Crucial correction of the Faix method for the determination of carbonyls in pyrolysis and hydrothermal liquefaction bio-oils with high nitrogen content

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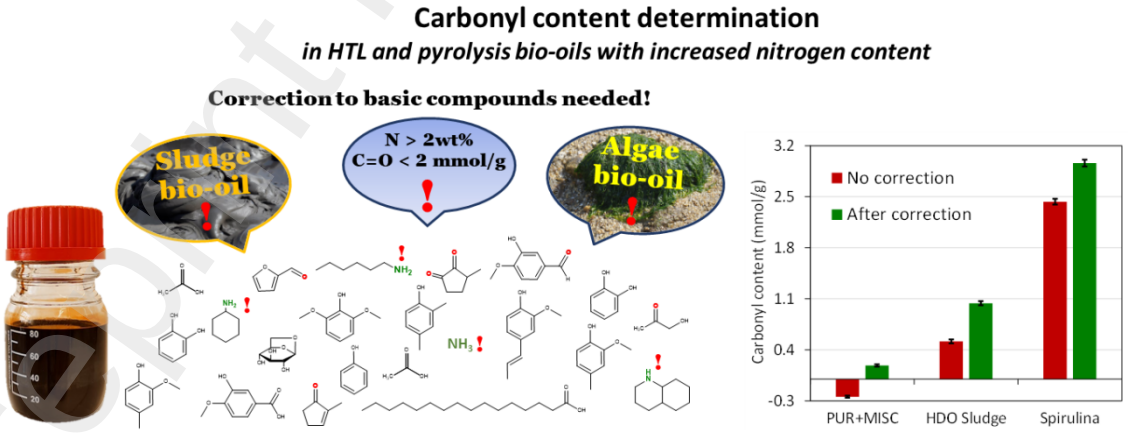
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Abstract

Accurate and reliable determination of carbonyl content in bio-oils from pyrolysis and hydrothermal liquefaction (HTL) of biomass is crucial not only for estimation of sample storage stability but also to create a corresponding design of the hydrotreatment process allowing long-term operation. The composition of bio-oils from various feedstocks can, however, significantly differ from woody bio-oils, which were utilized to develop the Faix method, the most frequently used for carbonyl determination. In this study, we present a limitation of this method when used for the analysis of bio-oils prepared from protein-rich biomass. These bio-oils are known to have a high nitrogen content, which can be present in the form of possibly interfering compounds. In this study, nitrogen interferences were identified, and a correction step mitigating the underestimation of carbonyl content was introduced. This novel approach was tested for 13 crude bio-oils prepared by pyrolysis or HTL from 10 different feedstocks with a wide range of nitrogen content. Besides crude bio-oils, five hydrotreated HTL oils from sewage sludge were also analyzed. Statistical analysis of results showed that the introduction of the correction step can be crucial for bio-oils containing more than 2 wt% of nitrogen. For bio-oils or hydrotreated products with increased nitrogen content but containing less than 0.5 mmol/g of carbonyls, without the correction step, the method can provide zero and in extreme cases even negative values.

Graphical Abstract



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1. Introduction

Liquid products from biomass thermal decomposition like bio-oils from pyrolysis and hydrothermal liquefaction (HTL) are promising intermediates for hydrocarbon fuels production [1]. Although the bio-oils from these two processes differ in physicochemical properties, the typical compounds found in pyrolysis oils are also present in HTL oils [2], and thus, the same analytical methods are usually applied for the analysis of the chemical composition of both bio-oil types [3].

These oils are very complex mixtures of thousands of mostly oxygenated compounds, differing significantly in polarity and molecular weight. Aldehydes and ketones, further called carbonyls, are a very abundant group of compounds in bio-oils creating up to 25 wt% in pyrolysis bio-oils [1,4]. These compounds are very reactive and the main reason for bio-oils low thermo-oxidative stability [5,6]. Carbonyl content determination is thus the main metric for the bio-oil accelerated aging test for the prediction of storage stability [7]. When bio-oils are upgraded to hydrocarbon fuels by hydrotreatment, the content of reactive carbonyls should be reflected in the design of the hydrotreatment process as a catalyst zone operated at a lower temperature is needed to prevent polymerization reactions of carbonyls [8,9]. For this purpose, it is crucial to have an accurate and reliable method for the determination of the carbonyl group content.

Although several bands of different carbonyl groups can be easily identified by FTIR [10], the application of FTIR for quantitative analysis is very problematic because of the different responses of various carbonyl compounds [4]. It is also possible to utilize ^{19}F-NMR method for the determination of carbonyl compounds after derivatization [11], however, the simplest and most affordable option is the titration method developed by Faix [12]. This method was optimized for pyrolysis bio-oils by Black and Ferrell [13] and finally standardized as ASTM E3146 in 2018 [14]. The method is based on the oximation reaction of carbonyl group with hydroxylamine hydrochloride in the presence of excess triethanolamine. The presence of triethanolamine forces the reversible oximation to completion as it reacts with HCl, which is released during the reaction. After the reaction, the content of carbonyls is determined by back titration of the excess triethanolamine using HCl. More details about the possible methods for carbonyls determination can be found elsewhere [4].

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In contrast to the original Faix method optimized for the analysis of lignin, the correction step for strong inorganic acids (pH<2) content, which would overestimate the results, is not needed for bio-oils as strong inorganic acids are not typically present, unless externally contaminated [13]. However, to the best of our knowledge, so far no one has taken into account basic compounds present in bio-oils possibly reacting with HCl. The presence of basic compounds would result in the lower consumption of HCl and underestimation of carbonyl content. The most probable reason is that most of the methods used for bio-oils characterization were developed utilizing pyrolysis bio-oils from wood and are just further applied for analysis of all bio-oils without further validation.

In the case of pyrolysis oils, the majority of biomass ash (possibly containing inorganic bases) remains in the solid product (biochar) as the majority of ash can be removed from pyrolysis bio-oil before its condensation [15]. Regarding the HTL process, the solid product removal from oil is much more difficult because of the high pressure applied in the process [16]. Moreover, basic inorganic salts like Na_2CO_3 and KOH are sometimes used to promote biomass decomposition and increase yields of HTL bio-oil [17,18]. Although the majority of these basic inorganics should remain in the aqueous phase, their presence in bio-oil cannot be excluded. Basic compounds can be, however, present also in form of organic compounds, mostly the ones containing nitrogen. Although lignocellulosic biomass contains usually a low amount of nitrogen (at ppm level), bio-oils prepared from herbaceous biomass like straw can contain significantly more nitrogen (around 1 wt%) [19]. Much more nitrogen can however be present in bio-oils from biomass rich in proteins like algae, sludge, manure, food waste, etc. The oils from these feedstocks can contain up to 10 wt% of nitrogen present in various compounds, mainly amines, amides, nitriles, and heterocyclic compounds, more details about nitrogen in HTL bio-oils can be found in the latest reviews [20,21]. A detailed study of nitrogen transformation during algae pyrolysis was published e.g. by Chen et al. [22]. Many nitrogen compounds present in bio-oils are basic in nature and thus can possibly react with HCl when analyzing carbonyl content.

To overcome the above-mentioned issues and achieve more accurate and reliable results for carbonyl content, we introduced a simple correction step based on analyzing the content of basic compounds in the sample. This approach was tested for various bio-oils and their hydrotreated products, coming from pyrolysis and HTL processes, differing significantly in nitrogen content.

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2. Material and Methods

2.1. Studied model compounds and bio-oil samples

The list of 65 tested nitrogen compounds is shown in the supporting material **Table A1**. The overview of the composition of a model mixture of nitrogen-containing compounds, reflecting average interfering (12%) and basic nitrogen fraction (34%) in studied samples, can be found in **Table 1**.

Table 1 Composition of a model mixture of nitrogen-containing compounds used for HTL straw bio-oil spiking. PYR = bio-oil from pyrolysis, HTL = bio-oil from hydrothermal liquefaction, WS = wheat straw, A = algae, SS = sewage sludge, CM = cow manure.

Compound	Basic nitrogen	Interference carbonyls	Amount in the mixture (wt%)	Identified in bio-oils ([reference] bio-oil type-feedstock)
Quinoline	YES	NO	7.41	[23] PYR-WS; [24] HTL-A; [25] PYR-A; [26] HTL-SS; [27] PYR-SS; [28] PYR-SS
N-Methylpyrolidon	NO	NO	7.62	[23] PYR-WS; [24] HTL-A; [29] HTL-A
Indole	NO	NO	7.54	[22] PYR-A; [23] PYR-WS; [24] HTL-A; [25] PYR-A; [30] HTL-A; [29] HTL-A; [26] HTL-SS; [27] PYR-SS; [28] PYR-SS; [31] PYR-SS
Cyclohexylamine	YES	YES	8.15	[30] HTL-A
Piperidine	YES	YES	7.70	[30] HTL-A
4-Methylquinoline	YES	NO	7.35	[32] HTL-WS, CM; [24] HTL-A; [25] PYR-A; [26] HTL-SS
Benzonitrile	NO	NO	7.60	[22] PYR-A; [23] PYR-WS; [25] PYR-A; [27] PYR-SS; [28] PYR-SS; [31] PYR-SS
Pyrrole	NO	NO	8.15	[22] PYR-A; [23] PYR-WS; [24] HTL-A, [25] PYR-A; [30] HTL-A; [27] PYR-SS; [31] PYR-SS; [33] PYR-A; [28] PYR-SS
Urea	NO	NO	7.41	[32] HTL-WS, CM

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Acetamide	NO	NO	7.96	[23] PYR-WS; [25] PYR-A; [27] PYR-SS; [33] PYR-A; [28] PYR-SS
Pyrazine	YES	NO	7.66	[23] PYR-WS; [24] HTL-A; [25] PYR-A; [29] HTL-A
Pyridine	YES	NO	7.70	[22] PYR-A; [23] PYR-WS; [24] HTL-A; [25] PYR-A; [28] PYR-SS; [33] PYR-A
2-Pyrrolidone	NO	NO	7.74	[23] PYR-WS; [25] PYR-A; [28] PYR-SS

Pyrolysis bio-oils were prepared in Fraunhofer UMSICHT from four different feedstocks by laboratory-scale ablative fast pyrolysis (5 kg/h) equipped with staged condensation of pyrolysis vapors. Biomass in form of briquettes with outer diameter of about 5 cm was pressed onto a hot rotating disc. Bio-oil (organic phase) was condensed in the first condenser operated at 70 °C except for hay, where the cooling water supply temperature was 75 °C. More details about the pyrolysis unit can be found elsewhere [34]. The studied pyrolysis oils overview and respective pyrolysis conditions can be found in **Table 2**.

Table 2 Pyrolysis bio-oils overview

Biomass type	**Designation**	**Pyrolysis temperature (°C)**	**Elemental composition (wt%)**				
			C	**H**	**S**	**N**	**Basic N**
Wheat/barley straw (1:1, m/m)	PYR-S	550	58.59	7.47	0.07	1.39	0.38
W/B straw and autumn leaves (25:75 m:m)	PYR-S+L	550	54.67	8.05	0.09	2.12	0.64
Bush cutting	PYR-BC	550	60.26	7.02	0.03	0.70	0.14
Hay	PYR-HAY	550	48.64	7.71	0.12	2.16	0.49

Nine HTL bio-oils were prepared at Aarhus university using a pilot scale continuous flow reactor. More details about the HTL unit can be found elsewhere [35]. In short, the reactor used is a plug flow reactor with integrated heat exchanger, it has a throughput of 50–100 kg/h using 5–20 wt% dry matter slurries. It typically operates at temperatures of 300–350 °C and pressures of 150–230 bar. The studied HTL oils overview and respective HTL conditions can be found in **Table 3**.

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Table 3 HTL bio-oils overview

Biomass type (article reference)	Designation	Temperature (°C)	Elemental composition (wt%)				
			C	H	S	N	Basic N
Wheat straw **[32]**	HTL-WS	330	63.23	8.05	0.06	0.75	1.14
Cow manure **[32]**	HTL-CM	320	66.09	8.23	0.68	3.43	0.30
Miscanthus + polyurethane (22 78 m:m) **[36]**	HTL-M+PUR	316	63.75	9.53	0.03	2.71	1.05
Primary sludge	HTL-PS1		60.90	10.12	0.90	3.14	0.96
Primary sludge	HTL-PS2	318	75.05	11.14	0.67	2.78	0.99
Primary sludge	HTL-PS3	326	70.05	11.11	0.56	2.64	0.87
Primary sludge + food waste (50:50 m:m)	HTL-PS+FW	315	73.69	11.37	0.34	2.81	0.95
Food waste	HTL-FW	315	66.78	11.98	0.22	2.50	0.97
Spirulina **[35]**	HTL-SP	350	69.1	8.6	0.85	6.6	1.96

Five hydrotreated HTL bio-oils were prepared in laboratory scale hydrotreatment set up from CACTU Solutions (www.cactu.eu). HTL bio-oil from sewage sludge (HTL-PS1) was hydrotreated over a two-zone catalytic bed created by commercial $CoMo/Al_2O_3$ and $NiMo/Al_2O_3$ catalysts. Hydrotreatment was carried out at constant pressure of 4 MPa, bio-oil flow rate of 25 g/h and hydrogen flow rate of 45 L/h. In the CoMo catalyst bed a gradual temperature ramp was kept from ca 145 °C up to 340 °C or the same temperature as in NiMo bed when it was lower. More details about the hydrotreatment process applied can be found in our previous publication utilizing the same reactor and catalyst bed filling [37]. The studied hydrotreated HTL oils overview and respective temperatures of hydrotreatment in NiMo catalyst bed can be found in **Table 4**.

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Table 4 Hydrotreated HTL bio-oils overview

Designation	Average temperature in NiMo catalyst bed (°C)	Elemental composition (wt%)				
		C	H	S	N	Basic N
HTL-PS1-HDO1	376	84.31	12.95	0.08	1.42	0.62
HTL-PS1-HDO2	339	83.64	11.95	0.25	2.41	1.00
HTL-PS1-HDO3	303	79.24	11.39	0.54	3.25	1.20
HTL-PS1-HDO4	303	80.13	11.58	0.36	3.19	1.09
HTL-PS1-HDO5	288	76.96	10.82	0.58	3.13	0.92

2.2. Analytical methods

For all titrations, a potentiometric titrator Titrando 888 (Methrom AG) equipped with a combined pH electrode (LL-Solvotrode easy clean) for non-aqueous titration was used. **Basic nitrogen content** was determined in agreement with the UOP 269 standard. Sample (in the case of pure nitrogen compounds ca 0.2 mmol and in the case of bio-oil ca 0.1-0.2 g) was weighted into the beaker, dissolved in 50 mL of glacial acetic acid and titrated using 0.02 M solution of perchloric acid in glacial acetic acid.

Approximately 0.5 mmol of each nitrogen compound tested was weighted into a beaker, dissolved in 50 mL of 80% ethanol and titrated by 0.1 M HCl standardized by Na_2CO_3 to identify possible interferents in the determination of carbonyls. When reactivity with HCl in this method was observed for direct titration, the analysis was repeated in the presence of triethanolamine (1 mL of solution B for carbonyl content determination). Only compounds, for which equivalence point was determined sooner than for triethanolamine or was joined with triethanolamine equivalence point, can be considered as interferences.

Measurement of **carbonyl content** was carried out in accordance with the ASTM E3146 method. Sample (ca 0.2 g for crude bio-oil or 0.4 g for hydrotreated bio-oils) was weighed into an 8 mL vial. Then 2 ml of hydroxylamine hydrochloride 0.4 N solution in 80% ethanol (solution A), 2 mL of 0.5 N triethanolamine solution in ethanol (solution B), 1 mL of dimethyl sulfoxide and a magnetic stirrer was added into the vial. Each sample batch included 4 vials for blank determination (containing everything except for the sample). The vial was closed and placed into a water bath preheated to 80 °C. The reaction

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proceeded at continuous stirring using a magnetic stirrer for 2 h. After that, the vials were left cooled down to ambient temperature. The content of the vial was then quantitatively transferred to a beaker using 50 mL of 80% ethanol and titrated using 0.1 M HCl. For maintaining the electrode clean, ethanol without added water was found to be a better solvent when hydrotreated bio-oils were analyzed, as they contain preferably hydrocarbons, which form an emulsion in 80% ethanol. As evident from the oximation reaction (**Figure 1**), the carbonyl content in mmol/g is stoichiometrically equivalent to the decrease in triethanolamine content.

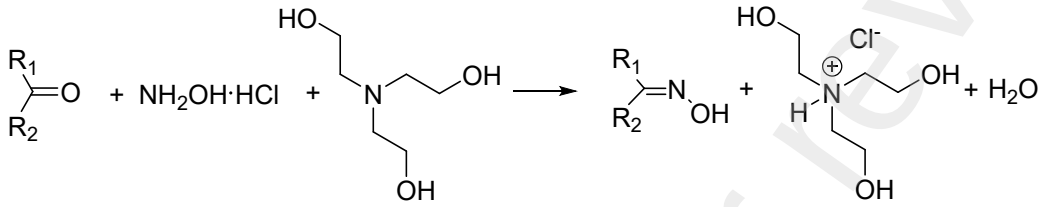


Figure 1: Oximation of carbonyl compounds

To validate the effect of nitrogen compounds on the determination of carbonyl content, HTL bio-oil from straw (containing only 0.75 wt% of nitrogen and 3.80 mmol/g of carbonyls) was spiked by nitrogen-containing compounds model mixture specified in **Table 1**. Three different spiking levels were tested by mixing HTL from straw dissolved in ethanol with the above-mentioned nitrogen compounds mixture to reach ca 5, 10 and 14 wt% of nitrogen in the HTL bio-oil. Prepared samples were measured by the ASTM E3146 method to determine the effect of the nitrogen compounds addition on the determined carbonyl content.

As clear from the oximation reaction (**Figure 1**) and the whole determination principle in the carbonyl content analysis, the amount of HCl released by oximation in mmol/g reacted with basic compounds present in the vial is equivalent to the amount of carbonyl content. When other basic compounds than triethanolamine reagent reactive with HCl will be present in the vial, the carbonyl content cannot be determined reliably without a correction step. To eliminate the possibility of underestimation of the carbonyl content, three different correction approaches to determine reactive basic compounds content in the sample were tested and compared.

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Further called **direct titration of sample** was, as clear from the designation, based on bio-oil (0.2-0.4 g) sample weighing directly into the beaker. After dissolving in 50 mL of 80% ethanol, the sample was titrated by 0.1M HCl. From the equivalence point, the correction value in mmol/g which must be added to the result of carbonyls was calculated.

The **correction** approach intended for inorganic acid content described in **ASTM E3146** (Appendix X2) could in principle serve also as the correction for basic compounds content. The only difference would be that in contrast to acids, the presence of which results in overestimation (negative correction), the correction for basic compounds responsible for underestimation would be positive. The correction (Blank B) is based on the same principle as carbonyl content determination including also sample stirring at 80 °C for 2 h, with the only difference, that no solution A (hydroxyl amine hydrochloride) is added to the vial. Additional blank (Blank C) must be prepared consisting of 2 mL of solution B and 1 mL of DMSO. All the following steps (from stirring at 80 °C up to the titration by HCl) are the same as in the case of carbonyl content determination. The correction value that must be added to carbonyl content is calculated based on Equation 1:

$$correction\ value\ ASTM\ E3146\ (\frac{mmol}{g}) = \frac{EP_C - EP_B}{w_B} \times [HCl] \quad (1)$$

where:

EP_B = equivalence point of Blank B in mL

EP_C = equivalence point of Blank C in mL

w_B = weight of bio-oil added to Blank B in g

$[HCl]$ = concentration of HCl in mol/L.

The third correction approach further referred to as **"solution B + sample"** was developed to help eliminate problematic determination of direct correction for samples containing a low amount of basic compounds. To overcome this problem, bio-oil (0.2 g for crude bio-oil or 0.4 g for hydrotreated bio-oil) was weighed into a beaker together with 1 mL of solution B (0.5 N solution of triethanolamine) used for carbonyls determination, dissolved in 50 mL of 80% ethanol and titrated by 0.1M HCl. The base

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content in solution B in mmol/g was determined before the analysis. The correction value was determined using Equation 2:

$$correction\ value\ "solution\ B + sample"\ (\frac{mmol}{g}) = \frac{EP_s \times [HCl] - [solution\ B] \times w_{solB}}{w_s} \quad (2)$$

where:

EP_s = the strongest equivalence point of triethanolamine and interfering compounds in mL

$[HCl]$ = concentration of HCl in mol/L

$[solution\ B]$ = concentration of triethanolamine in solution B in mmol/g

w_{solB} = weight of solution B added to a beaker (ca 1 mL) in g

w_s = weight of bio-oil added to a beaker in g

The corrected value of carbonyl content was calculated as the sum of the value of carbonyl content measured without a correction and the **correction value** determined by one of the three above-mentioned methods.

2.3 Statistical evaluation and validation of the results

All bio-oil samples were analyzed at least in triplicates and a standard deviation was calculated. For carbonyl content after correction, a combined standard deviation (*cobm.SD*) was calculated according to Equation 3:

$$comb.\ SD = \sqrt{SD_{BC}^2 + SD_{CV}^2} \quad (3)$$

where:

SD_{BC} = standard deviation of carbonyl content determination before correction

SD_{CV} = standard deviation of correction value determination.

For comparison of the results for carbonyl content without correction with the results after correction, Welch's t-tests were used for all statistical calculations with α=0.05. The Welch's t-test (t) was calculated according to Equation 4:

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$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}} \quad (4)$$

where:

$\bar{x_1}$ = mean of corrected carbonyl content

$\bar{x_2}$ = mean of carbonyl content without a correction

s_1 = standard deviation of corrected carbonyl content

s_2 = standard deviation of carbonyl content without a correction

n_1 = number of repetitions of corrected carbonyl content determination

n_2 = number of repetitions of carbonyl content determination without a correction.

3. Results and Discussion

3.1. Interfering nitrogen containing compounds

A wide range of nitrogen-containing model compounds was tested to identify the possible interferences in carbonyl content determination. The overview of all tested compounds (a reactivity in basic nitrogen and carbonyl content determination) together with pKa values and the information about respective compound identification in bio-oils are shown in supporting material **Table A1**. The interfering compounds created a relatively narrow subset of compounds determinable by the basic nitrogen method. However, in contrast to basic nitrogen determination (most of the reactive compounds have pKa in the range 4–12), no clear correlation between pKa value and reactivity during carbonyl determination was observed. For the compound to be considered as an interferent in carbonyls determination, an equivalence point of the respective compound should be joined with triethanolamine or contribute to its shift. See examples of titration curves for three different compounds in **Figure A1**. Out of the 65 tested nitrogen compounds, 18 of them can be considered as interference. Based on the data and the average bio-oil composition, we can assume that three main groups of nitrogen compounds can contribute to the determination of carbonyl content: i) aliphatic primary amines (e.g. n-hexylamine or cyclohexylamine), ii) cyclic aliphatic secondary amines that can be considered as side products of hydrogenation of nitrogen heterocyclic compounds (e.g. piperidine or decahydroquinoline) and iii) heterocycles with rich

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alkyl substitution increasing electron density and thus basicity of the nitrogen-containing functional group (e.g. 2,4,6-trimethylpyridine).

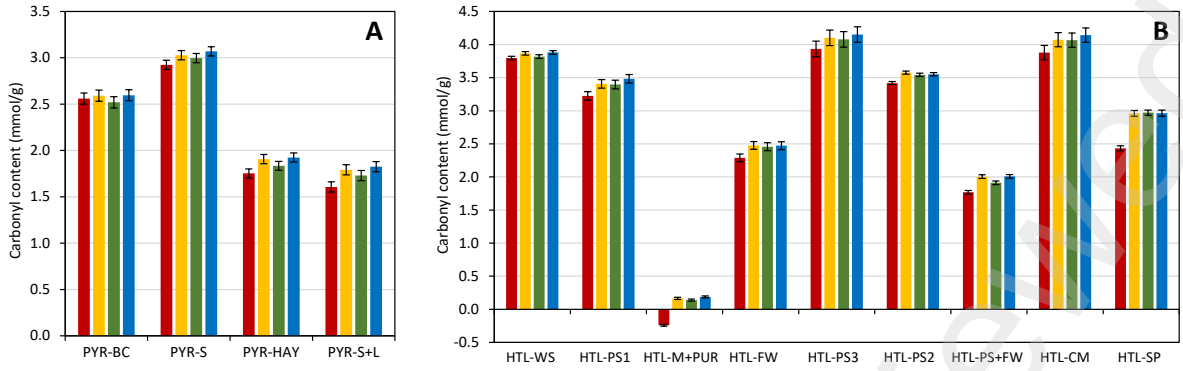
3.2. Comparison of carbonyl content results before and after correction

The studied pyrolysis oils summarized in **Table 2** contained 1.5–3 mmol/g of carbonyl groups and a maximum of around 2 wt% of nitrogen. All pyrolysis bio-oils had a very low ratio of basic nitrogen to total nitrogen and the standard deviation of carbonyl content determination was high (samples had high water content and were not homogenous). All these factors resulted in the observation of only small differences in the value of carbonyl content before and after correction, results were close to the standard deviation value (**Figure 2A**). Although it might seem from this limited scope of samples that for pyrolysis bio-oils correction step is not needed, the opposite is true as pyrolysis bio-oils from e.g. sewage sludge and algae have typically higher content of nitrogen than equivalent oils from HTL [38]. Pyrolysis bio-oils from sewage sludge and algae can contain typically up to 10 wt% of nitrogen [25,27,31], which would result in the significant underestimation of carbonyl content (see section 3.3).

The tested HTL bio-oils summarized in **Table 3** covered a broad range of nitrogen content from 0.75 wt% up to 6.6 wt% for straw and spirulina bio-oils, respectively. Also, the carbonyl content in the bio-oils covered a very broad range from 0.2 mmol/g up to 4.1 mmol/g for miscanthus liquified together with polyurethane and the primary sludge sample 3, respectively. Although the highest absolute value of correction (0.53 mmol/g, underestimation by ca 18%) was determined for spirulina bio-oil containing the highest amount of nitrogen, the highest relative underestimation was observed for bio-oil from a mixture of miscanthus and polyurethane (**Figure 2B**). Only 22% of feedstock was created by biomass (miscanthus) contributing to carbonyl content and bio-oil was produced four years before this study. Originally low carbonyl content was thus even reduced by the aging of bio-oil. The combination of the lowest carbonyl content (ca 0.2 mmol/g) and the highest fraction of interfering nitrogen (over 20%) in this sample, resulted in the determination of a negative value for carbonyl content without the correction step.

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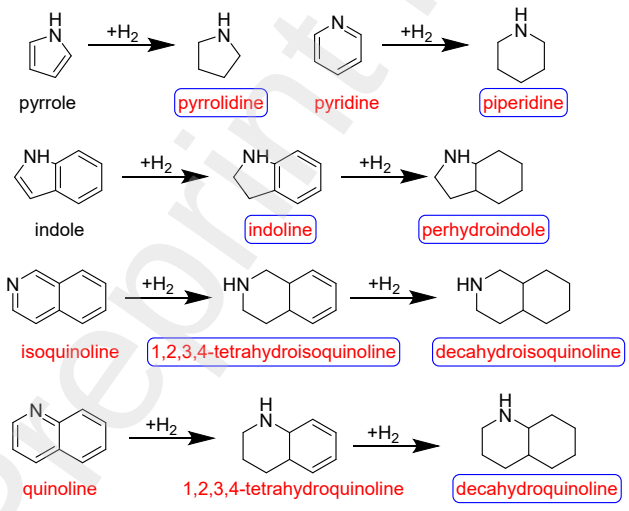


269 **Figure 2**: Comparison of carbonyl content before and after correction determined by various methods
270 for crude pyrolysis oils (**A**) and HTL oils (**B**), samples sorted by increasing nitrogen content. Red bar –
271 carbonyl content as measured (no correction). Yellow bar – correction direct titration. Green bar –
272 correction based on ASTM E3146. Blue bar- correction solution B + sample.

273

274 As clear from the observations of model compounds, the carbonyl content determination in hydrotreated
275 bio-oils produced from nitrogen rich biomass should be even more affected by the presence of nitrogen-
276 containing compounds. The interference in this case is caused not only by ammonia produced during
277 hydrotreatment and absorbed in the product, but also by the heterocycle rings being saturated and
278 becoming more reactive during the analysis. Examples of model nitrogen heterocycles hydrotreatment
279 and reactivity of side products in studied titration methods is displayed on **Figure 3**.

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Figure 3: The reaction scheme of hydrotreatment of chosen nitrogen heterocycles to secondary amines. In the next step of hydrotreatment ring opening to primary amines follows and in the final step ammonia is released (both primary amines and ammonia are basic and interfering nitrogen compounds). Black compounds = non-basic nitrogen compounds, non-interfering compounds in carbonyl determination. Red compounds = basic nitrogen compounds, non-interfering compounds in carbonyl determination. Red compounds in blue frame = basic nitrogen compounds, interfering compounds in carbonyl determination.

As clear from the results in **Figure 4**, for all hydrotreated bio-oils, a significant underestimation of results for carbonyl content was observed. For the sample having the lowest concentration of carbonyls and nitrogen (HDO1 prepared at the highest hydrotreatment temperature), without a correction step, even zero amount of carbonyls was determined. This error can provide misleading information about the thermal stability of the sample, as carbonyls are the main negative contributor to this parameter.

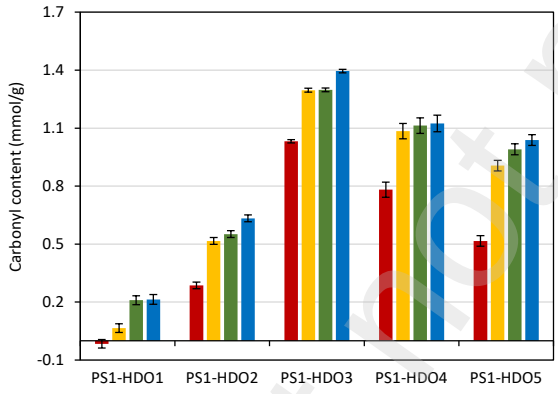


Figure 4: Comparison of carbonyl content before and after correction determined by various methods for hydrotreated HTL bio-oil from primary sewage sludge (HTL-PS1), samples sorted by increasing nitrogen content. Red bar – carbonyl content as measured (no correction). Yellow bar – correction direct titration. Green bar – correction based on ASTM E3146. Blue bar- correction solution B + sample.

All three correction approaches gave for most samples very consistent results. Direct titration of the sample is the simplest and fastest, but for samples containing low amounts of interfering compounds, a higher sample size is needed to get reliable and accurate results. The correction approach according to

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ASTM E3146 method is clearly the most laborious and time-consuming approach. Moreover, this correction approach showed the highest error rate, as it covers several less precise steps (pipetting of reagents mainly solution B and quantitative transfer of vial content into a beaker). Based on our experience, the correction method consisting of sample titration in the presence of solution B, seems to be the best approach not only from the repeatability perspective but also from the point of procedure speed.

3.3. Dependance of correction value on nitrogen content in the sample

To eliminate the effect of other possibly present basic compounds in bio-oil, we spiked the straw HTL bio-oil, for which the correction value was at the level of measurement repeatability, with nitrogen compounds model mixture with known composition. This mixture consisted of 13 nitrogen compounds from various groups previously identified in bio-oils; in total the composition of this mixture reflected the average fraction of basic and interfering nitrogen identified in the studied bio-oils (see composition in **Table 1**). We took into account the bio-oil dilution by nitrogen mixture and from determined and theoretical carbonyl content a graph was plotted. As clearly seen from **Figure 5A**, the fraction of determined carbonyl content gradually decreased with increasing nitrogen content in the sample.

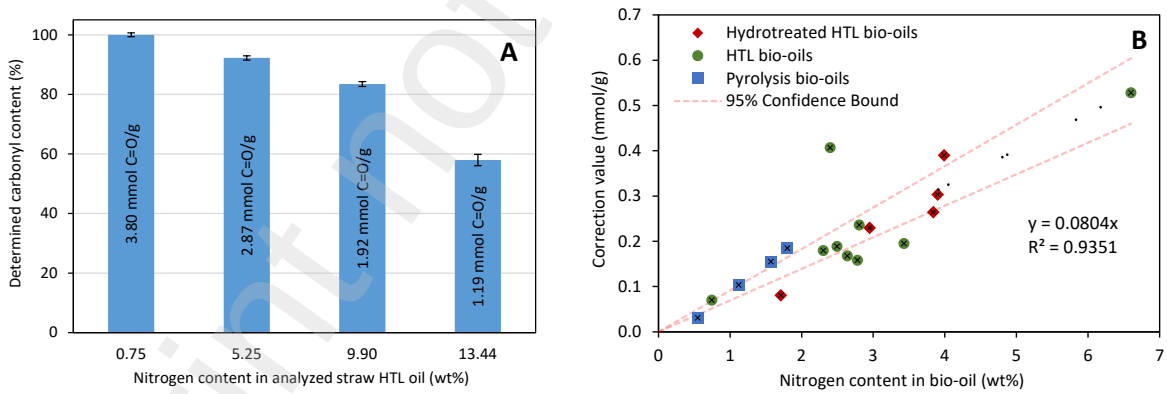


Figure 5: A – The fraction of determined carbonyl content vs. theoretical carbonyl content in % calculated after taking into account dilution by nitrogen mixture for straw HTL bio-oil. Dependance on nitrogen content increased by spiking with nitrogen compounds model mixture. The determined value of carbonyl content and standard deviation are shown in the respective bar. B – dependence of correction value for carbonyls on nitrogen content in bio-oils.

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Although nitrogen speciation in different bio-oils can differ significantly, when we plot the dependence of correction value on nitrogen content for the studied bio-oils, it seems to be clear that there is a linear correlation (**Figure 5B**). The corrected carbonyl content could be thus simplified to a simple calculation from the measured nitrogen content using Equation 5:

$$corrected\ carbonyl\ content\ (\frac{mmol}{g}) = [C=O] + 0.08 \times [N] \quad (5)$$

where:

[C=O] = carbonyl content measured by the ASTM E3146 method in mmol C=O/g

[N] = nitrogen content in respective bio-oil in wt%

This equation has obviously a limitation as not all bio-oils contain the average fraction of interfering nitrogen compounds. However, especially for bio-oils with nitrogen content above 2 wt% (for which the correction can be crucial), this equation could serve a corrected carbonyl content closer to reality without any additional laborious measurement. As clear from the **Figure 5B**, the majority of data points are within or close to 95% confidence interval of the correlation, the most outlier is HTL bio-oil from the mixture of miscanthus and polyurethane, which was a special sample in many ways.

3.4. Statistical data evaluation

Statistical data evaluation using Welch´s t-test (see **Table A2**) showed that for 11 analyzed samples, statistically different results were obtained after a correction step by all three methods. Statistically insignificant differences were observed for 7 samples. All pyrolysis bio-oils and HTL bio-oil from straw contained a relatively low amount of nitrogen (< 2 wt%). Both HTL bio-oils from cow manure and sewage sludge (sample HTL-PS3) showed relatively high carbonyl content (around 4 mmol/g) and increased the standard deviation of the determination. Based on the results obtained, it is possible to state that for samples containing more than 2 wt% of nitrogen, it would be each time recommended to determine a correction value and thus validate the result of carbonyl content.

4. Conclusions

In this paper, we demonstrated using 65 model nitrogen compounds that some of these compounds, when present in bio-oils, can cause significant interferences in the carbonyl content determination. By

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spiking the straw HTL bio-oil with a model mixture of nitrogen compounds, we have proven that with the increase in nitrogen content, underestimation of the result increases as well. Three correction approaches were, therefore, tested and compared for the analysis of 18 different bio-oils derived from different biomass and processes. Both pyrolysis and HTL bio-oils as well as bio-oils upgraded by hydrotreatment were tested. All three methods serve mostly well comparable results of the correction value for carbonyl content. The method called "solution B + sample" was identified as the most suitable correction approach, mostly for the shortest analysis time and the best repeatability. Alternatively, the carbonyl content can be corrected using the equation based on the found correlation between the correction value and nitrogen content. The statistical data evaluation showed that for 11 bio-oils, statistically different results were obtained before and after the proposed correction. The results of this paper will help researchers and industrial companies in the reliable and accurate determination of carbonyl content in bio-oils containing an increased amount of nitrogen. The information on the correction for basic compounds should be incorporated into ASTM E3146.

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