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Effects of environmental conditions on aerobic degradation of a commercial naphthenic acid

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HIGHLIGHTS

- Added nutrients (including N & P) significantly increased NA degradation rates.
- [DO]>8 mg L^{-1} increased NA degradation rates compared to [DO]<5 mg L^{-1}.
- Temperatures of 23.4 °C increased NA degradation relative to 6.9 & 16.9 °C.
- pH 7–9 increased NA degradation relative to pH~6.

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ABSTRACT

Naphthenic acids (NAs) are problematic constituents in energy-derived waters, and aerobic degradation may provide a strategy for mitigating risks to aquatic organisms. The overall objective of this study was to determine the influence of concentrations of N (as ammonia) and P (as phosphate), and DO, as well as pH and temperatures on degradation of a commercial NA in bench-scale reactors. Commercial NAs provided replicable compounds necessary to compare influences of environmental conditions on degradation. NAs were quantified using high performance liquid chromatography. Microbial diversity and relative abundance were measured in treatments as explanatory parameters for potential effects of environmental conditions on microbial populations to support analytically measured NA degradation. Environmental conditions that positively influenced degradation rates of Fluka NAs included nutrients (C:N 10:1–500:1, C:P 100:1–5000:1), DO (4.76–8.43 mg L^{-1}), pH (6–8), and temperature (5–25 °C). Approximately 50% removal of 61 ± 8 mg L^{-1} was achieved in less than 2 d after NA introduction, achieving the method detection limit (5 mg L^{-1}) by day 6 of the experiment in treatments with a C:N:P ratio of 100:10:1, DO > 8 mg L^{-1}, pH ~8–9, and temperatures >23 °C. Microbial diversity was lowest in lower temperature treatments (6–16 °C), which may have resulted in observed slower NA degradation. Based on results from this study, when macro- and micronutrients were available, DO, pH, and temperature (within environmentally relevant ranges) influenced rates of aerobic degradation of Fluka NAs. This study could serve as a model for systematically evaluating environmental factors that influence NA degradation in field scenarios.

1. Introduction

Aerobic degradation can alter structural compositions and decrease concentrations of naphthenic acids (NAs), a complex group of carboxylic acids associated with crude oils (Seifert and Teeter, 1969; Tomcyzk et al., 2001) and energy-derived waters (Dorn, 1992; Allen, 2008). NAs are sources of toxicity in energy-derived waters such as refinery effluents and oil sands process-affected waters (OSPWs; Dorn, 1992; Schramm, 2000), with adverse effects observed for fish, macro- and microinvertebrates, aquatic macrophytes, and a bacterium, *Vibrio fischeri* (Dorn, 1992; Nero et al., 2006; Frank et al., 2008; Armstrong et al., 2009;

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Kavanagh et al., 2012; Leclair et al., 2013; Marentette et al., 2015). NAs are described by the formula $C_nH_{2n+Z}O_2$, where n is the number of carbons and Z is either zero or a negative even integer representing the hydrogen deficiency of the molecule due to rings or double bonds (Holowenko et al., 2002; Clemente and Fedorak, 2005). Fluka commercial NAs have been used for assessing aerobic degradation of NAs (Scott et al., 2005; Headley et al., 2010) and have also been evaluated as a simplistic and replicable analogue to understand more compositionally complex mixtures of NAs present in energy-derived waters (Rudzinski et al., 2002; Headley and McMartin, 2004; Barrow et al., 2004; Scott et al., 2005; Lo et al., 2006; Armstrong et al., 2007; Headley et al., 2010).

Previous studies have evaluated effects of NA structural compositions on rates and extents of aerobic degradation with NAs from various sources. These studies included comparisons between commercial NA sources (Clemente et al., 2004), comparisons between commercial and energy-derived NA sources (Scott et al., 2005; Headley et al., 2010), and correlations of cyclicity of structures with half-lives of NAs (Han et al., 2008). Half-lives of less than 10 d have been reported for commercial NAs (Clemente et al., 2004; Scott et al., 2005; Headley et al., 2010), relative to 25% removal of OSPW-derived NAs (initial concentration 37 mg L^{-1}) in 49 d (Scott et al., 2005). Han et al. (2008) measured half-lives ranging from 1 to 8 d for Merichem commercial NAs and from 44 to 240 d for OSPW-derived NAs, illustrating that commercial NAs are relatively labile compared to energy-derived NAs. Consequently, commercial NAs are often relatively potent in terms of aquatic toxicity compared to energy-derived NAs (Marentette et al., 2015; Kinley et al., 2016).

Environmental conditions that may influence aerobic degradation of NAs include nitrogen and phosphorus concentrations, dissolved oxygen (DO) concentrations, pH, and temperatures. Changes in rates and extents of aerobic degradation by microorganisms can be due to effects on degradation pathways, microbial abundance and diversity, shifts in microbial species, or bioavailability of the targeted compound to operative species as a function of the aforementioned environmental conditions (Paris et al., 1981). Within the constraints of the compositional and structural differences among commercial and energy-derived NAs, environmental conditions affecting microbial populations and subsequent aerobic degradation of commercial NAs are also expected to affect degradation of energy-derived NAs.

Sufficient nitrogen (N) and phosphorus (P) relative to carbon (C) concentrations (from NAs) must be achieved to provide macronutrients for microbes capable of degrading complex organic molecules (e.g. NAs). Provision of essential aqueous molar ratios and concentrations is critical for establishing and maintaining biological activity for aerobic degradation (Herbert, 1976; Brock and Madigan, 1988). Compositional proportions of these nutrients necessary to sustain growth of microbial populations have been well studied. Redfield (1958) and Goldman et al. (1987) estimated requisite C:N:P ratios of 105:15:1 and 106:12:1, respectively. Based on nutrient requirements observed for microorganisms, C:N and C:P ratios of approximately 10:1 and 100:1, respectively, should be sufficient to establish and maintain microbial growth, assuming that all other necessary macro- and micronutrients are available. Bushnell-Haas aqueous culture medium (Bushnell and Haas, 1941) is widely used for biodegradation studies (Clemente et al., 2004; Scott et al., 2005; Del Rio et al., 2006; Han et al., 2008; Headley et al., 2010) because it does not supply an organic carbon source, so the potential for the targeted compound (e.g. NAs) to serve as a carbon or energy source for microbes can be evaluated and compared. To minimize effects of limited N and P as a confounding variable, measures of degradation for a range of C:N and C:P ratios preceded evaluation of other treatment factors.

Once questions regarding C:N and C:P ratios are resolved, DO is the next critical parameter to evaluate, since a primary degradation pathway for aliphatic and alicyclic carboxylic acids by microorganisms is β-oxidation (Taylor and Trudgill, 1978; Trudgill, 1984), which utilizes molecular oxygen. Diatomic oxygen is sparingly soluble in water and rates of DO consumption by microorganisms (as a macronutrient and as an electron acceptor in degradation) can exceed rates of atmospheric oxygen diffusion into water (Brock and Madigan, 1988). Aerobic degradation of NAs results in biochemical oxygen demand (BOD) in water, and estimates of moles O_2/mole carbon necessary for NA degradation can provide data to ensure sufficient oxygen for sustainable aerobic degradation.

Somewhat unique to NAs as a class of compounds, pH can precipitously influence their solubility and bioavailability. Solubility of NAs in water is influenced by pH, with log K_{ow} values ranging from about 1.8 at pH 6 to 0.8 at pH 8 for NAs found in OSPWs (Schramm, 2000). Susceptibility of organic constituents to biodegradation often correlates with water solubility and octanol-water partition coefficients (Alexander, 1999). Clearly, pH can be a confounding factor in measuring the rates and extents of removal of NAs. For evaluating degradation in bench-scale studies, maintaining pH of approximately 8 ensures solubility of NAs (Schramm, 2000) while remaining within environmental requirements and tolerances of microorganisms (USEPA, 1986). Decreasing pH from 8, within the range of requirements for microbes, provides information about potential effects on degradation, presumably due to decreased bioavailability of NAs.

Finally, ambient temperatures can affect aerobic degradation of NAs by altering microbial metabolic rates of mesophilic organisms and physical state of the compounds (Atlas, 1981; Alexander, 1999). Increases in temperature (above freezing) can correlate with increases in enzymatic activity, improving hydrocarbon degradation rates (Leahy and Colwell, 1990; Atlas and Bartha, 1972; Gibbs et al., 1975). In addition, as temperature increases, viscosity of hydrocarbons decreases (Leahy and Colwell, 1990), improving transport and bioavailability to microorganisms (Atlas, 1981). Increased temperatures are anticipated to correlate with increased NA degradation rates by mesophilic organisms. In the context of cold-climate environmental situations, psychrophilic (i.e. cold-loving) organisms would be naturally present and therefore, a shift in degradation rates may not occur with decreased temperatures as a more heterogeneous population including psychrophiles would be present. Psychrophiles can have similar metabolic rates at cold temperatures as mesophilic organisms in ambient temperatures and therefore are adapted to these environmental conditions (Knoblauch et al., 1999).

Measurements of diversity and relative abundance of microbial populations among different environmental conditions (i.e. experimental treatments) can serve as explanatory parameters for potential effects on microorganisms, supporting analytically measured NA degradation. In addition, environmental conditions (C:N and C:P ratios, DO, pH, and temperature) can be ranked in terms of relative influence on degradation with time. Ranking the influence of these environmental conditions on degradation using model Fluka NAs may provide context for managing these conditions to promote efficacious degradation of NAs associated with energy-derived waters.

The overall objective of this experiment was to determine the relative influence of nitrogen (as ammonia) and phosphorus (as phosphate) concentrations, DO concentrations, temperatures, and pH on aerobic degradation of Fluka NAs in bench-scale laboratory reactors. To achieve this overall objective, specific objectives were to 1) measure and compare changes in Fluka NA concentrations with time for a range of C:N (10:1–500:1) and C:P ratios (100:1–5000:1), 2) measure and compare changes in Fluka NA concentrations with time for a range of DO concentrations