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Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor

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ABSTRACT

Generation of process waters contaminated by naphthenic acids is a serious environmental concern associated with processing of the oil sands. This together with the necessity for sustainable use of water highlights the need for development of effective technologies such as bioremediation for treatment of these contaminated waters. In this work, a circulating packed bed bioreactor and a culture developed in our laboratory were used to study batch and continuous biodegradation of *trans*-4-methyl-cyclohexane carboxylic acid (*trans*-4MCHCA), a mixture of *cis*- and *trans*-4-methyl-cyclohexane acetic acid (4-MCHAA), and mixture of these three naphthenic acids. Experimental results revealed that the biodegradability of the naphthenic acids was influenced by both carbon number and the spatial arrangement of the alkyl side branch. The maximum biodegradation rate of *trans*-4MCHCA observed during the continuous operation (209 mg/L h at a residence time of 0.15 h) was significantly higher than those reported for CSTR and packed-bed bioreactors. The biodegradation rates of *cis*- and *trans*-4-MCHAA were much lower than *trans*-4MCHCA, with the maximum biodegradation rates determined for the two isomers being 4.2 and 7.8 mg/L h, respectively (residence time: 3.3 h).

1. Introduction

A large portion of Canadian oil reserves exists as oil sands in the Athabasca Basin in Alberta and in Saskatchewan. The oil sands in northern Alberta have a proven reserve of 174 billion barrels and are considered to be one of the world's largest oil deposits [1]. The bitumen from the shallow deposits of oil sands is currently recovered by surface mining which accounts for approximately 65% of total production, while the deeper deposits are subjected to in situ techniques [1,2]. Extraction of bitumen from the surface mined oil sands is achieved by modified versions of the Clark hot water process, where caustic (NaOH) is used to improve the separation of bitumen from the ore [1,3]. This process results in extremely large volumes of tailings which consist of oil sand process water, sand, clay, and unrecovered hydrocarbons including bitumen and diluent used in the process [3]. The generated tailings are currently transferred to settling ponds where following the separation of large and fine particles a portion of water is recycled back to the extraction process. The zero discharge policy due to toxic nature of these tailings dictates the storage of the remaining waters in large ponds until they are reclaimed by an environmentally acceptable. The absence of an effective treatment process has led to accumulation of large volumes of contaminated tailings in enormous ponds which covers an area of at least 130 km^2 [4]. In Canada the volume of tailing water is predicted to exceed 1 billion m^3 by 2025 [5]. The toxicity of these tailing waters has been largely attributed to naphthenic acids (NAs) which are transferred to the aqueous phase during the extraction process. NAs are a mixture of naturally occurring alicyclic carboxylic acids described by the general formula $C_nH_{2n+Z}O_2$, where n indicates the number of carbon atoms and Z represents hydrogen deficiency for cyclic molecules. Recent works on oil sand process water, however, has found this definition inadequate for describing the total fraction of toxic organic acids, due to presence of more complicated molecules with multiple carboxyl groups, as well as hetroatoms such as N and S [6,7].

The current practice of storing the contaminated waters in tailing ponds and reliance on the natural biological and physicochemical processes occurring in the ponds for the removal of NA is not practical in the long term. This is due to limited treatment capacity offered in these ponds which could not cope with the increases in the volume of the processed ore and produced tailings. The tailing ponds impact the environment severely through changing the natural landscape, their detrimental effects on wildlife, and the possibility for leakage from the tailing ponds and contamination of the nearby soil, ground and surface waters. To tackle these environmental issues associated with the oil sand

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tailings various methods such as ozonation [8,9], advanced oxidation [10,11], photolysis [12–14], microwave treatment [15] and bioremediation [2,3,5,7,16–19] have been evaluated, using surrogate (pure) and commercial mixtures of naphthenic acids, as well as naphthenic acids originated from the oil sand process waters. Among these methods, bioremediation has been the centre of attention and is deemed as one of the most practical and economical approaches. Bioremediation studies aiming at treatment of naphthenic acids have been conducted generally in the microcosms and small scale culture systems. Furthermore, the focus of these studies has been mainly on biodegradability of naphthenic acids, impacts of molecular structure on the extent of biodegradation, and finally identification of biodegradation pathways. As a result information on the engineering aspects of the process especially biokinetics, mass transfer (both oxygen and organics), and bioreactor configuration which are critical in the design, operations and control of a large scale biotreatment process is very limited.

As part of our earlier works, we have studied biodegradation of a surrogate naphthenic acid in batch and continuous reactors with free cells and have determined the governing biokinetics [20]. Application of immobilized cells in a packed bed bioreactor resulted in 100-fold enhancement of biodegradation rate when compared with that obtained in the continuous reactor with free cells [21]. One of the major drawbacks of the packed-bed bioreactors is inefficient mass transfers, due to low fluid velocity, absence of mixing and lack of efficient contact between the gas and liquid phases [22]. Plugging of the bioreactor with growing biomass is another serious problem associated with the packed-bed bioreactors [22]. These problems could be alleviated by modifying the configuration of the bioreactor through addition of an external downcomer tube and inducing circulation of the liquid content by injection of the air to the bottom of the bioreactor. In this modified design which is referred to as circulating packed-bed bioreactor (CPBB), the diffusional limitations is reduced significantly and efficient mass transfer can be achieved. The use of high porosity, high surface area packing in the riser of the bioreactor permits high biomass hold-up, while high air flow rates and resulting shear forces reduce or eliminate the plugging by biomass. In the present work, a circulating packed bed bioreactor and a mixed microbial culture developed in our laboratory were used to study batch and continuous biodegradation of three surrogate naphthenic acids. Effects of molecular structure, NA concentration, temperature and loading rate on the extent of biodegradation and performance of the bioreactor were investigated.

2. Materials and methods

2.1. Model naphthenic acids

In this study *trans*-4-methyl-1-cyclohexane carboxylic acid (*trans*-4MCHCA; CAS NO. 13064-83-0), a mixture of approximately 35% *cis*- and 65% *trans*-geometric isomers of 4-methylcyclohexane-acetic acid (*cis*-4-MCHAA and *trans*-4-MCHAA; CAS NO. 6603-71-0) were used as model compounds. NA compounds which were analytical grade obtained from Sigma–Aldrich (Sigma–Aldrich Canada Ltd., Ontario).

2.2. Microbial culture and medium

The microbial culture used in this study was a mixed culture, developed in our laboratory [20]. McKinney's modified medium containing *trans*-4MCHCA (100 mg/L), KH_2PO_4 (840 mg/L), K_2HPO_4 (750 mg/L), $(NH_4)_2HPO_4$ (474 mg/L), NaCl (60 mg/L), $CaCl_2$ (60 mg/L), $MgSO_4{\cdot}7H_2O$ (60 mg/L), $Fe(NH_4)_2SO_4{\cdot}6H_2O$ (20 mg/L) and 1 ml of micronutrient solution was used for the growth and maintenance of the microbial culture. The micronutrient solution contained: H_3BO_3 (600 mg/L), $CoCl_3$ (400 mg/L), $ZnSO_4{\cdot}7H_2O$ (200 mg/L), $MnCl_2$ (60 mg/L), $NaMoO_4{\cdot}7H_2O$ (60 mg/L), $NiCl_2$ (40 mg/L), $CuCl_2$ (20 mg/L). The cultures were prepared in 250 ml flask containing 100 ml of sterilized medium and 10% (v/v) inoculum. Flasks were maintained on a rotary shaker (100 rpm) at 25 °C and subcultured on a biweekly basis.

2.3. Circulating packed-bed bioreactor

The circulating packed-bed bioreactor was made of glass and its main part which contained the packing material (riser) had height and diameter of 35 and 4.5 cm, respectively. The corresponding dimensions for the downcomer (external tube) were 32 and 0.5 cm, respectively. A peristaltic pump was used to transfer the feed into the bioreactor (only for continuous experiments) and effluent left the system through an overflow tube. Filter sterilized air with a flow rate of 0.5–0.6 L/min was introduced to the bottom of the bioreactor through a digital mass flow controller. The applied air flow rate was high enough (>1 vvm) to ensure oxygen was not limiting. Stainless steel wool (coarse grade) with an approximate porosity of 80% and a surface area per unit mass of 3.4×10^{-3} m^2/g was used as matrix for establishment of the biofilm. A schematic diagram of the experimental set-up is presented in Fig. 1.

2.4. Development of biofilm

The biofilm was established in the bioreactor by trickling the modified McKinney's medium containing 50 mg/L *trans*-4MCHCA and 10% (v/v) inoculum (31.7 ± 2.9 mg dry weight biomass/L) at a flow rate of 0.04 L/h over the stainless steel packing, while partially degraded effluent was recycled back at a rate of 2.4 L/h over a period of 1 month [21]. Following this period a substantial amount of biofilm was formed on the packing. The bioreactor was then drained and filled with fresh medium containing the naphthenic acid at the designated concentration in order to initiate the batch experiments.

2.5. Batch experiments

As part of batch experiments biodegradation of *trans*-4MCHCA alone, 4-MCHAA alone (mixture of *cis* and *trans* isomers), and a mixture of *trans*-4MCHCA and 4-MCHAA (mixture of *cis* and *trans*) were studied. The range of evaluated concentrations for *trans*-4MCHCA alone and 4-MCHAA alone were 50–500 mg/L, and 25–350 mg/L, respectively. In the batch experiments with all three NAs, *trans*-4MCHCA concentration was kept at 100 mg/L and various 4-MCHAA concentrations (25, 50, 75 and 100 mg/L) were tested. In order to evaluate the ultimate potential of the bioreactor, these applied concentrations reached values higher than 40–120 mg/L which is usually observed in the tailing pond waters [23]. The progress of biodegradation was monitored by regular sampling of the liquid contents and measuring the residual concentration of NAs. The bioreactor was operated at room temperature (25 ± 2 °C). To assess the reproducibility of the results, batch experiment with 250 mg/L 4-MCHAA was repeated. The effect of temperature on performance of the system was evaluated by conducting additional experiments with 100 mg/L 4-MCHAA at various temperatures of 15, 20, 30, and 35 °C. The experiment at 25 °C was previously done as part of the experiments aiming to study the effect of 4-MCHAA concentration.

2.6. Continuous experiments

Continuous biodegradation of *trans*-4MCHCA was investigated by pumping modified McKinney's medium containing 50 mg/L

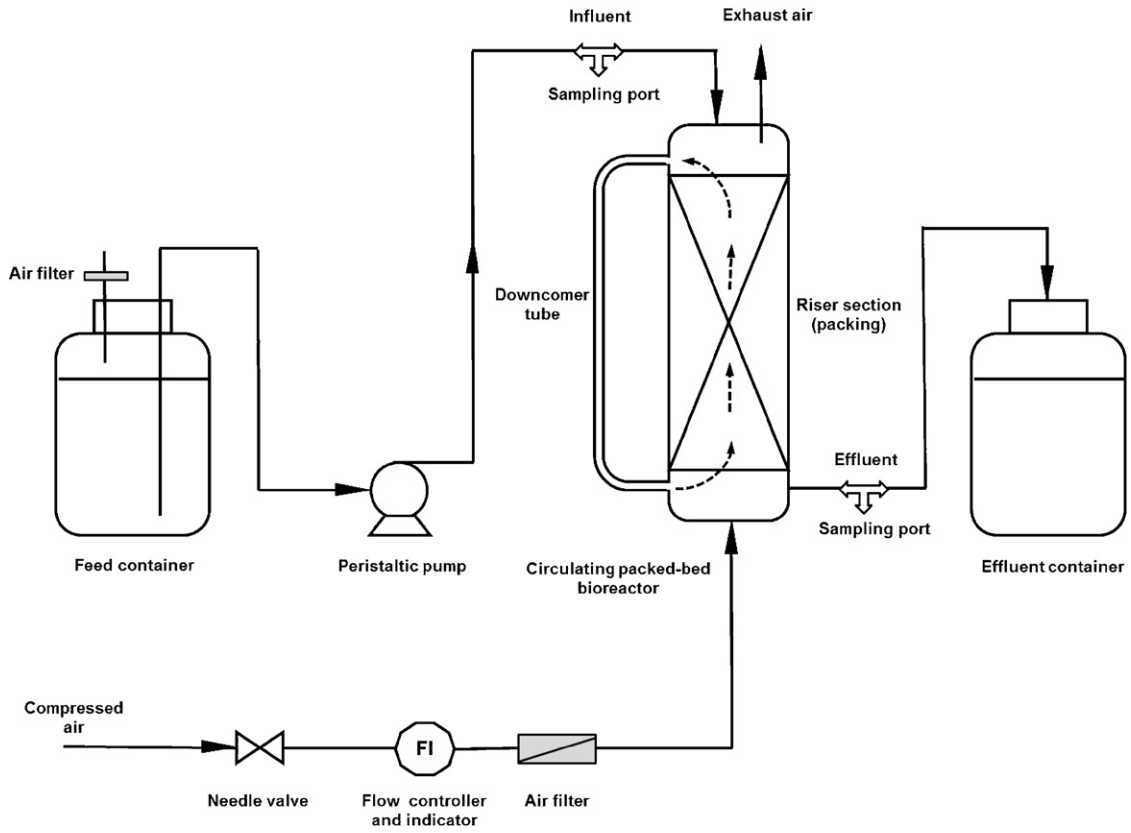

Fig. 1. Schematic flow diagram of the circulating packed bed bioreactor (CPBB) experimental set-up.

tran-4MCHCA into the bioreactor at incrementally increased flow rates of 0.375, 0.75, 1.5, 3, and 6 L/h (corresponding residence times: 1.2, 0.6, 0.3, 0.15, and 0.075 h). At each flow rate sufficient time was given for establishment of steady state conditions. Steady state conditions were assumed when the variation in residual concentration of naphthenic acid was less than 10%. The effluent from the bioreactor was sampled on a regular basis and tested for naphthenic acid concentration using a gas chromatograph. Naphthenic acid concentration in the feed tank and at the delivery point to the bioreactor was also checked frequently to ensure no contamination occurred. Upon completion of experiment with 50 mg/L *trans*-4MCHCA, flow rate was maintained at 6 L/h (corresponding residence time: 0.075 h) and feed concentration was increased to 100 mg/L to evaluate the performance of the bioreactor with a higher naphthenic acid concentration. Finally to assess the reproducibility of results when steady state data with 100 mg/L *trans*-4MCHCA were collected, feed concentration and flow rate were decreased to 50 mg/L and 1.5 L/h and performance of the bioreactor was evaluated again. The bioreactor was operated at room temperature ($25 \pm 2\,^{\circ}C$). In the next set of experiment the bioreactor was fed with modified McKiney's medium containing 50 mg/L of 4-MCHAA (mixture of *cis* and *trans* isomers). Batch results indicated that 4-MCHAA was less amenable to biodegradation. Thus, lower flow rates of 0.06, 0.10, 0.13 and 0.17 L/h (corresponding residence times: 8.0, 4.6, 3.3, and 2.7 h) were evaluated. Other applied conditions and procedures were similar to those described earlier.

To determine the dominant species of the microbial community in the bioreactor, several samples were taken from the biofilm and the effluent and analyzed for composition of the microbial community. Finally the potential stripping of naphthenic acids by injected air was tested using a similar circulating packed-bed bioreactor containing fresh packing but no biofilm. This control bioreactor was charged with McKinney's medium containing 150 mg/L *trans*-4-MCHCA and 250 mg/L 4-MCHAA (mixture of *cis* and *trans*) and injected 0.5–0.6 L/h air. Samples were taken from the liquid contents regularly for 24 h and tested for naphthenic acid concentration.

2.7. Analytical methods

Concentration of naphthenic acids was determined using a Varian-430 gas chromatograph, equipped with an FID and an HP-INNOWAX high resolution gas chromatography column (19091N-133). Helium at a flow rate of 29 ml/min was used as carrier. The detector was maintained at 250 °C, while the column temperature was ramped from 90 to 210 °C at a rate of 40 °C/min. Standard solutions of 1–100 mg/L of *trans*-4MCHCA and 4-MCHAA (mixture of *cis* and *trans* isomers) in McKinney's modified medium were prepared and used to develop the required calibration curves.

To analyze the microbial community, agar plates containing 3 g of DIFCO Bacto agar, 3 g DIFCO Bactotryptose phosphate, and 250 mg of *trans*-4MCHCA in 100 ml of McKinney's medium were inoculated with the sample collected from the bioreactor and maintained in an incubator at 32 °C. The developed colonies were then used for microbial identification which was conducted at a commercial laboratory (EPCOR-Quality Assurance Lab, Edmonton, Canada).

At the end of experiments, the liquid content of the bioreactor was drained and the packing material containing the biofilm was removed. The volume of the drained liquid was determined and used as working volume in calculating the residence time, naphthenic acid loading and removal rates for continuous experiments. The biomass hold-up was determined by drying the packing material containing the biomass in a vacuum oven maintained at −70 kPa and 65 °C. The weight of the packing containing the dried

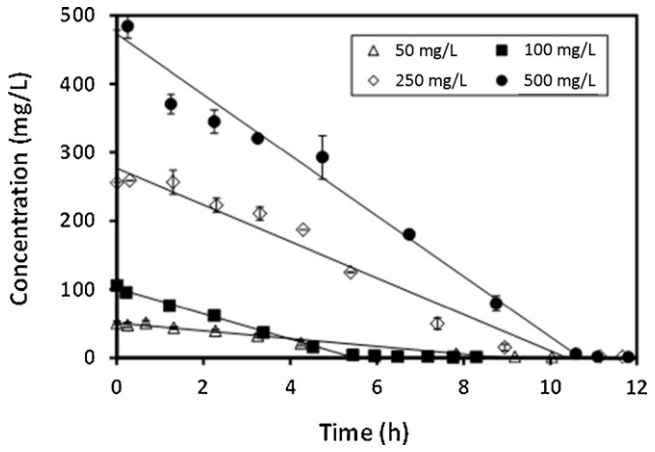

Fig. 2. Batch biodegradation of *trans*-4MCHCA in the CPBB at initial concentrations of 50, 100, 250, and 500 mg/L at 25 °C.

biomass was measured. The biomass was then removed from the packing and the dry weight of the clean packing was determined. These two measurements were used to determine the dry weight of the biomass and the biomass hold-up in the bioreactor. During batch experiments, liquid samples were taken in duplicate. Each sample was injected into the GC 3 times to determine the naphthenic acid concentration. The average value of these measurements were determined and used in presentation of results. Standard deviation for each set of measurements is included as error bar accompanying the data point. In case of continuous runs, following the establishment of steady state conditions at each applied flow rate, the bioreactor was operated at the same flow rate for at least three additional residence times. Data obtained from the samples taken after establishment of steady state conditions were used to calculate the average residual concentration and standard deviation. Optical densities (OD) of the liquid samples were determined at 620 nm.A calibration curve was developed and used to convert the OD to biomass concentration in the liquid phase.

3. Results and discussion

3.1. Batch biodegradation

Results of biodegradation of *trans*-4MCHCA in the CPBB at initial concentrations of 50, 100, 250, and 500 mg/L are shown in Fig. 2. In all cases and regardless of NA initial concentration, the microbial activity resulted in an immediate and linear decrease in concentration of NA until it was completely consumed. Regular pH measurements revealed that the pH in the bioreactor decreased slightly from an initial value in the range 6.8–7.2 to 6.3–6.7. The biodegradation rate for each tested concentration was determined using the slope of the NA concentration profile (Table 1). The calculated rates indicated that the biodegradation rate of *trans*-4MCHCA increased from 5.7 to 43.5 mg/L h due to the increase of initial concentration in the range 50–500 mg/L. Using batch reactors with freely suspended cells, Paslawski et al. [20] reported a similar dependency of biodegradation rate on initial concentration of *trans*-4MCHCA. However, the maximum biodegradation rate of 43.5 mg/L h obtained in the present work was around 9-fold higher than the maximum rate of 4.7 mg/L h reported by Paslawski et al. [20] for an initial concentration of 500 mg/L.

Results for biodegradation of 25, 50, 75, 100, 250, and 350 mg/L of 4-MCHAA (mixture of *cis* and *trans*) in the CPBB are presented in Fig. 3. Under all tested concentrations biodegradation of *cis*- and *trans*-4-MCHAA occurred simultaneously and without a marked lag phase, resulting in a continuous and linear decrease in concentration of each isomer. Using the slope of the concentration profiles, biodegradation rates for *cis*- and *trans*-4-MCHAA were calculated at each concentration and are presented as part of Table 1. For the range of concentrations tested in this work the dependency of biodegradation rate on initial concentration of 4-MCHAA (both *cis* and *trans* isomers) was somewhat different from that observed with *trans*-4-MCHCA. With 4-MCHAA increase in initial concentration from 25 to 100 mg/L led to increases of the biodegradation rate from 0.9 to 1.9 mg/L h to maximum values of 2.2 and 4.0 mg/L h for *cis* and *trans* isomers, respectively. Further increases in initial concentration led to lower biodegradation rates, indicating potential inhibitory effect of higher concentrations. In case of 4-MCHCA for the entire range of tested concentrations (50–500 ppm) biodegradation rate increased as concentration was increased. One could speculate that application of concentrations higher than 500 ppm could have resulted in decreased biodegradation rate, and an inhibitory pattern similar to that observed with 4-MCHAA. Kumar et al. [24] who studied biodegradation of 1,4-benzoquinone by *Pseudomonas putida* in batch system reported that increase of 1,4-benzoquinone concentration in the range 25–100 mg/L increased the biodegradation rate from 2.5 to 6.7 mg/L h but much lower rates (0.9–1.1 mg/L h) were observes at higher concentrations.

Data presented in Table 1 also revealed that for the entire range of tested concentrations, biodegradation of *trans*-4-MCHAA was 1.8–2 times faster than that of the *cis* isomer. Headley et al. [16] studied biodegradation of 7.5–9 mg/L of 4-MCHAA in a batch reactor and reported biodegradation rates of 0.005 and 0.019 mg/L h for *cis*- and *trans*- isomers, respectively. These authors attributed the slower biodegradation rates of the *cis* isomer to intramolecular hydrogen bonding which occurs only in the *cis* isomer. Significant improvement in biodegradation rates achieved in the present study (at least 2 orders of magnitude) showed the superior performance of the circulating packed-bed bioreactor in terms of biodegradation rate and its capability in treating high concentrations of naphthenic acids. Finally comparison of the biodegradation rates obtained for *trans*-4-MCHCA and *trans*-4-MCHAA highlighted the impact of structural differences, and the fact that biodegradation of *trans*-4MCHCA where carboxylic acid is directly attached to the cyclohexane ring was much faster (2–11-folds depending on the initial concentration) than that of *trans*-4-MCHAA in which the carboxylic acid is attached to the ring via a $-CH_2$ group. Headley et al.

Table 1
Batch biodegradation rates of *trans* 4-MCHCA, and 4-MCHAA (mixture of *cis* and *trans* isomers) as sole substrates at various initial concentrations at 25 °C.

trans-4-MCHCA concentration (mg/L)	*trans*-4-MCHCA biodegradation rate (mg/L h)	4-MCHAA total concentration (mg/L)	*cis*-4-MCHAA biodegradation rate (mg/L h)	*trans*-4-MCHAA biodegradation rate (mg/L h)
50	5.7 (R^2 = 0.98)	25	0.9 (R^2 = 0.98)	1.9 (R^2 = 0.93)
100	18.5 (R^2 = 0.99)	50	1.1 (R^2 = 0.90)	2.5 (R^2 = 0.99)
250	28.9 (R^2 = 0.96)	75	1.3 (R^2 = 0.89)	2.8 (R^2 = 0.88)
500	43.5 (R^2 = 0.98)	100	2.2 (R^2 = 0.97)	4.0 (R^2 = 0.98)
		250	1.3 (R^2 = 0.96)	2.5 (R^2 = 0.99)
		350	0.4 (R^2 = 0.96)	0.7 (R^2 = 0.94)

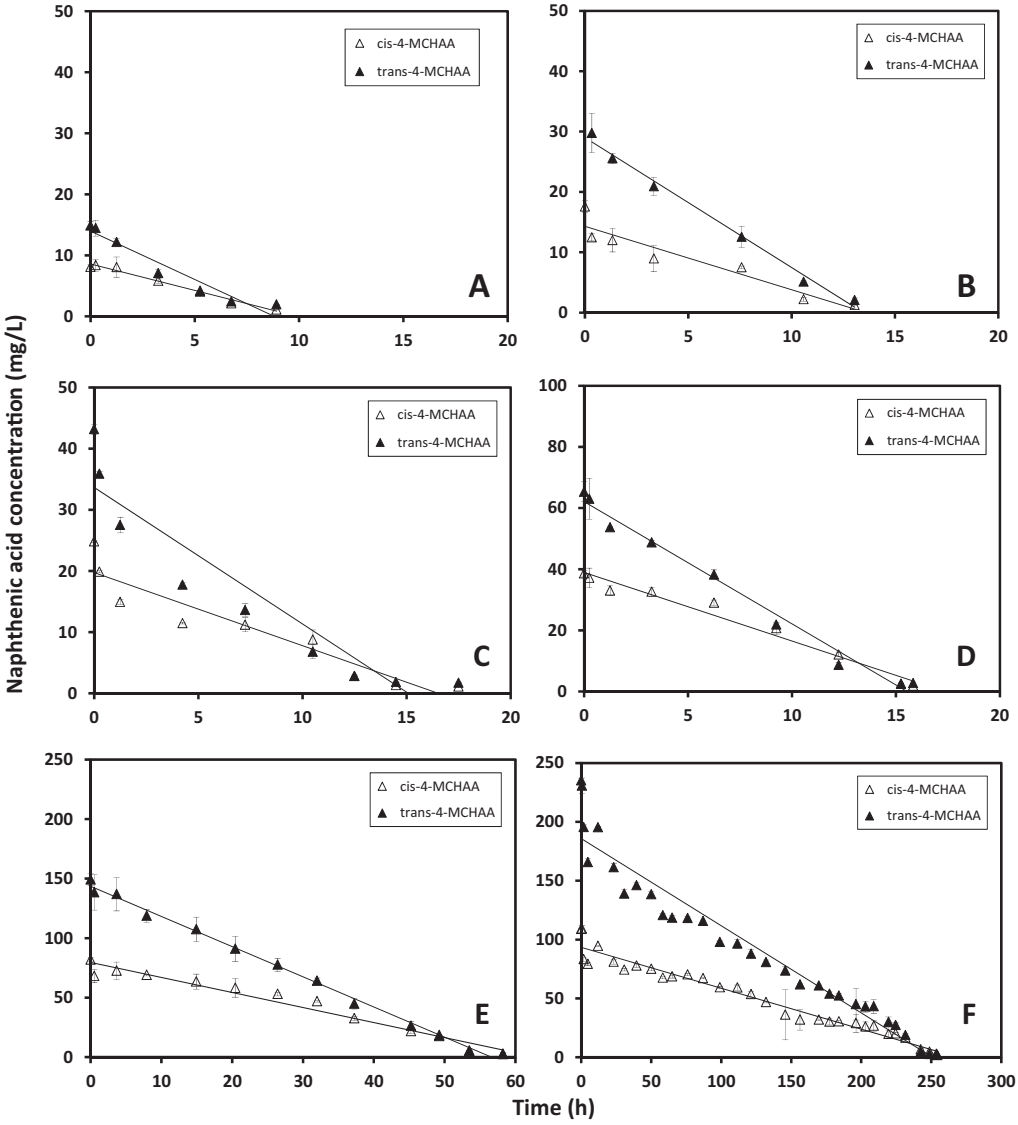

Fig. 3. Batch biodegradation of 4-MCHAA(mixture of *cis* and *trans* isomers) in the CPBB at initial concentrations of (A) 25, (B) 50, (C) 75, (D) 100, (E) 250, and (F) 300 mg/L at 25 °C.

[16] also reported a similar pattern, though the difference was not as significant, possibly due to the much lower concentrations used in their work. As indicated earlier, to assess the reproducibility of the results, the biodegradation of 250 mg/L 4-MCHAA was carried out twice. The small variations in biodegradation rates of *cis*- and *trans*-4-MCHAA (1.6% and 4.7%, respectively) and similar concentration profiles observed in both runs confirmed the reproducibility of the results (data for second run not shown).

The effect of temperature on the biodegradation of 100 mg/L 4-MCHAA (mixture of *cis* and *trans* isomers) is shown in Fig. 4. As seen simultaneous biodegradation of both isomers started without any marked lag phase even at the lowest temperature of 15 °C. The required time for complete biodegradation of each isomer and their corresponding biodegradation rate, however, was dependent on the applied temperature. Increase of temperature in the range 15–25 °C enhanced the biodegradation rate of *cis* and *trans* isomers from 0.4 to 0.6 mg/L h to 2.2 and 4.2 mg/L h, respectively. Further increase of temperature led to lower biodegradation rates (1.1 and 1.9 mg/L h, respectively at 35 °C). Regression coefficients (R^2) for the calculated rates were in the range 0.94–0.99. Using a mixed culture dominated by *Pseudomonas aeruginosa,* Paslawski et al. [20] studied the biodegradation of *trans*-4MCHCA at temperatures ranging from 4 to 40 °C and achieved the highest biodegradation at room temperature (23 ± 2 °C). A similar trend was observed by Kumar et al. [24] during the biodegradation of 1,4-benzoquinone with *P. putida*, where temperatures in the range 10–25 °C were tested and the optimum temperature was reported as 15 °C.

Fig. 5 summarizes the results for biodegradation of a mixture of 100 mg/L 4-MCHCA and 4-MCHAA (*cis* and *trans* isomers) at concentrations of 25, 50, 75 and 100 mg/L. In all cases biodegradation of all three compounds occurred simultaneously but biodegradation of *trans*-4-MCHCA was much faster than that of 4-MCHAA (both *cis* and *trans* isomers). The biodegradation rates calculated for each individual naphthenic acid (Table 2) show that consistent with patterns observed with *trans*-4-MCHAA alone, increases in initial concentration of 4-MCHAA in the range 25–100 mg/L led to faster biodegradation rates for both *cis* and *trans* isomers and that biodegradation of *trans* isomer was 1.5–2-fold faster than that its *cis* counterpart. No specific dependency between the biodegradation rate of *trans*-4-MCHCA (100 mg/L) and initial concentration of 4-MCHAA was apparent but the highest rate of 10.1 mg/L h observed in the mixture was lower than 18.5 mg/L h obtained during biodegradation of 100 mg/L *trans*-4-MCHAA alone.

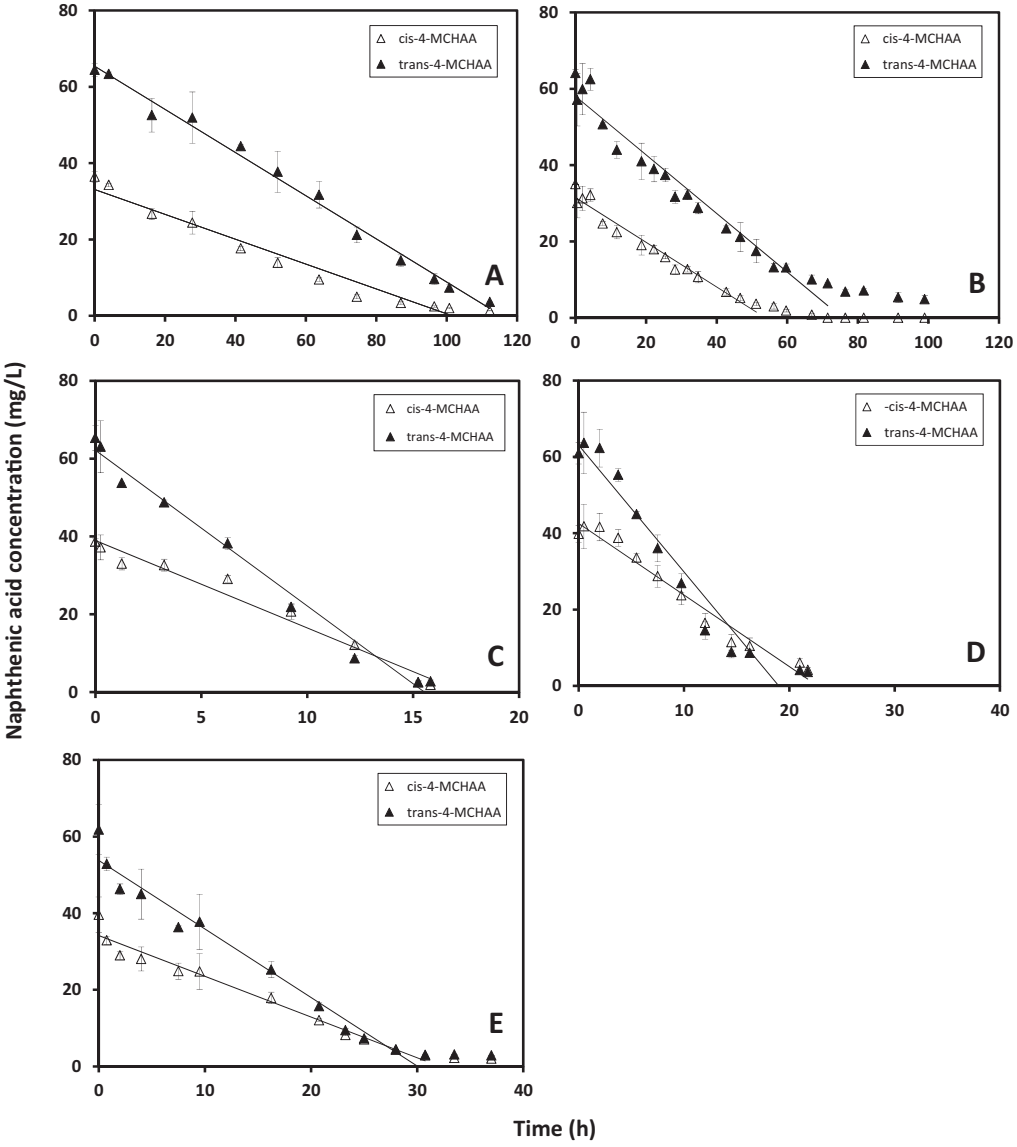

Fig. 4. Batch biodegradation of 100 mg/L 4-MCHAA(mixture of *cis* and *trans* isomers) in the CPBB at (A) 15, (B) 20, (C) 25, (D) 30, (E) 35 °C.

3.2. Continuous biodegradation

Results of continuous biodegradation of 50 and 100 mg/L *trans*-4MCHCA in terms of volumetric loading rate, removal percentage and volumetric removal rate are presented in Fig. 6 (panel A). The reactor was initially fed with medium containing 50 mg/L *trans*-4MCHCA and operated at a loading rate of 39.2 mg/L h (residence time: 1.2 h). Under these conditions removal percentage and removal rate were 83.0% and 32.5 mg/L h, respectively. Increase of loading rate up to 319.8 mg/L h (residence time: 0.15 h) increased the removal rate to a maximum value 208.8 mg/L h, with the corresponding removal percentage being 65%. Further increase of loading rate to 642 mg/L h (residence time: 0.075 h) led to a lower removal rate of 163.2 mg/L h. The biomass concentration in the liquid phase at the lowest and highest applied loading rates were 33.7 ± 1.2 and 21.8 ± 0.5 mg dry weight/L, respectively. Performance of the bioreactor with 100 mg/L *trans*-4MCHCA at the residence time of 0.075 h followed the same pattern observed with 50 mg/L (data not shown). As indicated in Section 2.6 at the end of this run the feed concentration and flow rate were decreased to 50 mg/L and 1.5 L/h, respectively to assess the reproducibility of the results. The variation in removal percentages and removal rates for the original and repeated runs were 8.6% and 3.2%, respectively which indicate good reproducibility of the results. The biofilm hold up in the bioreactor was 0.047 g dry weight biomass/g of the dry packing or 6290 mg/L of working volume, while the working volume was 450 ml.Paslawski et al. [21] studied continuous biodegradation of 500 mg/L *trans*-4MCHCA in a packed-bed bioreactor and achieved a maximum biodegradation rate of 916.7 mg/L h (specific biodegradation rate: 8.78×10^{-3} mg of substrate/mg biomass h) at a residence time 0.6 h. The maximum specific biodegradation rate obtained in the circulating packed-bed bioreactor (33.2×10^{-3} mg of substrate/mg biomass h) was approximately 4-fold faster than the rate reported by Paslawski et al. [21], while the required residence time was 4-fold shorter. It should be pointed out that due to use of 500 mg/L *trans*-4MCHCA in a packed bed bioreactor with no circulation and over a much longer period of operation, the biomass hold-up in the earlier work by Paslawski et al. was much higher than that in this present work.

Fig. 6 (panel B) shows the removal rates of *cis*- and *trans*-4-MCHAA as a function of their loading rates in the CPBB. Included in this figure is also the removal percentage of each isomer. It

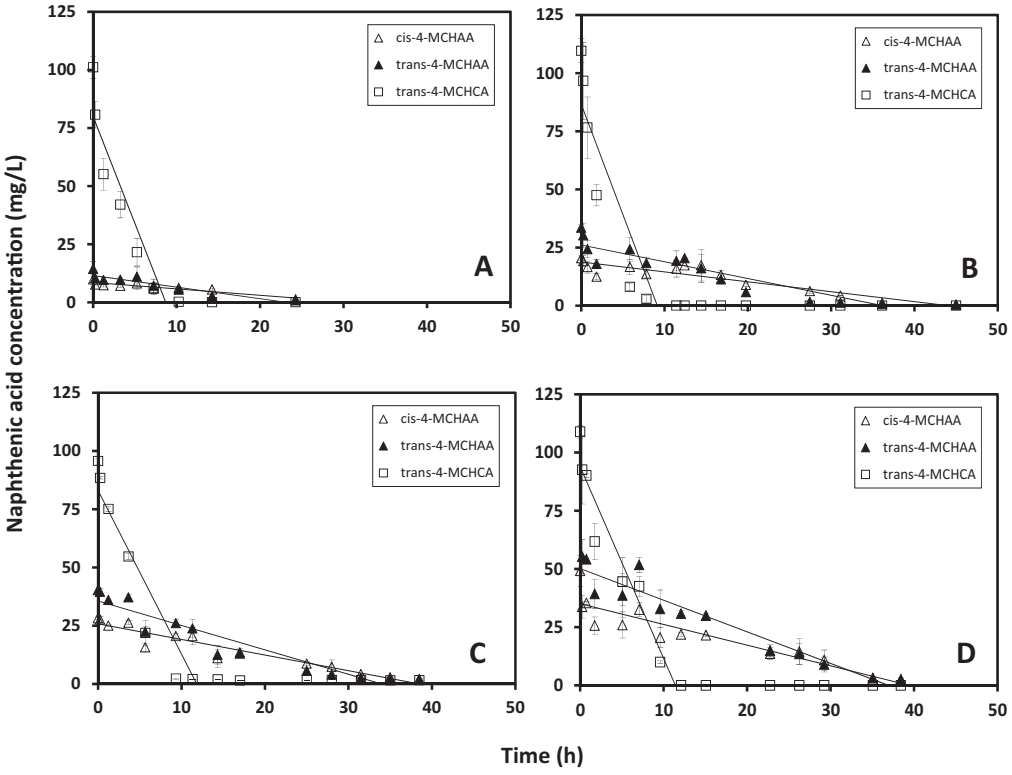

Fig. 5. Batch biodegradation of mixtures consisting of 100 ppm 4-MCHCA and (A) 25, (B) 50, (C) 75 and (D) 100 ppm 4-MCHAA (mixture of *cis* and *trans* isomers) in the CPBB at 25 °C.

should be reiterated that the 4-MCHAA used in this study was a mixture *cis* and *trans* isomers (35% and 65%, respectively). Thus, application of a constant feed flow rate or residence time resulted in different loading rates for *cis* and *trans* isomers. As shown in Fig. 6 the highest removal percentages of 94.5% and 88.4% were obtained at the lowest applied loading rates of 2.0 and 3.9 mg/L h for *cis* and *trans* isomers, respectively (corresponding residence time: 8 h). Biodegradation rates of both isomers increased with increases of loading rate to 4.7 mg/L h for *cis*-4-MCHAA and 10.7 mg/L h for *trans*-4-MCHAA (residence time: 3.3 h), with the maximum biodegradation rates being 4.2 and 7.8 mg/L h, respectively (corresponding removal percentages: 87.0% and 72.7%, respectively). pH in the bioreactor reactor fluctuated in the range 6.4–6.5. The biomass concentration in the liquid phase at the lowest and highest applied loading rates were 33.7 ± 2.5 and 30.3 ± 2.1 mg dry weight/L, respectively.

Operation of the bioreactor in the continuous mode in general resulted in biodegradation rates which were much higher than those during the batch operation but the impact of structural differences in both modes of operation were consistent. With *trans*-4MCHCA the maximum biodegradation rate of 208.8 mg/L h was achieved at a loading rate of 319.8 mg/L h (residence time: 0.15 h), while the with *cis*- and *trans*-4-MCHAA much lower maximum biodegradation rates of 4.2 and 7.8 mg/L h achieved at loading rates of 4.2 and 7.8 mg/L h, respectively (residence time: 3.3 h). Finally analysis of the microbial community in the bioreactor singled out *Pseudomonas aeruginosa* and *Alcaligenes paradoxus* as the dominant species. Control experiments conducted in the absence of biofilm did not show a marked decrease in concentration of naphthenic acids (1.9–4.5%), indicating that stripping of the naphthenic acids by flowing air was not significant.

As indicated earlier, a review of the literature dealing with biodegradation of naphthenic acids highlights the lack of concerted effort on engineering aspects of the biodegradation process. In fact the majority of earlier studies have focused on biodegradability of naphthenic acids, impacts of molecular structure on the extent of biodegradation, and identification of biodegradation pathways. Using enrichment cultures obtained from the rhizosphere of plants native to Alberta, Biryukova et al. [25] studied biodegradation of a commercial naphthenic acid mixture in shake flasks. The preferential degradation of low molecular mass and increase in proportion of high molecular mass naphthenic acids were reported by these authors. The overall biodegradation rate obtained in this work are in the range 0.4–0.6 mg/L h. However, biodegradation rates of only 0.04–0.06 mg/L h for a NA mixture extracted from the process water of the Mildred lake settling basin were reported by Videla et al. [2].

Effect of molecular structure on biodegradability of naphthenic acids has been the subject of a number of studies. Using

Table 2
Batch biodegradation rates of mixtures of *trans* 4-MCHCA and 4-MCHAA at various concentrations at 25 °C.

trans-4-MCHCA concentration (mg/L)	4-MCHAA total concentration (mg/L)	*trans*-4-MCHCA biodegradation rate (mg/L h)	*cis*-4-MCHAA biodegradation rate (mg/L h)	*trans*-4-MCHAA biodegradation rate (mg/L h)
100	25	9.2 (R^2 = 0.88)	0.3 (R^2 = 0.86)	0.5 (R^2 = 0.85)
100	50	9.5 (R^2 = 0.83)	0.4 (R^2 = 0.85)	0.8 (R^2 = 0.85)
100	75	10.1 (R^2 = 0.89)	0.7 (R^2 = 0.92)	1.2 (R^2 = 0.90)
100	100	8.8 (R^2 = 0.93)	0.9 (R^2 = 0.84)	1.4 (R^2 = 0.92)

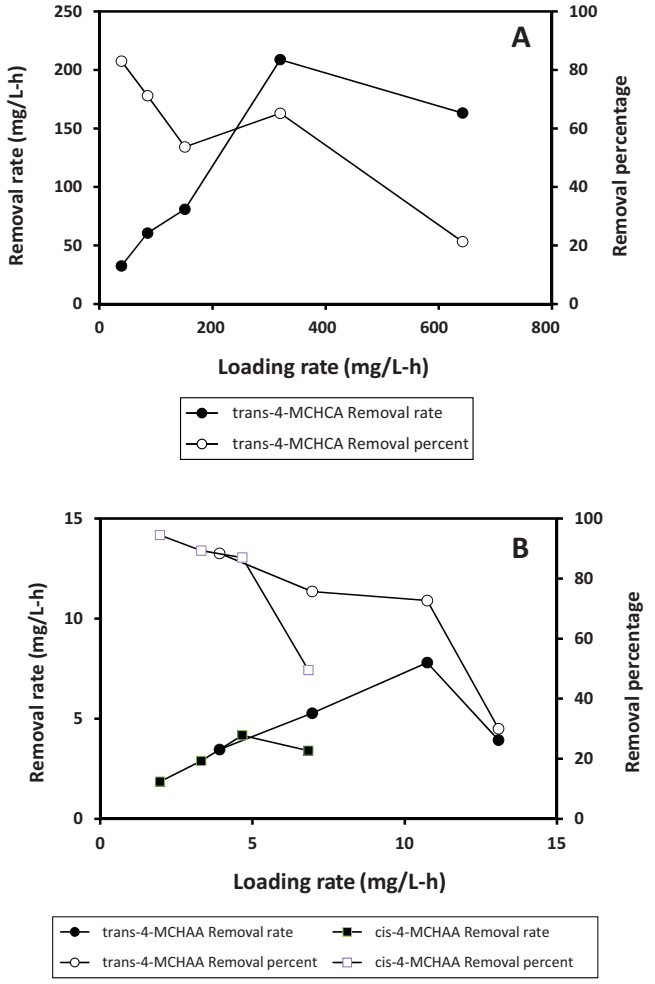

Fig. 6. Continuous biodegradation of 50 and 100 ppm *trans*-4MCHCA (A) and 50 ppm (mixture of *cis* and *trans* isomers) 4-MCHAA (B) in the CPBB.

^{14}C-labeled mono- and bi-cyclic NA surrogates, and sediments from eleven wetlands, both natural and process affected, Del Rio et al. [17] reported that degradation of monocyclic NAs by sediment microbial community occurred regardless of sediment exposure history, while in the case of bicyclic NAs effective degradation was observed only with sediments derived from the wetland exposed to oil sand process water. Comparing the biodegradation of a commercial NA mixture and NA in the oil sand process water, Han et al. [19] reported that commercial NA mixture consisted of a substantial labile fraction amenable to fast biodegradation and a recalcitrant fraction composed of highly branched compounds. By contrast NAs in oil sand process water were predominantly recalcitrant. In either case increased cyclization (z) decreased the biodegradation, while carbon number (n) had little effect. These authors concluded that biodegradation of both commercial and oil sand process water NAs likely occur through β-oxidation, while α-oxidation and aromatization may contribute to the degradation of any one compound, and that increased cyclicity and alkyl branching negatively impact the biodegradation of NAs. Further evidence on the effect of cyclicity was observed in a follow-up work [3], where comparison of the NA signatures in fresh oil sands ore extracts and those from oil sand process water suggested that the least cyclic fraction might undergo rapid biodegradation in settling ponds. Effects of alkyl chain branching on biodegradation of NAs was also studied by Smith et al. [18], using a sedimentary bacterial community, butylcyclohexylbutanoic acids (BCHBAs) with variously branched butyl side chains, and 4-4′-isobutylcyclohexylpentanoic acid (iso-BCHPA), with both branched butyl and branched alkanoate chains. In case of BCHBAs, biodegradation decreased as side chain branching increased, while iso-BCHPA was found resistant to degradation. Johnson et al. [5] confirmed the effect of alkyl side chain while studying the biodegradation of 4 aromatic alkanoic acid isomers with different alkyl side branching, namely 4′-n-butylphenyl-4-butanoic acid, 4′-iso-butylphenyl-4-butanoic acid, 4′-sec-butylphenyl-4-butanoic acid and 4′-tert-butylphenyl-4-butanoic acid.

The results of the present study have highlighted the spatial arrangement of the alkyl side branch as another important factor that affects the biodegradability of naphthenic acid. This was clearly demonstrated during biodegradation of 4-MCHAA ($C_9H_{16}O_2$) where biodegradation of the *trans* isomer proceeded at a rate much faster than that of *cis* counterpart. Furthermore, it appears that the carbon number also influenced the biodegradability of the naphthenic acids studied in this work, where biodegradation rates obtained for *trans*-4-MCHCA ($C_8H_{14}O_2$) was significantly higher than that of *trans*-4-MCHAA ($C_9H_{16}O_2$).

4. Conclusions

Generation of process waters contaminated by naphthenic acids is a serious environmental problem associated with the processing of oil sands for production of bitumen and other petroleum products. This together with necessity for sustainable use of water highlights the need for development of effective and feasible technologies such as bioremediation for treatment of these contaminated waters. Success of a large scale bioremediation process depends significantly on a thorough understanding of the engineering aspects such as biokinetics, mass transfer (both oxygen and organics), as well as bioreactor design. Results of the present study with surrogate naphthenic acids demonstrated that application of a circulating packed bed bioreactor could result in biodegradation rates which are much faster than those reported for conventional CSTR or packed-bed bioreactors. Furthermore, batch and continuous biodegradation of surrogate naphthenic acids in this system revealed that the biodegradability of the naphthenic acids is influenced by both carbon number, as well as the spatial arrangement of the alkyl side branch. The biodegradation results obtained in the present work would certainly assist in future studies aiming at biodegradation and treatment of naphthenic acids in oil sand process water.

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References

[1] E.W. Allen, Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives, J. Environ. Eng. Sci. 7 (2008) 123–138.
[2] P.P. Videla, A.J. Farwell, B.J. Butler, D.G. Dixon, Examining the microbial degradation of naphthenic acids using stable isotope analysis of carbon and nitrogen, Water Air Soil Pollut. 197 (2009) 107–119.
[3] X. Han, M.D. MacKinnon, J.W. Martin, Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS, Chemosphere 76 (2009) 63–70.
[4] S. Kean, Eco-alchemy in Alberta, Science 326 (2009) 1052–1055.
[5] R.J. Johnson, B.E. Smith, P.A. Sutton, T.J. McGenity, S.J. Rowland, C. Whitby, Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching, ISME J. 5 (2011) 486–496.
[6] D.M. Grewer, R.F. Young, R.M. Whittal, P.M. Fedorak, Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? Sci. Total Environ. 408 (2010) 5997–6010.

[7] D.M. Quensel, L.M. Bhaskar, L.M. Gieg, G. Chua, Naphthenic acid biodegradation by the unicellular alga *Dunaliella tertiolecta*, Chemosphere 84 (2011) 504–511.
[8] A.C. Scott, W. Zubot, M.D. MacKinnon, D.W. Smith, P.M. Fedorak, Ozonation of oil sands process water removes naphthenic acids and toxicity, Chemosphere 71 (2008) 156–160.
[9] J.W. Martin, T. Barri, X. Han, P.M. Fedorak, M. Gamal El-Din, L. Perez, A.C. Scott, J.T. Jiang, Ozonation of oil sands process-affected water accelerates microbial bioremediation, Environ. Sci. Technol. 44 (2010) 8350–8356.
[10] A. Zhang, Q. Ma, K. Wang, X. Liu, P. Shuler, Y. Tang, Naphthenic acid removal from crude oil through catalytic decarboxylation on magnesium oxide, Appl. Catal. A 303 (2006) 103–109.
[11] X. Liang, X. Zhu, E.C. Butler, Comparison of four advanced oxidation processes for the removal of naphthenic acids from model oil sand process water, J. Hazard. Mater. 190 (2011) 168–176.
[12] D.W. McMartin, J.V. Headley, D.A. Friesnen, K.M. Peru, J.A. Gillies, Photolysis of naphthenic acids in natural surface water, J. Environ. Health Sci. 39 (2004) 1361–1383.
[13] P. Drzewicz, A. Afzal, M. Gamal El-Din, J.W. Martin, Degradation of model naphthenic acids by vacuum UV (172 nm) and UV (254 nm)/H_2O_2, Phys. Chem. 114 (2010) 12067.
[14] S. Mishara, V. Meda, A.K. Dalai, D.W. McMartin, J.V. Headley, K.M. Peru, Photocatalysis of naphthenic acids in water, J. Water Resour. Prot. 2 (2010) 644–650.
[15] J.V. Headley, K.M. Peru, S. Mishra, V. Meda, A.K. Dalai, D.W. McMartin, M.M. Mapolelo, R.P. Rogers, A.G. Marshal, Characterization of oil sands naphthenic acids treated with ultraviolet and microwave radiation by ion electroscopy Fourier transform ion cyclotron resonance mass spectroscopy, Rapid Commun. Mass Spectrom. 24 (2010) 3121–3126.
[16] J.V. Headley, S. Tanapat, G. Putz, K.M. Peru, Biodegradation kinetics of geometric isomers of model naphthenic acids in Athabasca river water, Can. Water Resour. J. 27 (2002) 25–42.
[17] L.F. Del Rio, A.K.M. Hadwin, L.J. Pinto, M.D. MacKinnon, M.M. Moore, Degradation of naphthenic acids by sediment micro-organism, J. Appl. Microbiol. 101 (2006) 1049–1061.
[18] B.E. Smith, C.A. Lewis, S.T. Belt, C. Whitby, S.J. Rowland, Effects of alkyl chain branching on the biotransformation of naphthenic acids, Environ. Sci. Technol. 42 (2008) 9323–9328.
[19] X. Han, A.C. Scott, P.M. Fedorak, M. Batineh, J.W. Martin, Influence of molecular structure on the biodegradability of naphthenic acids, Environ. Sci. Technol. 42 (2008) 1290–1295.
[20] J. Paslawski, J. Headley, G.A. Hill, M. Nemati, Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid, Biodegradation 20 (2009) 125–133.
[21] J. Paslawski, M. Nemati, G.A. Hill, J. Headley, Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid in continuously stirred tank and immobilized cell bioreactors, J. Chem. Technol. Biotechnol. 84 (2009) 992–1000.
[22] M. Nemati, C. Webb, Bioreactors-Applications, Immobilized cell bioreactors, in: M. Moo-Young (Ed.), Comprehensive Biotechnology, vol. 2, 2nd Ed., Elsevier, 2011, pp. 331–346.
[23] J.S. Clemente, P.M. Fedorak, A Review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60 (2005) 585–600.
[24] P. Kumar, M. Nemati, G.A. Hill, Biodegradation kinetics of 1,4-benzoquinone in batch and continuous systems, Biodegradation 22 (2011) 1087–1093.
[25] O.V. Biryukova, P.M. Fedorak, S.A. Quideau, Biodegradation of naphthenic acids by rhizosphere microorganisms, Chemosphere 67 (2007) 2058–2064.