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A Preparative Method for the Isolation and Fractionation of Dissolved Organic Acids from Bitumen-influenced Waters

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A preparative method for the isolation and fractionation of dissolved organic acids from bitumen-influenced waters☆

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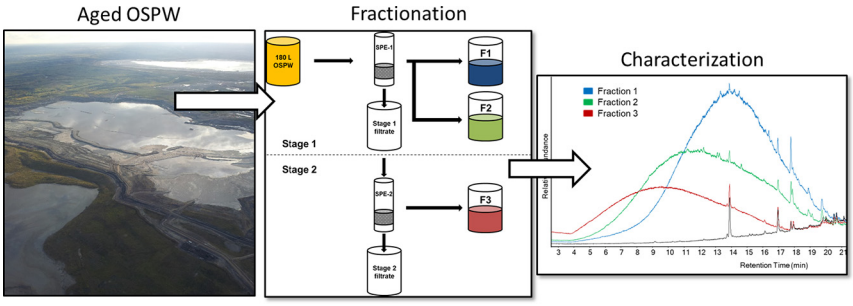
HIGHLIGHTS

- Dissolved organics are considered principal toxic components in OSPW.
- A novel method for extraction and fractionation of dissolved organics was developed.
- Three large volume organic fractions were generated.
- Isolated fractions increased in polarity and degree of aromaticity.
- Aged OSPW was primarily composed of polyoxygenated dissolved organics.

GRAPHICAL ABSTRACT



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ABSTRACT

The surface mining of oil sands north of Fort McMurray, Alberta produces considerable tailings waste that is stored in large tailings ponds on industrial lease sites. Viable strategies for the detoxification of oil sands process affected water (OSPW) are under investigation. In order to assess the toxic potential of the suite of dissolved organics in OSPW, a method for their extraction and fractionation was developed using solid phase extraction. The method successfully isolated organic compounds from 180 L of an aged OSPW source. Using acidic- or alkaline-conditioned non-polar ENV+ resin and soxhlet extraction with ethyl acetate and methanol, three fractions (F1–F3) were generated. Chemical characterization of the generated fractions included infusion to electrospray ionization ultrahigh-resolution mass spectrometry (ESI-UHRMS), liquid chromatography quadrupole time-of-flight mass spectrometry, gas chromatography triple quadrupole time-of-flight mass spectrometry, and synchronous fluorescence spectroscopy (SFS). Additionally, ESI-UHRMS class distribution data and SFS identified an increased degree of oxygenation and aromaticity, associated with increased polarity. Method validation, which included method and matrix spikes with surrogate and labelled organic mono carboxylic acid standards, confirmed separation according to acidity and polarity with generally good recoveries (average 76%). Because this method is capable of extracting large sample volumes, it is amenable to thorough chemical characterization

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and toxicological assessments with a suite of bioassays. As such, this protocol will facilitate effects-directed analysis of toxic components within bitumen-influenced waters from a variety of sources.

1. Introduction

The Canadian oil sands region, located in northern Alberta, contains one of the largest petroleum deposits worldwide, and extraction of the mineable bitumen has increased nearly 1000% in the last 4 decades (Royal Society of Canada Expert Panel, 2010). Bitumen is a viscous hydrocarbon mixture that is extracted via in-situ or surface mining methods and is ultimately upgraded to a synthetic crude oil. Extraction of bitumen results in the generation of oil sands process-affected water (OSPW) and tailings waste. According to the Alberta Government, in 2013 the surface area of associated oil sands tailings in containments reached 77 km^2 (Alberta Energy, 2013). In accordance with the Alberta Environment Protection and Enhancement Act, the release of substances that may cause adverse effects to the environment isprohibited and Crown-leased land must be reclaimed (Government of Alberta, 2017, FTFC, 1995). To address the growing reserve of OSPW on industrial leases, the development and testing of large-scale landscape reclamation strategies has begun.

Much of the difficulty associated with oil sands research is due to the complexity of OSPW. There is a plethora of inorganic and organic constituents present as a complex mixture, many of which are not fully characterized and remain undiscovered. Among the most toxic constituents of OSPW is the polar organic fraction (Allen, 2008; Royal Society of Canada Expert Panel, 2010), which includes a subclass of O_2 compounds which is referred to as naphthenic acids (NA) (Headley and McMartin, 2004; Clemente and Fedorak, 2005). Industrially-derived NA have been shown to be acutely toxic to a wide variety of aquatic organisms (Headley and McMartin, 2004; Allen, 2008; Royal Society of Canada Expert Panel, 2010) and also to elicit reproductive impairment in fish (Kavanagh et al., 2012). As a result, research during the past decade has focussed on NA characterization and toxicity. More recently, NAs have been considered as a chemical class among the broader acid extractable organics (AEOs), which themselves comprise the totality of bitumen-derived dissolved organics. Acid extractable organics have been shown to contain heteroatoms in addition to dicarboxyl, hydroxyl, dihydroxy, and aromatic moieties (Bataineh et al., 2006; Barrow et al., 2009; Grewer et al., 2010; Headley et al., 2011; Rowland et al., 2011; Bauer et al., 2015). Recent toxicological assessments have begun to identify the toxic chemical classes, and O_2-containing substances (which includes NA) have emerged as the principal toxicants within OSPW (Morandi et al., 2015; Hughes et al., 2017). Accordingly, the Alberta government has advocated for Canadian Council of Ministers of the Environment (CCME) water quality guidelines for NA (Energy Resources Conservation Board and Canadian Environmental Assessment Agency, 2011; Minister of Environment, 2013). To create these guidelines, a more comprehensive understanding of the chemical composition and toxicity of all bitumen-derived dissolved organics is required. This is critical in developing methods to reduce tailings toxicity and demonstrate end pit lakes as a viable reclamation strategy.

Effects-directed analysis (EDA) approaches are well suited to investigate the classes of compounds associated with toxicity to aquatic species that are present in complex mixtures (Brack et al., 2016). However, when applied to the oil sands, these investigations have been hampered by obstacles of scale with regard to bioassay requirements. A typical 7-day, static-renewal embryo-larval fish bioassay with a 5-concentration dilution series and three replicates can require up to 1 L of sample volume. Bioassays involving flow-through systems, juvenile/adult fish, chronic test durations, etc., require even greater quantities (USEPA, 2002). As a result, varying degrees of success in characterizing bioactive substances have been achieved, and it is worth noting that the advances that have been made were primarily with single bioassays (Armstrong et al., 2009; Frank et al., 2006; Johnston et al., 2017; Kavanagh et al., 2012; Lo et al., 2006; Nero et al., 2006; Scarlett et al., 2013) in an in vitro scale that requires extrapolation to effects at the individual or population levels.

Previous attempts to fractionate OSPW based on solubility have generated fractions with a substantial degree of overlap with regard to NA species present (Lo et al., 2006; Grbovic et al., 2012; Huang et al., 2015). One solubility-based study incorporated anion-exchange chromatography, eluting with buffers encompassing a range of pH values (Lo et al., 2006). While this study was still limited in its capacity to generate distinct fractions, it was able to observe a pK_a-dependent trend where higher pK_a NAs exhibited lower potencies using Microtox assays (Lo et al., 2006). Unfortunately, fractionation attempts based on solubility have not led to the assignment of structure-toxicity relationships for specific compound classes. Argentation solid phase extraction (SPE) achieved separation of dissolved acids based on aromaticity through the use of different eluents (Jones et al., 2012). A separate study by Borgund et al. (2007) used cyano HPLC column chromatography to separate NAs into four fractions based on polarity using different solvent mixtures (hexane, dichloromethane, and methanol): non-polar compounds, carboxylic acids, phenols, and polyfunctional compounds. Both of these methods were suitable for the analytical characterization of acids, however neither generated sufficient quantities required for toxicity testing using ecologically relevant species. Lack of sufficient fraction volume in these studies is due to the absence of commercially available preparative chromatography columns, and associated preparative extraction methodology. Success in this regard was initially achieved with a study utilizing preparative fractional distillation to produce 5 fractions of bitumen-derived dissolved organics isolated from fresh OSPW (Frank et al., 2008). Chemical characterizations of the fractions generated according to boiling point ranges were associated with increasing mean molecular weight, aromaticity, and heteroatom content (Frank et al., 2008; Bauer et al., 2015). Although this method produced sufficient material to conduct toxicity assays with several test organisms, the OSPW extraction method prior to distillation was biased toward O_2 species.

In order to advance the understanding of the principal toxic components of OSPW and other bitumen sources, EDA studies need to utilize large volumes of source waters for use in bioassays of relevant vertebrate and invertebrate species. Therefore, the challenge remains to develop a method capable of extracting all soluble organics and produce chemically distinct fractions in the large quantities required. The most successful preparative fractionation approach to date involved OSPW collected from the oil sands industry's first end pit lake as the source material. This study incorporated an initial extraction at two different pH's, followed by preparative HPLC fractionation (Morandi et al., 2015). Using an in vivo bioassay with an ecologically relevant test species (96 h fathead minnow embryos), the authors determined that the toxicity of OSPW was largely attributable to the O_2 NA species, but non-acid species also contributed to the overall toxicity. While these findings highlighted the contributions of non-acid species to the toxicity of OSPW, there remains a paucity of information regarding the identity of bioactive substances in surface and groundwaters influenced by natural bitumen-derived dissolved organics. As such, the method developed herein evaluated the presence of metals and major ions in addition to dissolved organics.

To add further complexity, the majority of fractionation and toxicity analyses on OSPW have used fresh tailings as a source material. Unfortunately, this has led to major knowledge gaps regarding constituents present in aged tailings and the relative toxicities of components therein. There is ample research identifying both chemical and toxicological differences between fresh and aged tailings (Siwik et al., 2000; Bataineh et al., 2006; Han et al., 2009; Marentette et al., 2015; Bartlett et al., 2017), specifically with regard to natural degradation of dissolved organics by algae and bacteria (MacKinnon and Boerger, 1986; Herman et al., 1993; Lai et al., 1996; Clemente et al., 2004). With the advent of decommissioned tailings ponds and end-pit lakes, risk assessments of aged mixtures and comparisons to natural mixtures are needed. Previous attempts by the present authors to investigate dissolved organics from aged OSPW using a preparative acid precipitation method originally developed for fresh tailings (Frank et al., 2006) yielded low recoveries (<10%). This exemplified the chemical differences associated with aging and underscored the need for a revised method that could be used for all bitumen-influenced waters.

The objective of the present study was to develop a robust preparative-scale extraction and fractionation method that could be applied to the range of relevant bitumen-influenced water sources. Moreover, this objective required that the method be capable of producing large quantities of each fraction to enable chemical characterization and toxicity evaluations using a suite of ecologically relevant test organisms and endpoints.

2. Methods and materials

2.1. Chemicals and reagents

Chemicals used for the preparative scale fractionation and chemical analysis (methanol (MeOH), ethyl acetate (EtOAc), toluene, and hydrochloric acid (HCl)) were purchased from Fisher Scientific (Mississauga, ON). Sodium hydroxide (NaOH) was purchased from Sigma-Aldrich® (Oakville, ON). Chemicals used for method development (hexane, ethanol, acetonitrile (ACN), and dichloromethane (DCM)) were purchased from Fisher Scientific (Mississauga, ON). Chromatography resin Isolute® ENV+ was purchased from Biotage® (Charlotte, NC), Diaion® HP20 and Sepabeads® SP825 were supplied by Itochu Chemicals America (Farmington Hills, MI), Oasis® HLB, and Oasis® MAX resins were supplied by Waters Ltd. (Mississauga, ON).

2.2. OSPW sample source

An OSPW sample was collected from an aged test pond (Pond 9), on Syncrude Canada Ltd.'s lease, which contained OSPW similar to fine tailings present in current end-pit lakes that have low turbidity. This test pond was originally filled in 1993 with OSPW from an active tailings pond (Mildred Lake Settling Basin), with no subsequent water amendment other than natural precipitation and evaporative and degradative processes (Siwik et al., 2000). This sample therefore serves as an example for OSPW sources influenced by natural degradation processes. In 2011, approximately 2000 L was collected from the test pond into two 1000-L polypropylene totes. Following method development experiments and scale-up, the preparative scale fractionation was conducted on 180 L.

2.3. Bench scale: extraction method development

Three bench scale extraction techniques were evaluated. Throughout, "bench scale" is defined as small-scale experiments and involved sample volumes in the range of 30–3000 mL. The main objective of the bench-scale experiments was to determine which method and resin type provided the greatest dissolved organics yield. The results of these evaluations are presented in Fig. 1, where the y-axes indicate recovery of dissolved organics (mg/L). The three evaluated methods included a previously defined acid precipitation method (Frank et al., 2006), a resin settling/flocculent approach, and an SPE approach (Fig. 1B). The settling/flocculent approach involved mixing methanol pre-conditioned SPE resins (described below) with water samples and allowing them to settle or floc for 24 h or 48 h. The method followed SPE theory (International Sorbent Technologies, 2001; Argonaught Technologies, 2002) such that acidic compounds present in solution were expected to adsorb to the resin where they were extracted via acid precipitation methods (Frank et al., 2006). The SPE approach followed standard SPE protocols (International Sorbent Technologies, 2001; Argonaught Technologies, 2002) adapted for the concentration and clean-up of dissolved organics as described previously (Frank et al., 2008; Gagné et al., 2011). Briefly, the current SPE method involved the use of an organic solvent to pre-condition a porous resin (stationary phase) that was packed in a column, in order to adsorb organic compounds. Resins with different chemical and structural properties (hydrophobic, ionic, surface area, etc.) can be selected with the intention of best capturing an analyte of interest. In this case, the sample water was acidified to precipitate dissolved organics so that they adsorbed to the resin. Following loading and passage of the water sample through the column, sorbed organics were then removed and isolated by elution with an organic solvent through the same column. The latter two methods (settling/flocculent and SPE) were themselves evaluated with five different solid phase resin types: hydroxylated polystyrene divinylbenzene (ENV+, Biotage®, NC USA), unsubstituted polystyrene divinylbenzene copolymers (HP20 and SP825, Diaion®, MI USA), divinylbenzene copolymer and divinylbenzene copolymer modified with dimethylbutylamine (HLB and MAX, respectively, Oasis®, ON Canada), representing a range of adsorptive properties.

2.4. Scale-up to preparative extraction

Scale-up experiments of 10× and 100× relative to the bench scale 30 mL OSPW loading volume (300 mL and 3 L OSPW, respectively) were conducted using custom packed ENV+ columns for which recovery of organics was optimized at a preparative scale. The procedure was run in duplicate with the addition of a method blank, which involved conducting the extraction using 180 L de-ionized water as the source material. Importantly, in order to maintain high yields with increasing cartridge diameter, resin bed heights (resin mass) and loading/elution flow rates were altered according to equations, outlined previously (Rathore and Velayudhan, 2002), accordingly at each scale-up step. Experimentation to evaluate resin capacity was conducted using 60 mg of ENV+ resin with increasing OSPW volume. Subsequently, flow rates were assessed at each OSPW loading volume scale (1×, 10×, 100×) in order to optimize method efficiency.

Following optimization of an extraction method, established parameters were employed for development of a fractionation procedure. During fractionation method development, the separation of the least polar (neutrals and organic bases) from the most polar compounds (including organic acids) was ultimately achieved by conducting the extraction in two stages, initially at pH 11, and then at pH 2. For each stage, the previously optimized SPE conditions (flow rates, resin ratio) were applied and elutions with various solvents of different polarities were evaluated to achieve maximal solubilities and recoveries.

2.5. Preparative fractionation

Previous bench-scale and scale-up experimentation ultimately led to the development of a preparative fractionation method with the ability to process 200 L of OSPW. The preparative fractionation procedure was conducted in two stages; addition of base to the OSPW (Stage 1; pH 11) and the subsequent acidification stage (Stage 2; pH 2) (Fig. 2). The preparative fractionation apparatus consisted of a glass column with plunger (10 cm ID × 30 cm height, Spectrum Chromatography, Houston, TX), two 200-L HDPE barrels, and a controller and motor

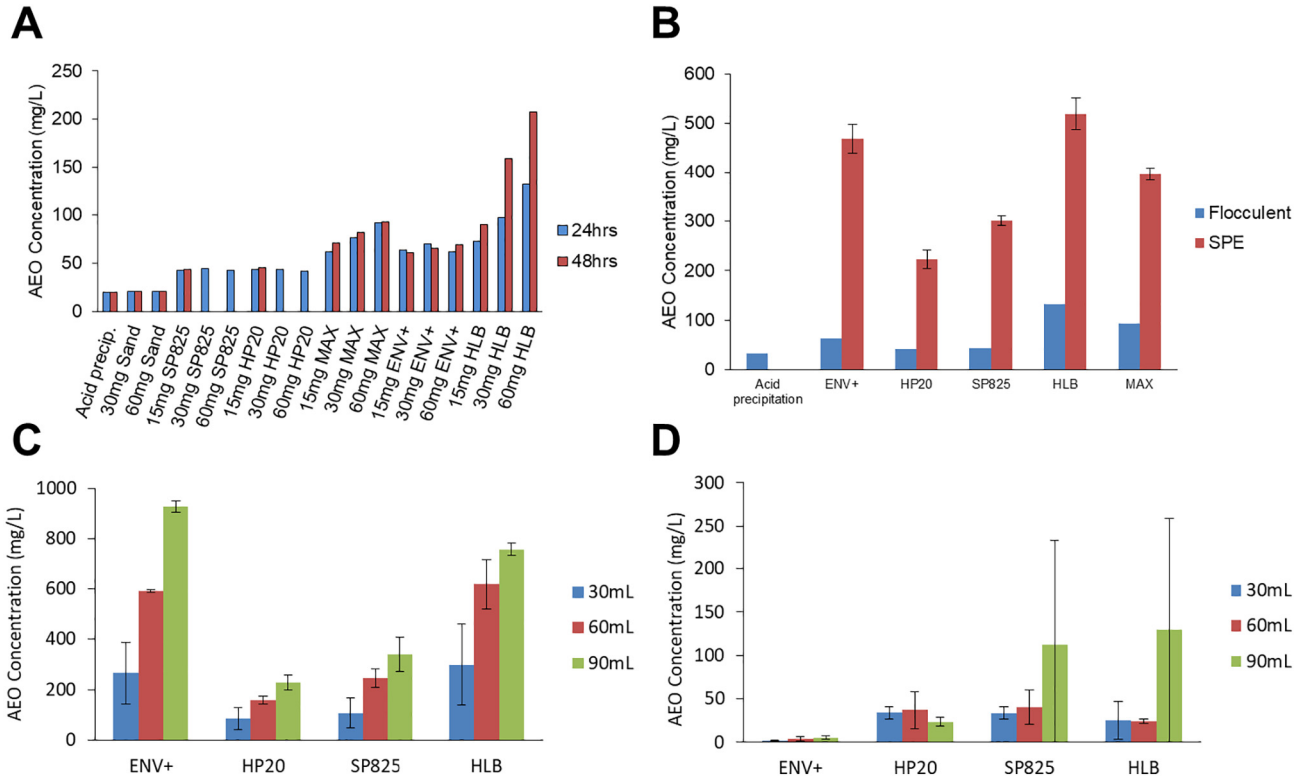

Fig. 1. Bench-scale method development for stationary phase evaluation. Dissolved organic concentrations using ESI-UHRMS represent equivalently concentrated sample volumes. Extraction of dissolved organics from 30 mL OSPW with different resin types was assessed using a settling/flocculent method at 24 h and, in some cases, 48 h settling times (A). The SPE method was assessed and compared to the settling/flocculent method using 30 mL of OSPW and 60 mg of different resins (B). SPE method dissolved organic yield (C), and breakthrough (D) using 60 mg resin with increasing OSPW volumes were compared across resin types.

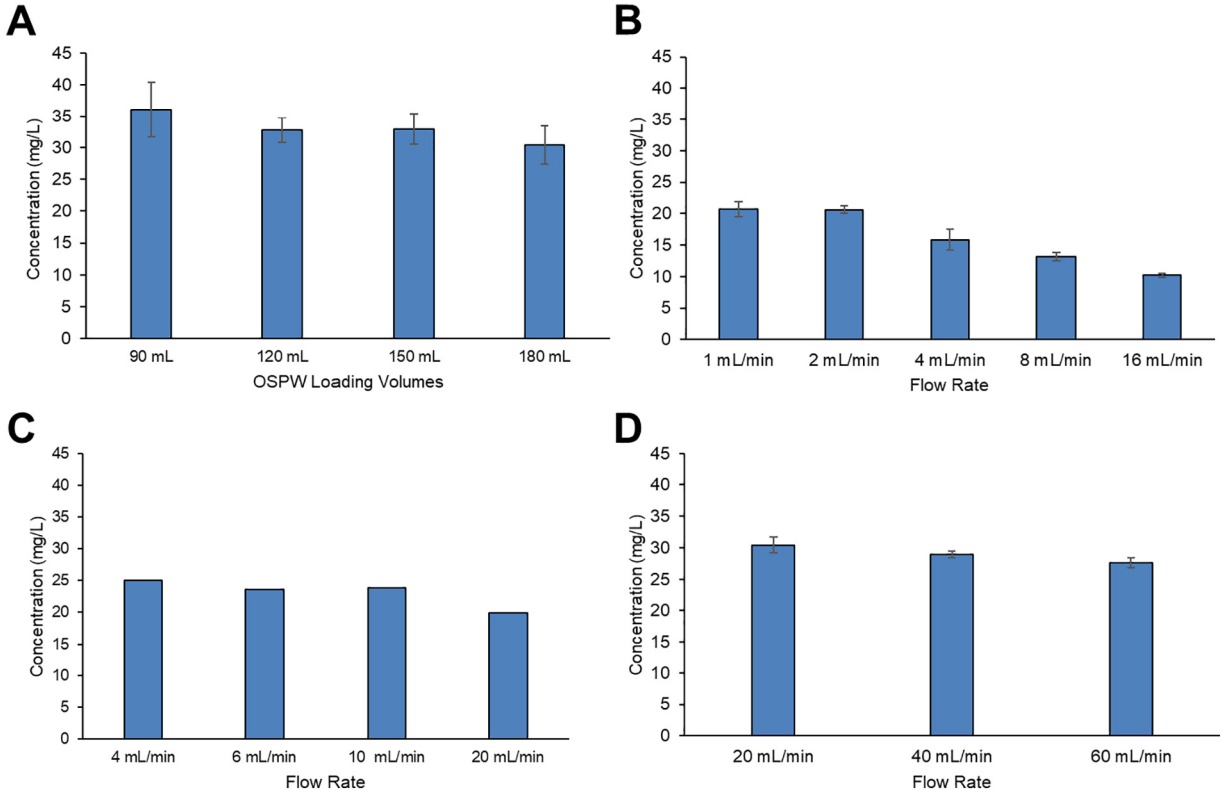

Fig. 2. Scale up of ENV+ SPE method showing dissolved organics yield (using 60 mg resin) with increasing OSPW volume (A). Scale up experiments were conducted at 1× (B), 10× (C), and 100× (D) the original 30-mL OSPW volume and 20 mg resin mass. Scale up experiments (A–D) were normalized to 1-L for comparison of dissolved organic concentrations (y-axes).

(Cole-Palmer) with a rotary vane pump head (Procon Pumps). The column was operated as an SPE cartridge where feedstock flow was directed onto the resin bed using an adjustable plunger. In place of a vacuum pump typical of SPE, a water pump was used to withdraw the sample from the sample barrel through the resin in the first column (SPE-1, Fig. 2) with negative pressure and transfer of the filtrate to a second barrel.

2.6. Stage 1: preparative neutrals and bases extraction

Using a water pump, a loading volume of 180 L of OSPW was transferred from 1000-L containers to 200-L barrels. The pH was directly measured from aliquots on a benchtop pH meter, and subsequently raised to 11.0 ± 0.5 with 10 M NaOH. Due to the fact that there were no particulates and the water was very clear, no filtration step was used. The OSPW was then mixed for approximately 1 h with a hand drill fitted with a teflon mixing rod and allowed to stand for 12 h. Following the equilibration period, the pH was adjusted, re-mixed, and left to stand for at least another 6 h until the pH was stable. The ENV + resin bed was added to the column as a slurry (120 g in 600 mL EtOAc) and was first conditioned with 1.5 L of EtOAc, a second solvent wash of 1.5 L of MeOH, and a final step with 6 L of pH 11 de-ionized (DI) water.

The column (SPE-1) was then plumbed to two barrels (one containing OSPW, one empty) in order for the OSPW to be extracted through the conditioned column and the filtrate to flow into an empty barrel. Throughout all conditioning, equilibration, and OSPW-loading steps, the solvent/water in the column was maintained at a height of 10 cm above the resin bed and the plunger at a height of 1 cm above the solvent/water to avoid disturbance of the resin. Likewise, all conditioning, equilibration, and loading steps were conducted using a flowrate of 100 ± 10 mL/min. Following extraction, the column was disassembled and the resin carefully transferred into a 4-L glass beaker. The beaker containing the resin was covered with a large Kimwipe® left in a fume hood for 12–24 h to dry.

Analytes were extracted from SPE-1 using a large soxhlet apparatus. The dried resin was split between two glass thimbles, sandwiched in each between 500 g sodium sulphate ($NaSO_4$). Analytes were extracted with 1.5 L of EtOAc in each soxhlet apparatus (3 L total) for 12 h. The soxhlet extraction method differs from standard SPE methods that involve solvent elution directly through the SPE cartridge, but still incorporates the dissolution of analytes into the solvent (discussed below). The EtOAc was pooled and filtered 4 times through 400 g $NaSO_4$ and 8 μm pore filter paper (Whatman grade 40 ashless, Sigma-Aldrich®, Oakville, ON) to remove any water, hereafter referred to as Fraction 1 (F1). The resin was then removed from the thimbles, allowed to dry, and placed in new thimbles with fresh $NaSO_4$. A second soxhlet extraction was performed as described above using 3 L of MeOH, hereafter referred to as Fraction 2 (F2).

2.7. Stage 2: preparative organic acids extraction

For extraction of more polar, and ionisable analytes from OSPW, the Stage 1 filtrate was acidified to pH 2 using HCl (12 M) in the second barrel with direct measurements by pH meter. For preparation of the SPE-2 stationary phase, 120 g fresh ENV+ resin was placed into the cleaned column, conditioned, and equilibrated as described previously for Stage 1, with the exceptions that only MeOH was used (1.5 L) and the final conditioning was with 6 L pH 2 DI water. Following SPE-2 conditioning, the acidified stage 1 filtrate was pumped from the second barrel, through the conditioned column, and back into barrel 1. The soxhlet extraction of SPE-2 was the same as Stage 1, but only required MeOH, hereafter referred to as Fraction 3 (F3). The extraction of 180 L of OSPW with 3 L of solvent for each fraction represented a 60-fold concentration of dissolved organics. Each fraction was stored at 4 °C until further use.

2.8. Chemical characterization

In addition to the 3 fractions, samples of OSPW were collected for chemical analysis at several points throughout the extraction/fractionation procedure. Samples were collected as follows: pre-Stage 1 pH 11 OSPW, Stage 1 pH 11 filtrate (after ~10 L, ~100 L, ~160 L), pre-Stage 2 pH 2 filtrate, Stage 2 pH 2 filtrate (after ~10 L, ~100 L, ~160 L). It is important to note that unlike water samples, fraction aliquots did not represent equivalent concentrations of organics, only equivalent fraction volumes. Moreover, although F1–F3 were aliquots from equivalent volumes of solvent and represented concentrated samples, all other samples were not concentrated and are only comparable to F1–F3 qualitatively. Chemical characterizations conducted on each sample employed several analytical techniques including: infusion negative-ion electrospray ionization ultrahigh-resolution mass spectrometry with Orbitrap (ESI-UHRMS), liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF) in negative-ion mode, gas chromatography triple quadrupole mass spectrometry operated in full scan mode (GC–MS), synchronous fluorescence spectroscopy (SFS), and analysis of total dissolved metals and major ions (see full details in Supplementary Information (SI #1). The resolving power of the ESI-UHRMS was >130000 and the LC-QToF was 4300. All chemical analyses were conducted in duplicate with respective solvent blanks and internal standards.

For quantitative analyses, including derivation of soluble organic concentrations, double-bond equivalents (DBE) of O_2, and ion class distributions, ESI-UHRMS with Orbitrap was conducted at Environment and Climate Change Canada's (ECCC) National Hydrology Research Centre in Saskatoon, SK according to methods outlined in Bauer et al. (2015) and described further in the Supplemental Information (SI #1). Qualitative profiling of soluble organics fractions for comparisons of relative polarity and abundance were conducted at ECCC's Canadian Centre for Inland Waters in Burlington, ON using LC-QToF and GC–MS. Additional qualitative analyses of soluble organic aromaticity using SFS was conducted at ECCC's National Water Research Institute in Burlington, ON as outlined previously (Kavanagh et al., 2009), with minor modifications which are provided in greater detail in the Supplementary Information (SI #1).

2.9. Surrogate and matrix spike validations

In order to validate the method and investigate the types of organics eluting in each fraction, two different stock solutions containing commercial organic mono-carboxylic acid standards as surrogates were fractionated at bench scale according to the finalized method. In the first stock solution, surrogate organic compounds with a range in structural and chemical properties (Table 1) were spiked into deionized water (referred to as "surrogate standards"). The second stock solution contained isotopically labelled compounds (Table 1) spiked into aged OSPW ("matrix standards"). Treatments were prepared by adding 1 mL of each spiking solution into 99 mL of deionized water or aged OSPW (100 mL total loading volume), then mixing for 10 min at 30 °C. Fractionation parameters such as resin conditioning, elution solvents and solvent orders were conducted as described previously for the preparative fractionation above. Specifically, SPE cartridges were packed with 67 mg of ENV+ resin. All resin was conditioned and standards eluted with 10 mL of appropriate solvents at a rate of 1 mL/min. For further quality assurance, a method blank was included and all treatments were run in triplicate. For chemical analysis, each 10 mL fraction generated was evaporated under N_2 in a 30 °C water bath to dryness and reconstituted in 1 mL of MeOH. Samples were then analysed using a LC-QToF according to parameters described in Supplementary Information (SI #1). Recoveries were determined by comparison of sample results against the original spiking solutions.

Table 1
Method recoveries of surrogate standards spiked into deionized water and isotopically labelled standards spiked into an aged OSPW matrix. Mean recoveries ± standard error were derived from LC-QToF analysis against the spiking solutions for each fraction which were then summed for a total method recovery. Molecular masses provided are for the acid form of each compound.

Compound	Molecular formula	Molecular mass (g/mol)	Retention time (min)	Recoveries (%)[a]			
				F1	F2	F3	Total
Surrogate standards							
3,4-Dihydroxybenzoic acid	$C_7H_6O_4$	154.12	3.05	–	–	26.8 ± 0.5	26.8
Adipic acid	$C_6H_{10}O_4$	146.14	4.26	–	–	44.4 ± 5.4	44.4
3-Thiopheneacetic acid	$C_6H_6O_2S$	142.17	7.15	–	–	80.1 ± 5.8	80.1
1,4-Cyclohexanedicarboxylic acid	$C_8H_{14}O_2$	172.18	7.62	–	–	82.7 ± 1.4	82.7
3-Methyl-2-thiophenecarboxylic acid	$C_6H_6O_2S$	142.17	9.74	–	–	114.2 ± 7.9	114.2
Cyclohexane carboxylic acid	$C_7H_{12}O_2$	128.17	11.15	–	–	16.4 ± 0.5	16.4
Diphenic acid	$C_{14}H_{10}O_4$	242.22	11.43	0.3 ± 0.0	1.2 ± 0.0	109.7 ± 1.2	111.2
2-Naphthylacetic acid	$C_{12}H_{10}O_2$	186.21	12.82	1.7 ± 0.2	7.2 ± 0.2	66.1 ± 2.0	75.0
5-(2-Thienyl) pentanoic acid	$C_9H_{12}O_2S$	184.26	13.03	3.4 ± 0.3	3.7 ± 0.2	64.2 ± 3.4	71.3
3-Cyclopentylpropionic acid	$C_8H_{14}O_2$	142.20	13.29	–	–	36.0 ± 3.3	36.0
Decanoic acid	$C_{10}H_{20}O_2$	172.26	16.91	74.1 ± 2.2	17.9 ± 1.3	9.1 ± 0.8	101.1
Cyclohexanepentanoic acid	$C_{11}H_{20}O_2$	184.28	16.93	75.5 ± 3.6	15.4 ± 2.2	8.5 ± 1.0	99.4
Dehydroabietic acid	$C_{20}H_{28}O_2$	300.44	19.07	95.1 ± 1.9	21.1 ± 0.8	2.7 ± 1.1	118.9
Labelled standards							
Benzoic-d_5 acid	$C_7HD_5O_2$	122.12	8.72	–	–	93.6 ± 2.4	93.6
9-Anthracene-d_9-carboxylic acid	$C_{15}HD_9O_2$	222.24	13.76	6.8 ± 2.7	30.0 ± 2.2	75.3 ± 0.9	112.1
Decanoic-d_{19} acid	$C_{10}HD_{19}O_2$	172.27	16.81	21.3 ± 3.0	8.0 ± 1.4	2.4 ± 1.0	31.7

[a] Fraction percentage recoveries are means of three replicates.

3. Results and discussion

3.1. Extraction method development and scale-up

The objective of this study was to develop a robust extraction method suitable for the range of bitumen influenced sources in the Athabasca oil sands region that would also generate sufficient quantities of isolated organics for toxicological evaluations using a suite of test organisms. Bench-scale experimentation evaluated an acid/base precipitation method, solid phase settling/flocculent method, and a SPE method, and identified SPE using ENV+ resin as the method that provided optimal yields of dissolved organics. Although the settling/flocculent approach generally provided better results than the acid precipitation method (Fig. 1A), the SPE method provided the best recovery overall regardless of resin type (Fig. 1B). Based on these results, the SPE method was selected for further experimentation to determine the best resin. Throughout the SPE extraction tests, the ENV+ stationary phase outperformed all other resins tested, displaying the most consistently high recovery of organics (Fig. 1C) and diminishing loss associated with breakthrough (Fig. 1D), measured using high-resolution electrospray ionization mass spectrometry methods described in Supplementary Information (SI #1). In applying an SPE approach, separation of inorganics from organics was initially achieved. The result was an extraction method which utilized ENV+ resin as a stationary phase to recover dissolved organics from acidified aged OSPW, which were then eluted with methanol.

This method allowed for simple isolation of organics from the initial aged OSPW matrix and also provided the opportunity for fractionation. In so doing, it also avoided large solvent volumes associated with liquid/liquid extractions on a preparative scale, was amenable to scale-up, and a small-scale variation of this approach was already routinely used for sample clean-up prior, and directly during, NA analysis of OSPW samples (Verbeek et al., 1993; de Campos et al., 2006; Gagné et al., 2011; Headley et al., 2013a). Furthermore, a study that compared different solvents and SPE systems for the extraction of dissolved organics from OSPW reported that the ENV+ resin provided the highest recovery and was able to capture the greatest range in different oxygenated organic species (Headley et al., 2013a).

Experimental data from dissolved organic extraction methods are presented in Fig. 1 with dilution factors incorporated, for within-graph comparison. Initial applications of the original acid precipitation method to aged OSPW produced very low recoveries of bitumen-derived dissolved organics (20 mg/L) as determined by a mass balance approach and hi-res electrospray analysis (Fig. 2A, B). As the original method (Frank et al., 2006) was developed for isolating the acid-extractable organics from fresh OSPW, namely O_2, we hypothesize that the lower recovery for the aged source was due to compositional differences that occurred during aging. The first set of bench-scale experiments determined that the use of a resin dispersed in aged OSPW achieved >10-fold greater recovery (208 mg/L vs 19.8 mg/L) than the original method (Fig. 1A). Utilizing the adsorbent resins as a stationary phase for SPE achieved up to 16-fold greater recovery (519 mg/L) than the acid precipitation method (32 mg/L) (Fig. 1B). In order to determine which stationary phase resin possessed the highest capacity, 60 mg of the four resins were subjected to increasing OSPW volumes. The results for the ENV+ phase were superior, as it maintained the highest recoveries with increasing OSPW volume, exhibited low breakthrough and with little variability (Fig. 1C, D).

Experimental data assessing ENV+ capacity with increasing OSPW loading volume and flow rates are presented in Fig. 2 and are standardized to whole OSPW volumes to enable comparisons. In order to determine the resin capacity of ENV+ for dissolved organics, 60 mg was used to extract organics from increasing loading volumes of OSPW (90 mL, 120 mL, 150 mL, 180 mL) (Fig. 2A). From 120 mL to 150 mL, and 90 mL to 120 mL OSPW loading, dissolved organic recoveries dropped 2.6 mg/L and 3.2 mg/L, respectively (Fig. 2A). The 90 mL loading volume displayed the highest dissolved organics recovery overall (36 mg/L). Therefore, a conservative ratio of OSPW (mL) to resin (mg) of 3:2 was selected.

Finally, loading and elution flow rates were optimized. These were conducted as part of the scale-up work and were initiated using the 3:2 OSPW:resin ratio. As scale-up increased to 10× and 100×, the optimal flow rate chosen was 10 mL/min and 20 mL/min, respectively (Fig. 2B,C,D). Optimal flow rates did not increase linearly with OSPW volume and resin weight, likely due to changes in resin bed dimensions changing OSPW linear velocity, which required slower relative flow rates to prevent breakthrough (International Sorbent Technologies, 2001). These assessments determined that at the bench scale a conservative flow rate of 1 mL for every 30 mL of OSPW could be sufficiently processed every minute, provided the previously established resin mass ratio was used.

3.2. Fractionation method development

The fractionation method was designed to separate organic compounds by utilizing two properties; ionization states, and polarity. The alkaline extraction was performed first based on the great body of literature that shows organic acids as the primary toxic components in OSPW and comprise the greatest proportion of dissolved organics. By employing the alkaline extraction first, organic acids remain in solution, preventing fouling of the solid phase, resulting in efficient adsorption of basic and neutral substances and an optimal separation of all classes (Fig. 3).

The following briefly explains the experimentation leading to the final fractionation method, which is described in detail in the Methods and materials section above. With the development of an extraction procedure complete, subsequent experiments were conducted to develop a preparative scale fractionation method. Fractionation parameters such as conditioning/extraction solvents, extraction solvent order, flow rates, and number of stages (columns) were optimized. Specifically, extraction solvents hexane, EtOAc, and MeOH, representing a range in polarities, were assessed for their ability to recover dissolved organics and generate chemically distinct fractions. These were applied to a Stage 1 (pH 11) and Stage 2 (pH 2) OSPW treatment (Fig. 3) in order of increasing polarity (Hexane < EtOAc < MeOH). Samples from each solvent application were analysed using GC–MS and LC-QToF to determine overlap in fraction chemical composition, while ESI-UHRMS determined areas of potential loss in yield. This experimentation identified hexane as a poor extraction solvent with low recovery, resulting in its exclusion. Stage 1 extraction with EtOAc and MeOH generated distinct fractions containing an appreciable amount of organics. Conversely, extraction with EtOAc followed by MeOH in Stage 2 resulted in low dissolved organic recovery in both elutions. This experimentation identified that MeOH alone was able to capture the remaining dissolved organics in Stage 2.

Extraction of organic compounds from the solid phase resin was performed using a soxhlet apparatus. The soxhlet extraction allowed for continual extraction of organics from the resin over a longer duration ($12h$) compared to elution, as described in the final method above (Fig. 1). Quantitative analysis of resulting fractions confirmed scale up experiments (Fig. 3) that identified a 3:2 ratio of OSPW volume: resin weight capable of optimal recovery. At very conservative resin weights (60 mg resin for 30 mL OSPW), previous experimentation determined that re-use of resin up to three times resulted in loss of recovered organics and increased variability in yield (SI #2). Due to the modification to a soxhlet extraction step, Stage 1 and Stage 2 were therefore conducted using two separate batches of ENV+ resin. This ensured that the resin capacity was maintained throughout the procedure by reducing potential loss of organics associated with re-use of resin.

In order for organic compounds to be adsorbed, the resin required a conditioning step. Conditioning of the resin was performed using 1.5 L of the same solvents in the same order as applied for elution as described by solid phase resin manufacturers (International Sorbent Technologies, 2001; Argonaught Technologies, 2002). The conditioning solvent volume was determined by adherence to International Sorbent Technologies (IST) guidelines of 1–2 mL for every 100 mg of resin. Thus, the use of 120 g of resin in the final method allowed for 1.2–2.4 L of conditioning solvent. As further described by the IST guidelines (2001), during elution the resin should be saturated by the solvent for at least 1–4 min regardless of flow rate. This recommendation, in addition to flow rates established in the scale-up, guided conditioning and sample loading flow rates for the preparative-scale fractionation. Thus, for conditioning the resin, a flow rate for 1.5 L of solvent to saturate the resin for 4 min during conditioning allowed a flow rate of

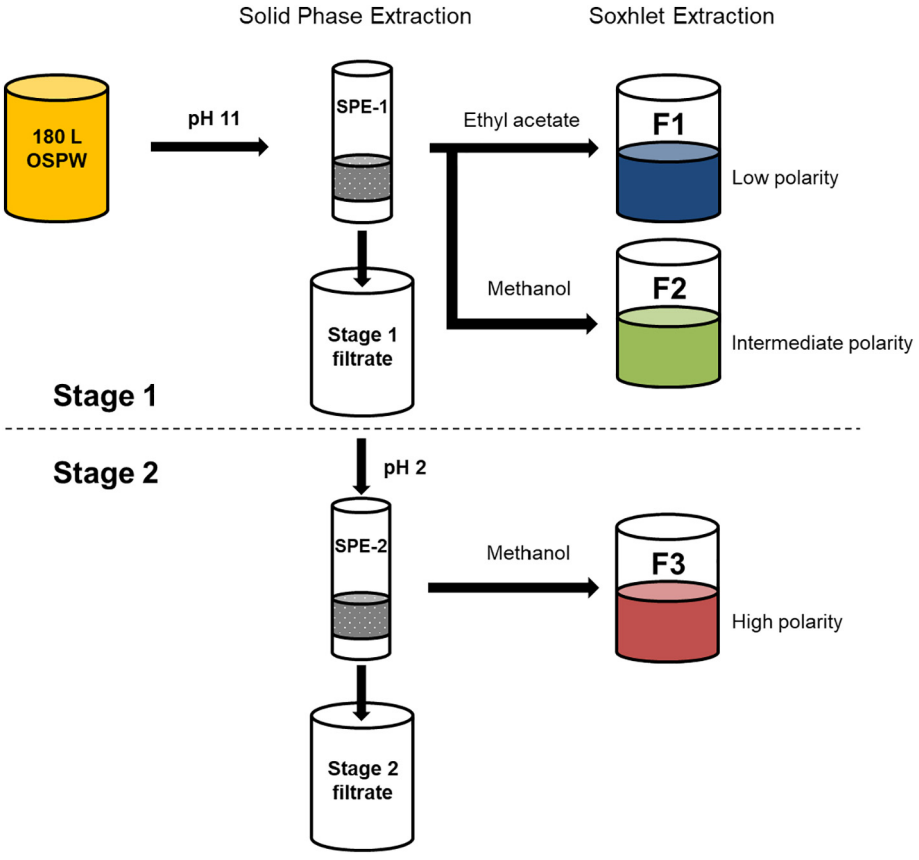

Fig. 3. Fractionation method schematic displaying Stage 1 and Stage 2 SPE loading followed by soxhlet extraction using solvents indicated. The fractionation resulted in the generation of fractions containing dissolved organic constituents of relative lower polarity (F1), intermediate polarity (F2), and higher polarity with ionisable protons (F3).

375–1500 mL/min. Consequently, a conservative flow rate of 100 mL/min was adopted for solvent conditioning of resin and within apparatus allowances. Resin manufacturer sample loading rates are typically provided for much lower sample volumes and resin weights, and are most dependent on resin bed height and width, which determine sample linear velocities. For this reason, IST (2001) and Argonaught (2002) guidelines suggest increasing flow rates until breakthrough of analytes is observed. Although previous scale-up experiments identified that loading of 300 L of OSPW could be processed at a flow rate of 10 L/min, this was not feasible with the apparatus set-up. Instead, IST (2001) guidelines which suggested 10–120 mL/min flow rates for a 6 mL sample were incorporated. For a conservative approach to the methodology and to maintain consistency with conditioning rates, a loading rate of 100 mL/min was applied. This new flow rate demonstrated the ability to capture organics at concentrations exceeding those observed in scale-up experiments as confirmed by ESI-UHRMS.

The resulting pH adjustments, solvents used, and solvent order were incorporated into the final method, as described in Methods and Materials (Fig. 3). Briefly, in Stage 1, ENV+ resin was conditioned using 1.5 L EtOAc and MeOH at a flow rate of 100 mL/min, followed by a pH 11 water wash. OSPW at pH 11 was then loaded onto the column and pumped through at a flow rate of 100 mL/min. In Stage 2, a fresh batch of ENV+ resin was conditioned using MeOH, followed by a pH 2 water wash at parameters identical to Stage 1. Stage 1 filtrate was then acidified (pH 2) and passed through the preconditioned ENV+. The ENV+ resin from Stage 1 and Stage 2 were soxhlet extracted for 12 h for each solvent, using 3 L of EtOAc/MeOH (separately) and MeOH, respectively. These extractions generated three fractions described herein as F1, F2, and F3. Methodology for separation of the dissolved organic analytes resulted in F1, F2, and F3 containing organics ranging from least polar (neutrals and organic bases), intermediate polarity, and most polar constituents (with acidic protons), respectively (Fig. 4).

3.3. Method validation

For quality assurance at the preparative scale, a complete fractionation method blank was undertaken using 180 L of deionized water. Analysis of samples from the method blank using LC-QToF and GC–MS displayed no detectable components above those within solvent blanks (SI #3). For further quality assurance and assessment of repeatability, the preparative scale OSPW fractionation was run in duplicate. There were no considerable differences between the first and second fractionation of Pond 9 OSPW as observed by LC-QToF (SI #4), GC–MS, and ESI-UHRMS. Although presented singly herein, all chemical analyses were run in duplicate which were not different in all cases.

To validate the method and investigate the type of organic compounds recovered in each fraction, authentic standards of various organic acids were fractionated at bench scale (100 mL sample volume). In two separate experiments, a method spike containing 13 standards was added to deionized water while a matrix spike containing 3 deuterated standards in MeOH was added to the aged OSPW. Table 1 displays the relative mean recoveries in each fraction as well as the total recoveries across fractions. Surrogates are listed in ascending order of LC-QToF retention time, where longer retention on the instrument reverse phase column indicates lower polarities. The decrease in relative compound polarities coincides with relative abundance in each fraction, as more polar and acidic compounds are more abundant in F3, while F1

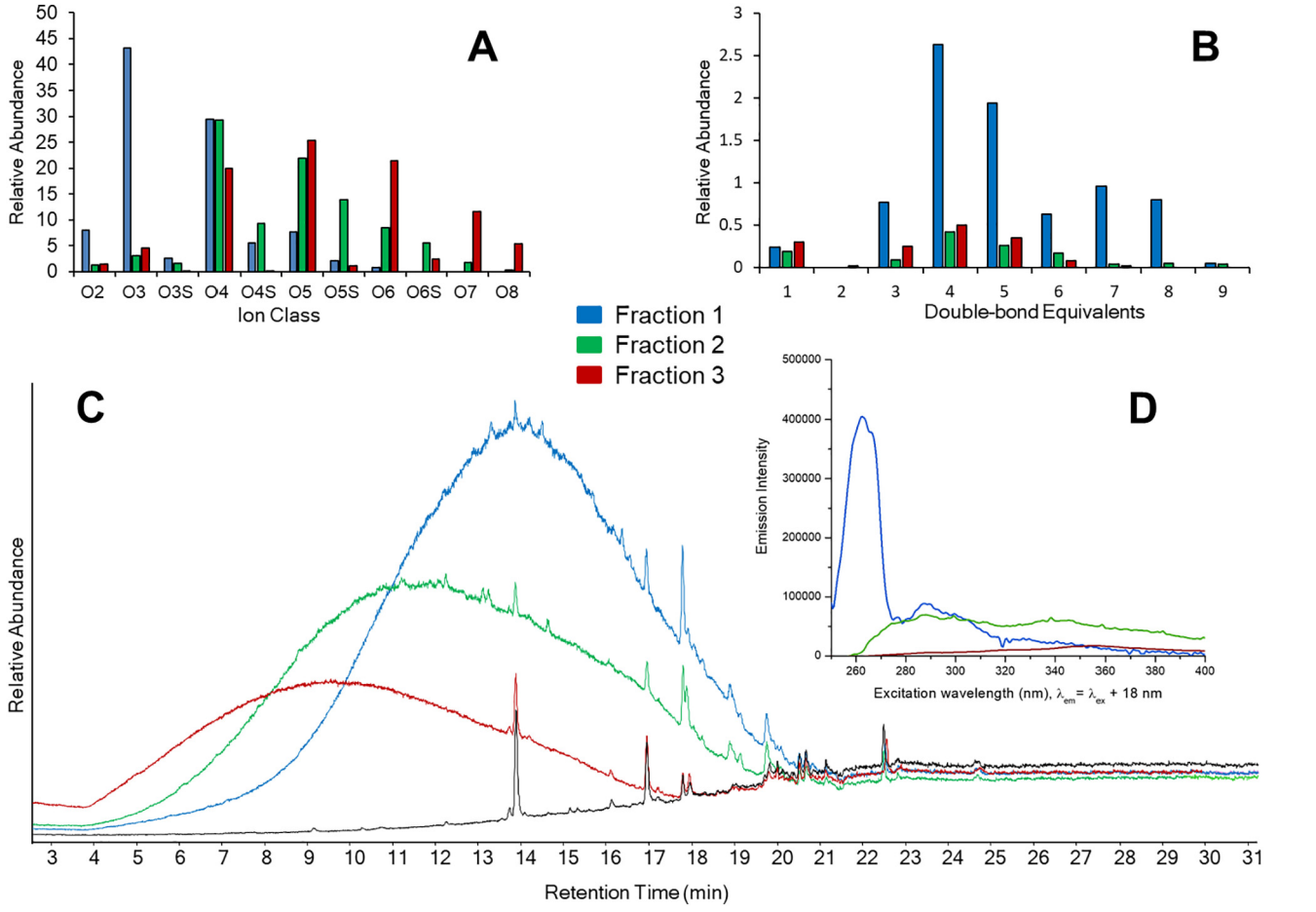

Fig. 4. Fraction characterization of F1–3 isolated from aged OSPW. Chemical properties analysed for F1–3 include ion class distribution for those species with >2% contribution (A), relative abundance of double-bond equivalents of O_2 ions (B), LC-QToF total ion chromatograms (C), and SFS fluorescence spectra indicating degree of aromaticity (D).

contains lower polarity compounds. This observation verifies the fractionation methodology in which subsequent fractions were designed to capture dissolved organics with increasing polarity. The majority of the compounds in the standard spike experiment displayed >70% recovery with the exception of 3,4-dihydroxybenzoic acid (26.8%), adipic acid (44.4%), cyclohexane carboxylic acid (16.4%), and 3-cyclopentylpropionic acid (36%). Overall, recoveries were very good (average 76%) and separation by acidity and polarity was demonstrated. In the matrix spiking experiment, two of the 3 labelled compounds displayed recoveries >90% (Benzoic-d_5 acid and 9-anthracene-d_9-carboxylic acid), while only 31% of the Decanoic-d19 acid standard was recovered. This low recovery for Decanoic-d_{19} acid may be due to its low water solubility and potential adsorption onto matrix components as the method recovery for native decanoic acid was optimal. Overall, both standards fractionation experiments validated that the method does show separation of organic compounds based on acidity and polarity with adequate recoveries of the acids.

Interestingly, results from the method validation experiments indicated that in addition to F3, acidic compounds were captured in F1 and F2, which incorporated a pH 11 pre-loading adjustment to sample water. With this adjustment, it would be expected that only neutral-basic compounds would be un-ionized, allowing adsorption to the resin. However, although Stage 1 involved an alkaline extraction, the resin was pre-conditioned to adsorb organic compounds, which was observed herein (Supplemental Information, SI#7). For example, class distribution data revealed that the majority of O_2 compounds (presumably including NA) were present in the high pH fraction F1. A similar fractionation study which utilized pH adjustments and SPE extractions, also observed an abundance of dissolved organics consistent with NA isolated in a high pH fraction (Morandi et al., 2015).

The analysis of major ions and metals was performed on water samples taken directly from pre- and post- Stage 1 and 2 steps in the procedure, providing a means to track inorganics in the method (SI #5). The fractionation design provided for the removal of soluble organics, with the final filtrate theoretically containing all inorganic species. The relatively constant concentration of all major ions and metals from unaltered OSPW to final Stage 2 filtrate after Stage 2 (SI #5), supports this assertion. The exceptions to this were sodium and chloride. Sodium increased slightly in the pre-Stage 1 sample and chloride increased substantially in the pre-Stage 2 sample, because NaOH was used to raise the pH prior to Stage 1 and HCl was used to acidify prior to Stage 2. The fact that all other ion/metal concentrations were relatively unaltered, and those that were could be accounted for in the final filtrate following SPE Stage 2, indicate the method was specific in its recovery of dissolved organics.

3.4. Fraction characterization

Chemical characterization of aged OSPW fractions consisted of a suite of instrumental applications including ESI-UHRMS which provided mass-charge distribution, ion class distribution, and double-bond equivalents (of O_2 ions), such as classical naphthenic acids. Additionally, GC–MS and LC-QToF profiling was conducted along with SFS, which provided an analysis of aromaticity.

Table 2
Concentration of dissolved organics in fractions and filtrate of aged OSPW determined by ESI-UHRMS. Values represent concentrations based on original 180 L sample.

	Dissolved organics	
	Concentration (mg/L)	Contribution (%)
Fraction 1	5.4	15.6
Fraction 2	0.4	1.1
Fraction 3	27.7	79.5
Post-stage 2 filtrate	1.3	3.8
Total	34.8[a]	100.0

[a] Represents a derived theoretical recovery based on all other measured values.

Dissolved organic concentrations for each fraction and the final filtrate were measured by ESI-UHRMS analysis (Table 2). Data indicate that the fractionation procedure was able to capture 96.2% of dissolved organics detectable by ESI-UHRMS with calculated 3.8% loss via breakthrough. Breakthrough represents a loss in recovery due to constituents which did not adsorb to the resin and, therefore, passed through at both stages. This breakthrough/loss was confirmed and quantified by a separate extraction of Stage 2 filtrate using SPE and extraction with MeOH. The distribution of dissolved organics could not be assigned with acceptable error using ESI-UHRMS analysis and is, therefore, not presented. In the present study, breakthrough was likely due to a combination of the resin reaching capacity in the area of the resin bed with highest linear velocity or the presence of very polar compounds. The bulk of dissolved organic compounds were captured in F3 (most polar, including ionisable protons) which comprised 79.5% of all organics observed by ESI-UHRMS, while only 1.1% were captured in F2 (intermediate polarity).

For determination of ion class distribution and DBE, ESI-UHRMS analysis was performed on the three generated fractions. This high-resolution analysis has been successfully used for analysis of bitumen-derived dissolved organics in previous research (Scarlett et al., 2013; Marentette et al., 2015; Morandi et al., 2015; Bauer et al., 2017). Generally, high-resolution analysis has been identified as producing more sensitive concentration determinations compared to low-resolution analyses such as FTIR and GC–MS (Brown and Ulrich, 2015). For this reason, and for comparison with some of our previous work (Marentette et al., 2015; Bartlett et al., 2017; Bauer et al., 2017), ESI-UHRMS was conducted. Infusion experiments with ESI-UHRMS showed qualitative differences between fraction spectra (SI #6). A comparison of sample mass spectra revealed very similar distributions between fractions with the majority of the compounds ranging from 200 to 400 m/z, analogous to observations made in previous work (Bauer et al., 2015). The notable exception was a minor “hump” appearing between 0 and 200 m/z in F3. The relative electronegativity of oxygen means that, in similar compounds, those containing more oxygen atoms are likely relatively more polar. However, this is dependent on the positions of oxygen atoms. Nonetheless, for class distribution data, F1 was comprised of predominantly O_3 and O_4 ions, F2 was dominated by O_4, O_5, and O_5S, and F3 displayed a major contribution from O_4, O_5, and O_6 ions (Fig. 4A). All fractions displayed minor contributions (<10% each) from O_2 (classical NA), and sulfur-containing ions (O_3S, O_4S, and O_5S). Degree of oxygenated ions increased with fraction number with F1, F2 and F3 displaying no oxygen class ions greater than O_6, O_7, and O_9, respectively (Fig. 4A). The relative increase in degree of oxygenation with fraction number suggests the methods ability to separate dissolved organics based on polarity and acidity.

Recent research (Ajaero et al., 2017) has suggested that WAX (weak anion-exchange) resin may provide a slightly improved recovery of O_2 classes relative to ENV+, however these differences appear to be minimal. Taken collectively and considering the margin of error, ENV+ was essentially shown to be equivalent to WAX in recovering the broad range of species within the dissolved organic mixture that was measured and may represent an alternate solid phase for extractions. In addition, our previous work identified a predominance of O_2 classes in fresh tailings using an ENV+ SPE cleanup (Bauer et al., 2015), therefore, the low relative contribution of O_2 ions observed for the aged source is likely due to compositional changes that occur through aging.

DBE analysis represents the degree of unsaturation of a molecule due to a double-bond, but can also signify a ring formation and degree of aromaticity. In the present analyses, only the O_2 class DBE were examined and the DBE are a percentage abundance relative to the total abundance of O_2 species (the total percent DBE equals the percent O_2 for class distribution; Fig. 4B). DBE data for F1-F3 ranged from 1 to 9 for F1 and F2, and 1–7 for F3 (Fig. 4B). Given that F1 contained a greater abundance of O_2 species compared to F2 and F3, it is no surprise that F1 contains a greater overall abundance of O_2 DBE. All three fractions

display the greatest percent abundance at DBE 4 and second highest at DBE 5.

LC-QToF, GC–MS, and ESI-UHRMS analyses verified the method's ability to isolate fractions with varying polarities. Negative ion electrospray LC-QToF profiles of fractions F1–3 are shown above in Fig. 4C. For the reverse phase LC conditions employed, the total ion chromatograms reveal the polarity differences exhibited by the fractions. All fractions present as individual complex mixtures that are chromatographically unresolved. The maxima of each fraction nevertheless are indicative of the differences in polarities of the components, with F3 maxima eluting first (most polar), F2 maxima intermediate (intermediate polarity) and F1 maxima eluting last (least polar). While it is possible for individual compounds to be present in one or more of the fractions, the differences apparent in these profiles suggest some degree of separation was achieved. Thus, we interpret the data as showing that from F1–F3, compound polarity increased, with ionisable compounds contained in F3. The LC-QToF results are supported by those obtained by GC–MS (SI #7). Fraction F1 exhibited the greatest signal intensities of the three fractions, consistent with its content of neutral dissolved organics obtained from pH 11 Stage 1 extraction and solubility in EtOAc (SI #7a). Although not present in the final procedure, a fourth EtOAc soxhlet extraction (final filtrate) step in the first Pond 9 fractionation was included (orange chromatogram in Supplementary Information, SI #7b). This extract clearly displayed several peaks between 6.6 and 7.5 min which were identified as phthalates and likely resulted from handling and possibly the OSPW storage barrels used during fractionation as these were observed in blanks prior to use. The presence of phthalates in the final Stage 2 EtOAc extraction verified that they were likely not present in other fractions and resulted in the exclusion of that extraction step.

Fig. 4D inset displays the SFS profiles of the three fractions. The excitation wavelength of F1 exhibits a narrow peak at ~265 nm, F2 displays a slightly bimodal plateau ranging from 260 to 400+ nm, and F3 displays a broad peak at ~360 nm. The SFS data suggests that from F1 – F3 there is an increase in the degree of aromaticity, and a reduction in abundance of aromatic compounds. According to previous SFS analyses on parent PAHs and other bitumen-influenced waters (Kavanagh et al., 2009; Rowland et al., 2011), F1 fluorescence appears to be composed primarily of monoaromatic compounds similar to toluene, while F2 and F3 contain mono- and polyaromatic compounds similar to naphthalene, fluorine, and anthracene. Finally, increases in fraction polarity were substantiated by ESI-UHRMS class distribution data (Fig. 4A) which displayed increases in degree of oxygenation, described in detail above. Because both aromatic content and oxygen content increases the polarity of a compound, both class distribution (ESI-UHRMS) and SFS data appeared to verify observations from LC-QToF and GC–MS. As high recovery of bitumen-derived dissolved organics was the primary objective for this method, losses due to breakthrough were assessed. ESI-UHRMS analysis of bench scale extraction alluded to possible breakthrough (Fig. 2D). It is, therefore, likely that the final filtrate (Stage 2) at the preparative scale contained some degree of breakthrough as well. We accounted for this possibility by characterizing samples from Stage 2 filtrate. GC–MS (SI #7b), LC-QToF (SI #8), and ESI-UHRMS (SI #9) analyses of post-Stage 2 samples indicate very low levels of organic content compared to method blanks. Analysis by ESI-UHRMS analysis showed that organics detected in the Stage 2 filtrate comprised <4% of the total (Table 2). This low level of breakthrough is consistent with ENV+ scale-up data (average 6.6 ± 7.2%).

It is understood that fraction analysis was limited by characterization in ESI negative-ion mode (ESI-) only. Positive-ion detection (ESI +) has been used in previous studies (Barrow et al., 2010; Headley et al., 2013b; Morandi et al., 2015), and has identified profile differences associated with compound classes (e.g. heteroatom containing species). Historically, organic acids have been identified as the components of primary toxicological concern in OSPW (e.g. Frank et al., 2008), and thus ESI- was used as a logical starting point. As such, the method was initially developed to fractionate organic acids, but in so doing also isolates neutrals and bases, as evidenced by GC–MS profiling (Supplemental Information, #S7). Accordingly, analyses using ESI+ can be incorporated in future studies, particularly as EDA investigations utilizing this protocol progress to characterizations warranting detailed profiling of toxic constituents.

4. Conclusion

According to theoretical design and analysis of data following fractionation, the procedure successfully separated organic compounds based on pH and polarity. The procedure performed well upon scale up, and was able to process 180 L of aged OSPW in 10 days. The LC-QToF data identified the three fractions as having a range in polarity from the least polar F1 fraction to the most polar F3 (Fig. 4C), with GC–MS data showing F1 contained the bulk of neutral organics (SI #7). The relative polarity of the fractions is also illustrated by ESI-UHRMS analysis, with respect to degree of oxygenated compounds, which increases from F1 – F3 (Fig. 4A). The success of the pH/polarity based fractionation is further substantiated by SFS data (Fig. 4D) which indicate that the greatest abundance of aromatic compounds appear in the least polar F1, as aromatics are generally nonpolar compounds. The success of the developed fractionation method allows for future work related to oil sands OSPW characterization. To further demonstrate the utility of this procedure, our future research will include processing large quantities of bitumen-influenced waters from a variety of sources for the creation of reference materials. With this method in place, we can now begin to identify the potential compounds responsible for toxicity and if these are consistent between industrial and natural sources (Bauer et al., 2019). To that end, our current research includes determining the relative toxicities of each fraction to a variety of aquatic species. An advantage of this method lies in the potential for each primary fraction to be sub-fractionated further using methods that require large volumes in an EDA approach.

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Appendix A. Supplementary Information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.244.

References

Ajaero, C., McMartin, D.W., Peru, K.M., Bailey, J., Haakensen, M., Friesen, V., Martz, R., Hughes, S.A., Brown, C., Chen, H., McKenna, A.M., Corilo, Y.E., Headley, J.V., 2017. Fourier transform ion cyclotron resonance mass spectrometry characterization of Athabasca oil sand process-affected waters incubated in the presence of wetland plants. Energy Fuel 31, 1731–1740.

Alberta Energy, 2013. Oil Sands Tailings Facts Sheet.

Allen, E.W., 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. J. Environ. Eng. Sci. 7, 123–138.

Argonaught Technologies, 2002. Step by Step Guide to SPE Method Development.

Armstrong, S.A., Headley, J.V., Peru, K.M., Germida, J.J., 2009. Differences in phytotoxicity and dissipation between ionized and nonionized oil sands naphthenic acids in wetland plants. Environ. Toxicol. Chem. 28 (10), 2167–2174.

Barrow, M.P., Headley, J.V., Peru, K.M., Derrick, P.J., 2009. Data visualization for the characterization of naphthenic acids within petroleum samples. Energy Fuel 23, 2592–2599.

Barrow, M.P., Witt, M., Headley, J.V., Peru, K.M., 2010. Athabasca oil sands process water: characterization by atmospheric pressure photoionization and electrospray ionization fourier transform ion cyclotron resonance mass spectrometry. Anal. Chem. 82, 3727–3735.

Bartlett, A.J., Frank, R., Gillis, P.L., Parrott, J., Marentette, J.R., Brown, L.R., Hooey, T., Vanderveen, R., McInnis, R., Brunswick, P., Shang, D., Headley, J.V., Peru, K.M., Hewitt, L.M., 2017. Toxicity of naphthenic acids to invertebrates: extracts from oil sands process-affected water versus commercial mixtures. Environ. Pollut. 227, 271–279.

Bataineh, M., Scott, A.C., Fedorak, P.M., Martin, J.W., 2006. Capillary HPLC/QTOF-MS for characterizing complex naphthenic acid mixtures and their microbial transformation. Anal. Chem. 78, 8354–8361.
Bauer, A.E., Frank, R.A., Headley, J.V., Peru, K.M., Hewitt, L.M., Dixon, D.G., 2015. Enhanced characterization of oil sands acid-extractable organics fractions using electrospray ionization-high-resolution mass spectrometry and synchronous fluorescence spectroscopy. Environ. Toxicol. Chem. 34 (5), 1001–1008.
Bauer, A.E., Frank, R.A., Headley, J.V., Peru, K.M., Farwell, A.J., Dixon, D.G., 2017. Toxicity of oil sands acid-extractable organic fractions to freshwater fish: *Pimephales promelas* (fathead minnow) and *Oryzias latipes* (Japanese medaka). Chemosphere 171, 168–176.
Bauer, A.E., Hewitt, L.M., Parrott, J.L., Bartlett, A.E., Gillis, P.L., Rudy, M.D., Vanderveen, R., Barrett, S.E., Campbell, S.D., Brown, L., Deeth, L.E., Farwell, A.J., Dixon, D.G., Frank, R.A., 2019. The toxicity of organic fractions from aged oil sands process-affected water to aquatic species. Sci. Tot. Environ. 669, 702–710.
Borgund, A.E., Erstad, K., Barth, T., 2007. Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils. J. Chromatogr. A 1149, 189–196.
Brack, W., Ait-Aissa, S., Burgess, R.M., Busch, W., Creusot, N., Di Paolo, C., Escher, B.I., Hewitt, L.M., Hilscherova, K., Hollender, J., Hollert, H., Jonker, W., Kool, J., Lamoree, M., Muschket, M., Neumann, S., Rostkowski, P., Ruttkies, C., Schollee, J., Schymanski, E.L., Schulze, T., Seiler, T.-B., Tindall, A.J., Umbuzeiro, G.D.A., Vrana, B., Krauss, M., 2016. Effect-directed analysis supporting monitoring of aquatic environments - an in-depth overview. Sci. Total Environ. 544, 1073–1118.
Brown, L.D., Ulrich, A.C., 2015. Oil sands naphthenic acids: a review of properties, measurement, and treatment. Chemosphere 127, 276–290.
Clemente, J.S., Fedorak, P.M., 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60, 585–600.
Clemente, J.S., MacKinnon, M.D., Fedorak, P.M., 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environ. Sci. Technol. 38, 1009–1016.
de Campos, M.C.V., Oliveira, E.C., Filho, P.J.S., Piatnicki, C.M.S., Caramão, E.B., 2006. Analysis of tert-butyldimethylsilyl derivatives in heavy gas oil from brazilian naphthenic acids by gas chromatography coupled to mass spectrometry with electron impact ionization. J. Chromatogr. A 1105, 95–105.
Energy Resources Conservation Board and Canadian Environmental Assessment Agency, 2011. Report of the Joint Review Panel Established by the Federal Minister of the Environment and the Energy Resources Conservation Board - Decision 2011–005: Total E&P Joslyn Ltd., Application for the Joslyn North Mine Project.
Frank, R.A., Kavanagh, R., Burnison, B.K., Headley, J.V., Peru, K.M., Van Der Kraak, G., Solomon, K.R., 2006. Diethylaminoethyl-cellulose clean-up of a large volume naphthenic acid extract. Chemosphere 64, 1346–1352.
Frank, R.A., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Solomon, K.R., 2008. Toxicity assessment of generated fractions from an extracted naphthenic acid mixture. Chemosphere 72, 1309–1314.
FTFC, 1995. Fine tails and process water reclamation. Advances in Oil Sands Tailings Research. Alberta Department of Energy, Oil Sands and Research Division, Edmonton, AB, Canada, FTFC (Fine Tailings Fundamentals Consortium). II.
Gagné, F., André, C., Douville, M., Talbot, A., Parrott, J.L., McMaster, M.E., Hewitt, L.M., 2011. An examination of the toxic properties of water extracts in the vicinity of an oil sand extraction site. J. Environ. Monit. 13, 3075–3086.
Government of Alberta, 2017. Environmental Protection and Enhancement Act - Revised Statutes of Alberta 2000 Chapter E-12 (Current as of March 31, 2017).
Grbovic, L., Pavlovic, K., Prekodravac, B., Kuhajda, K., Kevresan, S., Popsavin, M., Milic, J., Cirin-Novta, V., 2012. Fractionation of complex mixtures of naphthenic acids, their characterization and biological activity. Journal of Serbian Chemical Society 77 (2), 147–157.
Grewer, D.M., Young, R.F., Whittal, R.M., Fedorak, P.M., 2010. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? Sci. Total Environ. 408, 5997–6010.
Han, X., MacKinnon, M.D., Martin, J.W., 2009. Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere 76, 63–70.
Headley, J.V., McMartin, D.W., 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. Journal of Environmental Science and Health. Part A 39 (8), 1989–2010.
Headley, J.V., Barrow, M.P., Peru, K.M., Fahlman, B., Frank, R.A., Bickerton, G., McMaster, M.E., Parrott, J., Hewitt, L.M., 2011. Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun. Mass Spectrom. 25, 1899–1909.
Headley, J.V., Peru, K.M., Fahlman, B., Colodey, A., McMartin, D.W., 2013a. Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oil sands process waters by Orbitrap mass spectrometry. Int. J. Mass Spectrom. 345-347, 104–108.
Headley, J.V., Peru, K.M., Mohamed, M.H., Frank, R.A., Martin, J.W., Hazewinkel, R.R.O., Humphries, D., Gurprasad, N.P., Hewitt, L.M., Muir, D.C.G., Lindeman, D., Strub, R., Young, R.F., Grewer, D.M., Whittal, R.M., Fedorak, P.M., Birkholz, D.A., Hindle, R., Reisdorph, R., Wang, X., Kasperski, K.L., Hamilton, C., Woudneh, M., Wang, G., Loescher, B., Farwell, A., Dixon, D.G., Ross, M., Dos Santos Pereira, A., King, E., Barrow, M.P., Fahlman, B., Bailey, J., Mcmartin, D.W., Borchers, C.H., Ryan, C.H., Toor, N.S., Gillis, H.M., Zuin, L., Bickerton, G., Mcmaster, M., Sverko, E., Shang, D., Wilson, L.D., Wrona, F.J., 2013b. Chemical fingerprinting of naphthenic acids and oil sands process waters—a review of analytical methods for environmental samples. J. Environ. Sci. Health A 48 (10), 1145–1163. https://doi.org/10.1080/10934529.2013.776332.
Herman, D.C., Fedorak, P.M., Costerton, J.W., 1993. Biodegradation of cycloalkane carboxylic acids in oil sand tailings. Can. J. Microbiol. 39, 576–580.
Huang, R., Sun, N., Chelme-Ayala, P., McPhedren, K.N., Changalov, M., El-Din, M.G., 2015. Fractionation of oil sand-process affected water using pH-dependent extractions: a study of dissociation constants for naphthenic acids species. Chemosphere 127, 291–296.
Hughes, S.A., Mahaffey, A., Shore, B., Baker, J., Kilgour, B., Brown, C., Peru, K.M., Headley, J.V., Bailey, H.C., 2017. Using ultra-high resolution mass spectrometry and toxicity identification techniques to characterize the toxicity of oil sands process-affected water: the case for classical naphthenic acids. Environ. Toxicol. Chem. 36 (11), 3148–3157.
International Sorbent Technologies, 2001. Method Development in Solid Phase Extraction Using Non-polar ISOLUTE SPE Columns for the Extraction of Aqueous Samples.
Johnston, C.U., Clothier, L.N., Quesnel, D.M., Gieg, L.M., Chua, G., Hermann, P.M., Wildering, W.C., 2017. Embryonic exposure to model naphthenic acids delays growth and hatching in the pond snail Lymnaea stagnalis. Chemosphere 168, 1578–1588.
Jones, D., West, C.E., Scarlett, A.G., Frank, R.A., Rowland, S.J., 2012. Isolation and estimation of the 'aromatic' naphthenic acid content of an oil sands process-affected water extract. J. Chromatogr. A 1247, 171–175.
Kavanagh, R., Burnison, B.K., Frank, R.A., Solomon, K.R., Van Der Kraak, G., 2009. Detecting oil sands process-affected waters in the Alberta oil sands region using synchronous fluorescence spectroscopy. Chemosphere 76, 120–126.
Kavanagh, R.J., Frank, R.A., Burnison, B.K., Young, R.F., Fedorak, P.M., Solomon, K.R., Van Der Kraak, G., 2012. Fathead minnow (Pimephales promelas) reproduction is impaired when exposed to a naphthenic acid extract. Aquat. Toxicol. 116-117, 34–42.
Lai, J.W.S., Pinto, L.J., Kiehlmann, E., Bendell-Young, L.I., Moore, M.M., 1996. Factors that affect the degradation of naphthenic acids in oil sands wastewater by indigenous microbial communities. Environ. Toxicol. Chem. 15 (9), 1482–1491.
Lo, C.C., Brownlee, B.G., Bunce, N.J., 2006. Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. Water Res. 40, 655–664.
MacKinnon, M.D., Boerger, H., 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Water Pollution Research Journal of Canada 21 (4), 496–512.
Marentette, J.R., Frank, R.A., Bartlett, A.J., Gillis, P.L., Hewitt, L.M., Peru, K.M., Headley, J.V., Brunswick, P., Shang, D., Parrott, J.L., 2015. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (*Pimephales promelas*) embryos. Aquat. Toxicol. 164, 108–117.
Minister of Environment, 2013. Decision Statement for the Jackpine Mine Expansion Project.
Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W., Giesy, J.P., 2015. Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. Environ. Sci. Technol. 49, 12395–12404.
Nero, V., Farwell, A., Lee, L.E.J., Van Meer, T., MacKinnon, M.D., Dixon, D.G., 2006. The effects of salinity on naphthenic acid toxicity to yellow perch: gill and liver histopathology. Ecotoxicol. Environ. Saf. 65, 252–264.
Rathore, A.S., Velayudhan, A., 2002. Scale-up and Optimization in Preparative Chromatography: Principals and Biopharmaceutical Applications. CRC Press.
Rowland, S.J., West, C.E., Jones, D., Scarlett, A.G., Frank, R.A., Hewitt, L.M., 2011. Steroidal aromatic 'naphthenic acids' in oil sands process-affected water: structural comparisons with environmental estrogens. Environ. Sci. Technol. 45, 9806–9815.
Royal Society of Canada Expert Panel, 2010. Environmental and Health Impacts of Canada's Oil Sands Industry.
Scarlett, A.G., Reinardy, H.C., Henry, T.B., West, C.E., Frank, R.A., Hewitt, L.M., Rowland, S.J., 2013. Acute toxicity of aromatic and non-aromatic fractions of naphthenic acids extracted from oil sands process-affected water to larval zebrafish. Chemosphere 93, 415–420.
Siwik, P.L., Van Meer, T., MacKinnon, M.D., Paszkowski, C.A., 2000. Growth of fathead minnows in oilsand-processed wastewater in laboratory and field. Environ. Toxicol. Chem. 19 (7), 1837–1845.
USEPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA-821-R-02-012.
Verbeek, A., McKay, W., MacKinnon, M., 1993. Isolation and characterization of the acutely toxic compounds in oil sands process water from Syncrude and Suncor. Proceedings of Oil Sands - Our Petroleum Future Conference, Fine Tails Fundamentals Consortium. Edmonton, AB: F10,11-11.