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Article

Interlaboratory Study of Sample Homogeneity Impact on CHNS, Water, and ICP Analysis of Biomass Liquefaction Oils

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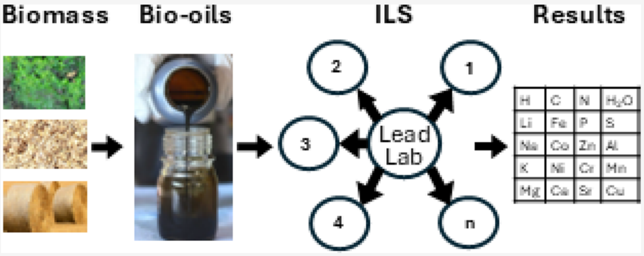
ABSTRACT: Five biomass liquefaction oils (BLOs) comprising four different lignocellulosic fast pyrolysis bio-oils (FPBO) and one algae-derived hydrothermal liquefaction (HTL) biocrude were subject to an interlaboratory study (ILS). Participating laboratories were asked to analyze all BLOs after gentle sample mixing and again but with vigorous mixing. Blind duplicates were included to improve the statistical power. Requested analysis included CHN, water, trace nitrogen, and sulfur (using solvent-miscible solvents) and ICP by an independently developed method. Blind duplicates applied in this study indicated that representative sampling was achieved to the extent required for bulk composition analysis. More vigorous sample mixing did not yield benefits for precision or reproducibility for composition analysis; however, the importance of consistent and precise sample mixing is underscored. Results of CHN and water showed performance similar to those of previous studies. However, adapted trace nitrogen methods consistently report 25−50% lower nitrogen content compared to ASTM D5291 and highlight the need for reconciliation. Poor performance of ICP across eight participant laboratories further underscores the need for standardization. ICP sample preparation by dissolution is generally ineffective for the recovery of more recalcitrant multivalent analytes. Digestion is recommended as the sample preparation of choice for ICP analysis of BLOs.



■ INTRODUCTION

As desire to achieve net-zero emissions grows,[1] application of direct thermochemical liquefaction (DTL) of biomass to produce renewable fuels and chemical feedstocks plays an important role and may help enable GHG emissions reductions.[2] DTL facilitates the valorization of underutilized materials from agriculture, forest, and waste sectors. DTL facilitates decoupling of energy consumption from petroleum extraction, without extensive retooling of existing carbon-based energy carrier infrastructure (i.e., shipping, aviation, medium- and heavy-duty land transport, rail, etc.). Prominent DTL technologies include fast pyrolysis (FP) (typically applied to lignocellulosic materials and dry waste)[3−6] and hydrothermal liquefaction (HTL).[7−9]

BLOs are complex mixtures of oxygenated hydrocarbons with wide-ranging properties, depending on the feedstock, process conditions (such as temperature and pressure), and conversion technology used.[10−13] Understanding the properties of these liquids is vital for their downstream upgrading for the production of fungible biofuels[14−18] or for their direct applications in heat and power generation.[19−22] Of specific interest here is the composition analysis of BLOs. Adoption of methods applied in petroleum product analysis for biomass-derived materials has been common practice. In some cases, however, methods are adapted for BLOs, or new methods are developed to ensure suitability.[23]

Compositions of BLOs play a critical role in determining their suitability for end use. Carbon and hydrogen are primary contributors to heating value, whereas oxygen limits heating value.[24] These elements are prevalent as major components of biomass constituents, from which BLOs are derived. These major biomass constituents include but are not limited to cellulose, hemicellulose, lignin, starch, sugars, extractives, lipids, and fats.[24,25] However, as more diverse biomass, such as algae, are considered from increased competition for biomass feedstocks, contributions to composition by nitrogen and sulfur become significant in the form of universal macronutrients.[24] Water is inherent to biomass in the form of moisture, in addition to being generated through dehydration reactions during BLO production.[3,26] In HTL biocrude production, most water is phase separated from the organic-rich phase.

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The large presence of oxygen and its removal in BLO by upgrading has been a major research focus.[27] However, the presence of nitrogen and sulfur also poses critical challenges. Regulations and environmental guidelines continue to demand stringent fuel specifications requiring desulfurization and denitrification to mitigate production of SO_x and high global-warming-potential NO_x.[28] For BLO upgrading, removal of sulfur is required to prevent downstream poisoning of noble metal catalysts.[29,30] However, with low sulfur feedstocks, denitrification becomes a priority for removal.[28] Nitrogen is particularly more difficult to remove than sulfur.[31]

Inorganics in BLOs originate from feedstock contamination with sand or soil,[3] heat transfer media,[32] feedstock preparation,[33] catalysts,[34] and attrition of process equipment.[35] These inorganics are also intrinsic to feedstocks as plant macronutrient (N, S, P, K, Ca, and Mg) or micronutrient (Fe, Mo B, Cu, Mn, Zn, Ni, and Cl) requirements. The disposition of BLO inorganics comprises soluble ions in solution, insoluble precipitates (i.e., salts) in condensed solid inclusions (i.e., small particles), organometallic compounds or complexes, and adsorbed species on charged or partially charged adsorption sites of condensed solid inclusions (i.e., char).[35]

The presence of inorganics has several implications. In prime mover applications (ICE and turbines)[22] and in stationary combustion applications, inorganics cause fouling on engine linings and boiler tubes and lead to corrosion.[22,32,36] Furthermore, inorganics poison catalysts and can promote coke formation during catalytic upgrading.[22] Thus, measurement of inorganics is vital to understanding and mitigating associated risks.

As DTL technologies have matured, standards have been developed that recommend specific test methods for evaluation of BLOs' suitability for specific applications such as ASTM D7544 (Standard Specification for Pyrolysis Liquid Biofuel) or EN 16900 (Fast Pyrolysis Bio-Oils for Industrial Boilers).[37,38] With important implications and decisions dependent upon measurements of BLO composition, it is critical that the performance of applied techniques is understood. Specifically, insights into the accuracy, repeatability, and reproducibility of measurement techniques for various properties of BLOs are fundamental to understanding and developing DTL technologies. The inability to measure BLO properties accurately and precisely can be a barrier to their market adaptation.

To better understand the analytical requirements of these BLO samples, the International Energy Agency's Bioenergy Task 34: Direct Thermochemical Liquefaction has conducted a number of interlaboratory studies (ILS), the last one reported in 2020.[39] This study purported to include a blind duplicate sample among those distributed to the laboratories. In fact, this blind duplicate had been prepared in a different subsampling campaign from that of the original sample. As a result, statistically significant differences were observed for the majority of physical and elemental properties tested on the supposed duplicates. This study raised concerns related to consistency in subsampling and sample homogeneity.

A further challenge raised by the 2020 study was the analysis of nitrogen using elemental analyzers, following ASTM D5291. This analysis technique has a lower reporting limit of 0.75 wt % (or 0.1% for nonvolatile samples by one specified instrument),[40] while many BLOs have nitrogen content below this threshold (particularly Fast Pyrolysis Bio-Oils (FPBO) from clean virgin stemwood feedstocks). Despite this, it is the recommended analysis method by EN 16900. As nitrogen content is an important factor for process sustainability during downstream upgrading,[31] accurate nitrogen analysis is critical.[41] For this reason, analytical methods developed for samples with a lower nitrogen content were considered in this study.

Similarly, determination of sulfur content by the often-practiced ASTM D1552 implies a method with a lower reporting limit of 0.22 wt % (although in our experience laboratories can typically achieve a limit of quantification (LOQ) around 0.05 wt %).[42] The European specification EN 16900 recommends sulfur determination by ISO 20846, a fluorescence method with a significantly lower LOQ. The sulfur content for BLOs derived from clean stem wood sources can be in the mg/kg concentration range, requiring fluorescence techniques for determination.[43] Furthermore, if BLOs are upgraded to or used directly as transportation fuels, then this process would require accurate sulfur determination (and possible desulfurization) to meet local limits on sulfur content.[41]

Inductively coupled plasma (ICP) techniques (including OES or MS) enable the detection and quantification of inorganics. To the best of the authors' knowledge, no existing standard on ICP specifically addresses BLOs.[22] However, the most closely related standards include CSA ISO or ISO 16967, IP501, and ISO 11885. The 16967 series of standards address solid biofuels, specifically their digestion or complete solubilization of sample inorganics or its calcined ash at 525−550 °C prior to application of ICP variants or flame spectroscopy.[44] IP 501 involves application of ICP to digestate of fused ash of residual fuel oil.[45] The final standard specifies determination of dissolved and particle-bound elements in various aqueous mixtures but may be extended to digests of water and wastewater sludges or sediments with careful consideration for additional interferences.[46] For this reason, a relatively new standard test method has emerged from NREL for BLOs[47] but has yet to be the subject of ILS to produce repeatability and reproducibility values.

The primary goal of this study was to develop precise guidance for achieving sufficiently representative sampling for BLO composition analysis. The scope of this study primarily considers sample mixing immediately before instrumental analysis. To this end, different degrees of sample mixing, one low and one high, were tested to understand the impact on repeatability and reproducibility. To assist with the possible mistaken attribution of poor homogeneity that may actually be a result of inconsistent subsampling and sample integrity or vice versa, blind duplicates were included for all BLOs and sample mixing. Thus, to a certain extent, the scope of the study also extends to BLO subsampling from an initial bulk quantity, which also includes impacts on integrity during sample storage and distribution.

The secondary goal of this study was the performance evaluation of emerging trace compositional analysis of BLOs not yet subjected to ILS before. There has yet to be trace analysis of a common set of BLOs that have been reproduced by two or more independent laboratories. This is fundamentally required for the validation of any proposed analytical method or set of methods.

It is also important to highlight that specifics around analytical methods and their protocols were not enforced. Method development, which constitutes a coordinated effort, falls beyond the scope of this study; therefore, insights into improved or worse performance because of different method specifications are limited. However, this study shares the meaningful recommendations that are supported by evidence.

■ STUDY DESIGN

Participating Laboratories. A total of 14 laboratories were enrolled in the round-robin (RR) study, although not all enrolled laboratories returned data. Some of these are listed alphabetically: CanmetENERGY in Ottawa (CE-O) (Canada), Cepsa (Spain), Chevron (USA), DBFZ Deutsches Biomasseforschungszentrum Gemeinnützige GmbH (Germany), Innotech Alberta (Canada), Karlsruhe Institute of Technology (Germany), Scion Research (New Zealand), and VTT (Finland). Additional laboratories from Europe and North America participated but requested to remain anonymous. Each participant lab was randomly assigned a unique identifier code (01−14).

Instructions to Laboratories. Laboratories were recommended to use ASTM D5291 and ASTM E203 for CHN and water analyses, respectively.[40,48] These methods have a long history of application to FPBOs and have been used with minimal modification for many years. For trace sulfur and trace nitrogen analyses, laboratories were advised to use methods such as ASTM D5453 for sulfur analysis (i.e., by combustion—UV fluorescence),[49] and D5762 or D4629 or similar methods for nitrogen analysis (i.e., by combustion—chemiluminescence).[50,51] Given the unique characteristics of BLOs, laboratories were instructed to use a polar solvent for sample dilution.

For ICP analysis, laboratories were invited to use a method developed by NREL.[47] However, most laboratories reported ICP results acquired through their own in-house method.

Laboratories were guided to use a Pasteur (or disposable) glass pipet, when possible, for taking analysis aliquots, instead of using a syringe. It is surmised that homogeneous subsampling of suspended solids in some high solids-loaded samples is prevented by insufficient bore of syringe needles normally used to obtain sample aliquots.

To determine the effect of mixing on sample results, laboratories were provided two sets of samples and instructed to either well-mix or gently mix the sample. The well-mixed samples were to be warmed to 50 °C for 30 min, followed by vigorous shaking or stirring for at least 30 s. If the sample had any indication of nonhomogeneity, they were to continue shaking, in 30 s intervals, until the appearance of uniformity was attained. The protocol for gently mixed samples prescribed gentle shaking for up to 10 s.

BLOs Studied. BLOs for this study were generously donated by five organizations from across Europe and North America derived from a diverse range of feedstocks and conversion technologies. Four of the oils were FPBOs and the fifth an algae-derived HTL biocrude.

BTG Biomass Technology Group BV provided a sample from their rotating cone reactor derived from clean sawdust.

CanmetENERGY-Ottawa (CE-O) of Natural Resources Canada provided a fluidized-bed FPBO derived from roadside forestry harvesting slash from Quebec, Canada. More than 80% of this residue comprised softwood branches. The FPBO was produced in their pilot plant described elsewhere.[3]

Ensyn provided an FPBO from Kelly Group's fast pyrolysis plant in Renfrew, Canada. This plant produces over 11 ML annually from forestry and sawmill residuals, including both mixed hardwoods and softwoods.

VTT of Finland contributed a wheat straw-derived FPBO produced in their fluidized-bed reactor pilot plant.[5]

Pacific Northwest National Laboratory (PNNL), part of the United States Department of Energy, provided an algae-derived HTL biocrude.

The properties of the BLOs (excluding those properties to be analyzed by each lab) were determined following ASTM Standard Test Methods (with modifications, as required for the sample type). Additionally, halogens were determined by high-pressure combustion followed by ion chromatography of the washings in a method similar to that of EPA 5050.

Sample Preparation and Distribution. Bulk samples of BLOs (approximately 3 L each, five samples in total) were amassed and stored at −20 °C until distribution to the participants. Prior to subsampling, bulk samples were thawed and then homogenized by heating to 50 °C in an oven for 1 h, followed by blending with a shear mixer for 15 min. For each oil, 64 subsamples, each with approximately 40 mL of size, were prepared and poured directly from the homogenized bulk sample bottle. Subsamples of each BLO were randomly assigned one of four letter codes; two subsamples for application of gentle mixing and the two others for vigorous mixing (see Supporting Information Figure S1 for a sampling and distribution diagram). This provided a blind duplicate of each oil type for each mixing protocol. Laboratories would receive a random numbered bottle from each lettered series, thus 20 bottles in total (for example, Lab 6 was given sample bottles F-16, I-31, Q-2, and T-21, which were the 31st, 61st, 5th, and 43rd pours of the CE-O oil, respectively). The identity of bottles shipped to each lab is included in Supporting Information Table S1.

Furthermore, each lab was provided a unique analysis order to remove any sample carryover effect that could be inadvertently observed had every lab performed analysis in alphabetical order (Supporting Information Table S2). Each lab was asked to analyze each bottle in triplicate and report all results (excluding those errors with assignable cause).

To summarize, participating laboratories were asked to analyze all BLOs in triplicate after gentle sample mixing and again but with vigorous mixing. This was effectively performed twice over all BLOs with the inclusion of blind duplicates.

Packages containing BLOs and documentation were shipped to laboratories at the end of January 2024. One lab required a reshipment of oils after the first was delayed by 2 weeks in local customs quarantine. Analytical results were returned by the laboratories in the spring and summer of 2024 and were decoded using guides produced in the planning and sample preparation phase.

Statistical Methods. Statistical calculations were performed using Microsoft Excel, Python, and R with various R packages[52−56] employed to streamline the analysis process. Wilcoxon and Student's *t* tests were utilized to assess differences in mean values for establishing significance. These tests effectively yield a statistic, a *p* value, which indicates the likelihood of two observed means to be different. Setting lower *p*-value thresholds or stricter significance levels makes it harder to conclude that the observed difference is unlikely to have occurred by chance alone. Identifiable errors, typically related to data entry, were resolved by cooperation with laboratories. ASTM E691 Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method was used to calculate statistics for CHN and water results.[57]

Table 1. Properties of Oils Used in the Round-Robin Study

			BLO				
property	units	test method	BTG	CE-O	Ensyn	VTT	PNNL
viscosity (40 °C)	cSt	ASTM D445	31.47	123.3	73.73	42.19	655.1
viscosity (60 °C)	cSt	ASTM D445	11.48	22.37	22.35	10.16	189.3
density (20 °C)	kg/m^3	ASTM D4052	1206	1196	1229	1193	998.6
solids	wt %	ASTM D7579	<0.01	0.926	0.011	1.389	0.079
carbonyl	mol/kg	ASTM E3146	4.7	3.8	4.3	4.5	2.8
gross calorific value	MJ/kg	ASTM D240	17.840	19.565	17.900	17.400	36.060
TAN	mg KOH/g	ASTM D664	76.1	78.1	60.5	83.5	45.5
pH		ASTM E70	2.32	3.25	2.84	3.31	7.72
halogens F/Cl	ppm	bomb combustion/ion chromatography	120/17	13/58	30/21	105/71	<10/18
ash (750 °C)	wt %	ASTM D482	0.014	0.182	0.177	0.859	0.307
oxygen	wt %	ASTM D5622	48.6	44.5	49.5	48.6	12.5

RESULTS

BLO Properties. The analysis results of the specific properties of five BLOs not analyzed by participating laboratories are shown in Table 1. All results are reported on an "as-analyzed" basis. The first four BLOs have properties within the range expected for FPBOs. Similarly higher viscosity, lower density, higher energy content, and lower oxygen content fall within expectations for the PNNL HTL biocrude.

CHN and Water. Laboratories were requested to perform water and CHN analyses. Presumably, these test methods are well-practiced and can serve as a reference for lab performance. Consequently, they would provide a clear indication of impacts from gentle and vigorous sample mixing.

CHN. A total of 11 laboratories returned CHN data. One laboratory's values were outliers for all reported parameters, and another laboratory's nitrogen results were outliers. Summary statistics are shown in Tables 2, 3, and 4, with the statistical outliers removed.

Table 2. Results of Carbon Analysis of Gently and Well-Mixed BLOs (10 Labs Included, One Lab Dropped)[a]

sample/mix	carbon (wt %)	standard deviation	Student's *t* *p* value	Wilcoxon *p* value
BTG FPBO gentle	43.4	0.624	0.295	0.247
BTG FPBO well	43.6	0.671		
CE-O FPBO gentle	47.0	0.897	0.581	0.581
CE-O FPBO well	46.9	0.454		
Ensyn FPBO gentle	43.6	0.864	0.00183	0.0000892
Ensyn FPBO well	44.0	0.613		
PNNL HTL gentle	72.0	0.528	0.572	0.371
PNNL HTL well	71.9	0.560		
VTT FPBO gentle	42.1	0.569	0.100	0.116
VTT FPBO well	42.3	0.673		

[a] Student's *t* test and Wilcoxon test *p* values are shown comparing gently and well-mixed datasets for difference.

Table 3. Results of Hydrogen Analysis of Gently and Well-Mixed BLOs (10 Labs Included, One Lab Dropped)[a]

sample/mix	hydrogen (wt %)	standard deviation	Student's *t* *p* value	Wilcoxon *p* value
BTG FPBO gentle	7.64	0.216	0.0142	0.0710
BTG FPBO well	7.52	0.322		
CE-O FPBO gentle	7.73	0.219	0.468	0.454
CE-O FPBO well	7.69	0.270		
Ensyn FPBO gentle	7.53	0.174	0.0329	0.0399
Ensyn FPBO well	7.43	0.288		
PNNL HTL gentle	10.4	0.196	0.0622	0.157
PNNL HTL well	10.3	0.297		
VTT FPBO gentle	7.78	0.271	0.410	0.399
VTT FPBO well	7.74	0.290		

[a] Student's *t* test and Wilcoxon test *p* values are shown comparing gently and well-mixed datasets for difference.

The measurements of carbon, hydrogen, and nitrogen for the BLOs in this study correlate well with the results observed in previous studies and reviews.[39,58]

When comparing multiple data sets and multiple parameters, the requirement for true significance (i.e., $p < 0.05$) becomes more stringent. In this situation, with five BLOs duplicated across two mixing parameters (20 tests total), true significance requires a p value of <0.0025.[59] Based on Tables 2, 3, and 4, only Ensyn's FPBO carbon analysis showed a statistically significant difference when comparing gentle and vigorous mixing (t test, $p = 0.00183$).

Though the hydrogen content was generally higher in the gently mixed samples, no statistically significant trend was detected. Similarly, the mixing method was not found to have a significant effect on nitrogen content. Notably, much higher relative standard deviation (RSD) is observed in nitrogen analysis of Ensyn and BTG's FPBOs that contain the lowest nitrogen content.

For each treatment comprising a unique combination of BLO and mix protocol, laboratories received two sample bottles. These two sample bottles presumably contained the same contents acting as blind duplicates for one another (with

Table 4. Results of Nitrogen Analysis of Gently and Well-Mixed BLOs (Nine Labs Included, Two Labs Dropped)[a]

sample/mix	nitrogen (wt %)	standard deviation	Student's *t* *p* value	Wilcoxon *p* value
BTG FPBO gentle	0.0896	0.0385	0.271	0.986
BTG FPBO well	0.102	0.0633		
CE-O FPBO gentle	0.505	0.0434	0.450	0.627
CE-O FPBO well	0.514	0.0716		
Ensyn FPBO gentle	0.0957	0.0465	0.948	0.531
Ensyn FPBO well	0.0964	0.0388		
PNNL HTL gentle	5.00	0.0779	0.490	0.508
PNNL HTL well	5.01	0.0915		
VTT FPBO gentle	0.596	0.0447	0.599	0.603
VTT FPBO well	0.590	0.0707		

[a] Student's *t* test and Wilcoxon test *p* values are shown comparing gently and well-mixed datasets for difference.

two mix protocols, laboratories actually received a total of four sample bottles with the same BLO). Laboratories were asked to perform each analysis in triplicate, and most returned three results per bottle analyzed (although some performed less or more replicates).

To assess the consistency of sample bottle preparation, variability in carbon analysis for every bottle was analyzed. To this end, the standard deviations (SD) of replicate carbon measurements from every sample bottle was compiled and averages shown in Table 5. For well-mixed samples, the average SD was only 0.18 wt.%. When replicate measurements are pooled across a pair of corresponding blind duplicate sample bottles, the average SD does increase but only marginally. For instance, the difference between single-bottle and two-bottle-pooled average SDs for more vigorous mixing was only 0.06 wt.%. Differences of means between blind-duplicate sample bottles were also compiled, and the average difference is of similar order of magnitude as the single- and two-bottle-pooled SD. Nearly identical trends and figures are obtained for both gently and well-mixed treatments.

Table 5. Comparison of Carbon Analysis Variability for Single- and Pooled-Blind-Duplicate-Sample Bottles[a]

mixing protocol	single-bottle $\overline{\mathrm{SD}}^{\mathrm{A}}$ (wt %, $n = 100$)	two duplicate-bottle $\overline{\mathrm{SD}}$ (wt %, $n = 50$)	average duplicate bottle difference (wt %, $n_{\mathrm{pairs}} = 50$)
well-mixed	0.18	0.24	0.24
gently mixed	0.20	0.26	0.25

[a] Average standard deviation.

Carbon analysis was also plotted as a function of actual pour order per subsample preparation applied in this ILS (see Supporting Information Figure S2). No obvious trends were observed, such as consistent results above or below the mean with increasing or decreasing pour order. The lack of trends suggests a limited impact from sample bottle pour order.

Water. Results from eight laboratories are shown in Table 6. One lab returned only single replicate results (Lab 12). Another lab returned multiple extra results. Both were still considered in calculation of summary statistics. Slightly higher water content of VTT's FPBO can be attributed to high ash content in the wheat straw feedstock (see Table 1), which increases water yields during FP.[5] This observation may also be a result of feedstock moisture content.[60]

Table 6. Results of Water Analysis of Gently and Well-Mixed BLOs (Eight Labs Included)[a]

sample/mix	water (wt %)	standard deviation	Student's *t* *p* value	Wilcoxon *p* value
BTG FPBO gentle	22.6	1.94	0.277	0.812
BTG FPBO well	22.9	1.08		
CE-O FPBO gentle	21.4	1.39	0.343	0.147
CE-O FPBO well	21.7	1.52		
Ensyn FPBO gentle	22.2	2.05	0.685	0.285
Ensyn FPBO well	22.1	1.55		
PNNL HTL gentle	6.83	0.273	0.00434	0.151
PNNL HTL well	7.15	0.669		
VTT FPBO gentle	25.5	1.17	0.591	0.442
VTT FPBO well	25.4	1.76		

[a] Student's *t* test and Wilcoxon test *p* values are shown comparing gently and well-mixed datasets for difference.

When comparing gentle and vigorous mixing, statistical significance is approached only by the PNNL oil at a significance of <0.0025 (*t* test, $p = 0.0043$). It appears that it was highly influenced by the results from two laboratories. For six laboratories, the difference in the average water content between well and gently mixed was lower than 0.22 wt %. For two laboratories, a well-mixed sample was found to have a higher water content by 1.52 and 0.88 wt %. Student's *t* test showed that the difference was significant with a *p*-value lower than 0.002.

Trace Nitrogen and Sulfur. An insufficient number of laboratories returned results for nitrogen or sulfur analysis for statistical determination of repeatability and reproducibility values. The results of nitrogen analysis are nonetheless shown in Table 7 with the standard deviations (SDs) of the analysis from four laboratories. Relative standard deviations (RSDs) ranged from 5 to 19%.

Table 7. Nitrogen (Trace) Average Reported Values and Standard Deviations for Each Well-Mixed BLO, Data from Four Laboratories

sample	nitrogen (trace) (wt %)	standard deviation
BTG FPBO	0.0533	0.0030
CE-O FPBO	0.3814	0.0715
Ensyn FPBO	0.0573	0.0042
PNNL HTL	3.625	0.500
VTT FPBO	0.4321	0.0688

An interesting observation from the nitrogen determination by D5291 analysis was consistently higher measurements when compared with those of trace nitrogen by chemiluminescence methods. Moreover, RSDs from trace nitrogen for two FPBOs with the lowest nitrogen content were within 10%, albeit with low statistical weight with only four responding laboratories. In contrast, RSDs for nitrogen from CHN of the same samples were within the range of 40−63%.

The effect of mixing on nitrogen is shown and discussed in the Supporting Information (see Table S3). Also shown there are the results of trace sulfur analysis; with data returned by only two laboratories, no conclusions can be drawn from the data. However, there are no inherent disadvantages to using polar, sulfur-free solvents in analysis of trace sulfur content.

ICP. Participating laboratories were recommended a method for ICP, but many used their own method. Many laboratories performed analysis by microwave-aided digestion in nitric acid, but two laboratories performed direct aspiration of BLO samples diluted in butanol (see Table 8). Some laboratories reported all results, while others restricted their reporting to what values were above their determined LOQ values, despite instructions requesting all data be reported. The statistical analysis focused on only the numeric values and could not consider any less-than values reported by laboratories (e.g., "<0.1").

Table 8. ICP Method Used by Each Participating Laboratory

lab ID	standard method (if provided)	method description
3		ICP-OES—direct—samples dissolved in butanol
6	NREL (modified)	microwave digestion in HNO_3—ICP-MS
8		microwave digestion in HNO_3—ICP-OES
9		microwave digestion in HNO_3—ICP-MS
11	DIN EN 16170 of digestate	microwave digestion in HNO_3—ICP-OES
12	AOCS Ca17-01	ICP-OES—direct—samples dissolved in 1-butanol
15		microwave digestion in HNO_3—ICP-OES

Results of ICP analysis (from well-mixed samples only) are shown in Table 9. As with nitrogen and sulfur, there are an insufficient number of laboratories for determining repeatability and reproducibility values, but interim statistics are shown in Table 12. Quantification of lithium was not reported by laboratories despite being on the reporting sheet as well in the recommended NREL standard test method.

DISCUSSION

CHN and Water. Similar hydrogen and carbon contents are observed for all FPBOs. According to Oasmaa and Czernik, carbon and hydrogen contents typically vary between 32 and 49 wt % and between 6.9 and 8.6 wt %, respectively.[61] Observations of 42−47 wt % for carbon and 7.4−7.8 wt % for hydrogen for FPBOs in this study fall within this range. Forest residues comprise more bark and a high extractive content. Thus, this explains the slightly higher carbon content observed in CE-O's FPBO.[25] The high carbon and hydrogen content observed in PNNL's HTL biocrude is consistent with algal feedstocks composed of up to 60 wt % lipids.[62] Nitrogen contents of FPBOs below 1 wt % can be explained by the limited amount of nitrogen in the various lignocellulosic feedstocks. High nitrogen content RSDs observed in FBPOs highlight the frustration of ASTM D5291 or similar methods at lower levels of nitrogen. In contrast, the HTL biocrude contained around 5 wt % of nitrogen, as typically reported for biocrude from algae, due to its high protein content.[39,62,63]

Subsampling and Blind Duplicate Consistency. Carbon, hydrogen, and water measurements are widely established methods. Therefore, analysis of results from these methods may be used to assess the quality of representative sampling applied in this ILS. Representative sampling is a critical prerequisite for any RR and was a concern raised in the previously mentioned 2020 study. The execution of representative sampling applied in this ILS, to the extent that enables consistent bulk composition analysis, is supported by analysis of carbon measurements from single sample bottles and corresponding blind duplicate bottles (Table 5). Pooling of replicate measurements across two sample bottles containing the same BLO and subject to the same mixing only marginally increased average SD compared to single bottle SD. In the case of nonrepresentative sampling, a much larger difference would have been expected. Mean differences between blind duplicate sample bottles were also within approximately 1 to $1^1/_3$ of single bottle and two-bottle-pooled standard deviations. For normally distributed data, this corresponds to ranges where 68 to 80% of values would be observed.

The preparation of subsamples prior to distribution to laboratories also included pouring into different bottles in random order. Arranging the reported results by the order in which the subsample was produced showed no apparent trends (see Supporting Information Figure S2 and discussion). During subsample preparation, unavoidable differences between each subsample preparation potentially led to variability between bottles. For instance, an unavoidable difference includes a time lag between pours and necessarily different portions of the bulk sample being included in each subsample. In nonhomogeneous subsample preparation, practically some degree of sample separation could occur resulting in contents at the top of the bulk sample being richer in one specific BLO fraction compared to contents at the bottom (such as water-rich fractions, or settleable solids). It would be expected to see some observable trend as a function of pour order as a result. However, spread of values consistently above and below the mean were observed with no obvious monotonic trends regardless of increasing or decreasing pour number.

The marginal increase in SD upon pooling of replicate measurements across two corresponding blind duplicate bottles is unsurprising. This can be attributed to an average difference of only 0.24−0.25 wt % carbon observed between blind duplicate bottles in this study. This observation does indicate a small variability of results between bottles. It could be related to different instrument conditioning at the time of analysis of the two different bottles, different times out of the fridge and in the oven to warm, or other laboratory environmental factors.

Impact of Sample Mixing on CHN and Water Content. In the vast majority of CHN and water analyses conducted, neither of prescribed mixing yielded any consistent benefits over the other. Moreover, the occurrence of outliers was still observed regardless of mixing. Our findings suggest that prescribed mixing is relatively unimportant for CHN and water analysis. Generally, samples are sufficiently homogeneous after gentle mixing alone. Exceptions were found,

Table 9. ICP Results for BLOs (Well-Mixed Only) Showing Result Mean (in mg/kg), Standard Deviation (SD), Number of Labs Reporting Results for That Parameter, and Total Number of (Numerical) Results Included[a]

	Al				Ca				Co				Cr			
sample	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results
CE-O FPBO	14.7	21.3	6	30	227	105	6	35	0.085	**0.230**	4	24	3.28	**6.59**	5	29
BTG FPBO	8.60	13.7	6	30	31.1	36.2	7	34	0.034	**0.128**	4	24	4.07	**18.4**	4	24
Ensyn FPBO	7.48	1.68	5	29	333	132	6	35	0.024	0.038	4	24	2.46	**6.96**	4	24
VTT FPBO	100.	27.8	6	29	534	217	6	34	0.100.	0.135	4	24	4.12	2.19	4	22
PNNL HTL	7.02	18.0	4	24	13.9	11.0	6	30	0.921	0.325	5	28	0.812	1.44	4	24

	Cu				Fe				K				Mg			
sample	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results
CE-O FPBO	0.785	0.448	5	29	85.0	40.5	6	31	55.4	33.0	5	25	141	22.6	6	35
BTG FPBO	0.338	0.384	5	29	13.1	**32.7**	6	32	7.00	4.60	4	23	7.26	5.39	7	32
Ensyn FPBO	0.076	**0.212**	4	24	35.8	29.5	5	29	134	81.5	5	25	95.5	18.8	6	35
VTT FPBO	1.83	0.925	5	28	255	57.0	6	34	818	51.6	5	28	164	26.1	6	34
PNNL HTL	17.5	1.91	5	28	703.2	69.2	7	36	96.7	56.0	6	26	2.07	0.986	5	28

	Mn				Na				Ni				P			
sample	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results
CE-O FPBO	18.0	4.06	6	33	2.52	2.02	4	23	3.34	5.20	5	29	18.7	4.68	5	29
BTG FPBO	2.99	1.34	5	29	3.79	5.24	5	25	2.76	**12.4**	4	24	1.74	0.760	5	29
Ensyn FPBO	38.6	6.54	6	35	10.3	19.1	4	23	1.56	**4.06**	4	24	26.7	29.9	5	29
VTT FPBO	10.4	1.70	6	29	23.5	15.0	4	22	5.37	9.38	5	28	103	18.7	6	30
PNNL HTL	1.47	0.157	5	28	480	54.9	6	30	14.2	2.53	6	30	2.98	0.987	5	28

	S				Si				Sr				Zn			
sample	mean	sd	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results	mean	SD	laboratories	results
CE-O FPBO	457	179	3	18	300		1	6	1.46	0.175	4	24	5.86	4.02	5	29
BTG FPBO	102	88.6	3	18	54.7	64.0	2	2	0.237	0.398	4	24	7.38	**19.8**	5	29
Ensyn FPBO	97.2	92.7	3	18	100		1	2	2.17	0.290	4	24	31.0	**78.8**	5	29
VTT FPBO	722	208	3	18	683	40.8	1	6	2.43	0.295	4	24	2.93	2.84	5	28
PNNL HTL	5089	895	3	18	3845	2371	2	8	0.166	0.0396	4	24	15.7	1.77	5	28

[a] Standard deviations larger than the reported concentration (i.e., those with RSD >100%) are underlined. Those of >200% are bolded.

Table 10. Calculated Repeatability (*r*)/Reproducibility (R) Statistics in This Study[a]

	C		H		N		water	
sample	W	G	W	G	W	G	W	G
BTG	1.70/1.94	1.54/1.80	0.83/0.93	0.52/0.62	0.13/0.28	0.13/0.14	2.36/2.98	3.72/5.38
CE-O	1.16/1.32	2.01/2.58	0.70/0.78	0.57/0.64	0.18/0.21	0.11/0.13	2.94/4.17	2.83/3.90
Ensyn	1.61/1.78	1.72/2.47	0.72/0.83	0.41/0.50	0.08/0.11	0.15/0.16	2.68/4.26	4.82/5.92
PNNL	1.44/1.62	1.43/1.54	0.67/0.86	1.06/1.56	0.18/0.26	0.20/0.23	1.81/1.89	0.63/0.78
VTT	1.76/1.95	1.59/1.66	0.78/0.84	0.73/0.79	0.18/0.21	0.12/0.13	2.82/4.76	2.83/3.29

[a] W—well mixed, and G—gently mixed. Outlier labs removed.

specifically statistical significance observed in Ensyn's FPBO for carbon and PNNL's HTL biocrude's water content.

The observed change in carbon results between well- and gently mixed analysis of the Ensyn FPBO was surprising, given that the sample has no evidently different properties in relation to the other oils (see Table 1). Indication of fundamentally different BLO carbon analysis is suggested, albeit marginal, and was reproduced in blind duplicates. Higher carbon content observed following vigorous mixing indicates an improved distribution of more carbonaceous compounds. Phase separation of FPBOs is a well-known behavior yielding an aqueous fraction and a more viscous organic-rich fraction. Gentle mixing, as prescribed in this ILS, may not have been sufficient to evenly distribute compounds of this organic-rich fraction. As a result, any sample aliquot from a gently mixed FPBO subsample may be more enriched with aqueous or hydrophilic compounds.

In the case of PNNL's HTL biocrude, whose water content approached statistical significance, a similar explanation may be synthesized. Higher water content observed following vigorous mixing may be attributed to a better distribution of water within any subsample and sample aliquot.

Among the samples used in this study, PNNL's biocrude was the sample with the highest viscosity in this study. In some ASTM test methods for petroleum fuels (such as D4870), the sample viscosity should be lower than 250 cSt to ensure representative sampling. With a viscosity above 650 cSt at 40 °C for PNNL's biocrude (Table 1), this value is much higher than that recommended value. Thus, the mixing regime was expected to influence the analysis variability. However, while the results did not demonstrate significant differences across the whole study, two laboratories demonstrated differences between their well-mixed and gently mixed sets. On average, the gently mixed protocol was sufficient even for this high-viscosity sample, but some laboratories may have been *too* gentle.

Despite a few examples of statistical significance attributed to sample mixing found in this ILS, specifically for CHN and water analysis, the impact is rather unremarkable and was not consistent across all BLOs. Upon comparison of Ensyn's BLO carbon content after gentle and vigorous mixing, the difference is merely 0.4 wt %. PNNL's HTL biocrude water content differs by 0.28 wt % when comparing sample mixing.

Moreover, aside from the result value itself, there is no clear trend that either mixing protocol yields better repeatability or precision. This is evidenced by shared instances of both higher and lower SDs when comparing mixing (see Tables 2, 3, 4, and 6). Depending on requirements for precision to detect differences in results, this ILS does indicate the need for consistency of sample mixing to rule out potential contributions to differences from unrepresentative sample aliquots. Future collaborative efforts by BLO producers and analysts should focus on developing standard sampling and subsampling guides similar to those of ASTM D4057. The latter provides recommendations for petroleum and petroleum product sampling for a wide range of analysis, from a variety of bulk sources.[64]

In line with ASTM E691, repeatability and reproducibility according to ASTM E691, with removed outliers, are shown in Table 10. Both repeatability and reproducibility values are significantly higher than the stated performance of the test method (as determined by ASTM D5291 and E203). This is also mirrored by RSDs reported in other BLO RR studies.[39,65−67]

Observations of outliers, some so severe that they can not only be explained by poor mixing of analysis aliquots, are concerning. In the carbon analysis, these outliers are exclusively higher than the observed mean. Method performance may benefit from further refinement to ensure better fit-for-purpose, specifically to BLOs by addressing primarily systematic errors. This is also evidenced by average *Z* scores greater than or equal to 1 shown in Table S5 of the Supporting Information.

Comparison of Nitrogen Results. An insufficient number of laboratories returned results of trace nitrogen analysis to allow statistical determination of repeatability and reproducibility values for these tests. Despite this, it is important to note the consistently higher nitrogen from CHN compared to the trace method (Table 11), even after removal of outlier results.

Table 11. Nitrogen (CHN) and Nitrogen (Trace) Values for Each Well-Mixed BLO, after Removal of Outlier Results from CHN Nitrogen

	nitrogen (wt %)	
sample	CHN	Trace
BTG	0.102	0.053
CE-O	0.514	0.381
Ensyn	0.096	0.057
PNNL	5.01	3.63
VTT	0.590	0.432

The difference may impact the evaluation of suitability of BLOs for end use. Potential reasons for differences include difficulty excluding atmospheric nitrogen from the sample capsules or errors from dilutions required for trace analysis. Furthermore, BTG and Ensyn BLOs, are near or below the reporting limits for the equipment used, per the ASTM D5291.[40] The difference between chemiluminescence and ASTM D5291 method results for nitrogen for the PNNL oil cannot be explained only by the limited data reported in this study and requires further efforts to reconcile.

The migration to polar organic solvents (miscible with the analytical sample) should have no inherent downsides when

Table 12. Comparison of ICP Repeatability and Reproducibility Values from ISO 16967 and This Study

	CAN ISO 16967			this study		
element	observed range (mg/kg)	CV$_r$ (%)	CV$_R$ (%)	observed range[a] (mg/kg)	CV$_r$[b](%)	CV$_R$[b](%)
Al				0–140 (210)	4–238 (57)	17–241 (72)
Ca	1500–14200	1.6–4.3	6.6–7.3	0–890	3–63 (13)	34–124 (53)
Co				0–2	5–331 (89)	38–335 (152)
Cr				0–7 (109)	18–338 (143)	31–341 (194)
Cu				0–22	1–183 (51)	8–485 (100)
Fe	54–1600	5.1–6.6	10.3–11.7	8–1091	2–375 (47)	9–375 (67)
K	691–24500	1.8–1.9	6.4–11.1	0–1088	0.5–24 (6)	6–73 (53)
Mg				0–218	2–105 (13)	12–108 (28)
Mn				0–52	1–60 (8)	9–64 (20)
Na	13–171	5.4–14	23–48	0–594	2–24 (7)	9–179 (74)
Ni				0–18 (78)	3–336 (118)	11–339 (153)
P	74–1490	3.4–3.9	6.7–8.5	0–181	2–122 (23)	12–124 (43)
S				60–5323	1–44 (12)	7–104 (37)
Si				0–5600	N/A	N/A
Sr				0–3	1–167 (19)	4–187 (29)
Zn				1–23 (260)	3–144 (42)	11–210 (70)

[a] Represents the range of most laboratories' measurements for the analyte. Values in parentheses are the largest values observed from laboratories reporting values outside the range. [b] In parentheses are averages from all samples for the given analyte.

using modern instrumentation with moisture scrubbers and excess oxygen in the analysis stream for D4629 nitrogen (as well as ASTM D5453 for sulfur).[68,69] Not only are trends between samples' nitrogen results reflected by both methods but also shortcomings of poor repeatability at low concentrations observed of nitrogen from CHN are attenuated with modified trace N methods.

ICP. Results of ICP analysis shown in Table 9 indicate low precision and reproducibility, in contrast to that of CHN and water analysis, which performed similarly to previous studies. These are also echoed by interim repeatability and reproducibility coefficients in comparison with those from CAN ISO 16967 for solids. As a result, the approach applied to assess the impact of mixing for CHN and water analysis is meaningless due to wide distributions that inevitably overlap.

Despite this, ICP repeatability may be inferred by within the laboratory relative standard deviations (RSDs). There was a low proportion (5% or less) of CHN and water RSDs exceeding 5%. In contrast, much greater proportions (15–71%) of ICP RSDs exceeding 5% were observed, indicating systematic challenges.

The degree of repeatability differed between laboratories, which considers differences between in-house methods. For instance, numerous RSDs of within 5% were observed across all analytes. This is on a similar order of repeatability reported in ISO 16967, although different material types are being compared (see Table 12). Coincidence of poor repeatability for the same samples by different laboratories may be rooted in limitations of in-house methods applied.

Furthermore, poorer repeatability is observed for analytes occurring below 100 mg/kg. This is also echoed by precision statistics presented in CAN ISO 16967, specifically sodium at lower levels.[44] This indicates challenges with representative sampling and homogeneity, specifically with low occurring metals.

Despite shortcomings of poor reproducibility, this ILS provides some confirmation that the status quo of ICP generally provides fair order-of-magnitude information on inorganics occurrence in BLOs. Relative proportions of inorganics were consistent with the provenance of BLOs.

Phosphorus results were relatively low across all of the BLO samples tested. While phosphorus is a critical macronutrient required for algal growth,[70] low quantities of phosphorus observed across five laboratories in PNNL's HTL biocrude may be explained through phosphorus preferentially partitioning to the aqueous and solid products.[71] However, both low and high phosphorus have been observed in other studies.[72] Appreciable amounts of phosphorus observed in FPBOs are rooted from plant micronutrients with higher amounts occurring in nonstem wood forest[73] and agriculture biomass.[74]

Levels of calcium, potassium, and magnesium observed in woody FPBOs are indicative of quantities of nonstem wood biomass inclusion in the converted feedstock. It is noteworthy that some levels of calcium surpass the limits demanded by marine residual fuels in ISO 8217. Overwhelmingly large concentrations of these inorganics observed in VTT's FPBO are characteristic of agriculture biomass.[75]

Low quantities of iron are observed in most FPBOs suggesting contributions from feedstock micronutrients. Particularly high amounts exist in VTT's oil, but coincidentally low chromium levels observed in all BLOs seem to suggest that iron is not deriving from stainless steel process equipment,[32] but rather inclusion of extraneous iron through feedstock contamination with soil or sand. Iron makes a large part of nutrients in algae,[76] explaining its high occurrence in PNNL's HTL biocrude.

High amounts of aluminum observed in VTT's FPBO and high silicon in most BLOs support the notion of feedstock inclusion or heat carrier derived silicon or aluminum.[32] Aluminum and silicon are abundant in earth's crust and their oxides are commonly used as heat carriers.[5,77] The marine residual fuel (ISO 8217) limits for these two elements (summed) is 60 mg/kg for heavy bunker fuels or lower for lighter grades of residual fuels. Should BLOs be considered for direct use in the marine industry, these inorganic components will require removal or abatement.

Sodium, manganese, nickel, zinc, copper, and cobalt, the majority of which are classified plant micronutrients,[33] are low for all BLOs with exception of specifically sodium in PNNL's HTL biocrude. Sodium may be present in relatively high

Table 13. Critical Gaps and Recommendations for BLO Composition Analysis

ID	method	issue	substantiation	limitation	potential remedies
A	water, ASTM E203	global result variability from inconsistent mixing	- difference approaching significance observed for PNNL HTL biocrude when comparing different mixing protocol - reproduced difference across respondent laboratories and blind duplicates	- no other HTL biocrude to compare to	- application of consistent and precise sample mixing procedures (i.e., gentle mixing by hand for 10 s) prior to introduction into analytical apparatus - develop standard sampling procedures similar to ASTM D4057
B	water, ASTM E203	outlier laboratories with consistently high and low measurements	- precision and reproducibility Z scores greater than one		- mitigation of matrix-dependent enhancement and suppression of signal - increased frequency of quality assurance/quality control (QA/QC) checks with reference materials - scrutiny of storage and sample handling impacting sample integrity
C	carbon, ASTM D5291	global result variability from inconsistent mixing	- statistical significance observed for Ensyn's FPBO when comparing different mixing protocol - reproduced difference across respondent laboratories and blind duplicates		- application of consistent and precise sample mixing procedures (i.e., gentle mixing by hand for 10 s) prior to introduction into analytical apparatus - develop standard sampling procedures similar to ASTM D4057
D	carbon and hydrogen, ASTM D5291	outlier laboratories with severely high or low measurements	- occurrence of unexplainable severe outliers uniformly above or below the mean		- increased frequency of quality control (QC) checks with reference materials - scrutiny of storage and sample handling impacting sample integrity
F	nitrogen, ASTM D5291	outlier laboratories with severely high measurements	- occurrence of unexplainable severe outliers uniformly above the mean		- validation of atmospheric nitrogen exclusion processes should be performed by laboratories - calibration with compounds or samples with nitrogen contents in target range (0.1 to 1 wt %) and use of quality control (QC) checks in measured range
G	nitrogen, ASTM D5291	poor repeatability with low measurements	- high standard deviation observed for FPBOs		- use of trace N method (post method validation and standardization) with improved repeatability within range of interest
H	nitrogen, ASTM D5291	doubt in accuracy at low range typical of FPBO	- consistent overestimation in comparison to trace N methods.	- low lab participation in trace analysis	- validate working range of calibration - matrix-matched calibration - validation with spike analysis or standard addition - validation of performance at low range with standard addition
I	trace N, ASTM D5762, or D4629	doubt in accuracy	- consistent underestimation in comparison to trace N methods	- low lab participation	- increased frequency of quality control (QC) checks with reference materials - matrix-matched calibration - validation with spike analysis or standard addition - consensus, standardization, and validation of dilution solvent performance
J	trace sulfur, ASTM D5453	suitability over other methods not known	- RSD within 10−20% observed for range of values between 50 and 7000 mg/kg (within 2% at higher end)	- only observed in two laboratories	- comparison with other methods required to evaluate suitability - validation with spike analysis or standards addition to ascertain precision and/or accuracy
K	ICP	low reproducibility, especially at lower occurrence below 100 mg/kg. Doubt in accuracy	- poor reproducibility and repeatability statistics	-low lab participation	- standardization of precise and quantifiable BLO subsampling protocols - validation of solubility and digestion performance - consensus on digestion and instrumentation methods - method standardization and validation - matrix-matched calibration - address dilution errors - validation with spike analysis or standards addition - establish working range
L	ICP	effect on results of sample preparation	- suppression or enhancement on results for various elements		- clarification of requirement to perform whole sample digestion instead of dissolution.

amounts in algae, explaining the high sodium content observed in PNNL's HTL biocrude.[76]

Impact of Sample Preparation on ICP Results. Separating ICP results by sample preparation type (either digestion in nitric acid or dissolution in butanol) does not meaningfully improve the repeatability of the reported results (see a breakdown in Supporting Information Table S4). Some striking differences exist for elements such as Al, Ca, Cr, Fe, Mn, P, and Zn, where digestion results are routinely higher than those reported by dissolution. This may imply that these elements are more typically bound in phases of oil that are poorly nebulized, atomized, and ionized in the instrument and benefit from being digested into the analysis liquor. The majority of these elements, being multivalent, are known to be found in biomass predominantly in solid forms (i.e., insoluble salts and oxides).[35] Moreover, they typically have poor solubility in polar solvents in the absence of an acid or base. The differences in recovery between two ICP sample preparations support the notion that ICP by dissolution alone is not adequate for complete recovery of these recalcitrant elements.

Counter to this finding is the higher reported concentration of potassium and sodium by dissolution instead of digestion. While the dissolution result typically falls within the (very large) standard deviation of the digestion sample results, this trend holds across all samples for potassium and for all but one sample for sodium. It may be that, in contrast to the elements discussed above, the majority of these elements' concentration are present as salts in the BLO liquid portion and are preferentially sampled through the nebulizer of the ICP.[35]

Finally, for Co, Cu, and Ni, no obvious difference in concentration was observed when comparing the digestion and dissolution analysis.

Variability of Blind Duplicate in ICP Results. Poor reproducibility across laboratories yielded global statistics with little value that masked any insights that could be gained concerning impact of mixing. However, some insights are possible with a comparison of within-lab results from the application of blind duplicates.

Within-lab results from blind duplicates of well-mixed aliquots were compared with one another by Student's *t* test. Difference marked by significance of 0.05 was observed at rates of 25% (see Supporting Information Table S7). This level of disagreement is concerning and indicates high variability even across blind duplicates, despite applying the same mix protocol. This may indicate either systematic errors in ICP methods applied by certain laboratories or challenges with homogeneous sampling of inorganics in BLOs. Validation of ICP results will inherently require the assertion of representative sampling of inorganics. It is suggested that subsampling applied in this study, although mostly satisfactory for CHN and water, may not be sufficient for ICP.

CONCLUSIONS

This study explores issues concerning the precision and reproducibility of BLO composition analysis. The capacity to certify the quality of the BLOs is invaluable. It helps inform decision-making and risk mitigation that affect the development and adoption of DTL in the marketplace. Significant losses, damages, and lost opportunities are at stake without validated methods. Critical gaps and recommendations identified from this study by method are summarized in Table 13.

Gentle mixing of sample bottles for 10 s is generally sufficient for most BLO composition analysis. Regardless of preference of mixing protocol, statistical differences or those approaching statistical difference for some analytes and BLOs attributable to mixing highlight the need for precise and consistent mixing. For ICP, however, it is difficult to assess the efficacy of any prescribed mixing. The extent to which a subsample is representative of any BLO of interest in terms of the distribution of inorganics is potentially a confounding factor for ICP validation efforts. Additional variability in the ICP is related to sample preparation. The results indicate dissolution alone, as sample preparation is inadequate for most multivalent inorganics and digestion is required for these recalcitrant analytes. It was also observed that monovalent inorganics are mostly present as solubilized ions in BLOs prior to digestion or dissolution of the sample preparation. However, sample preparation by dissolution alone unexpectedly yields consistent estimates of sodium and potassium abundances greater than those obtained by digestion. This may suggest matrix-related signal enhancement of sodium and potassium. Bias is observed when comparing the few trace nitrogen results with nitrogen from conventional CHN. However, promise in trace nitrogen is shown due to better repeatability than CHN nitrogen at lower levels observed in FPBOs. Trace sulfur methods were reproduced by two laboratories yielding RSDs within 20% even for measurements below 100 mg/kg and serves to inform suitability over sulfur by widely practiced ASTM D1552.

The status quo for BLO composition analysis comprises several laboratories independently adapting techniques and developing in-house methods for their own requirements. These practices pose challenges if not validated or reproducible and highlight the need for standardization. They potentially limit confidence of any reported result and ultimately raise unneeded concerns, doubts, and uncertainty in DTL technologies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.energyfuels.5c01309.

Raw data (XLSX)

Further information on sample preparation and distribution; discussion of trace nitrogen and sulfur results; information on pour order impact on results; ICP result statistics by sample prep method; laboratory *Z* scores by analyte; and frequency of disagreement of blind duplicate ICP (PDF)

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■ REFERENCES

(1) IPCC. Summary for Policymakers. In *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Core Writing Team, Lee, H., Romero, J., Eds.; IPCC: Geneva, Switzerland, 2023; pp 1−34. .

(2) Fonseca, F. G.; Funke, A. Modeling Fast Pyrolysis of Waste Biomass: Improving Predictive Capability. *Energy Fuels* **2024**, *38* (10), 8762−8776.

(3) Mazerolle, D.; Rezaei, H.; Bronson, B.; Nguyen, L.; Preto, F. Sieving and Acid Washing as a Pretreatment to Fast Pyrolysis of a High Ash Hog Fuel. *Energy Fuels* **2019**, 5352.

(4) Qureshi, M. S.; Oasmaa, A.; Pihkola, H.; Deviatkin, I.; Tenhunen, A.; Mannila, J.; Minkkinen, H.; Pohjakallio, M.; Laine-Ylijoki, J. Pyrolysis of Plastic Waste: Opportunities and Challenges. *J. Anal. Appl. Pyrolysis* **2020**, *152* (October 2019), No. 104804.

(5) Oasmaa, A.; Solantausta, Y.; Arpiainen, V.; Kuoppala, E.; Sipilä, K. Fast Pyrolysis Bio-Oils from Wood and Agricultural Residues. *Energy Fuels* **2010**, *24* (2), 1380−1388.

(6) Alcazar-Ruiz, A.; Dorado, F.; Sanchez-Silva, L. Fast Pyrolysis of Agroindustrial Wastes Blends: Hydrocarbon Production Enhancement. *J. Anal. Appl. Pyrolysis* **2021**, *157*, No. 105242.

(7) Pecchi, M.; Baratieri, M.; Maag, A. R.; Goldfarb, J. L. Uncovering the Transition between Hydrothermal Carbonization and Liquefaction via Secondary Char Extraction: A Case Study Using Food Waste. *Waste Manag.* **2023**, *168* (May), 281−289.

(8) Cronin, D.; Schmidt, A. J.; Billing, J.; Hart, T. R.; Fox, S. P.; Fonoll, X.; Norton, J.; Thorson, M. R. Comparative Study on the Continuous Flow Hydrothermal Liquefaction of Various Wet-Waste Feedstock Types. *ACS Sustain. Chem. Eng.* **2022**, *10* (3), 1256−1266.

(9) Cheng, F.; Cui, Z.; Chen, L.; Jarvis, J.; Paz, N.; Schaub, T.; Nirmalakhandan, N.; Brewer, C. E. Hydrothermal Liquefaction of High- and Low-Lipid Algae: Bio-Crude Oil Chemistry. *Appl. Energy* **2017**, *206* (April), 278−292.

(10) Tirumareddy, P.; Patra, B. R.; Borugadda, V. B.; Dalai, A. K. Co-Hydrothermal Liquefaction of Waste Biomass: Comparison of Various Feedstocks and Process Optimization. *Bioresour. Technol. Reports* **2024**, *27* (March), No. 101898.

(11) Haarlemmer, G.; Guizani, C.; Anouti, S.; Déniel, M.; Roubaud, A.; Valin, S. Analysis and Comparison of Bio-Oils Obtained by Hydrothermal Liquefaction and Fast Pyrolysis of Beech Wood. *Fuel* **2016**, *174*, 180−188.

(12) Pittman, C. U.; Mohan, D.; Eseyin, A.; Li, Q.; Ingram, L.; Hassan, E.-B. M.; Mitchell, B.; Guo, H.; Steele, P. H. Characterization of Bio-Oils Produced from Fast Pyrolysis of Corn Stalks in an Auger Reactor. *Energy Fuels* **2012**, *26* (6), 3816−3825.

(13) Staš, M.; Chudoba, J.; Kubička, D.; Blažek, J.; Pospíšil, M. Petroleomic Characterization of Pyrolysis Bio-Oils: A Review. *Energy Fuels* **2017**, *31* (10), 10283−10299.

(14) Alvarez-Majmutov, A.; Badoga, S.; Chen, J.; Monnier, J.; Zhang, Y. Co-Processing of Deoxygenated Pyrolysis Bio-Oil with Vacuum Gas Oil through Hydrocracking. *Energy Fuels* **2021**, *35* (12), 9983−9993.

(15) Stankovikj, F.; McDonald, A. G.; Helms, G. L.; Garcia-Perez, M. Quantification of Bio-Oil Functional Groups and Evidences of the Presence of Pyrolytic Humins. *Energy Fuels* **2016**, *30* (8), 6505−6524.

(16) Elliott, D. C.*Analysis and Upgrading of Biomass Liquefaction Products*. Richland, WA (USA), 1988. https://www.osti.gov/biblio/6833650.

(17) Krutof, A.; Hawboldt, K. Blends of Pyrolysis Oil, Petroleum, and Other Bio-Based Fuels: A Review. *Renew. Sustain. Energy Rev.* **2016**, *59*, 406−419.

(18) Ohra-aho, T.; Oasmaa, A.; Koponen, P.; Nyyssönen, S.; Tuominen, V.; Aakko-Saksa, P. Quality and Storage Properties of Upgraded Fast Pyrolysis Bio-Oil for Marine Transport. *Energy Fuels* **2024**, *38* (20), 19566−19583.

(19) Oasmaa, A.; Lehto, J.; Solantausta, Y.; Kallio, S. Historical Review on VTT Fast Pyrolysis Bio-Oil Production and Upgrading. *Energy Fuels* **2021**, *35* (7), 5683−5695.

(20) Kass, M. D.; Armstrong, B. L.; Kaul, B. C.; Connatser, R. M.; Lewis, S.; Keiser, J. R.; Jun, J.; Warrington, G.; Sulejmanovic, D. Stability, Combustion, and Compatibility of High-Viscosity Heavy Fuel Oil Blends with a Fast Pyrolysis Bio-Oil. *Energy Fuels* **2020**, *34* (7), 8403−8413.

(21) Lindfors, C.; Oasmaa, A.; Välimäki, A.; Ohra-aho, T.; Punkkinen, H.; Bajamundi, C.; Onarheim, K. Standard Liquid Fuel for Industrial Boilers from Used Wood. *Biomass Bioenergy* **2019**, *127* (October2018), No. 105265.

(22) Lienemann, C.-P.; Quignard, A.; Texier, N.; Charon, N. Measuring Inorganics in Biomass Fast Pyrolysis Oils. *J. Anal. Appl. Pyrolysis* **2021**, *159*, No. 105313.

(23) Ferrell, J. R.; Olarte, M. V.; Christensen, E. D.; Padmaperuma, A. B.; Connatser, R. M.; Stankovikj, F.; Meier, D.; Paasikallio, V. Standardization of Chemical Analytical Techniques for Pyrolysis Bio-Oil: History, Challenges, and Current Status of Methods. *Biofuels, Bioprod. Biorefining* **2016**, *10* (5), 496−507.

(24) Gollakota, A. R. K.; Kishore, N.; Gu, S. A Review on Hydrothermal Liquefaction of Biomass. *Renew. Sustain. Energy Rev.* **2018**, *81*, 1378−1392.

(25) Hon, D. N. S.; Shiraishi, N.*Wood and Cellulosic Chemistry, Revised, and Expanded*; CRC Press, 2000. .

(26) Westerhof, R. J. M.; Kuipers, N. J. M.; Kersten, S. R. A.; van Swaaij, W. P. M. Controlling the Water Content of Biomass Fast Pyrolysis Oil. *Ind. Eng. Chem. Res.* **2007**, *46* (26), 9238−9247.

(27) Iliopoulou, E. F.; Triantafyllidis, K. S.; Lappas, A. A. Overview of Catalytic Upgrading of Biomass Pyrolysis Vapors toward the Production of Fuels and High-value Chemicals. *WIREs Energy Environ.* **2019**, *8* (1), No. e322.

(28) Ortega, E.An Overview of Hydrotreating. *CEP Magazine*. American Institute of Chemical Engineers October 2021; pp 29−33. https://www.aiche.org/resources/publications/cep/2021/october/overview-hydrotreating.

(29) Wang, H.; Lee, S.-J.; Olarte, M. V.; Zacher, A. H. Bio-Oil Stabilization by Hydrogenation over Reduced Metal Catalysts at Low Temperatures. *ACS Sustain. Chem. Eng.* **2016**, *4* (10), 5533−5545.

(30) Mortensen, P. M.; Grunwaldt, J.-D.; Jensen, P. A.; Knudsen, K. G.; Jensen, A. D. A Review of Catalytic Upgrading of Bio-Oil to Engine Fuels. *Appl. Catal. A Gen.* **2011**, *407* (1−2), 1−19.

(31) Prado, G. H. C.; Rao, Y.; de Klerk, A. Nitrogen Removal from Oil: A Review. *Energy Fuels* **2017**, *31* (1), 14−36.

(32) Meers, E.; van de Beld, L.; Prins, W.; Leijenhorst, E. J.Fate of Minerals in the Fast Pyrolysis Process. In *22nd European Biomass Conference: Setting the Course for a Biobased Economy*; ETA-Florence Renewable Energies: Florence, Italy, 2014. .

(33) Giudicianni, P.; Gargiulo, V.; Grottola, C. M.; Alfè, M.; Ferreiro, A. I.; Mendes, M. A. A.; Fagnano, M.; Ragucci, R. Inherent Metal Elements in Biomass Pyrolysis: A Review. *Energy Fuels* **2021**, *35* (7), 5407−5478.

(34) Gardner, D. W.; Huo, J.; Hoff, T. C.; Johnson, R. L.; Shanks, B. H.; Tessonnier, J.-P. Insights into the Hydrothermal Stability of ZSM-5 under Relevant Biomass Conversion Reaction Conditions. *ACS Catal.* **2015**, *5* (7), 4418−4422.

(35) Leijenhorst, E. J.; Wolters, W.; van de Beld, L.; Prins, W. Inorganic Element Transfer from Biomass to Fast Pyrolysis Oil: Review and Experiments. *Fuel Process. Technol.* **2016**, *149*, 96−111.

(36) Kurzemann, F. R.; Fernández-Delgado Juárez, M.; Probst, M.; Gómez-Brandón, M.; Partl, C.; Insam, H. Effect of Biomass Fly Ashes from Fast Pyrolysis Bio-Oil Production on Soil Properties and Plant Yield. *J. Environ. Manage.* **2021**, *298*, No. 113479.

(37) *D7544 - Specification for Pyrolysis Liquid Biofuel*; ASTM International, 2023. .

(38) *EN 16900:2017 - Fast Pyrolysis Bio-Oils for Industrial Boilers - Requirements and Test Methods*; CEN/TC *19*, 2017.

(39) Bulsink, P.; de Miguel Mercader, F.; Sandström, L.; van de Beld, B.; Preto, F.; Zacher, A.; Oasmaa, A.; Dahmen, N.; Funke, A.; Bronson, B. Results of the International Energy Agency Bioenergy Round Robin on the Analysis of Heteroatoms in Biomass Liquefaction Oils. *Energy Fuels* **2020**, *34* (9), 11123−11133.

(40) *D5291 - Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants*; ASTM International, 2021. .

(41) Nanda, S.; Pattnaik, F.; Borugadda, V. B.; Dalai, A. K.; Kozinski, J. A.; Naik, S. Catalytic and Noncatalytic Upgrading of Bio-Oil to Synthetic Fuels. *An Introductory Review* **2021**, *1379*, 1−28.

(42) *D1552 - Test Method for Sulfur in Petroleum Products by High Temperature Combustion and Infrared (IR) Detection or Thermal Conductivity Detection (TCD)*; ASTM International, 2021. .

(43) Auersvald, M.; Kejla, L.; Eschenbacher, A.; Thi, H. D.; Van Geem, K. M.; Šimáček, P. Detailed Characterization of Sulfur Compounds in Fast Pyrolysis Bio-Oils Using GC × GC-SCD and GC−MS. *J. Anal. Appl. Pyrolysis* **2021**, *159*, No. 105288.

(44) *ISO 16967:2015 Solid Biofuels — Determination of Major Elements — Al, Ca, Fe, Mg, P, K, Si, Na and Ti*; ISO/TC *238*, 2015.

(45) *IP 501: Determination of Aluminium, Silicon, Vanadium, Nickel, Iron, Sodium, Calcium, Zinc and Phosphorous in Residual Fuel Oil by Ashing, Fusion and Inductively Coupled Plasma Emission Spectrometry*; Energy Institute, 2019.

(46) *ISO 11885:2007 Water Quality — Determination of Selected Elements by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)*; 2007.

(47) Christensen, E.; Deutch, S.; Paeper, C.; Ferrell, III, J.*Elemental Analysis of Bio-Oils by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Laboratory Analytical Procedure (LAP)*; Golden, CO (United States), 2022. .

(48) *E203 - Test Method for Water Using Vol.tric Karl Fischer Titration*; ASTM International, 2024. .

(49) *D5453 - Test Method for Determination of Total Sulfur in Light Hydrocarbons, Spark Ignition Engine Fuel, Diesel Engine Fuel, and Engine Oil by Ultraviolet Fluorescence*; ASTM International, 2019. .

(50) *D5762 - Test Method for Nitrogen in Liquid Hydrocarbons, Petroleum and Petroleum Products by Boat-Inlet Chemiluminescence*; ASTM International, 2018. .

(51) *D4629 -Test Method for Trace Nitrogen in Liquid Hydrocarbons by Syringe/Inlet Oxidative Combustion and Chemiluminescence Detection*; ASTM International, 2024. .

(52) Wickham, H.; François, R.; Henry, L.; Müller, K.; Vaughan, D.*Dplyr: A Grammar of Data Manipulation*. 2023; p v1.1.4.

(53) Wickham, H.; Vaughan, D.; Girlich, M.*Tidyr: Tidy Messy Data*. 2024; p v1.3.1.

(54) Wickham, H.*Ggplot2: Elegant Graphics for Data Analysis*; Springer-Verlag: New York, 2016.

(55) Wilke, C.*Ggridges: Ridgeline Plots in ̀ggplòt2*. 2024; p v0.5.6.

(56) Steenwyk, J. L.; Rokas, A. Ggpubfigs: Colorblind-Friendly Color Palettes and Ggplot2 Graphic System Extensions for Publication-Quality Scientific Figures. *Microbiol. Resour. Announc.* **2021**, *10* (44), 1−3.

(57) *E691 - Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*; ASTM International, 2023. .

(58) Oasmaa, A.; van de Beld, B.; Saari, P.; Elliott, D. C.; Solantausta, Y. Norms, Standards, and Legislation for Fast Pyrolysis Bio-Oils from Lignocellulosic Biomass. *Energy Fuels* **2015**, *29* (4), 2471−2484.

(59) Feise, R. J. Do Multiple Outcome Measures Require P-Value Adjustment? *BMC Med. Res. Methodol.* **2002**, *2* (1), 8.

(60) Fonseca, F. G.; Funke, A.; Niebel, A.; Soares Dias, A. P.; Dahmen, N. Moisture Content as a Design and Operational Parameter for Fast Pyrolysis. *J. Anal. Appl. Pyrolysis* **2019**, *139*, 73−86.

(61) Oasmaa, A.; Czernik, S. Fuel Oil Quality of Biomass Pyrolysis OilsState of the Art for the End Users. *Energy Fuels* **1999**, *13* (4), 914−921.

(62) Leow, S.; Witter, J. R.; Vardon, D. R.; Sharma, B. K.; Guest, J. S.; Strathmann, T. J. Prediction of Microalgae Hydrothermal Liquefaction Products from Feedstock Biochemical Composition. *Green Chem.* **2015**, *17* (6), 3584−3599.

(63) Usman, M.; Cheng, S.; Boonyubol, S.; Cross, J. S. From Biomass to Biocrude: Innovations in Hydrothermal Liquefaction and Upgrading. *Energy Convers. Manag.* **2024**, *302*, No. 118093.

(64) *D4057 - Standard Practice for Manual Sampling of Petroleum and Petroleum Products*; ASTM International, 2013. .

(65) Black, S.; Ferrell, J. R. Determination of Carbonyl Groups in Pyrolysis Bio-Oils Using Potentiometric Titration: Review and Comparison of Methods. *Energy Fuels* **2016**, *30* (2), 1071−1077.

(66) Oasmaa, A.; Meier, D. Norms and Standards for Fast Pyrolysis Liquids 1. Round Robin Test. *J. Anal. Appl. Pyrolysis* **2005**, *73* (2), 323−334.

(67) McKinley, J. W.; Overend, R. P.; Elliott, D. C. The Ultimate Analysis of Biomass Liquefaction Products: The Results of the IEA Round Robin #1. In *Proceedings of the Biomass Pyrolysis Oil Properties and Combustion Meeting, 26−28 September 1994, Estes Park, Colorado*; NREL, 1994; pp 34−53.

(68) Matthews, R. D.; Sawyer, R. F.; Schefer, R. W. Interferences in Chemiluminescent Measurement of Nitric Oxide and Nitrogen Dioxide Emissions from Combustion Systems. *Environ. Sci. Technol.* **1977**, *11* (12), 1092−1096.

(69) Tarkanic, S.; Crnko, J.Rapid Determination of Suifur in Liquid Hydrocarbons for At-Line Process Applications Using Combustion/Oxidation and UV-Fluorescence Detection. In *Elemental Analysis of Fuels and Lubricants: Recent Advances and Future Prospects*; ASTM

International: 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428−2959, 2005; pp 137−151. .

(70) Shakya, R.; Adhikari, S.; Mahadevan, R.; Shanmugam, S. R.; Nam, H.; Hassan, E. B.; Dempster, T. A. Influence of Biochemical Composition during Hydrothermal Liquefaction of Algae on Product Yields and Fuel Properties. *Bioresour. Technol.* **2017**, *243*, 1112−1120.

(71) Valdez, P. J.; Nelson, M. C.; Wang, H. Y.; Lin, X. N.; Savage, P. E. Hydrothermal Liquefaction of Nannochloropsis Sp.: Systematic Study of Process Variables and Analysis of the Product Fractions. *Biomass and Bioenergy* **2012**, *46*, 317−331.

(72) Yu, G.; Zhang, Y.; Guo, B.; Funk, T.; Schideman, L. Nutrient Flows and Quality of Bio-Crude Oil Produced via Catalytic Hydrothermal Liquefaction of Low-Lipid Microalgae. *BioEnergy Res.* **2014**, *7* (4), 1317−1328.

(73) Paré, D.; Bernier, P.; Lafleur, B.; Titus, B. D.; Thiffault, E.; Maynard, D. G.; Guo, X. Estimating Stand-Scale Biomass, Nutrient Contents, and Associated Uncertainties for Tree Species of Canadian Forests. *Can. J. For. Res.* **2013**, *43* (7), 599−608.

(74) Li, L.; Ren, Q.; Li, S.; Lu, Q. Effect of Phosphorus on the Behavior of Potassium during the Co-Combustion of Wheat Straw with Municipal Sewage Sludge. *Energy Fuels* **2013**, *27* (10), 5923−5930.

(75) Pienihäkkinen, E.; Lindfors, C.; Ohra-aho, T.; Oasmaa, A. Improving Fast Pyrolysis Bio-Oil Yield and Quality by Alkali Removal from Feedstock. *Energy Fuels* **2022**, *36* (7), 3654−3664.

(76) Lu, J.; Watson, J.; Liu, Z.; Wu, Y. Elemental Migration and Transformation during Hydrothermal Liquefaction of Biomass. *J. Hazard. Mater.* **2022**, *423*, No. 126961.

(77) Foth, H. D.; Turk, L. M.*Fundamentals of Soil Science*, 5th ed.; Wiley, 1972.