermal hydrothermal liquefaction of chitin. Energy Fuels 33, 11328–11338. https://doi.org/10.1021/acs.energyfuels.9b03209.

Gollakota, A., and Savage, P.E. (2020). Fast and isothermal hydrothermal liquefaction of polysaccharide feedstocks. ACS Sustain. Chem. Eng. 8, 3762–3772. https://doi.org/10.1021/acssuschemeng.9b06673.

Gollakota, A.R.K., Kishore, N., and Gu, S. (2018). A review on hydrothermal liquefaction of biomass. Renew. Sustain. Energy Rev. 81, 1378–1392. https://doi.org/10.1016/j.rser.2017.05.178.

Gunduz, S., and Dincer, S. (1980). Solubility behaviour of polystyrene: thermodynamic studies using gas chromatography. Polymer 21, 1041–1046. https://doi.org/10.1016/0032-3861(80)90035-X.

Jiang, J., and Savage, P.E. (2019). Using solvents to reduce the metal content in crude bio-oil from hydrothermal liquefaction of microalgae. Ind. Eng. Chem. Res. 58, 22488–22496. https://doi.org/10.1021/acs.iecr.9b03497.

Lin, X., Zhang, Z., Wang, Q., and Sun, J. (2020). Interactions between biomass-derived components and polypropylene during wood–plastic composite pyrolysis. Biomass Convers. Biorefinery, 1–13. https://doi.org/10.1007/S13399-020-00861-4/FIGURES/7.

Lu, J., Liu, Z., Zhang, Y., and Savage, P.E. (2018). Synergistic and antagonistic interactions during hydrothermal liquefaction of soybean oil, soy protein, cellulose, xylose, and lignin. ACS Sustain. Chem. Eng. 6, 14301–14309. https://doi.org/10.1021/acssuschemeng.8b03156.

Scheuer, C., Boot, E., Carse, N., Clardy, A., Gallagher, J., Heck, S., Marron, S., Martinez-Alvarez, L., Masarykova, D., Mcmillan, P., et al. (1989). Methylene chloride permeation in polycarbonate using a 14C tracer. Ind. Eng. Chem. Res. 28, 1494–1497. https://doi.org/10.1021/ie00034a010.

Seshasayee, M.S., and Savage, P.E. (2021a). Identifying and modelling interactions between biomass components during hydrothermal liquefaction in sub-near- and supercritical water. ACS Sustain. Chem. Eng. 9, 13874–13882. https://doi.org/10.1021/acssuschemeng.1c04810.

Seshasayee, M.S., and Savage, P.E. (2021b). Synergistic interactions during hydrothermal liquefaction of plastics and biomolecules. Chem. Eng. J. 417, 129268. https://doi.org/10.1016/j.cej.2021.129268.

Seshasayee, M.S., Stofanak, R., and Savage, P.E. (2021). Component additivity model for plastics—biomass mixtures during hydrothermal liquefaction in sub-near- and supercritical water. Science 24, 103498. https://doi.org/10.1016/J.ISCI.2021.103498.

United States Environmental Protection Agency. (2018). Advancing Sustainable Materials Management. 2015 Tables and Figures Assessing Trends in Material Generation, Recycling, Composting, Combustion with Energy Recovery and Landfilling in the United States (United States Environmental Protection Agency, Office of Land and Emergency Management): http://EPAS38F-18-004.

Williams, P.T., and Williams, E.A. (1998). Interaction of plastics in mixed-plastics pyrolysis. Energy Fuel. 13, 188–196. https://doi.org/10.1021/EF980163X.

Yang, J., He, Q., Niu, H., Corscadden, K., and Astatkie, T. (2018). Hydrothermal liquefaction of biomass model components for product yield prediction and reaction pathways exploration. Appl. Energy 228, 1618–1628. https://doi.org/10.1016/j.apenergy.2018.06.142.

Yang, J., He, Q., Corscadden, K., Niu, H., Lin, J., and Astatkie, T. (2019). Advanced models for the prediction of product yield in hydrothermal liquefaction via a mixture design of biomass model components coupled with process variables. Appl. Energy 233-234, 906–915. https://doi.org/10.1016/j.apenergy.2018.10.035.