Naphthenic Acids in Athabasca Oil Sands Tailings Waters Are Less Biodegradable than Commercial Naphthenic Acids

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Naphthenic acids (NAs) are natural constituents in many petroleum sources, including bitumen in the oil sands of Northern Alberta, Canada. Bitumen extraction processes produce tailings waters that cannot be discharged to the environment because NAs are acutely toxic to aquatic species. However, aerobic biodegradation reduces the toxic character of NAs. In this study, four commercial NAs and the NAs in two oil sands tailings waters were characterized by gas chromatography—mass spectrometry. These NAs were also incubated with microorganisms in the tailings waters under aerobic, laboratory conditions. The NAs in the commercial preparations had lower molecular masses than the NAs in the tailings waters. The commercial NAs were biodegraded within 14 days, but only about 25% of the NAs native to the tailings waters were removed after 40–49 days. These results show that low molecular mass NAs (C ≤ 17) are more readily biodegraded than high molecular mass NAs (C ≥ 18). Moreover, the results indicate that biodegradation studies using commercial NAs alone will not accurately reflect the potential biodegradability of NAs in the oil sands tailings waters.

Introduction

Naphthenic acids (NAs) are complex mixtures of predominately alkyl-substituted cycloaliphatic carboxylic acids (containing cyclopentane and cyclohexane rings) and small amounts of acyclic acids (1). They are described by the general chemical formula CₙH₂ₙ₊₂O₂, where n indicates the carbon number and Z is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation. Although simple saturated fatty acids found in biological membranes fit this formula for Z = 0, these acids are very susceptible to biodegradation, so they would not persist as NAs. Some possible NAs structures are given elsewhere (2).

NAs occur naturally in a variety of petroleum oils (1, 3–10) and are thought to have originated from aerobic microbial degradation of petroleum hydrocarbons (11–13). NAs are also found in Athabasca oil sands ores (14, 15). Commercial NA preparations, obtained via extraction of petroleum distillates (1, 16), are used as textile and wood preservatives, emulsifiers, surfactants, paint driers, and adhesion promoters in the manufacture of tires (1).

The complexity of NA mixtures provides a major challenge in the development of suitable analytical methods for them. Separation and identification of individual compounds have not been achieved, and most studies refer to NAs as a group. Accepted quantification methods include Fourier transform infrared (FTIR) spectroscopy (17–19) and high-performance liquid chromatography (HPLC) (19, 20). Characterization of NAs by mass spectrometry (MS) (5–7, 21–24) and gas chromatography—electron impact mass spectrometry (GC-MS) (18, 25, 26) can provide qualitative data useful for comparing NAs from different sources. The total ion chromatogram (TIC) of a NA preparation is an unresolved “hump” (25, 27). Holowenko et al. (26) presented GC-MS data by plotting relative ion intensities as a function of n and Z values. In this case, only ions having mass-to-charge ratios consistent with plausible NA structures were included. The resulting three-dimensional bar graphs illustrate the distribution of compounds in a particular NA mixture.

Syncrude Canada Ltd. and Suncor Energy Inc. (Fort McMurray, Alberta, Canada) employ a caustic hot water extraction method for the separation of bitumen from oil sands ore (16). During this process, the release of NAs from the bitumen into the aqueous phase is enhanced (16, 28). The resulting process water is transported to on-site ponds where tailings water (TW) is retained and a portion of the released waters is recycled back to the plant (16, 29). Storage of process-affected waters is part of the “zero discharge” policy specified in the licenses of operating companies. Currently, there is more than 600 × 10⁶ m³ of process-affected waters stored at Syncrude’s Mildred Lake site.

NAs are acutely toxic to a range of organisms (30, 31). MacKinnon and Boerger (28) demonstrated that with chemical and microbiological treatment approaches, the toxicity of TW could be reduced, presumably by removal or biodegradation of NAs, although this was not shown directly. Herman et al. (32) followed biodegradation of NAs extracted from Mildred Lake settling basin (Syncrude) in laboratory cultures and also observed detoxification, as determined by the Microtox method. Clemente et al. (2) used enrichments of NA-degrading microorganisms to biodegrade commercially available NAs (Kodak Salts and Merichem). Microtox analyses of culture supernatants revealed a reduction in toxicity after less than 4 weeks of incubation (2).

On the basis of the findings of previous studies, it was hypothesized that NAs in the oil sands TW would be readily biodegraded. However, repeated attempts to extensively biodegrade NAs from Syncrude, Suncor, and Albion Sands Energy Inc. were unsuccessful using laboratory cultures of TW bacteria (Clemente, Scott, Fedorak, unpublished results).

Comparison of three-dimensional plots from GC-MS analyses of some commercial NAs (2, 27) with those of NAs in oil sands TW (26) clearly shows differences in the relative distributions of high and low molecular mass acids; commercial NAs tend to have n from about 7 to 17, whereas NAs in TW have a broader range of n from about 7 to 28. There are also differences in the distribution of Z families within a group of acids sharing the same n. These dissimilarities may account for the differing biodegradation rates, and have prompted the current study in which four commercial NAs preparations were individually added to TW from two tailings ponds in order to follow the biodegradation pattern resulting from the activities of microbial communities indigenous to these TWs. Biodegradation was monitored by measuring the...
decrease in NA concentrations by HPLC and by following changes in the NA profile of each mixture using GC-MS.

**Experimental Section**

**Naphthenic Acids.** Kodak naphthenic acids ("Kodak acids") (lot 11575A) and Kodak naphthenic acids sodium salt ("Kodak salts") (lot B14C) were purchased from The Eastman Kodak Company (Rochester, NY). The sodium content of the salt preparation was 9 wt % (20). Merichem naphthenic acids ("Merichem acids") were provided by Merichem Chemicals and Refinery Services LLC (Houston, TX). Fluka naphthenic acids ("Fluka acids") were obtained from Fluka Chemie (Buchs, Switzerland). Total acid number (TAN) for each commercial preparation was determined at the National Centre for Upgrading Technology (Devon, Alberta, Canada) according to American Standard Test Method D664 (33). The Kodak salts were converted to their acid form prior to submission for TAN analysis.

**Tailings Waters.** Samples of TW from active settling basins were provided by Syncrude Canada Ltd. and Suncor Energy Inc. in June 2004. Syncrude TW was collected from the clarified water zone of its West In Pit, whereas the Suncor TW was sampled from its Consolidated Tailings Pond.

**Incubation Methods.** Biodegradation experiments were conducted to monitor the loss of NAs from viable incubations and changes in the NAs composition during incubation for a total of 40 days (Syncrude TW experiment) or 49 days (Suncor TW experiment). Microbial communities indigenous to TW were the sources of microorganisms used in these experiments.

Individual stock solutions of the four commercial NA mixtures were prepared at approximately 1 g L\(^{-1}\) in dilute NaOH. Solution pH was adjusted to between 10 and 11, to dissolve the NAs as sodium naphthenates.

All incubations had a final liquid volume of 200 mL in 500-mL Erlenmeyer flasks. Incubations of each combination of the TW samples and the four commercial NAs were prepared in triplicate with 180 mL of well-mixed Syncrude or Suncor TW plus 10 mL stock NA solution. Each incubation was also supplemented with 10 mL modified Bushnell–Haas medium (34) to ensure nitrogen and phosphorus were not limiting nutrients. The initial concentrations of N and P were 1 and 0.7 mM, respectively, and the total NAs concentration ranged from 30 to 100 mg L\(^{-1}\).

Four sets of positive control flasks (one for each commercial preparation) containing only commercial NAs were prepared in triplicate by adding 20 mL stock NA solution to 170 mL sterile MilliQ water. An inoculum for each of these flasks was prepared by centrifuging 200 mL TW at 12 000 g for 15 min, discarding the supernatant, and resuspending the resulting pellet in 10 mL modified Bushnell–Haas medium. The entire 10-mL suspension was then transferred to a positive control flask, providing nitrogen and phosphorus as well as viable microorganisms in approximately the same proportion as incubations set up directly in TW. Negative controls contained 20 mL filter-sterilized (using Millex-GS, 0.22 µm, Millipore, Bedford, MA) stock NAs solution and 180 mL sterile MilliQ water. Filter-sterilized TWs could not be used for this purpose because they contain NAs.

Viable and sterile controls, with either Syncrude or Suncor TW as the only source of NAs, were also prepared in triplicate. In this case, 190 mL TW and 10 mL medium were added to 500-mL Erlenmeyer flasks. TW used for the sterile controls was heat-killed by autoclaving at 121°C, 15 psi, twice for 20 min, with 24 h between treatments.

Incubations were carried out under aerobic conditions at room temperature (approximately 20°C) on a shaker at 200 rpm. Samples were taken from the incubations and stored at −20 or 4°C prior to analysis by HPLC or GC-MS.

**Analysis of Incubation Supernatants.** NAs quantification was carried out using the derivatization protocol and HPLC method described earlier (19). Individual calibration curves were prepared with each commercial NA preparation, and the specific slopes and intercepts from each calibration curve were used to calculate the NAs concentration in samples containing the corresponding commercial NAs.

Prior to analysis by GC-MS, NAs were extracted from incubation supernatants and derivatized using previously published methods (2), A GC-MS protocol (26) was used to generate TICs and average mass spectra of the unresolved "humps" of NAs.

**Results**

**Incubations with Kodak Acids and Syncrude TW.** Figure 1 summarizes the NAs concentrations in various incubations with Kodak acids and Syncrude TW. These concentrations were determined by HPLC using Kodak acids to prepare the calibration curves. The Kodak acids were readily biodegraded by bacteria in the Syncrude TW. For example, when the Kodak acids were the only source of NAs in the incubations, the concentration was rapidly depleted between days 4 and 7 (Figure 1). Sterile controls containing only the Kodak acids showed no change in NAs concentration after 40 days (Figure 1).

In contrast, the NAs that occurred naturally in the Syncrude TW proved to be more recalcitrant than the commercial NAs. The concentrations of these native NAs in the viable incubations remained almost equivalent to those in the sterile control (Figure 1). At the end of the 40-day incubation time, NAs concentrations in the viable incubations with Syncrude TW were about 20% less than in the sterile control with Syncrude TW. In another set of incubations, Kodak acids were added to the Syncrude TW to give a total initial NAs concentration of 94 mg L\(^{-1}\). Within 7 days of incubation, the NAs concentration was depleted to 64 mg L\(^{-1}\); the concentration contributed by the Syncrude TW (Figure 1). By the end of the 40-day incubation period, the NAs concentration in these incubations was about 48 mg L\(^{-1}\) or 17% lower than in the sterile control with Syncrude TW. The results in Figure 1 suggest that the NAs in the Syncrude TW were more resistant to biodegradation than commercial NAs.

From the data in Figure 1, it appeared that, in the incubations with Kodak acids added to Syncrude TW, most of the commercial NAs were degraded leaving mainly those that originated in the TW. To test this hypothesis, extracts of the Kodak acids, Syncrude TW, and an incubation
containing Syncrude TW amended with Kodak acids were derivatized and analyzed by GC-MS. The “humps” that were observed in TICs of these samples were quite revealing and are shown in Figure 2. The TICs for Kodak acids (Figure 2A) and Syncrude NAs (Figure 2D) were clearly different. The shape of their respective “humps” and the retention times over which they occurred were visibly distinct. The Kodak acids sample eluted from the GC column with a shorter retention time than the NAs extracted from Syncrude TW. Using this same GC-MS method, Clemente and Fedorak (27) demonstrated that, in general, lower molecular mass NAs eluted earlier than higher molecular mass NAs. Thus, the Kodak acids mixture has a high proportion of low molecular mass NAs. The Kodak acids hump was also narrower, suggesting that the composition of NAs in this mixture is less diverse than the NAs from the Syncrude TW, which gave a wider hump.

The two middle panels of Figure 2 show the TICs of extracts from incubations that contained Syncrude TW supplemented with Kodak acids. Figure 2B is the analysis of the extract taken just after the incubation was started. It showed the presence of two overlapping “humps”, corresponding to Kodak acids and NAs from the Syncrude TW. By day 21, the Kodak acids hump had almost completely disappeared (Figure 2C). This was attributed to biodegradation because a decrease in the NAs concentrations also occurred between these two times (Figure 1). The hump that remained at day 21 (Figure 2C) more closely resembled the hump from NAs in the Syncrude TW (Figure 2D) than the hump from the Kodak acids (Figure 2A). These results, like those from the HPLC analyses (Figure 1), are consistent with the preferential biodegradation of the commercial NAs.

Data used to generate three-dimensional plots, as in (26), (not shown) indicated a shift in the NAs composition during incubation. For example, at time zero in incubation with a mixture of Syncrude NAs and Kodak acids, 65% of the ions corresponded to NAs with \( n \leq 17 \). On day 7, the proportion of ions in this \( n \) range was 51%, and on day 21 the proportion had decreased to 44%. These results corroborate the TICs in Figure 2B and 2C showing the preferential removal of lower molecular mass NAs.

**Incubations with Kodak Acids and Suncor TW.** Microorganisms from the Suncor TW were able to degrade the Kodak samples. Changes in the NAs concentrations (determined by HPLC with Kodak acids used for the calibration curves) followed a pattern similar to that in Figure 1. With only the Kodak acids present, the NAs concentration decreased quickly from 81 to 11 mg L\(^{-1}\) during the first 10 days, and then remained essentially constant over the rest of the 49-day incubation.

Biodegradation of NAs in the incubations of Suncor TW supplemented with Kodak acids was evident by the decrease in NA concentrations, similar to the trend observed in the experiment with Syncrude TW supplemented with Kodak acids (Figure 1). With the supplemented Suncor TW, the initial NAs concentration (68 mg L\(^{-1}\)) decreased rapidly during the first 14 days of incubation, and then the concentration reached the concentrations (37 mg L\(^{-1}\)) measured in the sterile control and viable incubation that contained only the Suncor TW. By day 49, the concentration of residual NAs in the Kodak acid-supplemented Suncor TW incubations had dropped to about 26 mg L\(^{-1}\), which was 30% less than that in the sterile control that contained only Suncor NAs.

Initially, there was little change in the concentration of NAs in the incubations that contained only the Suncor NAs. However, after 49 days, the concentration in these incubations had decreased to approximately 25% less than that in the sterile control, which was similar to the extent of biodegradation in the incubations with Suncor TW amended with Kodak acids. Overall, the results from the HPLC analyses suggested that the NAs in the Suncor TW were less susceptible to biodegradation than the Kodak acids.

Figure 3 compares the TICs from GC-MS analyses of the extracts from incubation supernatants. The “humps” shown in Figure 3A and 3B are from the Kodak acids and the NAs in the Suncor TW, respectively. The day-zero sample from the incubation containing Suncor TW amended with Kodak acids (Figure 3B) shows a combined hump composed of the NAs shown in Figure 3A and 3D. After 49 days of incubation, the NAs concentration had decreased to about one-third of the original concentration, and the composition had changed, as evident by the TIC (Figure 3C). The hump with the shorter retention time (corresponding to the Kodak acids) disappeared, and the residual hump had a different shape than the original Suncor NAs hump (Figure 3D), particularly in the material that eluted with retention times between 10 and 25 min. These losses of the early eluting NAs are consistent with the preferential biodegradation of the lower molecular mass NAs.

Analyses of the data from three-dimensional plots (Figure 4) illustrate the preferential removal of the lower molecular mass NAs over the 49-day incubation time. For example, at time zero, 60% of the ions corresponded to NAs with \( n \leq 17 \). On day 14, 53% of the ions corresponded to NAs with \( n \leq 17 \), and on day 49, 47% of the ions corresponded to NAs with \( n \leq 17 \).

**Results from Incubations with Other Commercial NAs.** During the early stages of these experiments, the Kodak acids were used to prepare the calibration curves for all of the
incubations, regardless of which commercial NAs preparation was added to the TW. However, this gave unreliable results. For example, when an incubation of Syncrude TW was amended with 50 mg Fluka acids L\(^{-1}\), the results from the calibration curve prepared with Kodak acids showed only 8 mg NAs L\(^{-1}\) above that in the TW. This discrepancy was rectified by preparing individual calibrations curves with each of the four commercial NAs preparations and using the appropriate calibration curve for analyses of incubations amended with the corresponding commercial NAs. Table 1 summarizes typical parameters from four calibration curves.

The Kodak acids gave a calibration curve with the highest slope, which was nearly double the slope of the Fluka preparation (Table 1). It was presumed that the slopes would vary with the TAN of the NAs preparations because the derivatizing reagent reacts with the carboxylic acid moiety. However, there is no correlation between TAN values and the slopes of the calibration curves (Table 1). The reason for the different slopes remains unknown. The consequence of using the four different calibration curves was that concentrations of NAs measured in the TW varied depending upon which commercial preparations were used (Table 1). For example, the concentrations of NAs in the Syncrude TW ranged from 60 to 97 mg L\(^{-1}\).

Despite the difficulties determining the true concentrations of NAs in these incubations, the HPLC method was useful for following changes in NAs concentrations over time to determine whether the commercial preparations were more susceptible to biodegradation than NAs in the oil sands.

**FIGURE 3.** TICs for (A) Kodak acids, (B) Kodak acids plus Suncor NAs on day 0, (C) this mixture on day 21, and (D) and Suncor NAs. Crosshatching illustrates possible overlapping areas of the two NA humps. Average NA concentration in incubation supernatants is given for both time-points.

**FIGURE 4.** Changes in the distribution of residual acids recovered from incubations grown on Suncor NAs plus Kodak acids. Results are from GC-MS analyses of samples taken after (A) 0, (B) 14, and (C) 49 days of incubation. The sum of all the bars in each panel is 100%. Average NA concentration is given for each time.

TW. Incubations containing each of the commercial NAs alone and in combination with the NAs in the two TW samples were established and monitored for NAs concentrations. Results from these eight incubations are summarized in Table 2. Plotting the NAs concentrations over time resulted in graphs that had the same general shape as Figure 1. For example, Figure 1 shows that after 10 days of incubation there was 88% removal of the Kodak acids when they were the only NAs in the incubations. This figure also shows that the concentration of NAs in incubations with Syncrude TW amended with Kodak acids reached the concentration in the Syncrude TW sterile control after 7 days incubation. The values “88%” and “7 days” are the entries in the first line of Table 2.
TABLE 1. Total Acid Number (TAN) Values, Parameters from Typical Calibration Curves of Four Different NA Standards, and NA Concentrations in Tailings Water Determined with the Different NA Standards

<table>
<thead>
<tr>
<th>NA standard</th>
<th>TAN (mg KOH g⁻¹)</th>
<th>slope a (mAU mg⁻¹ L⁻¹)</th>
<th>Y-intercept b (mAU)</th>
<th>R²</th>
<th>NA concentrations c (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kodak acids</td>
<td>264</td>
<td>12.7</td>
<td>212</td>
<td>0.9997</td>
<td>60 ± 1 35 ± 2</td>
</tr>
<tr>
<td>Merichem acids</td>
<td>268</td>
<td>10.4</td>
<td>243</td>
<td>0.9982</td>
<td>73 ± 2 42 ± 2</td>
</tr>
<tr>
<td>Kodak salts</td>
<td>195</td>
<td>9.1</td>
<td>234</td>
<td>0.9993</td>
<td>69 ± 1 41 ± 2</td>
</tr>
<tr>
<td>Fluka acids</td>
<td>235</td>
<td>6.7</td>
<td>225</td>
<td>0.9999</td>
<td>97 ± 4 61 ± 3</td>
</tr>
</tbody>
</table>

a mAU = milliabsorbance units; mg L⁻¹ = refers to the concentration of naphthenic acids. b Average of triplicates. c One standard deviation. d Y-intercepts were similar because the composition of reagent blanks was identical for each set of calibration standards.

Discussion

The various concentrations of NAs in a given TW sample determined using different commercial NAs for calibration curves (Table 1) demonstrate the difficulties associated with the analysis of NAs. During the development of the HPLC method, Clemente et al. (20) did not observe differences in slopes of calibration curves prepared with Kodak acids and Merichem acids as standards. Yen et al. (19) improved this HPLC method, but they did not determine the slopes of calibration curves with various commercial NAs preparations. The results in Table 1 are the first evaluation showing how the measured concentrations of NAs in TW samples determined using the HPLC method can be affected by the commercial NAs preparation chosen for the calibration curve. The reason for this difference is yet to be determined, but the slopes of the calibration curves are not related to the TAN values (Table 1) of the NAs preparations. The oil sands industry standard method for measuring NAs concentration in water samples uses a FTIR spectroscopy method (17) with Kodak acids as the calibration standard. Using the same calibration standard, Yen et al. (19) showed that the HPLC method was in good agreement with the FTIR method.

GC analyses of complex mixtures often produce “humps” in the chromatograms, commonly known as unresolved complex mixtures (35–38). Little detailed information can be obtained from these “humps”, but they can be quite different with respect to their shape and retention times. For example, Fryxinger et al. (38) presented two GC chromatograms of organic materials extracted from different marine sediments. The “humps” in both chromatograms were distinctly different from each other. Likewise, the TICs of NAs presented in Figures 2 and 3 show distinctly different shapes and retention times. These TICs were used to glean information about the NAs extracted from our various laboratory incubations.

On the basis of GC-MS analyses of Syncrude TW samples that had aged for various lengths of time in pits that receive no fresh input of TW, Holowenko et al. (26) hypothesized that NAs with n ≤ 21 are more susceptible to biodegradation than those with n ≥ 22. Figures 2 and 3 illustrate that the NAs with the shorter retention times and lower molecular masses are biodegraded more rapidly than those with the longer retention times.

Using three-dimensional plots to summarize the data from the GC-MS can be a convenient method to observe differences between NAs preparations (26). For example, the NAs in oil sands TW are typically composed of a wide range of molecular mass, with n = 5–28 (26) or even 40 (23). In contrast, commercial preparations are often composed of mainly low molecular mass NAs, with n = 10–14 (2, 14). This narrow range of molecular mass in the commercial NAs depends on the boiling range of the petroleum fractions from which the NAs are recovered. Kodak salts are somewhat different than the other commercial NAs because the majority (90%) of NAs in these salts fall in the n = 14–21 range. On the basis of the molecular mass distributions from GC-MS analyses, the Kodak salts more closely resemble the NAs in Syncrude TW (14) and Suncor NAs (data collected during this study) than do any of the other three commercial preparations.

Each of the commercial NAs preparations was biodegraded by microorganisms from the two TW samples. After 10 days of incubation, over 80% of the Kodak acids, Merichem acids, and Fluka acids was degraded when they were the only source of NAs in the incubations (Table 2). The Kodak salts were degraded more slowly than the other three commercial preparations, with less than 80% being degraded after 10 days of incubation. Similarly, the Kodak-salts-amended incubations required 21 days of incubation before the NAs concentrations decreased to those in the sterile controls with TW water. Incubation times of ≥14 days were required for the same decrease in incubations that were amended with the other commercial NAs (Table 2). On the basis of the results from HPLC analyses, all four commercial NAs preparations were more readily biodegraded than the NAs in the two TW samples.

Samples from each of the incubations amended with commercial NAs were extracted and analyzed by GC-MS. The time zero samples all yielded TICs that were similar to those of Figures 2B and 3B. That is, two “humps” were apparent: one that eluted early, composed of the commercial NAs, and one that eluted late, corresponding to the NAs in the TW. Extended incubations resulted in the loss of the early hump, as illustrated by Figures 2C and 3C. All of these results indicated that the NAs in the TW samples were more persistent than those in the commercial preparations.

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The data in Table 2 show that, among the commercial preparations, Kodak salts were most resistant to biodegradation by microorganisms in the TW from Syncrude and Suncor. This is consistent with the fact that the Kodak salts contain higher molecular mass NAs than the other three commercial preparations. In addition, the Kodak salts contain a high proportion of multi-ring acids, similar to the NAs in the TWs. The proportion of ions that correspond to 3-, 4-, and 5-ring acids (Z = –6, –8, and –10, respectively) comprises 23% of the ions detected in the GC-MS analysis of these salts, and the proportions of 3- to 5-ring acids in the Syncrude and Suncor NAs are 37% and 35%, respectively. By comparison, the proportions of ions that correspond to 3- to 5-ring acids in the Kodak, Merichem, and Fluka acids are only 9%, 8%, and 16% of the total ions, respectively. These comparisons suggest that the structures of the NAs in the Kodak salts are more complex than those in the other three commercial preparations.
In contrast to the data presented in Table 2 for the Kodak salts, Clemente et al. (2) showed that the Kodak salts were nearly completely removed from laboratory incubations within only 10 days. However, Clemente et al. (2) used an enrichment culture that had been maintained for several months by repeated transfer to fresh medium with Kodak salts, increasing the biodegradative capability of the culture. No enrichment procedure was used in the current study. Instead, the commercial preparations were inoculated on day 0 with a suspension of microorganisms taken directly from the TW samples.

In one study, carboxylic acid fractions were separated from 33 crude oils, including some crude acids that were biodegraded and some that were not biodegraded (12). In general, the greater the degree of biodegradation, the higher the concentration of carboxylic acids in the crude oils. Bitumen in the Athabasca oil sands deposit is known to be the residue of conventional crude that has undergone extensive biodegradation (39). Thus, the presence of NAs in the oil sands is expected. Recently, Clemente (15) reported that the average NAs concentration in seven oil sands ore samples from Syncrude was 200 mg kg⁻¹ of ore. Presumably, as NAs in the oil sands “incubated” over geological time, those acids that were most susceptible to biodegradation would have been degraded, leaving mainly the recalcitrant NAs in the ore. These would be released during the alkaline, hot water extraction process used to recover the bitumen (16), and the recalcitrant NAs would remain in the TW.

In laboratory studies, Watson et al. (13) subjected a weathered, light Arabian crude oil to microbial degradation under aerobic conditions for up to 80 days. They observed that, after extensive biodegradation of the crude oil, there was an increase in the concentrations of branched and cyclic carboxylic acids with n > 20. These eluted from the GC as a hump, and they were considered to be NAs. These acids resisted further biodegradation for the duration of the experiment. The observed persistence of the high molecular mass NAs in our incubations is consistent with the findings of Watson et al. (13).

Several biodegradation studies (2, 32, 40) have used commercial NAs preparations (mainly Kodak acids, Kodak salts, and Merichem acids) as surrogates for NAs in TW. At the time that Herman et al. (32) did their biodegradation studies, the GC-MS method used in the current study was not available for monitoring changes caused by microbial metabolism. Microbial activity was monitored by measuring microbial respiration and the release of CO₂ from the NAs (or other organic compounds) in their cultures (32). In retrospect, the results reported by Herman et al. (32) showed the same trends as those of the current study. For example, microbial cultures oxidized 48% of the carbon from the Kodak salts to CO₂, whereas they oxidized only 20% of the carbon in a NAs extract from TW to CO₂ (32). These results demonstrated that the commercial NAs are more susceptible to biodegradation than the NAs in TW, as shown in Figure 1. Because it is now known that commercial NAs generally have lower molecular masses than NAs in TW, it is not surprising that the latter are more resistant to biodegradation.

Our investigation has shown that commercial NAs, with predominately low molecular mass acids, are not appropriate surrogates for predicting the biodegradability of NAs in the TW because the commercial NAs are much more readily biodegraded than the NAs in the TW. Thus, subsequent studies should use NAs from oil sands sources to accurately assess their biodegradability. Because of the demonstrated recalcitrance of the high molecular mass NAs in TW, oil sands companies are exploring new methods to remove the toxicity of these compounds.

Acknowledgments
Funding was provided by Syncrude Canada Ltd. and the Canadian Water Network. We thank H. Dettman, L. Harrower, P. Kong, and R. Whittal for their assistance. A.C.S. gratefully acknowledges the support of an NSERC Scholarship.

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Received for review May 27, 2005. Revised manuscript received August 10, 2005. Accepted August 23, 2005.