FATE AND EFFECT OF NAPHTHENIC ACIDS ON THE BIOLOGICAL WASTEWATER TREATMENT PROCESSES IN OIL REFINERIES

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Final Report

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1. OBJECTIVES AND MAJOR ACCOMPLISHMENTS

The overall objective of the project was to assess the fate, effect, and biodegradation of naphthenic acids (NAs) on the biological wastewater treatment processes commonly used in oil refineries.

The specific objectives of the project were:

1) Assessment of the type and concentrations of NAs in ConocoPhillips refineries.
2) Identification of unit processes responsible for the release of NAs in the combined refinery wastewater.
3) Quantification of the extent of NAs removal by biological wastewater treatment units commonly used in oil refineries.
4) Quantitative assessment of the effect and biodegradation of NAs under different electron accepting conditions.
5) Development of (bio)-processes and strategies for the effective reduction of NAs in oil refineries effluents.

The following major accomplishments were achieved:

1) Developed a three-tier analytical methodology for the quantification and characterization of naphthenic acids.

2) Determined the NA concentration and congener distribution (i.e., MW, *Z* and *n* number) in crude oil, desalter brine and refinery wastewater from six refineries.

3) Assessed the fate and effect of naphthenic acids, as well as the overall toxicity in refinery unit processes and biological treatment systems in six refineries.

4) Established correlations between water quality and toxicity and naphthenic acid molecular descriptors (i.e., MW, *Z* and *n* number).

5) Determined the effect and biodegradation potential of NAs under different electron accepting conditions (aerobic respiration, nitrification, denitrification, fermentation/methanogenesis).

6) Conducted preliminary NA partitioning and sorption assays on biomass, granular activated carbon, and organoclays.

7) Developed a structure-activity relationship (SAR) methodology to model the toxicity, biodegradability and corrosivity of NA congeners.

2. MAJOR OUTCOMES

A summary of the major project outcomes is provided below along with mention of the corresponding quarterly reports (QR) where details have been reported before.

2.1 Development of NA Analytical Method (QR 1, 3, 4, and 5; Tezel et al., 2010)

A three-tier analytical methodology was developed to quantify total NAs, assess the NA distribution, and identify molecular structures of predominant NAs in aqueous solutions. The first step involves pair-ion extraction (PIX) using benzyl tributyl ammonium chloride (BTBA) as the ion-pairing agent. The second step involves quantification of total NAs by measuring the extracted amount of NA-BTBA pair with HPLC-UV/Vis and/or by quantifying the extracted NAs using the MS spectrum abundance relative to a surrogate standard (*p*-toluene sulfonate; *p*TS). The minimum detection limit of the PIX method is 0.5 mg/L, which is 2 to 40 times lower than the limit of conventional NA measurement methods. The method requires low sample volume and no sample pretreatment. A direct infusion electrospray ionization/mass spectrometry (ESI/MS) technique was developed and optimized to identify the NAs as well as their molecular formula and to determine their distribution (carbon number, n and hydrogen deficiency or cyclization, Z) in the NA extracts. In order to identify exact NA molecular structures, a third step involves the transformation of the NAs in the pair-ion extracts to their subsequent benzyl amides following a Vilsmeier reaction, which transforms NAs to NA chlorides selectively, and a nucleophilic substitution of chloride by benzyl amine. The structural identification of the resulting naphthene benzyl amides (NBAs) is performed by turbo spray ionization hybrid triple quadrupole linear ion trap mass spectrometry followed by interpretation of the fragmentation data. The methodology was initially tested on an aqueous solution of TCI NA salt, which was used as a model NA mixture, and then applied to quantify and characterize NAs in crude oil, desalter brine, and petroleum refining process wastewater samples.

2.2 Characterization of Refinery Samples

In the course of the two-year project, samples were received from six ConocoPhillips refineries as follows (Location; crude origin): Bayway (Linden, NJ; Sea), Billings (Billings, MT; Midcontinent)), Borger (Borger, TX; Midcontinent), Ferndale (Ferndale, WA; Alaskan), Sweeny (Old Ocean, TX; Venezuelan), and Wood River (Roxana, IL; Midcontinent). In addition, two Canadian blends were also included in the crude oil and desalter brine characterization.

Characterization of Crude Oil Samples (QR 6)

Crude oil characterization included total acid number (TAN), total ionizable constituents (TIC), total petroleum hydrocarbons (TPH), total naphthenic acids (TNA), and total extractable naphthenic acids (TENA). Based on the above parameters, strong correlations were obtained for TIC vs. TAN and TNA vs. TAN. The TIC values of crude oils tested ranged from 12 to 40 mg/g crude oil. The Venezuelan crude had the highest TIC and was followed by the Canadian, Midcontinent, Sea and Alaskan crudes. On the other hand, Sea crude (Bayway) has the lowest and Canadian crude (Canadian Blend-2) has the highest molecular weight ionizable constituents. The TNA of crude samples ranged between 900 to 4000 µg/g crude. The highest TNA value was measured for the crudes having the highest TAN values. The average MW of NAs in the crude samples ranged between 370 and 430 Da. The TNA is highly correlated with the TAN and represents 30 to 100% of the TAN. The TENA of crude oil samples ranged from 80 to 220 µg/g crude. The highest TENA was measured for the crudes having the highest TAN values. All

crudes are dominated with TENAs having Z = -2, -4 and -6 and n between 10 and 20 carbons. The average MW of TENAs ranged from 256 to 286 Da. The Bayway crude had the lowest MW TENAs whereas the Billings crude had the highest MW TENAs. The TENA represents only 2.5 to 25% of the TAN values and 7 to 20% of TNA; this ratio is higher for crudes having low MW NAs.

Characterization of Desalter Brine Samples (QR 6; Table 1)
The total NAs concentration in the desalter brine samples ranged from 4 to 45 mg/L. The desalter brine of the Canadian crude oils had the highest NA concentration followed by the brines of Midcontinent, Venezuelan, Sea and Alaskan crude oil. The NAs of Canadian crude oil brines had the highest average molecular weight, n and Z numbers, whereas Bayway and Ferndale refineries' desalter brines contained the lowest molecular weight NAs. The total chemical oxygen demand (COD) of desalter brine samples ranged between 400 and 5000 mg/L. The Bayway refinery desalter brine had the lowest and the Canadian crude oil desalter brines had the highest COD concentrations. NAs contribute 1 to 7 % of the total COD of the desalter brines which indicates that many other organic compounds are present in the desalter brine and NAs are a minor component. On average the liquid-phase NAs accounted for about 71% of the total NAs in the desalter brine samples analyzed. NAs with higher cyclization and lower carbon number are the predominant NAs in the brines aqueous phase.

Characterization of Waste Steams (QR 2, 3, 4; Table 2)
Wastewater samples taken from the influent and effluent steams of biological treatment units as well as corresponding mixed liquor samples from six ConocoPhillips refineries were analyzed for several water quality parameters as well as total and liquid-phase NAs. The influent total COD, soluble COD, total solids, volatile solids, total NAs, and liquid-phase NAs ranged from 467 to 1,117 mg/L, 26 to 966 mg/L, 1.05 to 11.95 g/L, 0.22 to 1.23 g/L, 4.5 to 16.6 mg/L, and 3.4 to 12.2 mg/L, respectively. The mixed liquor total COD, soluble COD, total solids, volatile solids, total NAs, and liquid-phase NAs ranged from 690 to 9,100 mg/L, 69 to 878 mg/L, 1.63 to 21.68 g/L, 0.38 to 6.20 g/L, 9.6 to 140.3 mg/L, and from 2.9 to 8.5 mg/L, respectively. The liquid-phase NAs in the mixed liquor of the biological units in the six refineries accounted for 3.2 to 30.2% of the total NAs (for the Sweeny and Borger refineries, respectively). Thus, a significant fraction of the mixed liquor NAs is associated with solids (biomass and powder activated carbon used in some refineries). The effluent total COD, soluble COD, total solids, volatile solids, total NAs, and liquid-phase NAs ranged from 114 to 847 mg/L, 40 to 831 mg/L, 1.39 to 12.75 g/L, 0.13 to 0.91 g/L, 2.8 to 11.6 mg/L, and from 2.9 to 9.5 mg/L, respectively. On a COD basis, calculated based on a mean NA theoretical oxygen demand of 2.82 mg O_2/mg NA, the effluent total NAs accounted for 2.4 to 15.5% of the total effluent COD and the effluent liquid-phase NAs accounted for 2.8 to 2.4% of the soluble COD. Thus, on a COD basis, NAs are a minor organic component of the effluents from the refinery biological treatment units.

2.3 NA Toxicity

Standard Acute Microtox® Toxicity (QR 6, 7)
The toxicity of the TCI NA mixture was measured by the standard acute Microtox® toxicity assay and the effective concentration which causes inhibition of 50% of *Vibrio fischeri* cells (EC_{50}) was between 8 and 10 mg NA/L (31 to 38 µM based on a mean MW of 261 g/mole). Thus, NAs are very toxic compared to other organic compounds. Attempts to fractionate the TCI

NA salts mixture were not successful. As a result, further delineation and correlation of NA Microtox® toxicity to NA molecular descriptors (i.e., MW, *Z* and *n* number, and structure) were not achieved.

The toxicity of desalter brine sample filtrates was measured by the standard acute Microtox® toxicity assay and the EC_{50} values are reported as sample strength (%) in Table 1 (Note: the lower the EC_{50} value, the higher the toxicity). The most toxic desalter brine sample was the one obtained from the Ferndale refinery followed by Sweeny, Wood River*, Bayway, Borger, Canadian-2, Wood River and Canadian-1. The EC_{50} values in terms of liquid-phase NA concentration ranged from 0.001 to 4.50 mg/L which are 2 to 10,000 times lower (more toxic) than the TCI NA salt (EC_{50} 8 to 10 mg NA/L). Thus, only a fraction of the desalter brine toxicity measured by the standard acute Microtox® toxicity assay is attributed to the liquid-phase NAs. With the exception of the two, laboratory preparations of Canadian desalter brine samples, the desalter brine EC_{50} Microtox® toxicity of other samples was well and positively correlated with the NA molecular weight, which indicates that desalter brines having lower molecular weight NAs are more toxic compared to the ones with higher molecular weight NAs. However, the broad range of EC_{50} values based on NA concentration as well as the significantly low contribution of NAs to the soluble COD of the desalter brines (see section 2.2 above) suggest that other desalter components contribute to the measured Microtox toxicity.

The toxicity of influent, mixed liquor, and effluent sample filtrates was measured by the standard acute Microtox® toxicity assay and the EC_{50} values are reported as sample strength (%) in Table 2 (Note: EC_{50} values higher than 100% were obtained by linear extrapolation and imply no toxicity). The EC_{50} values for influent, mixed liquor and effluent samples ranged from 5.9 to 160%, 16.2 to 271%, and from >100 to 540%, respectively. The most toxic influent samples were those from the Sweeny, Billings, and Bayway refineries, whereas the influent samples from the remaining refineries were either not toxic (Borger and Wood River) or slightly toxic (Ferndale). It is noteworthy that a significant toxicity reduction is achieved by the biological treatment units in all six refineries tested and all filtered effluent samples had EC_{50} values above 100%, implying no toxicity.

Culture-based Physiological Tests (QR 4, 5, 6, 7)
A comparison of the toxicity data obtained with the TCI NA mixture and refinery wastewater samples using the Microtox® assay and the microbial activity of an aerobic culture via oxygen uptake rate measurements showed that the Microtox® assay is much more sensitive leading to lower EC_{50} values. As a result of this observation, four types of bioassays were conducted in order to assess the potential inhibitory effect of NAs to biological processes of relevance to refinery biological treatment systems as summarized below.

Aerobic Respiration: A protocol, referred to as *micro dilution susceptibility testing* was developed to assess the inhibitory effect and biotransformation potential of surrogate NAs (TCI NA salts) by the native refinery activated sludge units (ASU) microbial communities. Nine ASU mixed liquor samples obtained from eight ConocoPhillips refineries were tested. The toxicity testing involved pre-growth of each microbial community in a rich nutrient broth and then incubation with the same nutrient broth and a range of NA concentrations from 0.5 to 500 mg/L along with a culture series without any NA addition (control). After incubation at 24°C

overnight, growth was quantified by absorbance measurements. None of the ASU heterotrophic microcosms was completely inhibited by the NAs up to 500 mg/L. A slight inhibition (20 to 25%) was observed in all the microcosms at and above 100 mg/L. Sweeny and Billings microcosms were affected by NAs more than the other ones at and above 100 mg/L (50 to 65%). In the absence of the rich nutrient broth, all refinery microcosms, except Rodeo and Bayway, achieved significant growth on NAs. However, at higher NA concentrations at which partial inhibition of heterotrophic growth was observed (at about 125 mg NA/L), growth on NAs also ceased. In conclusion, the biological activity in the refinery ASU samples would be sustained even at very high NA concentrations when a readily biodegradable substrate is present. Most of the ASU mixed liquors tested in this study are capable of NA degradation.

Nitrification: A mixed aerobic culture, which was not previously exposed to NAs, was used to conduct a batch nitrification test at initial NA concentrations of 0 (control), 20, 40, 80, 200, and 400 mg/L without and within a readily degradable substrate (dextrin). In the absence of dextrin, nitrification was not significantly inhibited by NAs up to an initial NA concentration of 80 mg/L. However, at an initial NA concentration of 200 and 400 mg/L, the nitrification rate decreased by 31 and 46%, respectively, compared to the nitrification rate of the control culture (without NA or dextrin). When dextrin was provided as an additional carbon source, a higher degree of nitrification inhibition by NAs was observed, starting from an initial NA concentration of 40 mg/L. Compared to the nitrification rate of the control culture (without NA, dextrin-amended), the nitrification rate was reduced by 22, 42, 57, and 69% for the cultures with initial NA concentration of 40, 80, 200, and 400 mg/L, respectively. The lower nitrification inhibition in the absence of dextrin is attributed to the higher extent of NA degradation by heterotrophic bacteria compared to the dextrin-amended culture series. Therefore, when NAs were present together with a readily degradable substrate (dextrin), the degradation of NAs was slower maintaining relatively higher NA concentrations for a longer duration, which in turn resulted in a higher degree of nitrification inhibition.

A similar batch nitrification test was conducted with an enriched nitrifying culture in the absence of a readily degradable organic substrate and at initial NA concentrations of 0 (control), 20, 40, 80, 200, and 400 mg/L. The nitrification activity by the enriched nitrifying culture was not significantly affected by NAs until an initial concentration of 400 mg NA/L, in which case the ammonia removal rate was reduced by 45% compared to the control (zero NAs) culture. As the fraction of heterotrophic bacteria in the enriched culture was negligible and the nitrosofying (ammonia oxidizing bacteria; AOB) and nitrifying (nitrite oxidizing bacteria; NOB) autotrophs do not degrade any organic matter, degradation of NAs was not observed during the test period. In contrast to the results obtained with the above-described aerobic mixed heterotrophic/autotrophic culture, in which a higher nitrification inhibition was observed even at lower residual NA concentrations, the nitrification activity was not significantly impacted in the enriched nitrifying culture series amended with 20 to 200 mg/L of NA. One possible reason for the difference in nitrification susceptibility to NAs is the higher population size of nitrosofying and nitrifying autotrophs in the enriched culture. The ratio of NA to AOB and NOB biomass was lower in the test conducted with the enriched culture compared to that conducted with the mixed aerobic culture. Thus, nitrification inhibition data must be normalized to the nitrifiers population size.

Nitrate Reduction: A batch assay was conducted to assess the potential inhibitory effect of NAs on nitrate reduction and the biotransformation potential of NAs (TCI NA salts) in a mixed culture fed only with dextrin and peptone (D/P) and with no previous exposure to NAs. Culture series containing NAs at concentrations of 0 (control), 20, 40, 80, 200, and 400 mg NA/L and dextrin/peptone mixture, which served as an external electron and carbon source (COD:N 6:1), were set up and incubated at room temperature (22 to 23^{o}C). The rate of nitrate reduction and nitrogen gas production were the same in all culture series and even slightly increased in culture series with initial NA concentrations of 200 and 400 mg/L, indicating that NAs are not inhibitory to denitrification. Based on initial and final NA concentrations in all culture series, it was concluded that that NAs were only slightly degraded under nitrate reducing conditions as used in this study. The overall NA congener distribution did not change in all culture series, further indicating that NAs were not degraded or transformed to a large extent.

Fermentation and Methanogenesis: A batch assay was performed to investigate the potential inhibitory effect and biotransformation potential of NAs (TCI NA salts) on a mixed, mesophilic (35^{o}C) methanogenic culture. For the NA inhibition test, a culture series was amended with a dextrin/peptone solution (D/P), which served as carbon/energy source and NAs at initial total concentrations of 0 (control), 20, 40, 80, 200 and 400 mg/L. Based on the extent of methane produced over the 60-day incubation and the levels of volatile fatty acids measured at the end of the incubation, NAs completely inhibited the methanogens at and above 80 mg/L. However, acidogens were not inhibited at all NA concentrations tested as indicated by the comparable carbon dioxide production in the NA-amended cultures and the control culture (without any NAs). Therefore, methanogenesis was more susceptible to NA inhibition than acidogenesis. Another culture series with the same initial NA concentrations was set up without any additional carbon source. Methane and carbon dioxide was measured at and below 40 mg/L initial NAs. Based on COD balances as well as measurement of NAs at the end of the incubation, it appears that a fraction of NAs was degraded and transformed to methane at and below 40 mg NA/L both in the presence and absence of D/P. However, for the conclusive confirmation of NA degradation and conversion to methane, further assessment is necessary.

2.4 Biodegradation and Fate of NAs under Aerobic Conditions

Assessment of the Aerobic Biodegradation of NAs (QR 7)

The biodegradation potential of NAs was assessed using two cultures, Sweeny NA-enriched and Sweeny NA-unacclimated, maintained fed-batch with media and an initial NA concentration of 200 mg/L and media plus synthetic wastewater (a mixture of organic substrates typically found in refinery wastewater), respectively. The details for the development and maintenance of these cultures were previously reported (QR 4, 5). For each culture type, two culture series were setup with and without addition of synthetic wastewater at an initial concentration of 700 mg COD/L. All four culture series received NAs (TCI NA salts) at an initial concentration of 200 mg NA/L (700 mg COD/L) and culture media. Incubation was carried out at room temperature (22 to 24^{o}C). The total NA concentration in both Sweeny NA-enriched culture series (with and without synthetic wastewater amendment) decreased from 200 to 50 mg/L within 3 days and remained stable for the rest of the 16-day incubation period. NA degradation was not observed in the two Sweeny NA-unacclimated culture series until day 7 when the culture without synthetic wastewater amendment began degrading NAs and within 3 days reduced the total NA concentration from 200 to 60 mg/L. In contrast, NA degradation was not observed throughout

the 16-day incubation period in the Sweeny NA-unacclimated culture series initially amended with synthetic wastewater. These results indicate that when a readily degradable carbon source is available, an NA unacclimated microbial population does not degrade NAs.

Relative to the NA congener distribution, initially monocyclic (Z = -2) and dicyclic (Z = -4) NAs were the most dominant NAs and were the two NA fractions degraded the most. As a result of degradation, an increase in the weighted average MW, Z, and carbon number (n) from 275 to 350, -4 to -5.5, and from 18 to 23 was observed, respectively. At the end of the incubation period, the concentration of all acyclic and cyclic NAs in all three cultures which degraded NAs was the same, around 10 mg/L. This observation also supports the hypothesis that the residual NAs are not inherently recalcitrant, but are below a concentration threshold, below which they are not degraded and thus, they are persistent. Microtox® acute toxicity measurements with initial and day 16 samples from each culture series, which achieved a similar extent of NA degradation, resulted in a 12- to 16-fold toxicity decrease.

Desorption and Degradation of NAs from Refinery ASU Mixed Liquors (QR 7)
Mixed Liquor NA Desorption: In order to assess NA desorption from two chronically-loaded refinery ASU mixed liquors, without and with powder activated carbon (PAC), a batch desorption assay, conducted in five successive desorption steps, was performed using Wood River and Sweeny (containing PAC) activated sludge mixed liquors. The details of this test were reported previously (QR 7). The initial total/liquid-phase NA concentrations in the Wood River and Sweeny mixed liquors were 82.6/3.6 mg NA/L and 83.4/2.4 mg NA/L, respectively. Relative to the NA congener distribution, acyclic and monocyclic NAs with carbon number 15 to 20 were predominant in the Sweeny mixed liquor, whereas dicyclic and tricyclic NAs with carbon number between 20 and 30 were predominant in the Wood River mixed liquor. Both mixed liquors exhibited poor desorption with greater than 80% of NAs remaining on the solid-phase at the end of the 10-day desorption period. The desorption kinetics were very similar for both sludge samples, indicating that desorption was limited in all mixed liquors, regardless of the presence or absence of PAC. The results of this assay indicate that the majority of the NAs in refinery activated sludge systems are associated with the solid-phase (greater than 80%). Thus, chronic exposure of biomass (and PAC) to NAs leads to their accumulation and perhaps persistence (bioavailability issue; see below).

Mixed Liquor NA Degradation: In order to determine if chronically-adsorbed NAs to the ASU biomass and PAC are bioavailable and thus can be degraded, an aerobic batch assay was performed to investigate the effect of microbial activity on the extent of NA degradation in two chronically NA-loaded mixed liquors, without and with PAC (Wood River and Sweeny and ASU mixed liquors, respectively). After the two mixed liquors were aerated and showed very low biological activity (measured via oxygen uptake), indicating a very low level of available biodegradable substrates, the two mixed liquors were incubated at room temperature (22 to 24^{o}C) with continuous aeration and fed with a synthetic wastewater four times over the 90-day incubation period. Similar to the results of the above-discussed desorption test, the NA liquid-phase concentrations were below 10 mg/L throughout the incubation period and their average MW was lower by 60 to 100 Da compared to the MW of the solid-phase NAs. In the Wood River mixed liquor, the total NA concentration decreased by 30% within the first 15 days of incubation and by 61% by the end of the 90-day incubation. In contrast, in the Sweeny mixed

liquor, the total NA concentration showed a slow and gradual decline reaching an extent of NA degradation of 33%. The bi-phasic NA degradation pattern in the Wood River mixed liquor is assumed to be due to NA fractions with a variable degree of bioavailability. On the other hand, the presence of PAC in the Sweeny mixed liquor, with its superior NA adsorption capacity compared to biomass, resulted in a much lower NA bioavailability. In view of the negative impact of degradable organic substrates may have on the NA degradation as discussed above, long starvation periods between the four feedings were maintained. However, an increase in the rate of NA degradation during the starvation periods was not observed. A comparison of the NA congener distribution in both mixed liquors initially and at the end of the incubation showed an increase in the average MW, *Z*, and carbon number, again indicating that the lower MW NA fraction was degraded.

3. MAJOR CONCLUSIONS

NA Occurrence

- Desalter brine is the major source of NAs in refineries wastewater.
- The wastewater NA concentration gradually decreases moving towards the refinery outfall.
- NAs concentrations in refinery ASU effluents are relatively low, though occasionally effluent NAs concentrations are higher than influent NAs concentrations (possible in-situ NA formation).

NA Characterization

- The NA concentration and congener distribution is related to the origin of the crude oil (Canadian ~ Venezuelan > Midcontinent > Alaskan > Sea).
- The NA molecular weight increases (i.e., both the carbon number and hydrogen deficiency increase) moving towards the refinery effluent.

NA Toxicity & Inhibition

- NAs are toxic; the TCI NA mixture Microtox® acute toxicity EC_{50} value is between 8 and 10 mg NA/L (31 to 38 µM).
- Wastewater related to refinery biological treatment systems (i.e., influent, mixed liquor, and effluent) had Microtox® acute toxicity EC_{50} values in the range of 6 to 218% (0.4 to 10.5 mg NA/L based on measured NA concentrations).
- Wastewater components other than NAs also contribute to the measured Microtox® acute toxicity.
- A low degree of NA inhibition was observed at and above 200, 80, 200, and 40 mg NA/L under aerobic respiration, nitrification, denitrification, and methanogenic conditions, respectively.
- Toxicity of bioprocesses related to the refinery ASU operation is low at observed wastewater NA concentrations.

NA Biodegradation & Fate

- Aerobic NA degradation was confirmed; competition with other organics may hinder NA degradation.
- A relatively high NA threshold (residual) concentration exists for the aerobic degradation of NAs.
- Biomass (and PAC)-bound NAs are persistent (bioavailability issue).

Overall Conclusions

- Although NAs contribute to less than 10% of refinery wastewater COD, their effect is significant in terms of toxicity and persistence in refinery wastewater and effluents.
- Overall, the ASU are effective in lowering the effluent COD and NA concentrations, especially in those refineries which use relatively low NA content crude oils.
- Development of mitigation technologies is warranted only if treated effluent is reused as process water and/or if use of high NA-content crude oils is expanded.

4. DISSEMINATION OF PROJECT RESULTS

Manuscripts (in preparation)

Tezel, U., T. Misiti, D. Bostwick, C. Sullards and S. G. Pavlostathis. Three-tier methodology for the quantification and characterization of naphthenic acids in aqueous solutions (to be submitted to *Analytical Chemistry*).

Misiti, T., U. Tezel, M. Tandukar and S. G. Pavlostathis. Aerobic biodegradation of naphthenic acids (to be submitted to *Water Research*).

Tandukar, M., T. Misiti, U. Tezel and S. G. Pavlostathis. Inhibitory effect of naphthenic acids on mixed nitrifying cultures (to be submitted to *Water Research*).

Misiti, T., U. Tezel, M. Tandukar and S. G. Pavlostathis. Fate and effect of naphthenic acids in a mixed methanogenic culture (to be submitted to *Water Research*).

Oral Presentations

Tezel, U., T. Misiti, D. Bostwick, C. Sullards, and S. G. Pavlostathis. Three-tier Methodology for Quantification and Characterization of Naphthenic Acids in Aqueous Solutions. ACS 240th National Meeting, Division of Fuel Chemistry, Boston, MA, August 2010.

Poster Presentations

Misiti, T., Tezel, U. and S. G. Pavlostathis. "Biotransformation potential of naphthenic acids under different electron accepting conditions", Annual AEES Symposium, Georgia Institute of Technology, Atlanta, GA April 2010 (Best Poster Award).

Tezel, U., T. Misiti, D. Bostwick, C. Sullards and S. G. Pavlostathis. "Three-tier methodology for quantification and characterization of naphthenic acids in aqueous solutions", ACS 240th National Meeting, Division of Fuel Chemistry, Science Mix. Boston, MA, August 2010.

Table 1. Characteristics of desalter brine samples collected from ConocoPhillips refineries.

Refinery	pH	Total NAs mg/L	Liquid NAs mg/L	Total COD mg/L	Soluble COD mg/L	EC_{50} Value % (v)	TS g/L	VS g/L	Chloride mg/L	Sulfate mg/L	Ammonia mg-N/L
Sweeny	7.40	17.0±0.6[c]	10.3±0.4	1160±66	1153±7	2.6 [2.5-2.9]	2.8±0.1	0.88±0.1	549	5	2.2
Wood River	8.52	37.0±4.1	28.2±0.6	1530±117	1224±19	9.5 [8.8-10.4]	2.40±0.03	0.67±0.03	655	53	20.2
Bayway	6.32	9.6±0.3	7.7±0.2	415±47	391±59	4.8 [4.5-5.1]	0.77±0.03	0.27±0.03	316	31	ND
Borger	7.66	28.0±0.7	21.4±0.8	1329±98	1311±113	6.7 [6.2-7.2]	8.37±0.09	1.56±0.02	3683	171	ND
Billings	8.38	40.4±5.3	22.6±1.0	1942±77	1805±59	12.8 [10.4-15.7]	2.57±0.04	0.65±0.01	631	26	ND
Ferndale	5.24	4.2±0.3	3.6±0.3	1306±22	951±28	0.03 [0.006-0.13]	0.37±0.06	0.22±0.05	107	8	ND
Wood River[a]	6.80	17.3±0.4	14.3±0.0	1120±29	1100±23	4.4 [4.0-4.7]	3.50±0.04	0.59±0.01	1716	66	50.5
Canadian-1[b]	7.85	43.0±3.6	36.9±1.7	5351±2	4467±46	12.2 [10.5-14.2]	4.2±0.06	2.0±0.02	964	43	ND
Canadian-2[b]	8.10	45.8±3.3	26.9±0.8	3291±46	1088±80	8.0 [7.4-8.7]	2±0.02	1.2±0.02	335	19	ND
Mean	7.36	26.9	19.1	1938	1499	5.7	3.0	0.9	995	47	8.1
Low	5.24	4.2	3.6	415	391	0.03	0.4	0.2	107	5.0	0
High	8.52	45.8	36.9	5351	4467	12.2	8.4	2.0	3683	171	50.5

[a] Repeat sample; [b] Laboratory prepared samples; [c] Mean ± std. dev. (n = 3); [d] ND, not detected
Note: nitrate, nitrite, and phosphate were not detected in any of the desalter brine samples

Table 2. Characteristics of ASU influent, mixed liquor and effluent samples collected from ConocoPhillips refineries.

Refinery & Sample	pH	Total NA mg/L	Liquid NA mg/L	Total COD mg/L	Soluble COD mg/L	EC_{50} Value % (v)	TS g/L	VS g/L	Chloride mg/L	Nitrate mg/L	Sulfate mg/L	Phosphate mg/L	Ammonia mg-N/L
Wood River													
Influent	6.86	12.5±0.8[a]	6.2±0.6	474±44	386±33	160	1.89±0.15	0.22±0.19	278	403	11	1.3	1.4
Mixed Liquor	7.55	125±9.0	7.4±1.1	3438±44	393±43	180	5.26±0.11	1.75±0.04	179	1253	9	ND	2.8
Effluent	7.81	10.6±1.7	6.1±0.4	193±18	207±72	182	3.36±0.06	0.29±0.02	185	1314	7	ND	ND
Sweeny													
Influent	6.89	9.0±0.1	6.1±0.2	467±34	211±68	5.9	1.05±0.04	0.22±0.02	318	440	ND	ND	16.8
Mixed Liquor	6.41	140.3±0.5	4.5±0.2	9100±252	338±73	16.2	8.30±1.5	6.20±1.4	550	258	ND	13.4	16.8
Effluent	7.33	5.3±0.5	4.8±0.2	341±48	278±51	218	1.51±0.04	0.15±0.01	401	335	ND	ND	ND
Bayway													
Influent	7.39	13.0±1.1	10.2±1.7	1117±61	966±109	13.6	11.95±0.10	1.23±0.05	4269	ND	ND	42.4	28
Mixed Liquor	7.45	91.6±3.4	8.5±0.8	7782±307	878±62	>100[c]	21.68±0.13	5.00±0.07	4277	ND	24	11.2	4.2
Effluent	1.99	11.6±0.6	9.5±0.5	847±138	831±42	>100	12.75±0.05	0.91±0.04	4198	ND	39	34.6	ND
Borger													
Influent	8.13	4.5±0.5	3.4±0.5	467±41	410±150	143	1.34±0.02	0.22±0.00	222	254	3	0.8	ND
Mixed Liquor	7.64	9.6±0.8	2.9±0.5	690±68	435±28	67.6	1.63±0.03	0.38±0.04	228	264	9	1.4	ND
Effluent	8.00	3.9±1.4	2.9±0.6	452±75	297±13	>100	1.39±0.04	0.25±0.07	197	289	17	1	ND
Ferndale													
Influent	7.58	5.6±0.2	3.4±0.2	691±12	26±45	82.1	4.91±0.01	0.50±0.02	220	221	ND	ND	ND
Mixed Liquor	7.66	14.2±1.6	3.0±0.3	2254±61	69±41	271	6.76±0.03	1.69±0.03	189	2126	12	0.5	2.8
Effluent	7.72	2.8±0.4	2.9±0.3	114±53	40±44	540	4.62±0.03	0.13±0.02	199	215	27	ND	ND
Billings													
Influent	8.31	16.6±0.4	12.2±0.9	1008±31	747±42	10	1.77±0.00	0.29±0.01	266	324	ND	2.6	22.4
Mixed Liquor	7.22	43.0±5.0	6.4±0.3	4584±42	139±113	48.5	6.06±0.17	3.78±0.16	231	522	ND	ND	30.8
Effluent	7.33	6.5±1.0	7.0±0.5	273±58	251±70	107	1.79±0.02	0.16±0.02	212	528	ND	1.4	ND
Influent													
Mean	7.53	10.2	6.9	704	457.7	69.1	3.82	0.45	928.8	273.7	2.3	7.9	11.4
Low	6.86	4.5	3.4	467	26	5.9	1.05	0.22	220	ND	ND	ND	ND
High	8.31	16.6	12.2	1117	966	160	11.95	1.23	4269	440	11.0	42.4	28.0
Mixed Liquor													
Mean	7.32	70.6	5.5	4641.3	375.3	116.7	8.28	3.13	942.3	747.2	9.0	4.4	9.6
Low	6.41	9.6	2.9	690	69	16.2	1.63	0.38	179	ND	ND	ND	ND
High	7.66	140.3	8.5	9100	878	271	21.68	6.20	4277	2126	24.0	13.4	30.8
Effluent													
Mean	6.70	6.8	5.5	370	317	262	4.24	0.32	899	446.8	15.0	6.2	ND
Low	1.99	2.8	2.9	114	40	107	1.39	0.13	185	ND	ND	ND	ND
High	8.00	11.6	9.5	847	831	540	12.75	0.91	4198	1314	39.0	34.6	ND

[a] Mean ± std. dev. (n = 3); [b] ND, not detected; [c] Higher than 100%, but not quantifiable by extrapolation.