

ENHANCED BIODEGRATATION OF A MODEL NAPHTHENIC ACID COMPOUND

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Abstract

Naphthenic acids are a complex mixture of organic acid compounds which naturally occur in crude oil. The naphthenic acids are corrosive to equipment during the extraction process from oil sands. They become concentrated in tailings pond water that is retained at the oil sands mining sites in northeastern Alberta. Environmental regulations require tailings water to be retained in large ponds to prevent release into the environment due to concerns about toxicity. A major source of toxicity in tailings pond water has been linked to naphthenic acids. While research to date indicates that natural biodegradations processes do decrease the acute toxicity of naphthenic acid compounds, the kinetics of the degradation and their rates are not clearly understood. Also, as a result of increases in capacity of the oil sand processing plants, improved and more timely alternatives for bioremediation of naphthenic acids are required. In this study, a model naphthenic acid compound (trans isomer of 4-methyl-1-cyclohexane carboxylic acid) and a microbial culture, developed in our laboratory, have been used to evaluate the kinetics of biodegradation in batch and continuous bioreactors. The effects of naphthenic acid concentration on the kinetics of microbial growth are presented. A comparison of the effects of two bioreactor configurations is demonstrated.

1. Introduction

Naphthenic acids (NAs) are a complex mixture of organic acid surfactant compounds which naturally occur in crude oils (1, 2). As a result of their acidity and hence corrosivity to refinery units, NAs are removed from the oil sands during the processing of bitumen oil production (3). Large volumes of tailings pond water produced during the extraction process are presently retained at the oil sands mining sites in northeastern Alberta in accordance with the “zero” discharge policies of the mining companies. Environmental regulations require the tailings to be retained in large ponds to prevent release into the environment due to concerns about their toxicity. The toxicity of the

fluid wastes from the tailings pond water has been largely attributed to the salinity and a mixture of organic acids believed to be NAs (2, 4-7).

The petroleum industry intended to reduce the toxicity of the mine tailings waste, in part, by natural biodegradation. However, the observed rates of biodegradation do not meet with the increased wastewater production rates predicted for the petroleum industry. Furthermore, repeated attempts to extensively biodegrade NAs from Syncrude, Suncor, and Albion Sands Energy Inc. were unsuccessful using laboratory cultures of tailings pond water bacteria (8).

In addition to the recalcitrant properties with regard to biodegradation, the complexity of the NA mixtures continue to pose a major challenge in the development of a suitable analytical method (2). Separation and identification of individual compounds have not been achieved to date. Only a few studies have looked at model compounds of commercial available NAs (9, 10).

The focus of the present study was on the quantification and improvement of the natural biodegradation processes. Various bioreactor configurations were evaluated at a laboratory scale for the purpose of quantifying the specific growth rates and residence times in various systems as well as the specific yield for the consortium. Initially, the NA mixture as a whole was studied, however, as a result of the complexity of the mixture, and the ongoing criticisms of using a commercial grade NA mixture as a surrogate (8, 11), the research activities conducted and presented herein focused on the kinetics of biodegradation of a model compound representing a single ringed NA structure described in more detail in Section 2.1.

2. Materials and methods

2.1 Selection of a model compound

NAs are a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+z}O_2$, where n indicates the carbon number and Z specifies the number of hydrogen atoms that are lost as the structure becomes more compact from those with linear hydrocarbon chains. It is understood that the more compact the NA compounds become, the more persistent the molecule becomes in the environment. Biodegradation of Fluka standard NAs occurs more readily than that of the NAs extracted from tailings pond water and this degradation occurs in the less complex structures (i.e. lower Z series and lower molecular mass NAs) (8).

Several model NA compounds were evaluated to find a suitable compound as a simple representative substrate for this kinetic study. On the basis of biodegradability, the model compound, 4-methyl 1-cyclohexane carboxylic acid (4MCHCA), was selected. 4MCHCA is available in dry state from Sigma-Aldrich for both a mixture of trans- and cis-isomers as well as individual isomers. The trans-isomer of 4MCHCA (referred to herein as trans-4MCHCA) was selected for this study. An early report of research carried

out on four model compounds, indicated that the trans-isomer would be more amenable to biodegradation due to the less compact nature of the molecular arrangement (9). The formula weight for trans-4MCHCA is 142.2 g/mol with a single ring structure fitting the formula of NAs where the Z series equals -2. The chemical formula for trans-4MCHCA is $\text{CH}_3\text{C}_6\text{H}_{10}\text{CO}_2\text{H}$. (CAS Number 13064-83-0).

2.2 Microbial culture and media

In general terms, the biodegradation of hydrocarbon waste takes place through utilization by predominant bacteria, such as *Pseudomonas*. The bacteria utilize carbohydrate and hydrocarbon wastes often together with a mixture of other organisms such as *Bacillus*, *Flavobacterium* and/or *Alcaligenes* which serve to consume protein wastes (12). *Pseudomonas putida*, which is one of the most ubiquitous and versatile microorganisms for metabolizing recalcitrant chemicals (13) is well known for use in the biodegradation of persistent and recalcitrant environmental contaminants.

Three microbial cultures developed during the various stages of previous work were tested for biodegradation studies. The first culture was developed in earlier experiments with Fluka technical NAs (manufactured and supplied by Sigma-Aldrich Inc., CAS No. 1338-24-5). The second culture originated from Syncrude tailings pond water. A third culture was pure *Pseudomonas putida* (ATCC 17484). After several months of experimentation in shake flasks, the culture originating from the Fluka NA standard was selected as the candidate for bioremediation studies.

In order to identify the dominant species of the developed microbial consortium, a sample from a culture growing exponentially in a shake flask (optical density of 0.2) was plated on agar. A medium known to support the growth and metabolism of *Pseudomonas putida* was used for the growth and maintenance of the microbial consortium. The resultant solution presents a buffered medium and could be sterilized at 121°C. 250 ml Erlenmeyer flasks were initially prepared for inoculation with 100 ml of media and 100 mg/L of dry trans-4MCHCA. The substrate was dissolved by vigorous mixing and the pH adjusted to 7 a suitable concentration of sodium hydroxide. After dissolution of the substrate, the flasks were inoculated with 10 ml of one of three of the established cultures. Optical densities were monitored and recorded daily over several days. Upon complete biodegradation of the substrate, the shake flasks were sub-cultured into clean aseptic shake flasks. Sub-culturing, typically was initially carried out between 10 and 14 days until such a time that the lag phase could be decreased to approximately 5 or 6 days at room temperature. Batch cultures were also studied at a range of trans-4MCHCA concentrations (50 mg/L to 800 mg/L), temperatures (4°C through 40°C) as well as with varying pH values (5 through 13) at room temperature.

3. Experimental systems and procedures

The effect of initial substrate (trans-4MCHCA) concentration on the activity of the microbial community was studied in two types of bioreactors including: batch reactors and a continuous stirred tank reactor (CSTR).

3.1 Method for analyses of trans-4MCHCA

Some of the current methods for detecting model NAs in water require complex preparation procedures including liquid-liquid extraction and solid-phase extraction (5, 14). An alternative method was developed for the analyses of the model naphthenic acid compound using gas chromatography with flame ionization detection. Development of this method was established in response to a need for a simple routine direct injection method of quantitative analyses of the substrate concentration. Time sensitive substrate concentrations are necessary to assess the biokinetic principles of degradation of a specific contaminant or substrate. Naphthenic acids have been identified as weakly biodegradable, thus observing any changes in concentration must be monitored consistently, precisely and in a timely manner. The analytical method used herein serves as a guide in the identification of biodegradation process.

3.2 Evaluation of data

The batch reactor data was compiled in order to identify the specific growth rate for each run at different initial substrate concentrations, temperatures and pH. The CSTR was evaluated for designed dilution rates with a feed concentration of 500 mg/L. The residual concentrations in the CSTR (steady state concentrations) were determined at each dilution rate and plotted to assess the dependency of specific growth rate on substrate concentration. The data was evaluated using spreadsheet tools available in Excel™ and Sigma Plot™ (V 8.0) software.

4. Results

The results of the microbial identification of the microorganisms capable of degrading the selected model NA compound indicated that the consortium was dominated by three microbial colonies: a dominant colony clear in colour; two less dominant colonies; one beige coloured and one brown identified as species within the *Alcaligenes* and *Pseudomonas* genera, respectively, which concur with the indigenous species dominating the tailings pond waters (7).

The results of the kinetic study of trans-4MCHCA carried out to date demonstrate that biodegradation of a single naphthenic acid compound with a single ring structure can be achieved at temperatures ranging between 4°C and 37°C. The maximum specific growth rate achieved for an initial substrate concentration of 500 mg/L was 0.5 day⁻¹.

Cell yield for the mixed culture capable of degrading the trans-4MCHCA ranges between 0.2 and 0.3 mg of biomass/mg of substrate at room temperature. Similarly, yield factors

for various species of *Pseudomonas* using various carbon sources such as glucose, ethanol and methanol were within the same range (15). The reported yield for *Pseudomonas* in acetate is 0.3 g/g which may be more representative of the model NA compounds.

Conducting the biodegradation of the model naphthenic acid compound, trans-4MCHCA, in a continuously stirred reactor resulted in a significant enhancement of the biodegradation rate for a comparable initial substrate concentration in the batch reactor. Both sets of experimental runs were conducted at room temperature in controlled reactors with an initial substrate concentration of 500 mg/L. The results indicate that the specific growth rate for a batch reactor is half that of the CSTR (0.5 day^{-1} in the batch reactor as compared with a specific growth rate of 1.0 day^{-1} for the CSTR). This result may also be described as a decrease in residence time from 2.2 days in a batch reactor to 1.0 day in a continuous reactor.

5. Discussion

In similar studies of bioreactors with simpler contaminants such as PAHs, the maximum specific growth rates may be up to 6 times greater. For example, for phenol the maximum specific growth rate was reported to be 0.09 hr^{-1} (2.1 day^{-1}) (16) and 0.13 hr^{-1} (3.1 day^{-1}) for naphthalene (17). Research on reaction kinetics for model NA compounds in Athabasca River water (both amended and non-amended) resulted in an optimum maximum specific growth rate of 0.07 day^{-1} for an initial batch substrate concentration of 9 mg/L of trans-4MCHCA (9). This would compare with a theoretical value from this study of approximately 0.11 day^{-1} which is close to double that of non-amended river water.

Further investigation of reactors (both continuous and immobilized film reactors) at varying temperatures (in particular at 4°C) would be beneficial to the evaluation of the limiting factors in the degradation of the in-situ tailings pond waters. As well, in-situ studies of the biokinetics of the NAs in enhanced systems such as nutrient additives, aeration and biofilms could be developed on the basis of the research presented herein.

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