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Title: Contribution of acidic components to the total acid number (TAN) of bio-oil

Article Type: Research paper

Keywords: Total acid number (TAN); bio-oil acidity; switchgrass bio-oil; biofuel

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Abstract: Bio-oil or pyrolysis oil — a product of thermochemical decomposition of biomass under oxygen-limited conditions — holds great potential to be a substitute for nonrenewable fossil fuels. However, its high acidity, which is primarily due to the degradation of hemicelluloses, limits its applications. For the evaluation of bio-oil production and treatment, it is essential to accurately measure the acidity of bio-oil. The total acid number (TAN), which is defined as the amount of potassium hydroxide needed to titrate one gram of a sample and has been established as an ASTM method to measure the acidity of petroleum products, has been employed to investigate the acidity of bio-oil. The TAN values of different concentrations of bio-oil components such as standard solutions of acetic acid, propionic acid, vanillic acid, hydroxybenzoic acid, syringic acid, hydroxymethylfurfural, and phenol were analyzed according to the ASTM D664 standard method. This method showed the same linear relationship between the TAN values and the molar concentrations of acetic, propionic, and hydroxybenzoic acids. A different linear relationship was found for vanillic acid, due to the presence of multiple functional groups that can contribute to the TAN value. The influence of the titration solvent on the TAN values has been determined by comparing the TAN values and titration curves obtained from the standard method with results from the TAN analysis in aqueous environment and with equilibrium modeling results. Aqueous bio-oil samples with a known amount of acetic acid added were also analyzed. The additional acetic acid in bio-oil samples caused a proportional increase in the TAN values. The results of this research indicate that the TAN value of a sample with acids acting as monoprotic acids in the titration solvent can be converted to the molar concentration of total acids. For a sample containing acids that act as diprotic and polyprotic acids, however, its TAN value cannot be simply converted to the molar concentration of total acids because these acids have a stronger contribution to the TAN values than the contribution of monoprotic acids.

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March 1, 2017

Dr. Eric Suuberg
Department of Chemical Engineering
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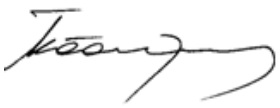
Dear Professor Suuberg:

RE: Manuscript # JFUE-D-16-04097R2
Title: "Contribution of acidic components to the total acid number (TAN) of bio-oil"

Thank you very much for sending us the reviews of our manuscript. We have carefully considered all the points raised by all four reviewers and addressed them to the best of our knowledge. Our responses have been included in a separate file named "Park et al_response to comments", where we also included our revisions to the manuscript based on the comments by the Reviewers. We hope that you will find our response satisfactory and the revised manuscript acceptable for publication.

Thank you again for your time and consideration. Please let me know if there are additional questions. I can be reached by phone (865-241-3246) or e-mail (tsourisc@ornl.gov). We are looking forward to hearing from you.

Sincerely,



Costas Tsouris, Ph.D.

JFUE-D-16-04097R2

Title: Contribution of acidic components to the total acid number (TAN) of bio-oil

Authors: Lydia K-E. Park; Jiaojun Liu; Sotira Yiacoumi, Ph.D.; Abhijeet P. Borole, Ph.D.; Costas Tsouris, Ph.D.

We sincerely appreciate the contributions of the Editor and the Reviewers toward enhancing the quality of our manuscript. Our responses follow the order of the comments provided by the Reviewers. Revisions in the manuscript are mentioned in our responses.

Reviewer 1

This article presents the relationship between the total acid number (TAN) and acidic compounds of bio-oil. From the detail of this article, there are some questions and comments for revising this manuscript as follows:

1. The objective of this article is to compare the measured TAN values of switchgrass bio-oil with theoretical ones. I question that the results obtained from the use of switchgrass bio-oil can be applied for bio-oil produced from other biomass or not since the different kinds of biomass can generate different acidic species in the bio-oil.

Response:
It is true that pyrolysis of different biomass materials would result in bio-oils that have different physical and chemical properties. The different biomass materials, as well as different pyrolysis conditions, may result in different types and amounts of acidic components in bio-oil. For this reason, a certain degree of variability in the acidity of bio-oils can be expected. The acidity is measured by a standard method, which is at the center of the current study. Based on the conclusion of this study, the total acid number (TAN) analysis is an acceptable method to determine the acidity of bio-oil, however, comparing TAN values of different types of bio-oil (produced from different sources of biomass or with different pyrolysis settings) should be done taking into consideration the type and concentration of acidic components in each bio-oil. In other words, the standard TAN analysis method should be used with caution when we want to compare different bio-oils. A statement has been added in the conclusions of the revised manuscript, on page 34, lines 590-596, to make this clarification.

2. In the experimental section, the method for measuring the water content in bio-oil is not explained since this value is needed for Table 3.

Response: The method for water content analysis has been inserted in the revised manuscript in **Section 2.2.1.**, page 12, lines 209-211: "For water content measurement, a Schott TitroLine Karl Fischer volumetric titrator was used according to the ASTM D4377 (2011) method."

Reference: ASTM D4377-00(2011), Standard test method for water in crude oils by potentiometric Karl Fischer titration, ASTM International, West Conshohocken, PA, 2011, www.astm.org.

3. How to evaluate type and quantity of all substances presented in Table 3? GC-MS or not. If the GC-MS was used to quantify the species in the bio-oil, the condition for GC-MS testing has to be given.

Response: In response to the Reviewer's question, the following statement has been added on page 14, lines 261-262 of the revised manuscript: "The chemical compositions of switchgrass crude bio-oil, aqueous bio-oil, and organic bio-oil obtained from GC-FID and HPLC analyses are presented in **Table 3**".

The methods involved in GC-FID and HPLC analyses are mentioned in the new **Section 2.2.1** on page 11, lines 195-197 of the revised manuscript**:** "The chemical composition of crude, aqueous and organic bio-oil (**Table 3**) was analyzed by gas chromatography with flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC). The methods involved in analyzing the chemical composition of bio-oil are found in Ren et al. [5, 38]."

Section 2.2.1. Bio-oil Analysis describing analytical methods used in the study was added on pages 11-12, lines 194-211 of the revised manuscript to provide more information and avoid confusion:

"2.2.1. Bio-oil Analysis
The chemical composition of crude, aqueous bio-oil, and organic bio-oil (**Table 3**) was analyzed by gas chromatography with flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC). The methods involved in analyzing the chemical composition of bio-oil are found in Ren et al. [5, 38]. Briefly, 2(5H)-furanone, 1-hydroxy-2-butanone, 1,3-propanediol, 3-methyl-1,2-cyclopentanedione, guaiacol, creosol, 2,6-dimethoxyphenol, and 3-ethylphenol were quantified using GC-FID with an HP-5 column (30 m × 0.32 mm, 0.25 μm film thickness) [5, 38]. The detailed settings for GC-FID are available in the literature [5, 38]. The identification of compounds was performed by comparing their mass spectra with those from the National Institute of Standards and Technology (NIST) mass spectral data library. Acetic acid, propionic acid, levoglucosan, hydroxymethylfurfural, furfural, phenol, and 1,2-benzendiol were analyzed using an HPLC, Jasco 2000Plus (Jasco Analytical Instruments, Easton, MD) with an MD-2018 plus photodiode array detector, an RI-2031 Plus intelligent refractive index detector, and an AS-2055 plus autosampler. The chemical analysis using HPLC was performed at 50°C with a Bio-Rad column HPX-87H (300 × 8 mm). The injected sample volume was 20 μL. Sulfuric acid (5 mM) in deionized water was used as the mobile phase with a flow rate of 0.6 mL/min. The compounds were quantified using external standards in both the HPLC and GC-FID analyses. For water content measurements, a Schott TitroLine Karl Fischer volumetric titrator was used according to the ASTM D4377 (2011) method [37]."

4. From Fig. 1, what was solvent to dissolve acetic or propionic acids for measuring the theoretical TAN?

Response: We used deionized water to prepare standard solutions of acetic and propionic acids for TAN measurements as described in **Section 2.2.2** of the revised manuscript. The theoretical TAN values presented in **Figure 1**, however, are not measured values. The theoretical TAN values represent the values from the conversion obtained using **Equation (1).** Thus, no solvents can be mentioned for the theoretical TAN values.

5. From Fig. 2, why did not use propionic acid same as shown Fig. 1 (this figure use formic acid)?

Response: The data presented in **Figure 2** are reproduced from Oasmaa et al. [4] by converting the x-axis from weight percent to molar concentrations. Following is the original figure published by Oasmaa et al. (2010).

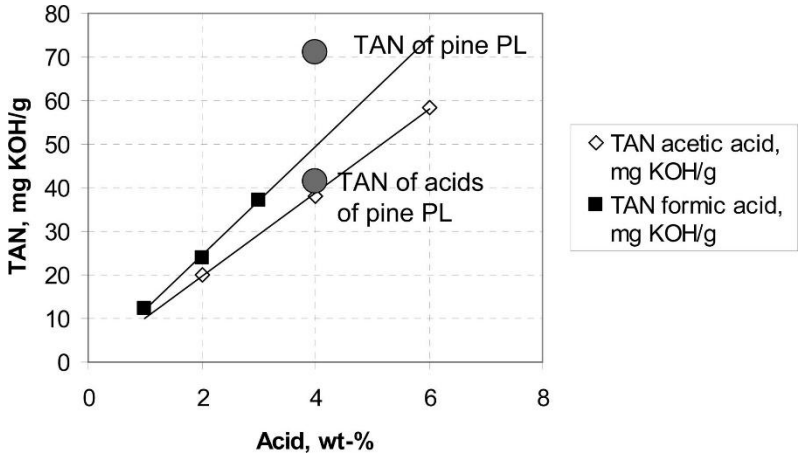


Figure 5. Correlation of TAN with the amount of volatile acids. Acid solutions of known concentrations were measured for TAN. The TAN value for an aqueous solution containing all of the acids in the pine PL is shown. For reference, the TAN of the whole pine PL has been added.
Reference: A. Oasmaa, D.C. Elliott, J. Korhonen, Acidity of Biomass Fast Pyrolysis Bio-oils, Energy & Fuels, 24 (2010) 6548-6554.

6. I think this manuscript has a lot of equations. The authors can group them to avoid the confusion.

Response: We thank the Reviewer for the suggestion. We initially numbered the equations, and sub-equations were labeled with a letter to avoid confusion. Following the suggestion of the Reviewer, we reviewed the equations again and grouped **Equations (6-a,b,c)** on page 25, lines 449-453 of the revised manuscript.

Conversion from [KOH] to TAN (mgKOH/g sample)

= [Concentration of KOH, (mol/L)] × [molecular weight of KOH, (g/ml)] × [unit conversion, (mg/g)] × [total volume of the system, (L)] / [weight of a sample, (g)] **(6-a)**
= 3.67×10^{-3} mol/L (KOH) × 56.1 g/mol × 1000 mg/g × 0.126 L / 1 g = **25.45 mgKOH/g (6-b)**
= 3.63×10^{-3} mol/L (KOH) × 56.1 g/mol × 1000 mg/g × 0.126 L/ 1 g = **25.66 mgKOH/g (6-c)**

7. The sign for phenol and HBA in Fig. 4 is the same. They should use different signs.

Response: As the Reviewer suggested, the symbol for HBA has been changed. We also realized that the marker shapes for HBA and phenol in **Figure 3** were the same, so we changed **Figure 3** as well. Please find the revised **Figures 3** and **4** on pages 19 and 23 of the revised manuscript.

8. The detail of pKa should provide the references following the international format for citation.

Response: Following the Reviewer's suggestion, we contacted ChemAxon inquiring about proper citation. The proper citation has been inserted in the title of **Table 5** as follows: "Table 5. Slopes of the linear relationships between TAN value and the molar concentration of various chemicals found in bio-oil with their chemical structure and pKa values obtained from the literature for HMF [42] and online chemical calculations from Chemicalize provided by ChemAxon [41] for other chemicals." on page 24, lines 422-425.

9. Is 0.126 in equation 6 correct or not? Should it be 0.125?

Response: In **Equation 6**, 0.126 L represents the total volume of the TAN analysis system including the amount of solvent and sample. Thus, (0.125 L from solvent) + (~ 0.001 L from a sample added) = 0.126 L was included in the calculation. To avoid any confusion, the following revision has been made on page 24, lines 433-436 of the revised manuscript: "This amount can be converted to TAN value using the following equation based on the assumption that 1 g of sample is titrated in 125 mL of water [i.e., a total volume of 0.126 L (0.125 L of water and ~ 0.001 L of sample) was used in the calculation] as described in **Equations (6-a, b)**."

10. What does it mean for –pH?

Response: The definition of pH is -log[H+], so –pH in the y-axis is just log[H+]. We plotted the experimental data (the TAN analyses in an aqueous system and the ASTM standard method) in the –pH scale for y-axis to compare the results from the MINEQL modeling. Following the Reviewer's question, however, we thought that the y-axis might be misleading. Therefore, for clarification, we replotted **Figure 5** on page 27 using y-axis in pH instead of –pH. The following revision has been made on page 25, lines 458-460 of the revised manuscript: "To compare with the results from MINEQL+ and the experimental aqueous TAN analysis, the data were plotted as pH vs. molar concentrations of KOH added."

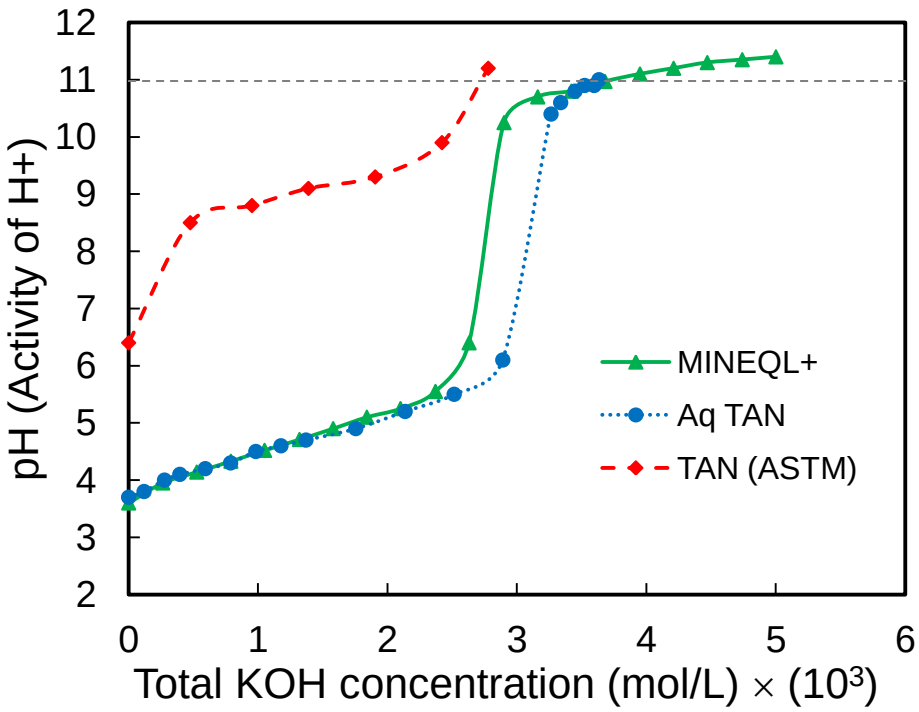


Figure 5. Titration curves of acetic acid (2 wt.%) solution from MINEQL+ modeling, aqueous titration, and standard titration (ASTM D664).

11. The author explained only TAN of aqueous bio-oil. How about the TAN of organic bio-oil?

Response: The reasons we focused on the TAN analysis of aqueous bio-oil are the following: (1) The aqueous fraction of bio-oil is much larger than the organic fraction, and is known to contain more acidic components than the organic bio-oil. (2) Organic bio-oil from switchgrass pyrolysis settles at the bottom of centrifuge tubes upon centrifugation. It is highly viscous and heterogeneous and contains solids. Thus, TAN analysis of organic bio-oil gives inconsistent values due to its heterogeneity. (3) The acidity of aqueous bio-oil has implications in separations and pH-neutralization studies [5, 8], which are part of our investigations. For these reasons, this study is focused on the acidity of aqueous bio-oil.

12. Form Fig. 5 and Table 5, the –pH values obtained from ASTM method were significantly different from MINEQL+ and aqueous system. Why? Is it from the different titration solvent or not?

Response: The ASTM method for measuring TAN is different from MINEQL modeling in the titration solvent. As mentioned in the text ("It is noteworthy that the pK_a values presented in **Table 5** are based on aqueous systems." on page 21 lines 385-386), the pK_a values are based on the aqueous system. The MINEQL+ modeling is only applicable to aqueous systems. Thus, a similar aqueous TAN measurement was performed, and it was found that the different titration solvent system led to significantly different TAN values. To clarify the discussion on different

TAN values in the aqueous system and standard solvent system, the following revision has been made on page 26, lines 472-480 of the revised manuscript: "As mentioned earlier, pK_a values (i.e., the logarithm of acid dissociation constants) that represent dissociation in acid-base reactions, are reported for the aqueous system. Therefore, the TAN values obtain from the MINEQL+ modeling, which is based on the pK_a values are comparable with results from aqueous TAN analysis. Since the ASTM method uses a mixture of toluene, isopropanol, and water as a titration solvent, the dissociations of compounds in terms of acid-base reactions would be different from those occurring in the aqueous system. In short, the pK_a values of compounds during the ASTM standard TAN analysis should be different from the known pK_a values for the aqueous system."

13. From Fig. 6, why did authors use 90% of measured TAN of the aqueous bio-oil? Why did not use 100%?

Response: We used 90% of the measured TAN value of the aqueous bio-oil for the calculation considering the dilution. The samples (overall 2, 4, and 6 wt.% of acetic acid in aqueous bio-oil) were prepared with 90% of aqueous bio-oil and 10% of the acetic acid solution as described in **Section 2.2.4**. The first data point (black circle marker) represents the aqueous bio-oil without any acetic acid added. Thus, if we compare the 100% of the measured TAN value of the aqueous bio-oil, it would not be comparable with other analyzed samples that had additional acetic acid because other samples only contained 90% of aqueous bio-oil. Thus, in order to demonstrate the relationship between TAN values and the amount of additional acetic acid in aqueous bio-oil samples, we took 90% of the measured TAN value of the aqueous bio-oil instead of 100%. To clarify, we revised the text on page 28, lines 503-510 of the revised manuscript as follows: "To incorporate the dilution effect due to added acetic acid solution, 90% of the measured TAN of the aqueous bio-oil was plotted with the measured TAN of aqueous bio-oil samples with acetic acid added, displayed by black circular markers in **Figure 6**. The samples (overall 2, 4, and 6 wt.% of acetic acid in aqueous bio-oil) were prepared with 90% of aqueous bio-oil and 10% of the acetic acid solution as described in **Section 2.2.4**. The first data point (black circle marker) represents the aqueous bio-oil without any acetic acid added, diluted by 10% with water to make it comparable with the other analyzed samples that had acetic acid because those samples contained only 90% aqueous bio-oil."

Reviewer 2

This paper examines how a number of important mono- and diprotic acid components contribute to the overall acidity of biofuels. They used an accepted ASTM potentiometric method for KOH titration of a variety of standard samples and switchgrass bio-oil. The analyses were performed in triplicate using both aqueous and organic solvents. Likewise, the bio-oil was separated into aqueous and organic fractions by centrifugation and analyzed accordingly. Appropriate recovery analyses were also performed. Their titration curves carefully demonstrate that the mono- and polyprotic acids have different slopes. Di- and polyprotic acids are determined to contribute more strongly than monoprotics to the TAN even when the polyprotic acid is a weaker acid. That is, the TAN analysis does not distinguish between weak and strong acids. Their results

indicate that TAN values determined for samples containing monoprotic constituents can be readily converted to molar acid concentrations, whereas this is not the case for TAN values determined for samples containing polyprotics.

I find this study to be well-focused, and carefully conceived and executed. The paper is well-written and clearly states both its methods and results. The results are useful and significant. I can find no errors nor make substantive suggestions for improvement.

Response: The Reviewer's summary above captured all the major points of the work described in the manuscript. We sincerely thank the Reviewer for the positive assessment of the manuscript.

Reviewer 3

In my opinion, the main problems of the previous manuscript was kept in this revised version. That is, the manuscript has reported only studies with standard solutions (some organic acids, 5-(hydroxymethyl)furfural and phenol in water or 2-propanol) and additions of acetic acid in the aqueous phase of a bio-oil sample. That is, only one acid was studied in aqueous phase of bio-oil and no study has been done with a full bio-oil sample (aqueous plus organic phases together). Therefore, it is clear to me that the results and conclusions obtained cannot be directly extended to bio-oil, since most studies were carried out in standard solutions and only one acid was studied in a pseudo-real sample (it was not a true real sample because the organic phase is not present in the aqueous phase of a bio-oil). That is why I think the manuscript does not contribute to understanding the "contribution of acidic components to the total acid number (TAN) of bio-oil" as stated in its title.

Response: We appreciate the time of Reviewer 3. Below, we have itemized the objections of the Reviewer and our responses.

1. The Reviewer questions the selection of the chemical components included in the study.

   Response: The components we have included represent the major components we have identified in switchgrass bio-oil.

2. The Reviewer added, "only one acid was studied."

   Response: As pointed out in the Experimental section on page 11, in this study we used acetic acid, propionic acid, vanillic acid, syringic acid, 4-hydroxybenzoic acid, 5-(hydroxymethyl)furfural, and phenol. These compounds have been identified in our analytical methods as components of switchgrass bio-oil, and this is the reason they were selected for the study. Reviewer 2 also pointed out that this paper examines how a number of important mono- and diprotic acid components, including weak and strong acids, contribute to the overall acidity of biofuels.

3. The Reviewer questions the use of aqueous bio-oil alone in the study.

   Response: Due to the heterogeneous nature of crude bio-oil, which contains aqueous and organic phases, it is very difficult to consistently analyze the TAN values given that the amount of a sample required for the TAN analysis is only 0.1 g. Sampling crude bio-oil

that contains a highly viscous, solid-like organic phase can lead to widely ranging results. Here, we excluded the much-lower-volume-fraction organic phase and only analyzed the aqueous bio-oil for consistency, as mentioned on page 13, **Section 2.2.4**.

4. The Reviewer suggests that the results and conclusions obtained cannot be directly extended to bio-oil since most studies were carried out in standard solutions and only one acid was studied in a pseudo-real sample.

   Response: We disagree with this statement. Acids in crude bio-oil are distributed between the aqueous and organic phases of bio-oil. By isolating and studying the aqueous bio-oil phase, which is over 76.2 wt.% of the switchgrass crude bio-oil, we were able to obtain consistent data and make concrete observations and conclusions that could be lost in fluctuating measurements if crude bio-oil were used. We strongly believe that the results are also applicable to the crude bio-oil because switchgrass crude bio-oil contains everything found in aqueous bio-oil. A statement has been added in the conclusions of the manuscript on page 34, lines 590-596 of the revised manuscript to better clarify the importance of the results.

Additional Revision:

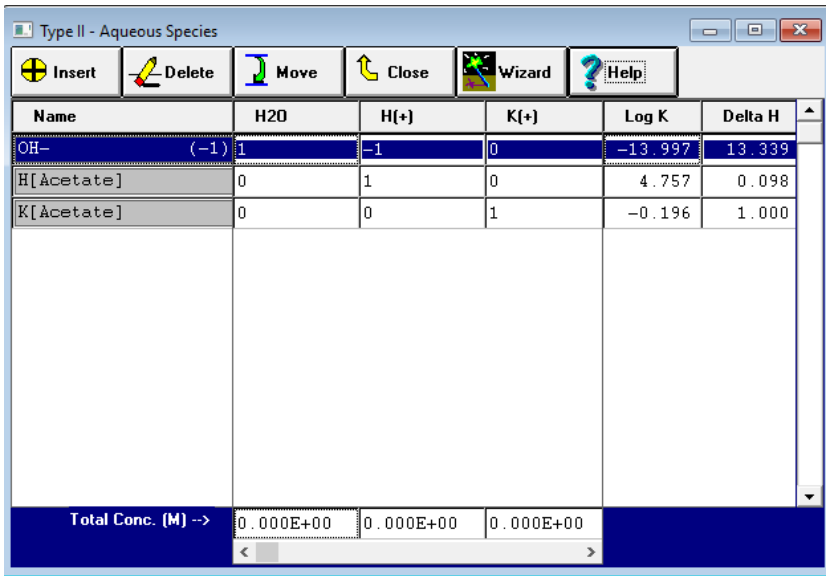
The details on the centrifugation setting have been inserted as follows: "Crude bio-oil obtained from UT was centrifuged at 3000 rpm (1673 g) for 30 minutes using Beckman Coulter Avanti J-E centrifuge with a JLA 10.500 rotor to separate the aqueous and organic components of bio-oil." on page 13, lines 243-245.

Reviewer 4

This manuscript deals with an obvious relation, i.e., the correlation between the acid number (mg of KOH per g of the sample) and the concentration of titratable acids in solution. Certainly this correlation is obvious. However, in some cases the obvious should be said (or written). In the matrix considered in the present work, the interaction among the present species there into is not completely understood and this aspect can justify the publication of the found data.
I found some problems (in my point of view) which are below reported in the table, together with suggestions and observations with them related.

1. "CH3COOK = K^+ + $CH3COO^-$ (log K = -0.196)" on page 12, line 214
Suggestion: Usually, due to its high solubility in water (about 270 g per 100 mL of water, at 25^oC), potassium acetate is considered completely dissociated in its ions in aqueous solution. Therefore, it is interesting to write some details in this case: 1- indicate clearly what is the reference; 2 – in what conditions this constant was determined.

Response: The log K value for potassium acetate was taken from the MINEQL+ library as mentioned in the revised manuscript on page 13, lines 233-234. The screen capture of the MINEQL+ modeling is shown below. The condition of the MINQEL+ modeling is at the room temperature (25°C) in the closed (not open to the atmosphere) aqueous system. The conditions of the MINEQL+ modeling were included in the revised manuscript for clarification as the Reviewer suggested. The following sentences were included in the revised manuscript on page 13, lines 234-236: "The conditions of the MINEQL+ modeling are: aqueous system, closed to the atmosphere (i.e., no carbonate species considered), and room temperature (25°C)."



2. [M] – in the figure on page 16, Figure 2
Suggestion: Please substitute by mol L^{-1} or mol/L. Please, along the manuscript, when it is the case, substitute always the symbol M by mol/L or mol L^{-1}.

Response: As the Reviewer suggested, we changed M in the manuscript to mol/L.

3. "… to the high pK_a…" on page 17, line 293
Suggestion: Please, introduce the value of pKa of the phenol, to clarify.

Response: On page 18, line 320 of the revised manuscript, we included the value of pK_a of phenol for clarification as the Reviewer suggested.

4. "The pKa of the hydrogen in phenol was less than 11, but phenol did not contribute to the TAN value" on page 20, lines 345-346.
Suggestion: "As the pKa of the hydrogen in phenol is high, phenol did not contribute to the TAN value."

Response: We thank Reviewer 4's comment on this part of the manuscript. We also understand the intention of the comment. It is true that the pKa of the hydrogen in phenol is high and phenol did not contribute to the TAN value. However, what we wanted to describe through the text was that, since a sample in the titration solvent is titrated to pH 11 during the TAN analysis, phenol with a pKa value of 10.02 was expected to be titrated. However, phenol did not contribute to the TAN value (0 mg KOH/g). As we demonstrated in a later section on results of the aqueous system and the MINEQL+ model, it is possible that the pKa value of phenol is higher in the titration solvent than in water.

To clarify this point, we revised the manuscript on page 20-21, lines 371-376 as follows: "Because the samples were titrated to pH 11 during TAN analysis, hydrogen atoms with pK_a values less than 11 were expected to be titrated. The pK_a of the hydrogen in phenol was less than 11, but phenol did not contribute to the TAN value. As discussed in the following section (**3.4**), the reason for this behavior could be that the actual acid-base constants in the TAN standard titration solvent are different from pK_a values reported for the aqueous system."

5. "… probably…" on page 20, line 357.
Suggestion: "… certainly …"

Response: The correction recommended by the Reviewer was made on page 21, line 389.

6. "…acids, then the TAN value of the sample can be converted to the molar concentration of total acids using the following relationship" on page 21, lines 364-366.
Suggestion: "… acids, then the TAN value of the sample can be converted to the molar concentration of total acids using the following relationship, where M is the concentration in mol L^{-1}."

Response: The correction recommended by the Reviewer was made on page 21, line 398.

7. "Due to limited solubility of syringic acid in water and isopropanol…on page 21, line 372.

Suggestion: "As the solubility of the syringic acid is low, its contribution to the TAN will be consequently negligible."

Response: We appreciate the Reviewer's comment for this part of the manuscript.
Due to limited solubility of syringic acid, we were not able to prepare the same concentrations (2, 4, and 6 wt%) of standard syringic acid solutions as for other chemicals. As mentioned in the manuscript, the maximum concentration we were able to prepare was 0.6 wt.% of syringic acid solution in the titration solvent (toluene: isopropanol: water = 100: 99: 1 v.). However, when this syringic acid solution was titrated to find the TAN value, the pH value of the titration system could not reach 11. Therefore, we were unable to obtain the TAN value of 0.6 wt% of syringic acid. An interesting observation during the TAN analysis of syringic acid solution was precipitation. As more KOH solution was added, the pH of the system initially increased. After the pH value reached 9.5, precipitation of solids occurred, and the pH value decreased significantly as more KOH solution was added.

Based on these observations, we think that the contribution of syringic acid to the TAN value is not negligible. Although the actual TAN value of the syringic acid was not obtained due to its limited solubility, its contribution to the TAN value could not be negligible. To clarify this point, we added the following text on page 22, lines 411-414:

"This means that, when a sample contains syringic acid, even at low concentrations, a strong contribution to the TAN value can be expected. Syringic acid in the sample will consume the KOH solution during titration; therefore, it would take more KOH solution for the system to reach pH 11, leading to a higher TAN value than that obtained in the absence of syringic acid."

8. Yellow line in Figure 4 on page 22.
Suggestion: As far I understood, the yellow line must be drawn in other color than green, violet, blue, black, yellow or red, as it represents a "mean" line. I suggest pink.

Response: The color of the combined line in Figure 4, on page 23, has been changed to pink as suggested by the Reviewer.

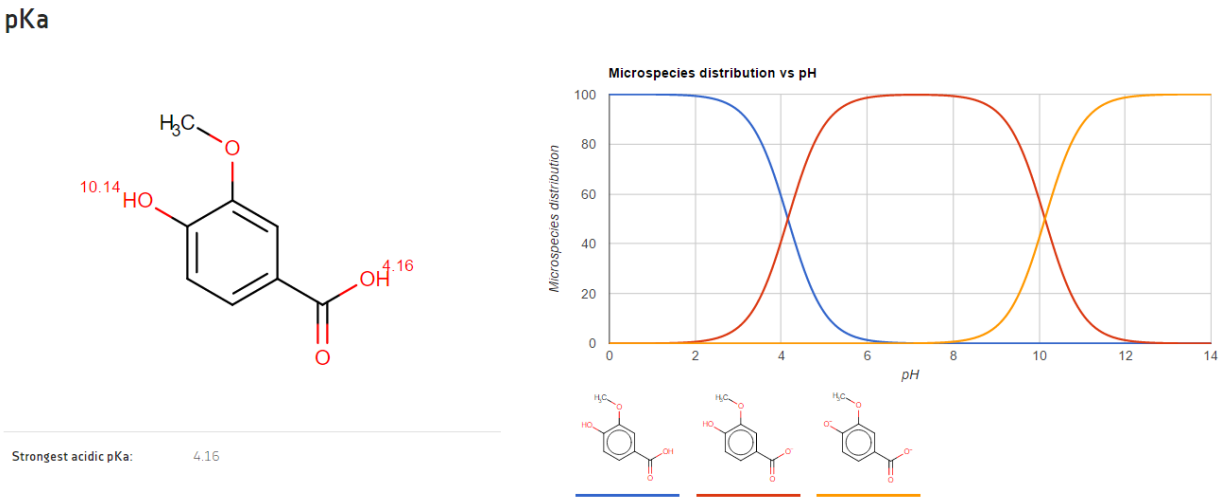
9. pK_a absent for HMF in Table 4 on page 23.
Suggestion: (pKa = 12.82). This value is easily found in the literature.

Response: The pKa value of HMF has been inserted in **Table 5** on page 24. We also included a reference: F. Liu, S. Sivoththaman, Z. Tan, Solvent extraction of 5-HMF from simulated hydrothermal conversion product, Sustainable Environment Research, 24 (2014).

10. pK_{a2} absent for vanillic acid in Table 4 on page 23.
Suggestion: pK_{a2}=8.96; please see Determination of pK_a values of some hydroxylated benzoic acids in methanol–water binary mixtures by LC methodology and potentiometry. F.Z. Erdemgila, S. Şanlib, N. Şanlib, G. Özkanb, J. Barbosac, J. Guiterasc, J.L. Beltránc,

http://dx.doi.org/10.1016/j.talanta.2006.11.007.

Response: We appreciate the Reviewer's comment on the pK_{a2} value of vanillic acid on Table 4. We also thank the Reviewer for providing the literature that has the pK_{a2} value of vanillic acid. We did include the pK_{a2} value (10.14) of vanillic acid in the manuscript. We understand that the pK_{a2} value (10.14) that we had was different from the pK_{a2} value (8.96) from the journal article provided by Review 4. While we were searching for pK_a values of chemicals, we noticed that some of the values vary in different papers. Therefore, to be consistent, we decided to obtain pK_a values from the single source, online chemical calculations from Chemicalize provided by ChemAxon. The pK_a values of the vanillic acid obtained from Chemicalize by ChemAxon are shown in the screen capture below.



Chemicalize by ChemAxon

For clarification, the following sentences have been inserted on page 21, lines 386-388 of the revised manuscript: "For consistency, the pKa values of chemicals were obtained from chemical calculations from Chemicalize provided by ChemAxon [41] except the pKa value of HMF, which was obtained from another source [42]."

11. "… at 3.67E-3 M." on page 23, line 396.
Suggestion: 3.67 x 10^{-3} mol/L. (Please see along the manuscript).

Response: The correction recommended by the Reviewer was made on page 24, line 433 of the revised manuscript.

12. "Molar concentration of total KOH × (10^3)" in Figure 5, on page 27.
Suggestion: Total concentration of KOH - (mol L^{-1}) x 10^3.

Response: The correction recommended by the Reviewer was made in Figure 5, page 27 of the revised manuscript.

13. “Most of the aqueous bio-oil samples showed acceptable repeatability (less than 12%) …” on page 27, lines 471-472.
Suggestion: Please justify why the repeatability is acceptable.

Response: According to ASTM (references 5 and 6), the definition of the repeatability is given as follows: ‘‘The difference between successive test results obtained by the same operator with the same apparatus under constant operating conditions on identical test material would in the long run, in the normal and correct operation of the test method, exceed the following values only in one case in twenty.” The repeatability equation (**Equation 2-a)** found in the manuscript is from the literature and ASTM standard method (references 1-6).

After receiving the Reviewer’s comment on the repeatability, we looked close into the acceptable repeatability. Previously, we stated that the acceptable repeatability is less than 12% according to the literature (references 2-4). However, we realized that this 12% of acceptable repeatability would only apply for automatic titration mode. The acceptable repeatability of acid number determination is found in **Table A** below. Our TAN analyses were performed manually. The titration curves did not have a defined inflection point. Therefore, the acceptable repeatability for the manual titration mode is 5% of the mean.

Table A. Repeatability of Acid Number Determination [Ref (5-6)].

Titration Mode	Fresh Oils and Additive Concentrates at Inflection Points		Used Oils at Buffer End Points	
	Manual	Automatic	Manual	Automatic
Percentage of Mean	7	6	5	12

We revised the manuscript accordingly. The revised manuscript includes an additional table shown below. Since detailed data are included in Table 4, the allowable repeatability lines are excluded in Figure 1. Moreover, the following sentences are inserted for clarification on pages 14-15, lines 283-282: “The mean of the measured TAN values, theoretical TAN values, and their repeatability are found in **Table 4**. According to ASTM [28], the definition of repeatability calculated from **Equation 2-a** [15, 28, 34, 40] is given as follows: “the difference between successive test results obtained by the same operator with the same apparatus under constant operating conditions on identical test material would in the long run, in the normal and correct operation of the test method, exceed the following values only in one case in twenty.” Since the titration curves of the manual TAN analyses did not have a defined inflection points, the acceptable repeatability for the manual titration mode is 5% of the mean based on the standard method [28]. Therefore, most of the data were within the acceptable repeatability (<5%) as shown in **Table 4**, while only one measurements exceeded the 5% limit.”

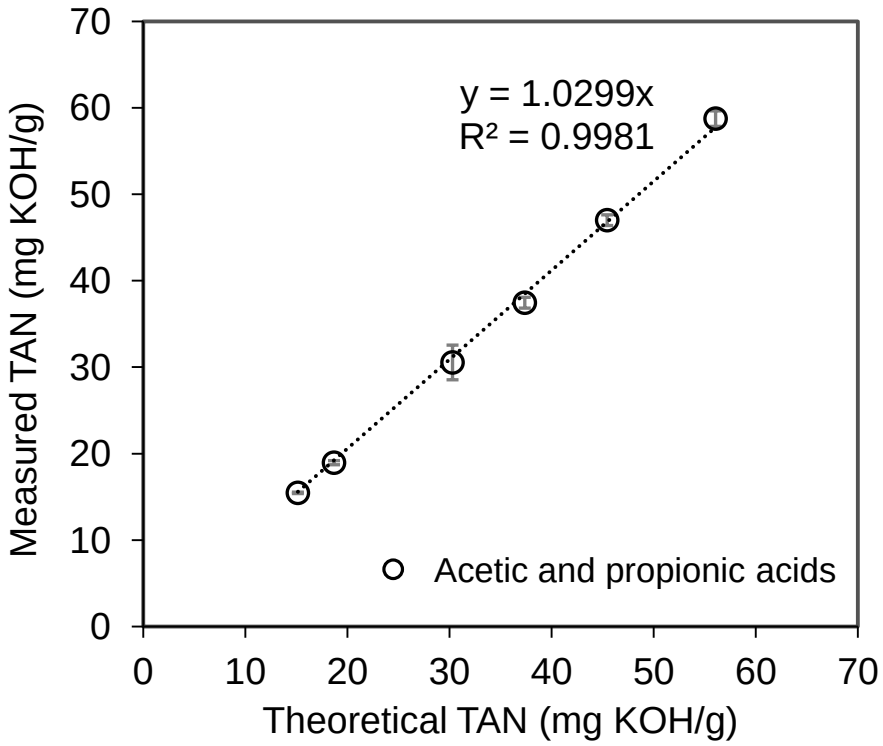


Figure 1. Relationship between measured TAN values of acetic acid and propionic acid solutions (2, 4, 6 wt.%) and their theoretical TAN values obtained from **Equation (1)**.

Table 4. Theoretical and measured TAN values of acetic and propionic acids standard solutions.

Chemicals	Concentration		Theoretical TAN (mgKOH/g)	Mean measured TAN (mgKOH/g)	Standard deviation	Repeatability (%)
	wt%	mol/L				
Acetic acid	2	0.33	18.69	18.96	0.24	3.5
	4	0.67	37.37	37.46	0.63	4.6
	6	1.00	56.06	58.75	0.90	4.2
Propionic acid	2	0.27	15.15	15.47	0.08	1.5
	4	0.54	30.30	30.55	2.00	18.2
	6	0.81	45.44	47.00	0.62	3.6

Since repeatability has been discussed in Section 3.2 of the revised manuscript, it was decided to delete the following text from Section 3.5: “Most of the aqueous bio-oil samples showed acceptable repeatability (less than 12%) except for the aqueous bio-oil samples with 4% acetic acid, which had a slightly higher repeatability of 15%.”

References:
(1) A. Baig, F.T. Ng, Determination of acid number of biodiesel and biodiesel blends, Journal of the American Oil Chemists' Society, 88 (2011) 243-253.
(2) H. Wang, H. Tang, J. Wilson, S.O. Salley, K.Y.S. Ng, Total Acid Number Determination of Biodiesel and Biodiesel Blends, Journal of the American Oil Chemists' Society, 85 (2008) 1083-1086.
(3) J. Shao, F. Agblevor, New Rapid Method for the Determination of Total Acid Number (Tan) of Bio-Oils, American Journal of Biomass and Bioenergy, 4 (2015) 1-9.
(4) P. Hamblin, I. Rapenne-Jacob, J. Reyes-Gavilan, P. Rohrbach, Standard test methods for TAN assessment and modifications ThereofE, Tribology & Lubrication Technology, 60 (2004) 40-46.
(5) ASTM D664-01. Standard Test Method for Acid Number of Petroleum Products by Potentiometric Titration, American Society for Testing Materials (ASTM) International, West Conshohocken, PA, 2011.
(6) ASTM D664-95. Standard Test Method for Acid Number of Petroleum Products by Potentiometric Titration, American Society for Testing Materials (ASTM) International, West Conshohocken, PA, 2011.

14. "… value is probably due to other chemicals …" on page 30, line 517.
Suggestion: "… value is surely due to other chemicals …"

Response: The word "probably" has been removed from page 32, line 564, of the revised manuscript.

15. "… other chemicals may be much smaller than the concentration of propionic …" on page 30, line 518.
Suggestion: "… other chemicals may be smaller than the concentration of propionic …"

Response: Following the Reviewer's suggestion, the manuscript has been revised on page 32, lines 564-565.

16. "… these chemicals on the TAN value may be stronger, showing a …" on page 31, line 520.
Suggestion: "… these chemicals on the TAN value may be important, showing a …"

Response: Following the Reviewer's suggestion, the manuscript has been revised on page 33, line 567.

17. "Thus, the TAN analysis does not discriminate between weak and strong acids" on page 32, lines 536-537.
Suggestion: "Thus, TAN analysis does not discriminate between weak acids and strong acids, since these are titratable, i.e., since the respective protons are titratable."

Response: Following the Reviewer's suggestion, the manuscript has been revised on page 34, lines 590-591.

18. "… and experiments, indicate that the pK_a values …" on page 32, line 538.
Suggestion: "… and experiments, as expected, indicate that the pKa values …"

Response: Following the Reviewer's suggestion, the manuscript has been revised on page 34, lines 598-599.

Contribution of acidic components to the total acid number (TAN) of bio-oil

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Abstract

Bio-oil or pyrolysis oil — a product of thermochemical decomposition of biomass under oxygen-limited conditions — holds great potential to be a substitute for nonrenewable fossil fuels. However, its high acidity, which is primarily due to the degradation of hemicelluloses, limits its applications. For the evaluation of bio-oil production and treatment, it is essential to accurately measure the acidity of bio-oil. The total acid number (TAN), which is defined as the amount of potassium hydroxide needed to titrate one gram of a sample and has been established as an ASTM method to measure the acidity of petroleum products, has been employed to investigate the acidity of bio-oil. The TAN values of different concentrations of bio-oil components such as standard solutions of acetic acid, propionic acid, vanillic acid, hydroxybenzoic acid, syringic acid, hydroxymethylfurfural, and phenol were analyzed according to the ASTM D664 standard method. This method showed the same linear relationship between the TAN values and the molar concentrations of acetic, propionic, and hydroxybenzoic acids. A different linear relationship was found for vanillic acid, due to the presence of multiple functional groups that can contribute to the TAN value. The influence of the titration solvent on the TAN values has been determined by comparing the TAN values and titration curves obtained from the standard method with results from the TAN analysis in aqueous environment and with equilibrium modeling results. Aqueous bio-oil samples with a known amount of acetic acid added were also analyzed. The additional acetic acid in bio-oil samples caused a proportional increase in the TAN values. The results of this research indicate that the TAN value of a sample with acids acting as monoprotic acids in the titration solvent can be converted to the molar concentration of total acids. For a sample containing acids that act as diprotic and polyprotic acids, however, its TAN value cannot be simply converted to the molar concentration of total acids because these acids have a stronger contribution to the TAN values than the contribution of monoprotic acids.

Highlights

- The ASTM D664 standard method can be used for acidity measurements of bio-oil
- TAN values of monoprotic acids are linearly related to their molar concentrations
- Vanillic acid has a stronger influence on TAN values than other monoprotic acids
- TAN values with water are higher than those with the standard titration solvent
- TAN values in bio-oil are proportional to the amount of acetic acid present

Keywords

Total acid number (TAN); bio-oil acidity; switchgrass bio-oil; biofuel

1. Introduction

Increasing energy demand, climate change, and carbon dioxide emissions from fossil fuels raise serious concerns. The exploration, marketing, and transportation of fossil fuels cause additional pollution as well as social and political unrest [1]. In light of these circumstances, carbon-neutral biofuels produced from various lignocellulosic materials such as grass, wood, agricultural, or forest residues appear to be a promising substitute for fossil fuels. The currently available forms of biofuels include bioethanol, biodiesel, and bio-oil (pyrolysis oil).

Bioethanol and biodiesel are commercially used in blends of gasoline and diesel, respectively, for vehicle-use and heating. Bio-oil is produced, along with byproducts such as char and syngas, from pyrolysis in which biomass is heated under oxygen-limited conditions. Currently, bio-oil is the cheapest biofuel produced from lignocellulosic materials, with good potential of becoming a sustainable energy source [2]. However, it has some application-hindering properties including high moisture content, high viscosity, high density, low heating value, and high acidity.

High acidity is especially problematic for the storage and transportation of bio-oil, which has a typical pH of 2-3 [3-5]). Acidic components can also cause instability by generating protons (H^+) that can promote condensation and polymerization reactions [6]. Hence, there have been various attempts to reduce the acidity through neutralization and catalytic reactions [6-8]. One approach to neutralizing acidic bio-oil is by adding alkaline solution [8]. To properly compare initial and final bio-oils after a neutralization treatment, however, the acidity of bio-oil must be accurately quantifiable.

Various techniques have been employed to measure the acidity of bio-oil including pH, ion chromatography (IC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS), though each technique has some limitations. The pH, which measures the concentration of protons, does not provide the concentrations of acidic components, and also is found to

be susceptible to errors [9]. Chemical analysis using IC, HPLC, and GC-MS can identify and quantify various chemicals including acids in bio-oil samples [5, 10]. There are, however, some disadvantages associated with these techniques. Small changes in pH can greatly affect IC results by altering the binding profiles of IC resins [11]. On the other hand, HPLC and GC-MS may underestimate the acidic components in bio-oil samples. Specifically, GC-MS is known to detect only volatile compounds (e.g., acetic acid and propionic acid), which comprise 25 – 40 wt% of bio-oil, while other heavy carboxylic acids may not be detected [12]. Moreover, due to a high diversity of chemicals in bio-oil, the use of HPLC and GC-MS has been linked to measurement inconsistencies among different laboratories, as demonstrated through the round-robin testing by Oasmaa et al. [9]. Although recently Oasmaa et al. [4] suggested that capillary electrophoresis (CE) can accurately measure the acidic components in bio-oil, CE has disadvantages of lower injection precision and sensitivity as compared to HPLC [13]. Moreover, chemical analysis, in general, cannot provide a single parameter that can be used for comparing samples with different chemical compositions.

Regardless of differing characteristics between various bio-oil samples, the total acid number (TAN) analysis can provide a single parameter that can be used for acidity comparisons. TAN is the amount of potassium hydroxide (KOH, in milligram) required to titrate one gram of a sample. The TAN analysis was originally developed for measuring the acidity of petroleum products but has been applied more recently to measure the acidity of biodiesel [14-23], biodiesel blends [15, 16, 20], vegetable oils [18, 24], lubricating oils [25], heavy oil [26], bitumens [26], fats [24], and bio-oil [4, 9, 12, 27].

The current standard on biodiesel acidity is 0.50 mg KOH/g, according to the American Society for Testing and Materials (ASTM) D644 and European Standard (EN) 14104 [20]. However, currently, there is no available standard for bio-oil. The typical TAN value of switchgrass intermediate pyrolysis bio-oil is 137.4 ± 3.0 mg KOH/g [5], which is high compared to the standard for biodiesel.

Today, various standard methods (summarized in **Table 1** for comparison) are available to measure the TAN. Largely, there are potentiometric (ASTM D664 [28]) and colorimetric methods (ASTM D974 [29], ASTM D3339 [30], and American Oil Chemists' Society, AOCS Cd 3d 63 [31]). The colorimetric methods are known to be simple and better than potentiometric methods [15, 22]. Moreover, the colorimetric methods (ASTM D974 [29], EN14104 [32], and AOCS Cd 3d-63 [31]) were found to be more accurate for biodiesel analysis because these methods avoid errors introduced by the electrode [15, 22]. The aqueous titration from EN14104, however, can cause some ester bonds to be hydrolyzed by the aqueous base, leading to consumption of base and elevating measurements [20]. Furthermore, the end point determination in colorimetric methods can be challenging [14, 19]. Although ASTM D974 is known to be compatible for a colored sample, the dark brown (nearly black) color of bio-oil would most likely interfere with the endpoint determination during the analysis. Thus, for bio-oil analysis, the potentiometric method (ASTM D664 [28]) would be the appropriate option.

Table 1. Standard methods of acidity analysis

Standards	ASTM D664-11a **[28]**	ASTM D974-14	ASTM D3339-12	AOCS Cd 3d 63
Methods	Potentiometric	Colorimetric	Colorimetric	Colorimetric
Alkaline of titrant	KOH	KOH	KOH	KOH
Solution for titrant	isopropanol	isopropanol	isopropanol	isopropanol
Concentration of titrant (M)	0.1	0.02	0.01	0.1
Titration solvent	toluene:isopropanol: water	toluene:isopropanol	toluene:isopropanol: water	toluene:isopropanol
Solvent volume Ratio	100:99:1	1:1	100:99:1	1:1
Titration solvent (mL)	125	10	40	125
Indicator	n/a	p-naphtholbenzein	p-naphtholbenzein	phenolphthalein
Amount of indicator	n/a	8 drops		until the appearance of a slightly pink color
Sample weight range (g)	0.1 - 20	0.2 - 20	0.1 - 5	Varies
TAN ranges (mg KOH/g)	0.05 - 260	0.0 - 250	0.0 – 3.0	

The potentiometric method, ASTM D664 [28], has some challenges. While this method requires an electrode, the variability of the electrode dehydration may result in mediocre reproducibility and a lack of accuracy in the acidity analysis [15, 22]. Additionally, a conventional pH electrode is originally designed to measure the pH in aqueous systems. Hence, the pH value would be different in non-aqueous systems (i.e., titration solvent) due to possible effects of the non-aqueous solvent on the pH and reference electrode, including effects on proton activity [33]. All current standard methods require KOH/isopropanol as the titrant solution and the mixture of toluene and isopropanol as a titration solvent. Thus, the measured pH refers to these conditions. Another challenge for using organic solvents is that toxic chemical waste is generated when analyzing the TAN value by standard methods due to the titration solvent. Therefore, many researchers have indicated that current standard methods are labor-intensive, expensive, error-prone, and harmful to the environment [14, 16, 17, 23].

To improve the currently available standard methods, many research groups have modified these methods to reduce the error in reproducibility. Furthermore, modified methods were quicker, simpler, cheaper, more environmentally friendly, and more accurate. Some modifications from other groups are summarized in **Table 2-a, b** for reference. An organic titrant solution was replaced by aqueous solutions (KOH in water [4, 34] or NaOH in water [14, 23, 24]. The toxic titration solvent was substituted with less harmful solvents (e.g., only isopropanol [19], solely acetone [4, 34], a mixture of ethanol and water [14, 23, 24], and a mixture of acetone and tert-butanol [12]). Additionally, decreasing the amount of titration solvent [4, 14-16, 19, 23, 24, 34] and the sample weight [15, 16, 34] were attempted by many groups. Other modifications include changing the electrode filling solution [19] and introducing different electrode cleaning procedures [23, 24] to address dehydration in the electrode.

150 **Table 2-a.** Modifications of total acid number analysis of biofuels

Modifications	Baig et al. [15, 16]	Aricetti et al. [14]	Tubino et al. [23, 24]	Gonçalves et al. [19]
Standards	ASTM D974	AOCS Cd 3d-63	ABNT-NBR 14448 [23], AOCS Cd 3d-63 and ABNT-NBR 14448 [24]	ASTM D664
Methods	Colorimetric	Colorimetric	Potentiometric	Potentiometric
Alkaline titrant	KOH	NaOH	NaOH	KOH
Solvent for titrant	isopropanol	Water	Water	isopropanol
Titrant conc. (M)	0.02	0.02	0.02	0.01
Titration solvent	toluene:isopropanol:water	ethanol:water	ethanol:water	isopropanol
Volume ratio	100:99:1	1:1	1:1	1
Solvent vol. (mL)	10	75	75	50
Indicator	p-naphtholbenzein	phenolphthalein	n/a	n/a
Amount of indicator	8 drops	n/a	n/a	n/a
Sample weight (g)	2	20	20	n/a
Sample type	Distilled biodiesel, biodiesel blends	Biodiesel	Biodiesel [23], oils and fats [24]	Biodiesel
Other modifications	a 5 mL burette with division of 0.02 mL	n/a	Rinsing the electrode with ethanol and soaked it in water for 1 min	Electrode filling solution (3.0 mol/L aqueous KCl solution)
Results	• Reduced max error (from 42.88% to 5.92 %); good accuracy and repeatability [15] • Reduced max error (from 101% to 18%); repeatability decreased (from 290% to100% [14]	• Reliable, accurate, and precise TAN • Same results as AOCS Cd 6d-63 with a little better precision	• Statistically equivalent results as ABNT NBR 14448 • Less dehydration of the electrode [23] • Easier endpoints determination [24]	• Good repeatability that was lower than that of colorimetric method • The difference in TAN for different solvents and filling solutions was < 5% • Statistically similar results from standard methods and modifications
Advantages	Easy, reproducible, cost-effective, environmentally friendly, and time-efficient	Greener solvent, less solvent, aqueous titrant, cheaper (82%)	Minimizing dehydration of the electrode by using aqueous solution of ethanol as the solvent; quicker analysis, which decreases the possibility of dehydration of the electrode; less organic solvents, lower cost, greener method, lower toxicity	Less toxic and lower probability of causing possible aqueous hydrolysis of methyl esters
Challenges	Color changes at endpoint of titration of dark-colored samples could not be observed.	Difficult endpoint determination as a function of the yellow color of biodiesel	n/a	n/a

151 [ABNT: the Brazilian Association of Technical Standards (*Associação Brasileira de Normas Técnicas*)]

152

153 **Table 2-b.** Modifications of total acid number analysis of biofuels (continued)
154

Modifications	Fuhr et al. [26]	Shao et al. [34] and Oasmaa et al. [4]	Wu et al. [12]
Standards	ASTM D664	ASTM D664	ASTM D664
Methods	Potentiometric	Potentiometric	Potentiometric
Alkaline titrant	KOH	KOH	tetramethylammonium hydroxide (TMAH)
Solvent for titrant	isopropanol	water	methanol and isopropanol (1:10 v.)
Titrant conc. (M)	0.05, 0.1, 0.15 (N)	0.1	0.12 mM
Titration solvent	toluene:isopropanol	acetone	acetone: tert-butanol
Volume ratio	75:25	1	1:9
Solvent vol. (mL)	125	50	n/a
Sample weight (g)	1 - 2	0.1 - 0.5	n/a
Sample type	Heavy oils and bitumens	Bio-oil	Bio-oil
Others	time < 30 min; pretreatment of samples = heating to 60°C	Used 0.1mol/L HCl in water as standard solution	Nonaqueous titration
Results	• Concentrations of titrant have no effect • Pretreatment is required for viscous samples • More toluene in titration solvent can dissolve samples • Good reproducibility	• Sample size (0.1 - 0.5 g) and solvent volume (50 - 125 mL) did not have effects on TAN analysis • Possible recycling titration solvent (up to three times)	• Detected heavy carboxylic acids and phenolics as well as strong acids • Differentiated the carboxylic acids and phenolic groups through a non-aqueous titration potentiometric titration
Advantages	Suitable analysis of viscous samples (perhaps this modification is applicable for organic phase of bio-oil) Titration without delay to avoid asphaltene precipitation	Less toxic, cost saving, and shorter analysis time	Nonaqueous titration: avoid leveling effect and obtain distinguishable endpoints Titration solution (acetone and tert-butanol) can dissolve bio-oil
Challenges	n/a	n/a	n/a

155 n/a: not available

Further steps have been made to develop new approaches to measuring acidity including a coulometric analysis [17], a 3D-printed flow injection analysis [35], and a sequential injection analysis with multivariate curve resolution-alternating least squares (SIA-MCR-ALS) [18]. Although these new methods were developed for analyzing the acidity of plant oils [18], biodiesel [17, 18], and thermal conductive oil [35], they may be applicable for analyzing the acidity of bio-oil as well.

Even though various modifications and new methods were recently developed for biofuels, it is important to understand the meaning of TAN values using the standard methods. Moreover, as compared to biodiesel, there are no in-depth studies on the TAN analysis of bio-oil, even though the TAN value has been primarily used for measuring the acidity of bio-oil. Understanding the relationship between TAN and the concentrations of acidic components found in bio-oil samples can help us develop methods to reduce the acidity of bio-oil. It is also important to determine if other non-acidic chemical species present in bio-oil (e.g., sugars, furans, ketones, aldehydes, phenolics) contribute to the TAN values. Therefore, the objectives of this study were to: (1) analyze the acidity of switchgrass bio-oil in terms of TAN value; (2) compare measured TAN values with theoretically calculated ones for standard solutions; (3) investigate the relationship between TAN values and the concentrations of different chemicals species found in bio-oil using standard solutions; (4) examine the effect of the titration solvent on the TAN measurement by comparing the TAN values and titration curves obtained from the standard method with results from the TAN analysis in aqueous environment and with equilibrium modeling results; and (5) explore whether a variety of chemicals present in bio-oil have any influence on the TAN analysis by examining the recovery of added acetic acid in aqueous bio-oil samples (recovery test) through the TAN values. Because D664 [28] was previously employed for bio-oil acidity analysis, while D974 (colorimetric) was limited by the dark color of bio-oil as discussed above, we attempted to investigate the relationship between TAN values and concentrations of different standard solutions using the D664 method [28]. In this study, a minor modification to the electrode cleaning procedure — the 1-minute water-spray method described in Baig et al. [16] — was adopted.

2. Experimental

2.1. Materials

Acetic acid (analytical standard), propionic acid (analytical standard), vanillic acid (≥ 97.0%), syringic acid (≥95%), 4-hydroxybenzoic acid (HBA, ≥99%), 5-(hydroxymethyl)furfural (HMF, ≥99%), phenol (≥ 99.5%), anhydrous 2-propanol (99.5 %) and toluene (≥ 99.5 %) were purchased from Sigma-Aldrich (Milwaukee, WI). A titrant solution, 0.1 mol/L KOH in 2-propanol was purchased from Fisher Scientific. Switchgrass bio-oil was produced via intermediate pyrolysis with a semi pilot-scaled auger pyrolysis system (Proton Power, Inc., Lenoir City, TN, USA) at the University of Tennessee (UT) Center for Renewable Carbon. More details on the production of bio-oil are available elsewhere [5, 8, 36]. Switchgrass bio-oil had pH of 2.5.

2.2. Methods

2.2.1. Bio-oil Analysis

The chemical composition of crude, aqueous bio-oil, and organic bio-oil (**Table 3**) was analyzed by gas chromatography with flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC). The methods involved in analyzing the chemical composition of bio-oil are found in Ren et al. [5, 37]. Briefly, 2(5H)-furanone, 1-hydroxy-2-butanone, 1,3-propanediol, 3-methyl-1,2-cyclopentanedione, guaiacol, creosol, 2,6-dimethoxyphenol, and 3-ethylphenol were quantified using GC-FID with an HP-5 column (30 m × 0.32 mm, 0.25 μm film thickness) [5, 37]. The detailed settings for GC-FID are available in the literature [5, 37]. The identification of compounds was performed by comparing their mass spectra with those from the National Institute of Standards and Technology (NIST) mass spectral data library. Acetic acid, propionic acid, levoglucosan, hydroxymethylfurfural, furfural, phenol, and 1,2-benzendiol were analyzed using an HPLC, Jasco 2000Plus (Jasco Analytical Instruments, Easton, MD) with an MD-2018 plus photodiode array detector, an RI-2031 Plus intelligent refractive index detector, and an AS-2055 plus autosampler. The chemical analysis using HPLC was performed at 50°C with a Bio-Rad column HPX-87H (300 × 8 mm). The injected sample volume was 20 μL. Sulfuric

acid (5 mM) in deionized water was used as the mobile phase with a flow rate of 0.6 mL/min. The compounds were quantified using external standards in both the HPLC and GC-FID analyses. For water content measurements, a Schott TitroLine Karl Fischer volumetric titrator was used according to the ASTM D4377 (2011) method [38].

2.2.2. Total Acid Number Analysis

For the TAN analysis, the ASTM D664 method [28] was followed except for the cleaning procedure. In brief, the titration solvent was prepared using toluene, anhydrous 2-propanol, and deionized water (100:99:1 v.). It should be mentioned here that one of the ASTM D664 modifications that have been suggested for biodiesel does not include toluene as one of the titration solvents. For the case of bio-oil, however, we did not follow that modification because, in contrast to biodiesel, bio-oil includes organic components that are not soluble in the 2-propanol/water mixture. For the titrant solution, a concentration of 0.1 mol/L KOH in 2-propanol was used as received from Fisher Scientific. The volume of titration solvent used was 125 mL. Sample amounts varied depending on the range of TAN values according to ASTM D664-11a [28]. The modified electrode cleaning procedure (spraying water for 1 min) was adapted from Baig et al. [16]. Samples were titrated to pH 11 [5], and the TAN values were calculated by following ASTM D664. Acetic acid, propionic acid, HMF, and phenol solutions were prepared in deionized water with concentrations of 2, 4, and 6 wt%. Sample analysis was preformed in triplicate. Solutions of HBA and vanillic acid were prepared in 2-propanol due to its limited solubility in water and higher solubility in 2-propanol [39]. Due to limited solubility of syringic acid in water or 2-propanol, syringic acid solution (0.6 wt%) was prepared in the titration solvent.

2.2.3. Equilibrium MINEQL+ Modeling and Aqueous Titration

The TAN analysis of acetic acid (2 wt%) was modeled using a solution equilibrium software called MINEQL+ for a closed system (with respect to air). The following chemical reactions were considered in modeling the TAN analysis of acetic acid (2 wt%). The equilibrium constants, K

values, of these reactions were provided by the MINEQL+ library. The conditions of the MINEQL+ modeling are: aqueous system, closed to the atmosphere (i.e., no carbonate species considered), and room temperature (25°C).

$$CH_3COOH = H^+ + CH_3COO^- \quad (\log K = 4.757)$$

$$CH_3COOK = K^+ + CH_3COO^- \quad (\log K = -0.196)$$

To imitate the MINEQL+ modeling experimentally, an aqueous TAN analysis was performed using water as titration solvent and 0.1 mol/L KOH solution in water as titrant solution.

2.2.4. Bio-oil Recovery Test

Crude bio-oil obtained from UT was centrifuged at 3000 rpm (1673 g) for 30 minutes using Beckman Coulter Avanti J-E centrifuge with a JLA 10.500 rotor to separate the aqueous and organic components of bio-oil. The chemical compositions of aqueous bio-oil (AqBO) and organic bio-oil (OrgBO) were analyzed. Due to the heterogeneity of crude bio-oil and organic bio-oil, AqBO was used in the recovery test for consistency. AqBO (9 g) was mixed with 1 g of acetic acid solutions (20, 40, and 60 wt% in deionized water) to yield an overall 2, 4, and 6 wt% of acetic acid, respectively. The TAN values of acetic-acid-added AqBO samples were analyzed in triplicate. Prepared samples were also analyzed using HPLC as described in Section 2.2.5.

2.2.5. High-Performance Liquid Chromatography (HPLC) Analysis for Bio-oil Recovery Test

Agilent 1100 was used as the HPLC system. A BioRad Aminex HPX-87H column and a refractive index detector were incorporated into this system. The mobile phase of the HPLC was 5 mM sulfuric acid with a flow rate of 0.6 mL/min. Standard solutions were prepared with the mobile phase, and bio-oil samples were diluted 1000 times with the mobile phase. A volume of 20 µL was injected, and each sample was analyzed for one hour.

3. Results and Discussion

3.1. Chemical Composition and TAN Analysis of Switchgrass Bio-oil

The chemical compositions of switchgrass crude bio-oil, aqueous bio-oil, and organic bio-oil obtained from GC-FID and HPLC analyses are presented in **Table 3**. When the heterogeneous crude bio-oil is centrifuged, there were two different phases: aqueous bio-oil (supernatant) and organic bio-oil (precipitate). Aqueous bio-oil contains mostly water, levoglucosan, acetic acid, and propionic acid. Organic bio-oil contains less water but more furfural and phenolics than aqueous bio-oil. More than 70 wt% of organic bio-oil was not quantified by GC-FID and HPLC analyses. The TAN values of crude bio-oil and aqueous bio-oil were found as 109.7 ± 4.3 and 138.6 ± 14.7 mgKOH/g, respectively.

Table 3. The chemical compositions of crude bio-oil, aqueous (centrifuged) bio-oil, and organic bio-oil.

	Crude Bio-oil	Aqueous Bio-oil	Organic Bio-oil
Water (weight%)	43.3	43.65	15.18
Levoglucosan	9.09	9.19	0.72
Acetic acid	7.71	8.06	6.16
Propionic acid	3.42	3.57	0.00
1-Hydroxy-2-butanone	1.37	1.08	0.86
1,3-Propanediol	0.29	0.07	0.06
HMF	0.59	0.58	0.19
Furfural	1.38	0.40	1.35
2(5H)-Furanone	0.38	0.41	0.26
3-Methyl-1,2-cyclopentanedione	0.25	0.17	0.25
1,2-Benzenediol	0.59	0.42	0.61
Phenol	0.26	0.12	0.31
Guaiacol	0.11	0.13	0.29
3-Ethylphenol	0.38	0.15	0.74
2-Methyl-4-methylphenol	0.42	0.24	0.47
2,6-Dimethoxyphenol	0.13	0.11	0.33
Quantified (wt%)	69.67	68.34	27.78
Non-quantified (wt%)	30.33	31.66	72.22

3.2. Comparison between Measured and Theoretical TAN Values

The relationship between measured TAN values of acetic acid and propionic acid solutions (2, 4, 6 wt%) and theoretical TAN values obtained from **Equation 1** are presented in **Figure 1**. The mean of the

measured TAN values, theoretical TAN values, and their repeatability are found in **Table 4**. According to ASTM [28], the definition of repeatability calculated from **Equation 2-a** [15, 28, 34, 40] is given as follows: "the difference between successive test results obtained by the same operator with the same apparatus under constant operating conditions on identical test material would in the long run, in the normal and correct operation of the test method, exceed the following values only in one case in twenty." Since the titration curves of the manual TAN analyses did not have defined inflection points, the acceptable repeatability for the manual titration mode is 5% of the mean based on the standard method [28]. Thus, most of the data were within the acceptable repeatability (<5%) as shown in **Table 4**, while only one measurements exceeded the 5% limit.

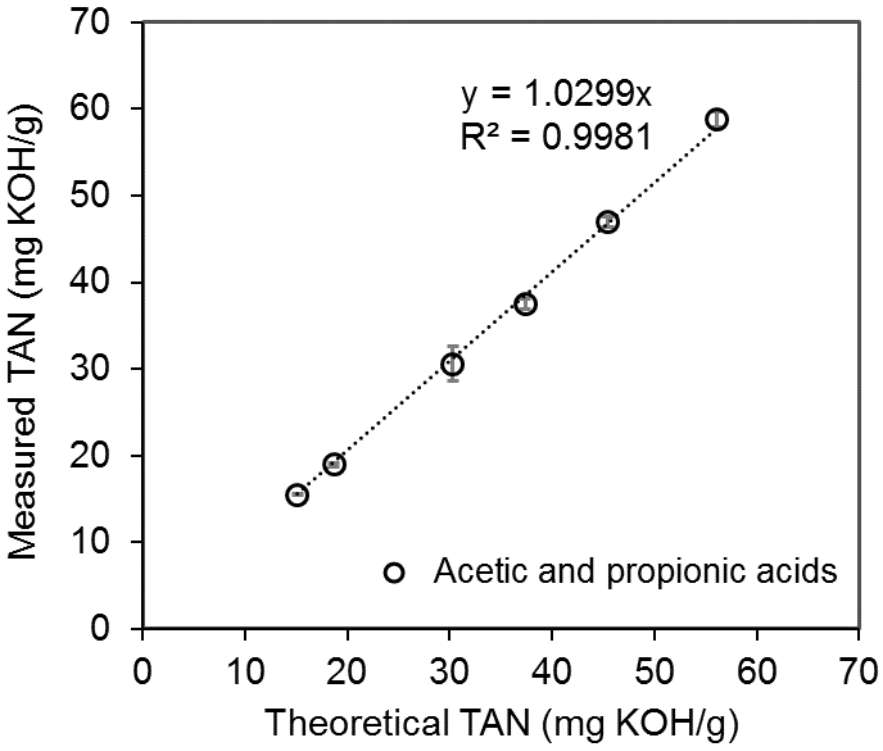


Figure 1. Relationship between measured TAN values of acetic acid and propionic acid solutions (2, 4, 6 wt%) and their theoretical TAN values obtained from **Equation (1).**

Theoretical TAN = (wt% of acid) × (MW of KOH)/(MW of acid) × 1000 (mg/g) **(1)**

where TAN is the total acid number (mg KOH/g) and MW is the molecular weight in g/mol (MW of KOH: 56.1056 g/mol).

$$\text{Repeatability (\%)} = [(2.77 \times SD) / (n \times \text{mean TAN})] \times 100\ \% \quad \textbf{(2-a)}$$

$$\text{Error (\%)} = (\text{mean of measured TAN} - \text{theoretical TAN}) / (\text{theoretical TAN}) \times 100\ \% \quad \textbf{(2-b)}$$

where n is the number of operators involved in the analysis (1 in this case), and SD is the standard deviation.

Table 4. Theoretical and measured TAN values of acetic and propionic acids standard solutions.

Chemicals	Concentration		Theoretical TAN (mgKOH/g)	Mean measured TAN (mgKOH/g)	Standard deviation	Repeatability (%)
	wt%	mol/L				
Acetic acid	2	0.33	18.69	18.96	0.24	3.5
	4	0.67	37.37	37.46	0.63	4.6
	6	1.00	56.06	58.75	0.90	4.2
Propionic acid	2	0.27	15.15	15.47	0.08	1.5
	4	0.54	30.30	30.55	2.00	18.2
	6	0.81	45.44	47.00	0.62	3.6

3.3. TAN Analysis of Standard Solutions

Oasmaa et al. [4] found that there were different linear relationships between TAN values and the concentrations of acetic acid and formic acid standard solutions (wt%). The reason for the different linear relationships, however, was not discussed. When the same data are replotted against molar concentrations, as shown in **Figure 2**, it is clear that both formic and acetic acids actually have the same linear relationship of TAN vs molar concentrations. The combined data resulted in a slope of 58.494, which is very close to the combined slope (58.059) discussed later in this paper.

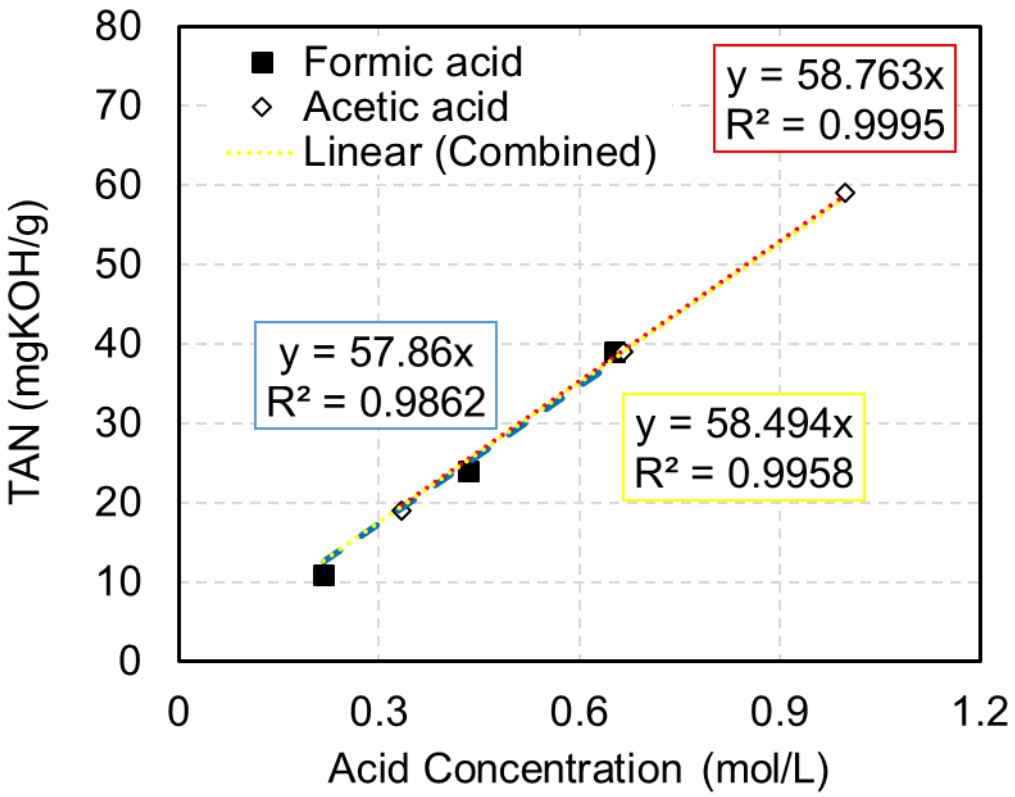


Figure 2. The same relationship is found when the TAN value is plotted vs molar concentrations of formic and acetic acid (data obtained from Figure 5 in Oasmaa et al. [4]).

To further investigate the relationship between the TAN value and concentrations of various chemicals, the TAN analysis of standard solutions was performed for different concentrations (2, 4, and 6 wt%). All standard solutions except hydroxybenzoic, vanillic, and syringic acids were prepared in deionized water. Solutions of HBA and vanillic acid were prepared in 2-propanol due to its limited solubility in water [39]. Preparing HBA and vanillic acid solutions in 2-propanol should not affect the TAN analysis because the titration solvent (125 mL) used contains 2-propanol (toluene: 2-propanol: water = 100:99:1 v.) at a relatively high concentration.

The measured TAN values are plotted vs weight percent in **Figure 3**. As the concentration of the acidic solutions increased, the TAN values also increased. Unlike the acidic solutions, the phenol and HMF

solutions did not influence the TAN measurements despite that phenols are more acidic than alcohols and may form phenoxide ions by reacting with hydroxide ions [4]. This is due to the high pK_a value (10.02) of the phenol, as well as the effect of the nonaqueous solvent. The low sensitivity of the standard method (ASTM D664) towards weak acids like phenol may be due to a suboptimal acidity of 2-propanol as Wu et al. [12] pointed out.

The linear trendline of acetic acid, which has the lowest molecular weight, had the highest slope, while the vanillic acid with the highest molecular weight had the lowest slope. The relationship between the TAN value and weight percent of standard solutions shown in **Figure 3** can be described by **Equations (3-a, b, c)**. As shown in **Equation (1-c)**, the slope of each line in **Figure 3** depends on the molecular weight of the sample.

Weight percent

$$TAN = [slope] \times [wt\%] \qquad \textbf{(3-a)}$$

$$TAN(mgKOH/g) = \frac{MW\ of\ KOH\ (g/mol)}{MW\ of\ Sample\ (g/mol)} \times 1000\ \left(\frac{mg}{g}\right) \times \frac{Sample\ (g)}{Total\ (g)}\% \times \alpha \qquad \textbf{(3-b)}$$

$$[slope] = \frac{MW\ of\ KOH\ (g/mol)}{MW\ of\ Sample\ (g/mol)} \times 1000\ \left(\frac{mg}{g}\right) \times \alpha \qquad \textbf{(3-c)}$$

where $[wt\%] = \frac{Sample\ (g)}{Total\ (g)}\%$, where $Total\ (g) = Sample\ (g) + Solvent(g)$

Molar concentration

$$TAN = [slope] \times [M] \qquad \textbf{(4-a)}$$

$$TAN = \frac{Sample\ (g)}{Total\ (L)} \times \frac{1}{MW\ of\ Sample\ (g/mol)} \times \frac{Total\ (L)}{Total\ (g)} \times MW\ of\ KOH\ (g/mol) \times 1000\ \left(\frac{mg}{g}\right) \times \alpha \qquad \textbf{(4-b)}$$

$$TAN\left(\frac{mgKOH}{g}\right) = \frac{Total\ (L)}{Total\ (g)} \times MW\ of\ KOH\ (g/mol) \times 1000\ \left(\frac{mg}{g}\right) \times \alpha \times [M] \qquad \textbf{(4-c)}$$

$$TAN\left(\frac{mgKOH}{g}\right) = \frac{1}{\rho Total} \times MW\ of\ KOH\ (g/mol) \times 1000\ \left(\frac{mg}{g}\right) \times \alpha \times [M] \qquad \textbf{(4-d)}$$

$$[slope] = \frac{1}{\rho Total} \times MW\ of\ KOH\ (g/mol) \times 1000\ \left(\frac{mg}{g}\right) \times \alpha \qquad \textbf{(4-e)}$$

where $[M]: molar\ concentration = mol/L = \frac{Sample\,(g)}{Total\,(L)} \times \frac{1}{MW\ of\ Sample\ (g/mol)}$

$\rho_{Total}: \frac{Total\,(g)}{Total\,(L)}$

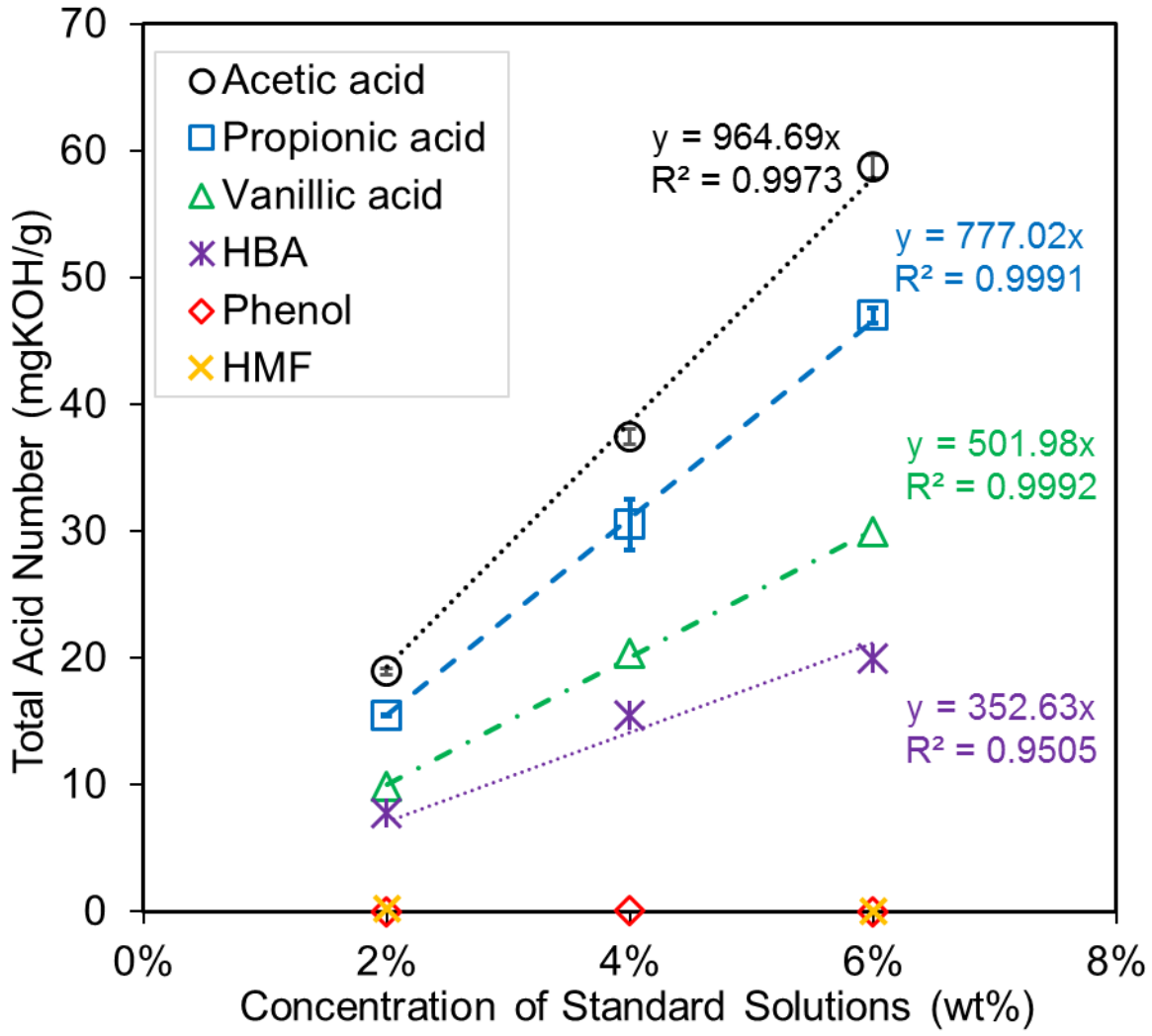


Figure 3. Different linear relationships between TAN values and weight percent concentrations of standard solutions of acetic acid, propionic acid, vanillic acid, and phenol.

Since the slopes of different linear relationships are inversely related to the molecular weight, the same data points were plotted with the x-axis as molar concentration as shown in **Figure 4**. Acetic, propionic, and hydroxybenzoic acids showed the same linear relationship; however, vanillic acid showed a different linear relationship. The slope of vanillic acid in **Figure 4** is approximately two times the slope of acetic, propionic, and hydroxybenzoic acids. The relationship between TAN value and molar concentrations of standard solutions from **Figure 4** is described by **Equations (4-a** through **4-e)**. The slope of each line in **Figure 4** is inversely related to the density of the standard solution. The different concentration standard solutions that were analyzed in this research were assumed to have similar density. However, at higher concentrations, the density of solutions may change causing also a change in the slope of the line.

The slope of linear relationships is also related to the alpha factor. The alpha factor (α) in **Equations (3)** and **(4)** is the number of functional groups that contribute to the TAN value or the number of protons that can be removed during the TAN analysis. For instance, if a compound had more than one functional group that could react with KOH, consuming KOH with multiple functional groups would lead to a stronger effect on the TAN value as compared to compounds with only one acidic functional group. Even though vanillic acid has one carboxylic acid group, its slope, which is double the slope of other monoprotic acids, indicates that two protons were removed during the TAN analysis. This is because vanillic acid loses two protons and acts as diprotic acid during the TAN analysis (titration to pH 11) in the titration solvent system. Therefore, the alpha factor for formic, acetic, propionic, and hydroxybenzoic acids is 1, while the alpha factor for vanillic acid is found to be 2.

The results in **Figure 4** are summarized in **Table 5** with chemical structures and pK_a values. As expected, HMF that has pK_a value of 12.82 with the slope of 0 did not contribute to the TAN values. Because the samples were titrated to pH 11 during TAN analysis, hydrogen atoms with pK_a values less than 11 were expected to be titrated. The pK_a of the hydrogen in phenol was less than 11; however, phenol did not contribute to the TAN value. As discussed in the following section (**3.4**), the reason for this behavior

could be that the actual acid-base constants in the TAN standard titration solvent are different from pK_a values reported for the aqueous system. Acetic, propionic, and hydroxybenzoic acids had a slope of 58.059, which is close to the molecular weight of KOH. HBA has two hydrogens that have pK_a values of 4.38 and 9.67. Based on the slope of the linear relationship, only the hydrogen in the carboxylic group contributed to the TAN value and was removed during the TAN analysis. Vanillic acid has a very similar chemical structure as HBA except for the presence of a methoxy group. The slope of the linear relationship (**Figure 4**) for vanillic acid was 114, approximately double that for other acids. The slope of vanillic acid indicates that the two protons were removed during the TAN analysis (one from its carboxylic group and the other from its hydroxyl group). Perhaps the presence of the methoxy group helped the hydrogen in the hydroxyl group to be removed in the titration solvent – the mixture of toluene, 2-propanol, and water. It is noteworthy that the pK_a values presented in **Table 5** are based on aqueous systems. For consistency, the pK_a values of chemicals were obtained from chemical calculations from Chemicalize provided by ChemAxon [41] except the pK_a value of HMF, which was obtained from another source [42]. In the titration solvent system (toluene, isopropanol, and water – 100:99:1 v.), the pK_a values are certainly changing due to interactions between the titration solvent molecules and the various chemical species. Further investigations, which are not in the scope of this research, are needed to fully understand those interactions.

The data points for the TAN values of the acetic, propionic, and hydroxybenzoic acids were combined in **Figure 4**, and these data are well represented by a solid line, as shown on the graph. The combined slope for acetic, propionic, hydroxybenzoic acids is 58.059, which is close to the molecular weight of KOH (56.1056 g/mol). Thus, if a sample contains formic, acetic, propionic, and hydroxybenzoic acids, then the TAN value of the sample can be converted to the molar concentration of total acids using the following relationship, where M is the concentration in mol/L.

$$[\text{M of total acids}] = [\text{TAN (mg KOH/g)}] / [58.059\ (\text{mg KOH M}^{-1}\ \text{g}^{-1})] \quad \textbf{(5)}$$

If a sample such as bio-oil, however, contains acidic components such as vanillic acid, which have a stronger contribution to the TAN value than monoprotic acids, **Equation (5)** cannot be used to convert the TAN measurement to the molar concentration of total acidic components.

The TAN analysis of syringic acid solutions was also attempted. Due to limited solubility of syringic acid in water and isopropanol, the TAN titration solvent (toluene: isopropanol: water = 100: 99: 1 v.) was used to prepare the syringic acid solution. The maximum concentration that we were able to prepare was 0.6 wt% of syringic acid in the titration solvent. Unfortunately, the TAN value of 0.6 wt% of syringic acid could not be obtained because, during the TAN analysis of syringic acid, the pH value of the titration system could not reach 11, which is the end-titration pH according to the standard method. Instead, the maximum pH reached was 9.5. After reaching pH 9.5, precipitation of solids occurred, and the pH value dropped significantly. This means that, when a sample contains syringic acid, even at low concentrations, a strong contribution to the TAN value can be expected because syringic acid in the sample will consume the KOH solution during titration; therefore, it would take more KOH solution for the system to reach pH 11, leading to a higher TAN value than that obtained in the absence of syringic acid.

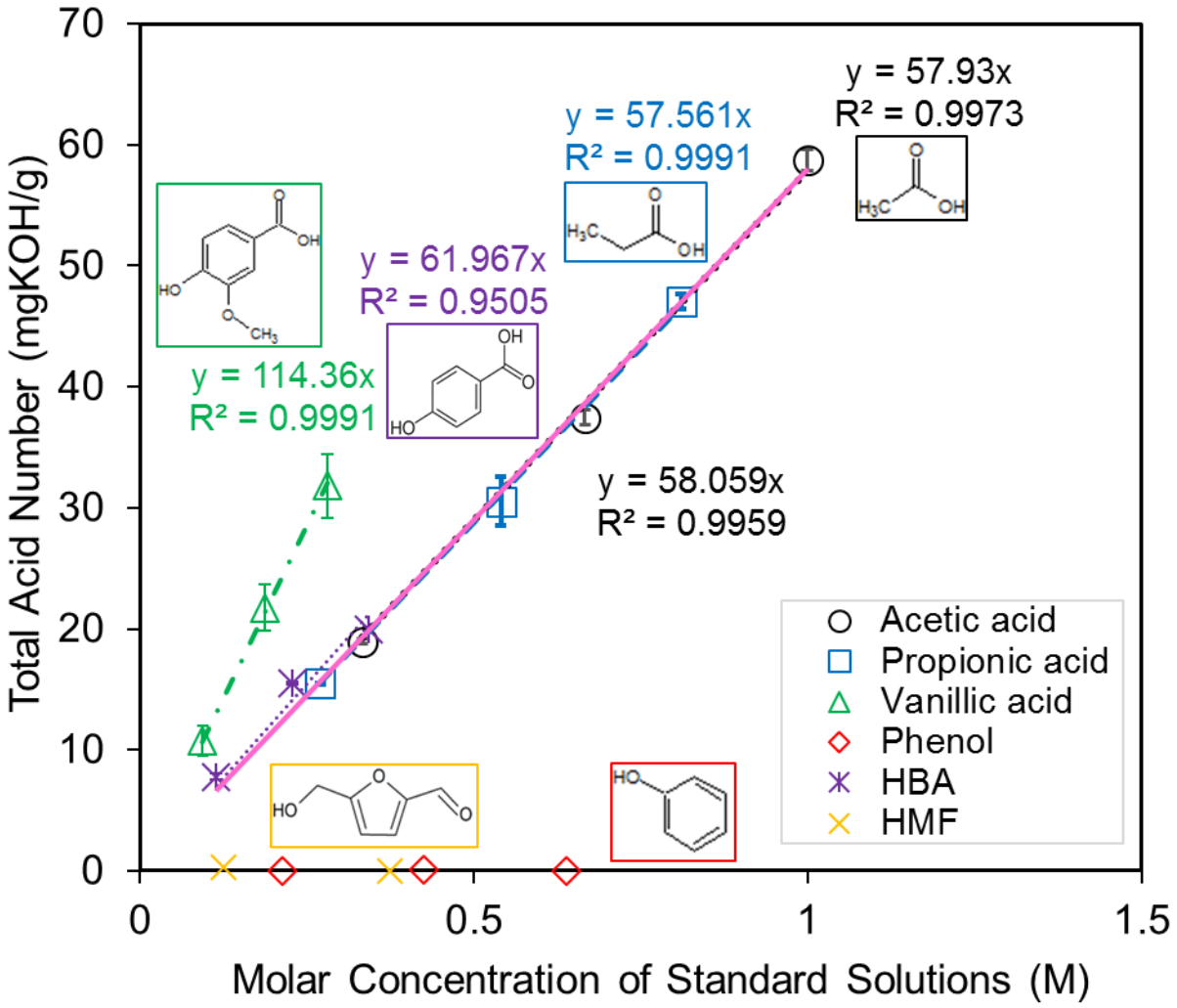
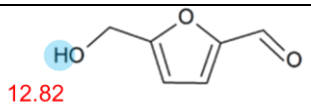
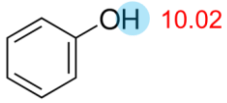
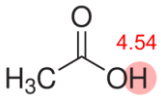
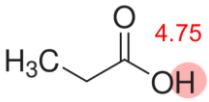
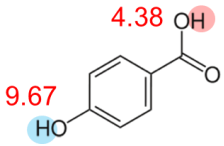
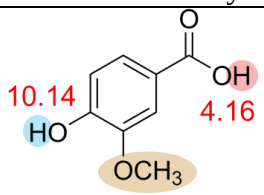


Figure 4. Relationship between TAN and molar concentrations of various bio-oil components. The TAN value of acetic, propionic, and hydroxybenzoic acids shows the same linear relationship vs. molar concentrations, represented by a solid trendline. The TAN vs. molar concentration line for vanillic acid shows a higher slope because of the release of 2 protons during titration to pH 11. Phenol and HMF show zero contribution to the TAN value.

Table 5. Slopes of the linear relationships between TAN value and the molar concentration of various chemicals found in bio-oil with their chemical structure and pK_a values obtained from the literature for HMF [42] and online chemical calculations from Chemicalize provided by ChemAxon [41] for other chemicals.

Slopes	Chemical structures and pK_a values		
0 (TAN = 0)	 Hydroxymethylfurfural (HMF)	 Phenol	
58 (TAN = 58 * [M])	 Acetic acid	 Propionic acid	 Hydroxybenzoic acid (HBA)
114 (TAN = 114 * [M])		 Vanillic acid	

[M] is the molar concentration in mol/L.

3.4. Influence of Titration Solvent on TAN Analysis - MINEQL+ Modeling of Aqueous TAN Analysis

To investigate the effect of the titration solvent (i.e., the mixture of toluene, 2-propanol, and water), we employed MINEQL+, a solution equilibrium software, to model the TAN analysis. The MINEQL+ software is designed to model aqueous systems. Results from the MINEQL+ modeling are presented in **Figure 5**, where the molar concentration of potassium ions needed for the system to reach pH 11 is estimated at 3.67×10^{-3} mol/L. This amount can be converted to TAN value using the following equation based on the assumption that 1 g of sample is titrated in 125 mL of water [i.e., a total volume of 0.126 L (0.125 L of water and ~ 0.001 L of sample) was used in the calculation] as described in **Equations (6-a, b).** Thus, the TAN value obtained from MINEQL+

modeling is 25.45 mgKOH/g **[Equation (6-b)]**, which is different from the actual TAN value obtained from the TAN analysis using the ASTM D664 method (18.96 mgKOH/g).

Since the TAN values from the ASTM method and the MINEQL+ modeling were different, the TAN analysis in an aqueous system was performed using 2 wt% of acetic acid, water as the titration solvent, and 0.1 mol/L KOH solution in water. The experimental TAN analysis in the aqueous system is also presented in **Figure 5**. The amount of KOH solution added was measured during this analysis, and for comparison with the results from MINEQL+, the volume of KOH solution added was converted to molar concentration of KOH. From **Figure 5**, the amount of KOH required to titrate the system to pH 11 is estimated at 3.63×10^{-3} mol/L. Similarly to **Equation (6-b)**, this concentration (3.63×10^{-3} mol/L) can be converted to TAN value as shown in **Equation (6-c)**.

Conversion from [KOH] to TAN (mgKOH/g sample)
= [Concentration of KOH, (mol/L)] × [molecular weight of KOH, (g/ml)] × [unit conversion, (mg/g)] × [total volume of the system, (L)] / [weight of a sample, (g)] **(6-a)**
= 3.67×10^{-3} mol/L (KOH) × 56.1 g/mol × 1000 mg/g × 0.126 L / 1 g = **25.45 mgKOH/g (6-b)**
= 3.63×10^{-3} mol/L (KOH) × 56.1 g/mol × 1000 mg/g × 0.126 L/ 1 g = **25.66 mgKOH/g (6-c)**

Hence, the TAN value obtained from the TAN analysis in the aqueous system is 25.66 mgKOH/g, which is very close to the TAN value from MINEQL+ (25.45 mgKOH/g) but different from the TAN analysis using the ASTM method. The TAN analysis of acetic acid (2 wt%) using the ASTM D664 standard method is shown in **Figure 5**. To compare with the results from MINEQL+ and the experimental aqueous TAN analysis, the data were plotted as pH vs. molar concentrations of KOH added. Unlike the titration curves from MINEQL+ modeling and aqueous TAN analysis, the starting pH value is around 6.4, which is higher than the MINEQL+ model and the aqueous TAN

462 analysis (~ pH 3.8). The TAN value from this analysis is 18.96 mgKOH/g, which is lower than the
463 results from the model and aqueous system.
464
465 The results from MINEQL+, aqueous TAN analysis, and standard TAN analysis are summarized in
466 **Table 6**. The MINEQL+ modeling results agree well with the experimental TAN analysis data for
467 the aqueous system; however, these TAN values were significantly higher than the TAN value
468 obtained from the ASTM D664 standard method. The initial pH value in the standard titration
469 solvent was higher than those in aqueous systems. Different titration curves between aqueous and
470 titration solvent systems also represent the different pK_a values in these systems. These differences,
471 as well as different TAN values, indicate that there may be different reactions and activities among
472 the solvent molecules, sample molecules (e.g., acetic acid), and the titrant (i.e., KOH). As
473 mentioned earlier, pK_a values (i.e., the logarithm of acid dissociation constants) that represent
474 dissociation in acid-base reactions, are reported for the aqueous system. Therefore, the TAN values
475 obtain from the MINEQL+ modeling, which is based on the pK_a values, are comparable with results
476 from aqueous TAN analysis. Since the ASTM method uses a mixture of toluene, isopropanol, and
477 water as a titration solvent, the dissociations of compounds in terms of acid-base reactions are
478 expected to be different from those occurring in the aqueous system. In short, the pK_a values of
479 compounds during the ASTM standard TAN analysis should be different from the known pK_a
480 values for the aqueous system. In order to fully understand these differences, the reactions or
481 activities among the sample and titration solvent molecules need to be further investigated.
482

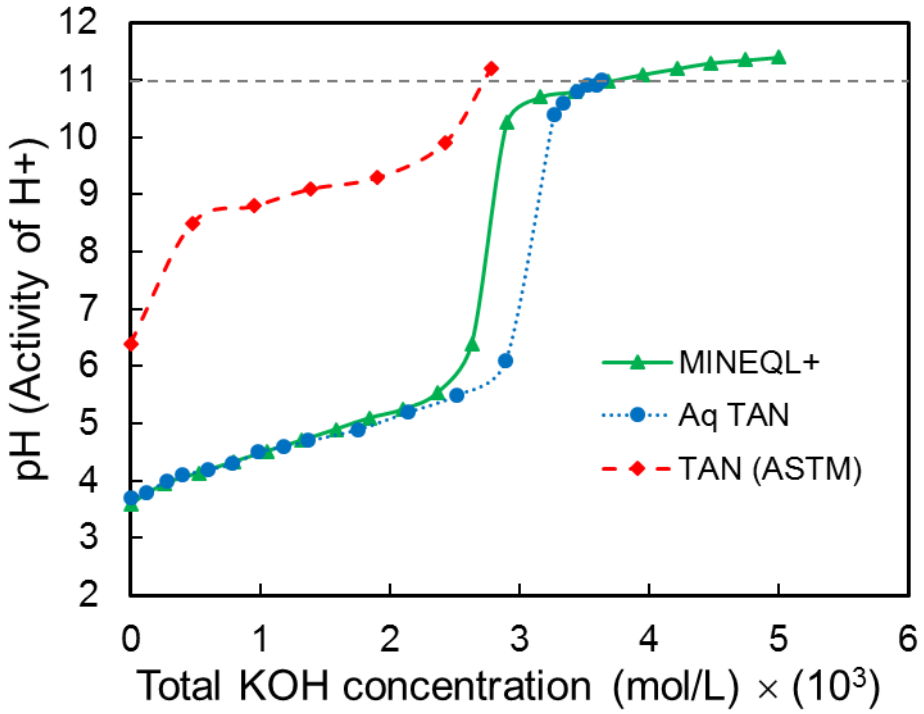


Figure 5. Titration curves of acetic acid (2 wt%) solution from MINEQL+ modeling, aqueous titration, and standard titration (ASTM D664)

Table 6. Summary of TAN values from MINEQL+ modeling and experimental TAN analysis in aqueous system and the titration solvent from ATSM D664

	MINEQL+	Aqueous System	ASTM TAN Analysis
Sample (Conc.)	Acetic acid (2 wt%)	Acetic acid (2 wt%)	Acetic acid (2 wt%)
Titration Solvent	Water	Water	Isopropanol: toluene: water (100:99:1 v.)
TAN (mgKOH/g)	25.45	25.66	18.96
[K+] (mol/L)	3.67E-3	3.63E-3	2.68E-3

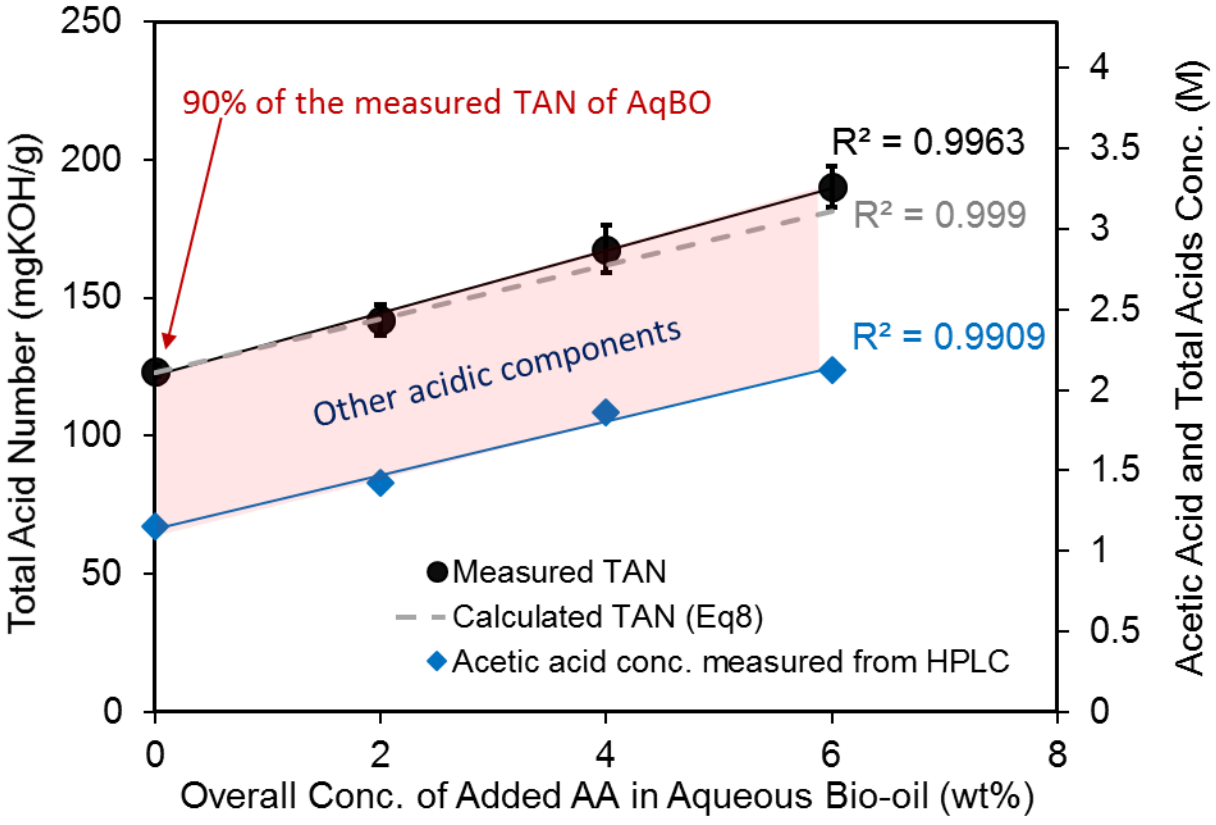
3.5. Recovery Test on Acetic Acid in Bio-oil Samples

Here, to verify whether the addition of acetic acid can be detected by TAN analysis and whether the various chemical species in switchgrass intermediate-pyrolysis bio-oil affect the TAN analysis, a recovery test was performed. Previously, a recovery test with biodiesel samples was performed by a coulometric

titration method [17]. In this study, a similar recovery test, which involved adding known amounts of acetic acid to aqueous bio-oil, was performed to verify whether the TAN analysis can accurately recover the mass of acetic acid added to aqueous bio-oil. Since known amounts of acetic acid were added to the aqueous bio-oil sample, the TAN values of aqueous bio-oil samples should be directly related to the increasing acetic acid concentration.

Results from the TAN and HPLC analyses of aqueous bio-oil samples with added acetic acid are presented in **Figure 6.** To incorporate the dilution effect after adding acetic acid solution, 90% of the measured TAN of the aqueous bio-oil (the first data point) was plotted with the measured TAN of aqueous bio-oil samples with acetic acid added, displayed by black circular markers in **Figure 6**. To incorporate the dilution effect due to added acetic acid solution, 90% of the measured TAN of the aqueous bio-oil was plotted with the measured TAN of aqueous bio-oil samples with acetic acid added, displayed by black circular markers in **Figure 6**. The samples (overall 2, 4, and 6 wt% of acetic acid in aqueous bio-oil) were prepared with 90% of aqueous bio-oil and 10% of the acetic acid solution as described in **Section 2.2.4**. The first data point (black circle marker) represents the aqueous bio-oil without any acetic acid added, diluted by 10% with water to make it comparable with the other analyzed samples that had acetic acid because those samples contained only 90% aqueous bio-oil. Based on the measured TAN of the aqueous bio-oil and the measured TAN of the acetic acid standard solution from **Section 3.3**, the calculated TAN values were estimated using **Equation (7),** similarly to Baig et al. [15], and represented by a gray dashed line in **Figure 6**. The measured TAN values of aqueous bio-oil samples were slightly higher than the calculated TAN values. The calculated TAN values, however, were still within the range of measured TAN values, which are marked with error bars that indicate the standard deviation. Moreover, the relationship of data points for aqueous bio-oil samples was linear with an R^2 value of 0.9963.

519 The TAN analysis of aqueous bio-oil samples with added acetic acid led to less than ± 5% error,
520 calculated using **Equation (2-b)** and based on the calculated TAN from **Equation (7)**.
521

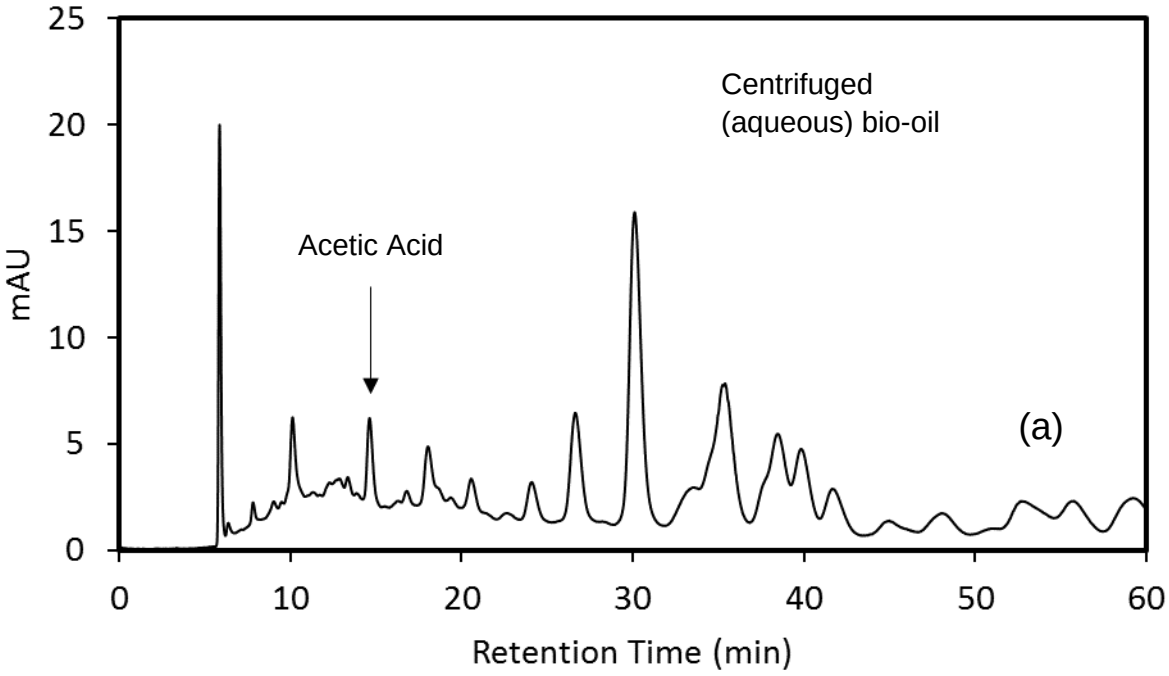


522

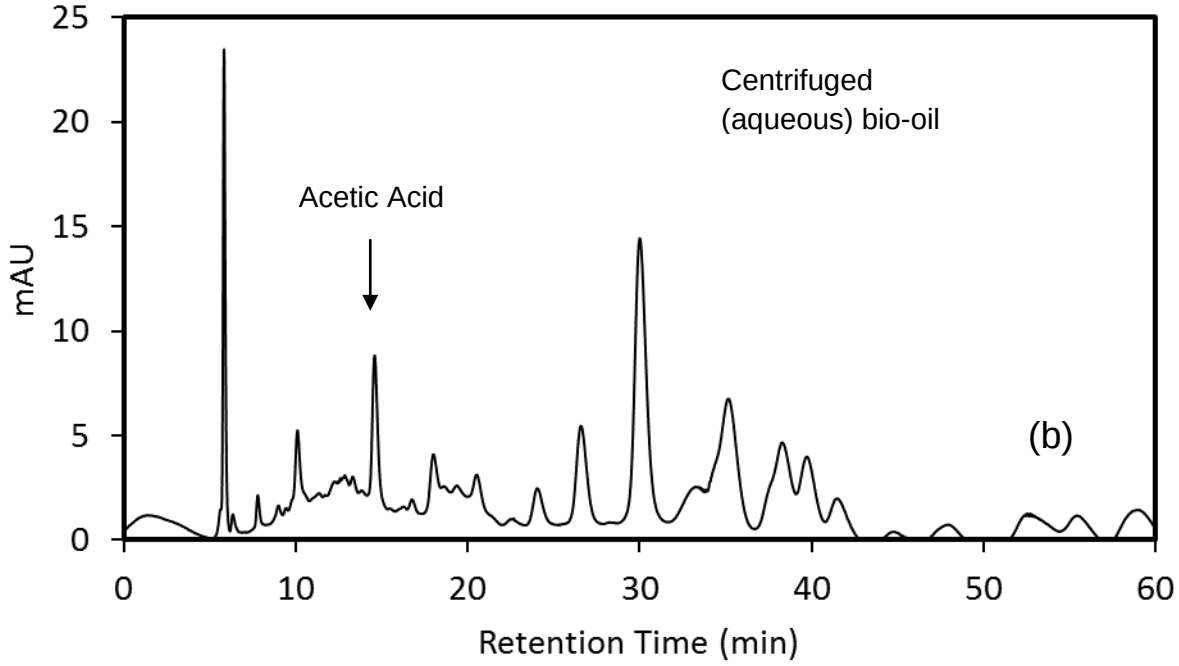
523 **Figure 6.** Measured TAN values of aqueous bio-oil (90% of the value) and aqueous bio-oil samples with
524 known amounts of acetic acid added, calculated TAN of aqueous bio-oil samples using the measured
525 TAN values of aqueous bio-oil (90%) and acetic acid standard solutions (2, 4, and 6 wt%) through
526 **Equation (7)** (broken line), and measured molar concentrations of acetic acid from HPLC analysis vs.
527 overall concentration of added acetic acid.
528

529 Calculated TAN = [(TAN of AqBO) × (wt% of AqBO) + (TAN of AA) × (wt% of AA)] / 100 **(7)**
530 AqBO: centrifuged or aqueous bio-oil, AA: acetic acid
531

Aqueous bio-oil samples were also analyzed by HPLC to quantify the acetic acid concentration. The molar concentrations of acetic acid in aqueous bio-oil samples were also included in **Figure 6**. Chromatographs from the HPLC analysis are found in **Figure 7**. As expected, the concentration of acetic acid detected by HPLC increased as more acetic acid was added to aqueous bio-oil samples. Using the known relationship between TAN and molar concentrations of acetic acid solutions **[Equation (5)]**, we converted the molar concentrations of acetic acid in aqueous bio-oil samples to the TAN values, represented as diamond markers in **Figure 6**. As shown in **Figure 6**, there is good agreement between measured and calculated TAN values for different amounts of acetic acid added. Also, the differences between TAN from total acids and TAN from acetic acid were consistent, as presented with the shaded area. It may be noteworthy to mention that the concentrations of the shaded area may be overestimated because some of these acidic components (e.g., vanillic acid) have a stronger effect on TAN values than monoprotic acids. The consistent difference between total acids and acetic acid indicates that acidic components, other than acetic acid, in aqueous bio-oil contributed similarly to the TAN value because no other acidic components were added besides acetic acid. From the recovery test results, it can be concluded that the presence of various chemicals, other than acetic acid, in bio-oil did not interfere the detection of additional acetic acid. Moreover, the TAN values of bio-oil samples are proportional to the amount of acetic acid present in bio-oil. In other words, the TAN values can reflect the amount of acetic acid—the major acidic component present in switchgrass bio-oil.


25
20
15
10
5
0
mAU
Centrifuged
(aqueous) bio-oil
Acetic Acid
(a)
0
10
20
30
40
50
60
Retention Time (min)

551


25
20
15
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5
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mAU
Centrifuged
(aqueous) bio-oil
Acetic Acid
(b)
0
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60
Retention Time (min)

552

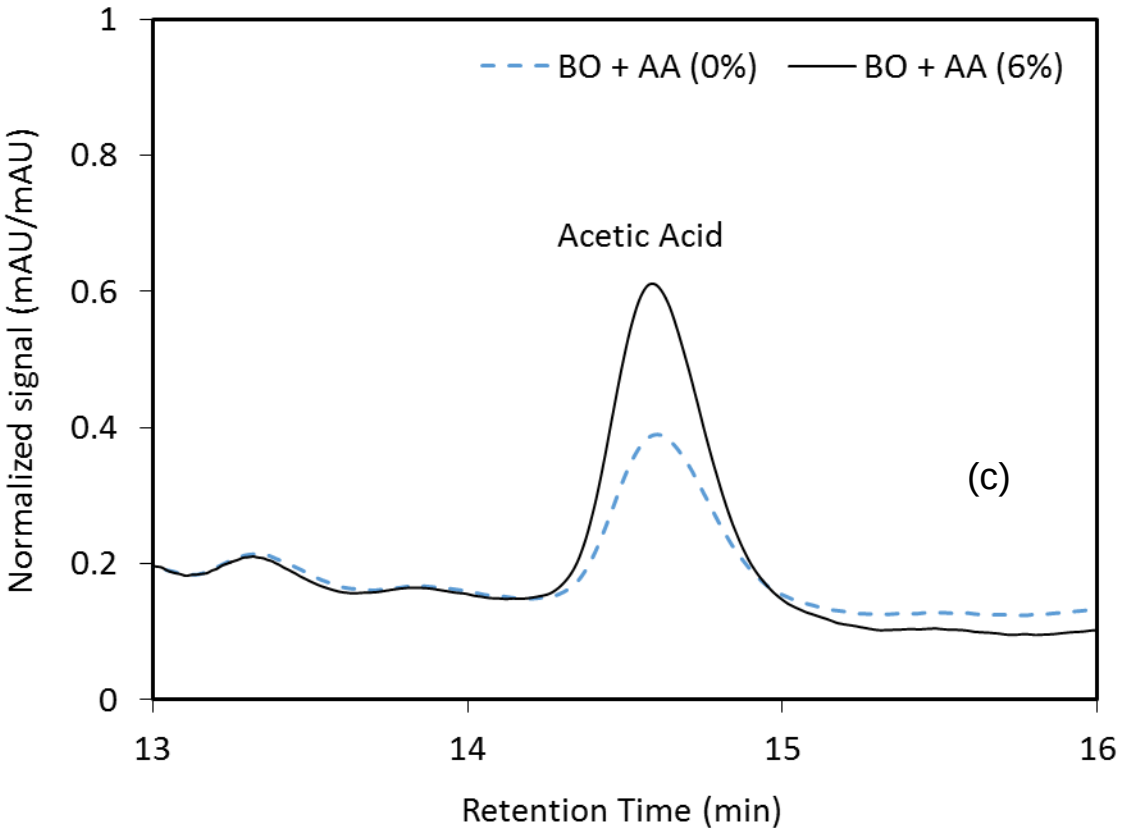
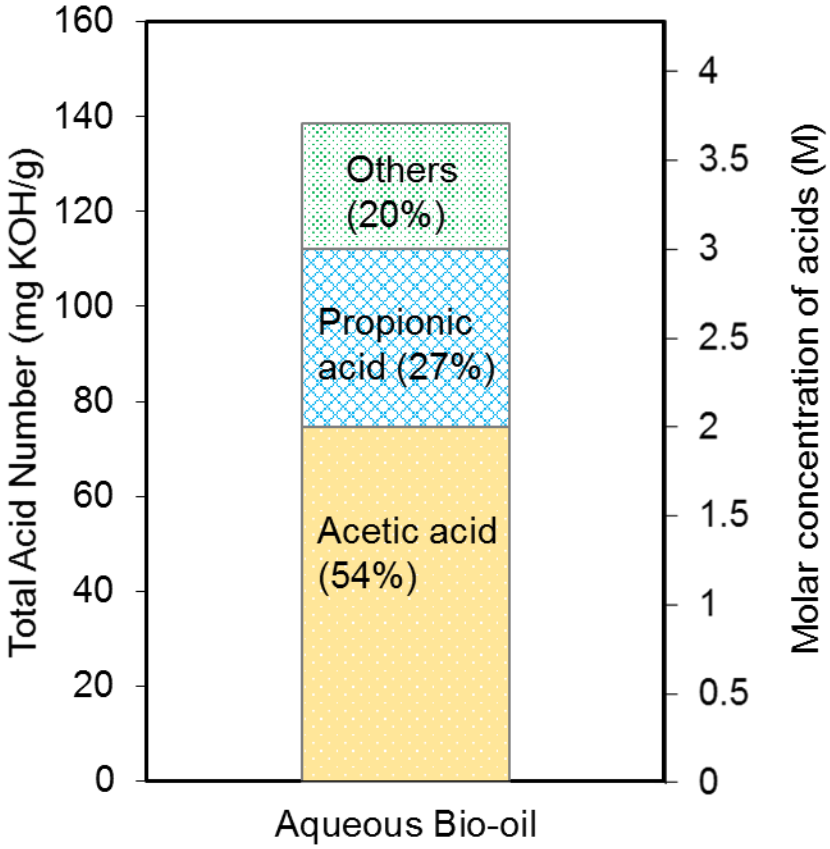


Figure 7. (a) HPLC chromatographs of aqueous bio-oil, (b) aqueous bio-oil with added acetic acid (overall 6 wt% in aqueous bio-oil), and (c) zoomed-in view for acetic acid peaks from aqueous bio-oil (dotted line) and aqueous bio-oil with added acetic acid (solid line).

Two major carboxylic acids—acetic and propionic acids—and other chemicals that contribute to the TAN value of aqueous bio-oil are shown in **Figure 8**. It was found that 54% of the TAN value of the aqueous bio-oil comes from the acetic acid. According to the chemical analysis of crude switchgrass bio-oil (prior to centrifugation), the propionic acid content in the switchgrass bio-oil is roughly half of the acetic acid content. Thus, assuming that the propionic acid content in the switchgrass bio-oil is half of the acetic acid content, 27% of the TAN value should be attributed to propionic acid. The remaining 20% of the TAN value is due to other chemicals, such as vanillic and syringic acids. The total molar concentration of other chemicals may be smaller than the concentration of propionic and acetic acids, however, as shown in

566 **Section 3.3** from the TAN analysis of standard acids, the effects of these chemicals on the TAN value
567 may be important, showing a fraction of 20% in **Figure 8.**
568



569

570 **Figure 8.** Carboxylic acids and other chemical components that contribute to the TAN value of the
571 aqueous bio-oil. The molar concentration equivalent to others (20%) is overestimated in the graph
572 because chemical species other than acetic and propionic acids (e.g., vanillic acid) have a stronger effect
573 on the TAN value than monoprotic carboxylic acids.
574

575 **4. Conclusions**

576 This study investigated how monoprotic and diprotic acid bio-oil components contribute to the overall
577 acidity of bio-oils. An accepted ASTM potentiometric method for KOH titration of a variety of standard
578 samples and switchgrass aqueous bio-oil has been employed to yield the TAN value of the samples. The

analyses were performed in triplicate using both aqueous and organic solvents. The bio-oil was separated into aqueous and organic fractions by centrifugation and analyzed accordingly to provide consistent measurements. An appropriate recovery analysis has also been performed.

A similar linear relationship was found between the TAN values vs. molar concentrations of acetic, propionic, and hydroxybenzoic acids, which act as monoprotic acids in the titration solvent. This result indicates that the TAN values can be converted to molar concentrations of total acids if a sample contains only these acids. For more complex organic acid molecules that act as polyprotic acids during the TAN analysis (e.g., vanillic and syringic acids), a higher slope of TAN values vs. molar concentrations was obtained. The higher slope indicates a stronger contribution to the TAN value than that of chemicals acting as monoprotic acids. The stronger effect on the TAN values from vanillic acid is interesting because vanillic acid is considered a weaker acid than acetic and propionic acids. Thus, the TAN analysis does not discriminate between weak and strong acids, since the respective protons are titrable. The TAN analysis is in general an acceptable method to determine the acidity of bio-oil; however, a comparison of TAN values of different types of bio-oil (produced from different sources of biomass or with different pyrolysis settings) should take into consideration the type and concentration of acidic components in each bio-oil. In other words, the standard TAN analysis method should be used with caution when we want to compare different bio-oils.

Different titration curves and TAN values found for aqueous systems, both in modeling and experiments, as expected, indicate that the pK_a values in the standard titration solvent are different from those in aqueous systems. These differences indicate different interactions and activities among chemical species analyzed and titration solvent molecules. In a recovery test, the TAN values of aqueous bio-oil samples increased proportionally to the amount of acetic acid added. Thus, the recovery test demonstrated that the TAN value is proportional to the acetic acid content in bio-oil samples, and the various chemicals present in AqBO do not interfere with the TAN analysis. This study demonstrates the usefulness of TAN analysis

605 in determining the acidity of bio-oil before and after treatment and helps us understand how strongly
606 different bio-oil components contribute to the TAN value and, therefore, to the acidity of a complex
607 chemical system like bio-oil.
608
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617
618 **References**
619 [1] M. Garcia-Perez, T.T. Adams, J.W. Goodrum, D.P. Geller, K. Das, Production and fuel properties of
620 pine chip bio-oil/biodiesel blends, Energy & Fuels, 21 (2007) 2363-2372.
621 [2] D. Chiaramonti, A. Oasmaa, Y. Solantausta, Power generation using fast pyrolysis liquids from
622 biomass, Renew Sust Energ Rev, 11 (2007) 1056-1086.
623 [3] A.V. Bridgwater, D. Meier, D. Radlein, An overview of fast pyrolysis of biomass, Organic
624 Geochemistry, 30 (1999) 1479-1493.
625 [4] A. Oasmaa, D.C. Elliott, J. Korhonen, Acidity of Biomass Fast Pyrolysis Bio-oils, Energy & Fuels, 24
626 (2010) 6548-6554.
627 [5] S. Ren, X.P. Ye, A.P. Borole, P. Kim, N. Labbé, Analysis of switchgrass-derived bio-oil and
628 associated aqueous phase generated in a semi-pilot scale auger pyrolyzer, Journal of Analytical and
629 Applied Pyrolysis, 119 (2016) 97-103.

[6] J.-J. Wang, J. Chang, J. Fan, Upgrading of bio-oil by catalytic esterification and determination of acid number for evaluating esterification degree, Energy & Fuels, 24 (2010) 3251-3255.

[7] L. Moens, S.K. Black, M.D. Myers, S. Czernik, Study of the neutralization and stabilization of a mixed hardwood bio-oil, Energy & Fuels, 23 (2009) 2695-2699.

[8] L.K.-E. Park, S. Ren, S. Yiacoumi, X.P. Ye, A.P. Borole, C. Tsouris, Separation of Switchgrass Bio-Oil by Water/Organic Solvent Addition and pH Adjustment, Energy & Fuels, 30 (2016) 2164-2173.

[9] A. Oasmaa, D. Meier, Norms and standards for fast pyrolysis liquids: 1. Round robin test, Journal of Analytical and Applied Pyrolysis, 73 (2005) 323-334.

[10] M.R. Rover, P.A. Johnston, B.P. Lamsal, R.C. Brown, Total water-soluble sugars quantification in bio-oil using the phenol–sulfuric acid assay, Journal of Analytical and Applied Pyrolysis, 104 (2013) 194-201.

[11] Ion Exchange Chromatography, Bio-Rad. http://www.bio-rad.com/en-us/applications-technologies/liquid-chromatography-principles/ion-exchange-chromatography.

[12] L. Wu, X. Hu, D. Mourant, Y. Wang, C. Kelly, M. Garcia-Perez, M. He, C.-Z. Li, Quantification of strong and weak acidities in bio-oil via non-aqueous potentiometric titration, Fuel, 115 (2014) 652-657.

[13] K.D. Altria, Overview of capillary electrophoresis and capillary electrochromatography, Journal of Chromatography A, 856 (1999) 443-463.

[14] J.A. Aricetti, M. Tubino, A green and simple visual method for the determination of the acid-number of biodiesel, Fuel, 95 (2012) 659-661.

[15] A. Baig, F.T. Ng, Determination of acid number of biodiesel and biodiesel blends, Journal of the American Oil Chemists' Society, 88 (2011) 243-253.

[16] A. Baig, M. Paszti, F.T.T. Ng, A simple and green analytical method for acid number analysis of biodiesel and biodiesel blends based on potentiometric technique, Fuel, 104 (2013) 426-432.

[17] F. Barbieri Gonzaga, S. Pereira Sobral, A new method for determining the acid number of biodiesel based on coulometric titration, Talanta, 97 (2012) 199-203.

655 [18] V. del Río, M.S. Larrechi, M.P. Callao, Sequential injection titration method using second-order
656 signals: Determination of acidity in plant oils and biodiesel samples, Talanta, 81 (2010) 1572-1577.
657 [19] M. Gonçalves, K. Cunha, S. Sobral, F. Gonzaga, I. Fraga, P. Borges, Acid Number Determination of
658 Biodiesel by Potentiometric Titration Using Different Methods, Biofuels, ASTM International, 2011.
659 [20] G. Knothe, Analyzing biodiesel: standards and other methods, Journal of the American Oil Chemists'
660 Society, 83 (2006) 823-833.
661 [21] K. Komers, F. Skopal, R. Stloukal, Determination of the Neutralization Number for Biodiesel Fuel
662 Production, Lipid / Fett, 99 (1997) 52-54.
663 [22] S. Mahajan, S.K. Konar, Determining the acid number of biodiesel, Journal of the American Oil
664 Chemists' Society, 83 (2006) 567-570.
665 [23] M. Tubino, J.A. Aricetti, A green method for determination of acid number of biodiesel, Journal of
666 the Brazilian Chemical Society, 22 (2011) 1073-1081.
667 [24] M. Tubino, J.A. Aricetti, A green potentiometric method for determination of the acid number of oils
668 and fats, Journal of the Brazilian Chemical Society, 24 (2013) 1691-1696.
669 [25] R. Kauffman, Rapid, portable voltammetric techniques for performing antioxidant, total acid number
670 (TAN) and total base number (TBN) measurements, Tribology & Lubrication Technology, 54 (1998) 39.
671 [26] B. Fuhr, B. Banjac, T. Blackmore, P. Rahimi, Applicability of Total Acid Number Analysis to Heavy
672 Oils and Bitumens, Energy & Fuels, 21 (2007) 1322-1324.
673 [27] D. Chen, J. Zhou, Q. Zhang, X. Zhu, Evaluation methods and research progresses in bio-oil storage
674 stability, Renewable and Sustainable Energy Reviews, 40 (2014) 69-79.
675 [28] ASTM D664. Standard Test Method for Acid Number of Petroleum Products by Potentiometric
676 Titration, American Society for Testing Materials (ASTM) International, West Conshohocken, PA, 2011.
677 [29] ASTM D974-14e2. Standard Test Method for Acid and Base Number by Color-Indicator Titration,
678 American Society for Testing Materials (ASTM) International, West Conshohocken, PA, 2014.

679 [30] ASTM D3339-12. Standard Test Method for Acid Number of Petroleum Products by Semi-Micro
680 Color Indicator Titration, American Society for Testing Materials (ASTM) International, West
681 Conshohocken, PA, 2012.
682 [31] AOCS Cd 3d-63. Acid Value, The American Oil Chemists' Society, Urbana, IL 2009.
683 [32] EN140104. Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of acid value
684 European Standard, 2003.
685 [33] The Theory of pH Measurement, Emerson Process Management, Irvine, CA, 2010, pp. 1-7.
686 [34] J. Shao, F. Agblevor, New Rapid Method for the Determination of Total Acid Number (Tan) of Bio-
687 Oils, American Journal of Biomass and Bioenergy, 4 (2015) 1-9.
688 [35] J. Wang, C. Xu, Q. Song, Rapid and Environmentally Friendly Three-Dimensional-Printed Flow
689 Injection Analysis System for the Determination of the Acid Number in Thermal Conductive Oil, Energy
690 & Fuels, 29 (2015) 1040-1044.
691 [36] P. Kim, A. Johnson, C.W. Edmunds, M. Radosevich, F. Vogt, T.G. Rials, N. Labbé, Surface
692 Functionality and Carbon Structures in Lignocellulosic-Derived Biochars Produced by Fast Pyrolysis,
693 Energy & Fuels, 25 (2011) 4693-4703.
694 [37] S. Ren, X.P. Ye, A.P. Borole, Separation of chemical groups from bio-oil water-extract via
695 sequential organic solvent extraction, Journal of Analytical and Applied Pyrolysis, 123 (2017) 30-39.
696 [38] ASTM D4377-00, Standard test method for water in crude oils by potentiometric Karl Fischer
697 titration, West Conshohocken, PA, 2011.
698 [39] Y. Zhang, F. Guo, Q. Cui, M. Lu, X. Song, H. Tang, Q. Li, Measurement and Correlation of the
699 Solubility of Vanillic Acid in Eight Pure and Water + Ethanol Mixed Solvents at Temperatures from
700 (293.15 to 323.15) K, Journal of Chemical & Engineering Data, 61 (2016) 420-429.
701 [40] H. Wang, H. Tang, J. Wilson, S.O. Salley, K.Y.S. Ng, Total Acid Number Determination of
702 Biodiesel and Biodiesel Blends, Journal of the American Oil Chemists' Society, 85 (2008) 1083-1086.
703 [41] Calculation, Chemicalize - Instant Cheminformatics Solutions, ChemAxon, Ltd, 2016.
704 http://www.chemicalize.org

[42] F. Liu, S. Sivoththaman, Z. Tan, Solvent extraction of 5-HMF from simulated hydrothermal conversion product, Sustainable Environment Research, 24 (2014).