Partitioning and bioaccumulation of metals from oil sands process affected water in indigenous *Parachlorella kessleri*

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HIGHLIGHTS

▶ Metal partitioning from oil sands tailings by indigenous *Parachlorella kessleri* is reported.
▶ FTIR scan showed presence of functional groups on *P. kessleri* cell wall.
▶ These functional groups contribute to extracellular bioaccumulation of metals.
▶ Yet, intracellular bioaccumulation played the main role in metal removal from TPW.

ARTICLE INFO

ABSTRACT

This paper studies the partitioning and bioaccumulation of ten target metals ([53]Cr, Mn, Co, [60]Ni, [65]Cu, [66]Zn, As, [88]Sr, [95]Mo and Ba) from oil sands tailings pond water (TPW) by indigenous *Parachlorella kessleri*. To determine the role of extracellular and intracellular bioaccumulation in metal removal by *P. kessleri*, TPW samples taken from two oil sands operators (Syncrude Canada Ltd. and Albian Sands Energy Inc.) were enriched with nutrient supplements.

Results indicate that intracellular bioaccumulation played the main role in metal removal from TPW; whereas extracellular bioaccumulation was only observed to some extent for Mn, Co, [60]Ni, [65]Cu, [88]Sr, [95]Mo and Ba. The FTIR scan and titration of functional groups on the cell surface indicated low metal binding capacity by indigenous *P. kessleri*. However, it is believed that the dissolved cations and organic ligand content in TPW (such as naphthenic acids) may interfere with metal binding on the cell surface and lower extracellular bioaccumulation. In addition, the total bioaccumulation and bioconcentration factor (BCF) varied during the cultivation period in different growth regimes.

1. Introduction

The Athabasca region in Alberta, Canada possesses the third largest oil deposits (in the form of oil sands) in the world after Saudi Arabia and Venezuala. For the extraction of bitumen, alkaline hot water is needed; where every 1 m^3 of produced crude oil is accompanied with 1.5 m^3 of process affected water containing clay, sand, unrecovered hydrocarbons and metals of environmental concern (Holden et al., 2011). To date, process affected water has been collected in designated tailings ponds and due to its toxicity cannot be released to the environment. With global oil demand increasing and unprecedented oil sands industry growth in the Athabasca region, it is predicted that oil production will surge to 3 million barrels per day in 2015. This will lead to considerable volumes of process affected water in the near future. Currently, total impounded tailings among all oil sands companies in Alberta have exceeded 700 million m^3 (Allen, 2008).

Along with unrecovered hydrocarbons, another environmental concern of TPW is metal content. The concentration of dissolved metals As, Cr, Mo and Cu in Syncrude's TPW and Cr and Cu in Albian's TPW exceeded Canadian Water Quality Guidelines for the protection of aquatic life (http://ceqg-rcqe.ccme.ca). Metals can be a threat to human health, aquatic life and the environment (Febrianto et al., 2009), yet there has been little research to find efficient methods for removing metals from TPW. Physical–chemical methods such as precipitation–filtration, ion exchange, reverse osmosis and oxidation–reduction reactions can be employed for the removal of metals from contaminated water. These methods are not economically cost effective or feasible when they are applied to a large volume of water (such as TPW) (Lourie et al., 2010). Biological methods for metal removal, however, are much more economical for this scenario, since there is no cost for operation and maintenance or addition of chemicals and equipment use, moreover microorganisms (biomass) can be regenerated.

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Bioaccumulation processes in algal cells occur through two mechanisms; extracellular where metals bind to functional groups on the cell wall, and intracellular where metals are taken inside the cell body. Ionic strength, pH, temperature, organic matter, multi-metal interaction and algal biomass weight impact the efficiency of metal removal and consequently the total bioaccumulation capacity (Terry and Stone, 2002; Peña-Castro et al., 2004; Andrade et al., 2005; Hassler et al., 2005; Chojnacka, 2007; Worms et al., 2010).

There are only three studies on TPW treatment using algae, two of these focus on the removal of naphthenic acids, the organic pollutant fraction of TPW (Headley et al., 2008; Quesnel et al., 2011). The third study was conducted by our research group and reported the removal of 10 target metals of environmental concern (^{53}Cr, Mn, Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, As, ^{88}Sr, ^{95}Mo and Ba) from two oil sands TPWs (taken from Syncrude's and Albian's ponds) using alga *Parachlorella kessleri* (Mahdavi et al., 2012). However, the fate of these removed metals, partitioning among algal cells and mechanism of removal, are still unknown. To address these knowledge gaps, this follow-up paper first considers the functional groups associated with metal removal on *P. kessleri*'s cell wall, and then investigates the intracellular and extracellular bioaccumulation of target metals and corresponding bioaccumulation parameters. The possible role of organic and inorganic fractions of TPW and their interference on the bioaccumulation and partitioning of target metals will be discussed. The results of this study elucidate many unknown aspects of metal uptake in algae and can be applied for further algae related investigations on metal removal.

2. Materials and methods

The preparation of material and facilities, *P. kessleri* identification and inoculum preparation, experimental condition, and the methodology of target metal removal confirmation have been described previously in detail (Mahdavi et al., 2012). Water chemistry analysis is shown in Table 1. A short explanation of the experimental set-up follows: Two oil sands TPWs taken from Syncrude and Albian ponds were filter sterilized (0.22 μm, Stericup® *Millipore*) and each TPW was enriched with two concentration levels of nitrate and phosphate (as low as 0.24 mM NO_3^- and 0.016 mM PO_4^{3-} or as high as 1.98 mM NO_3^- and 0.201 mM PO_4^{3-}). Three of these enriched TPW samples were inoculated with *P. kessleri* cells to a final concentration of 10^5 cells mL^{-1} (22.4 mg L^{-1} dry biomass), and two were left as sterilized blanks. The cultivations were performed in autoclaved 1000 mL flasks under continuous illumination at 4300 lux (cool light, 44 W, Philips, Markham, ON) at 28 °C with shaking (100 rpm). Over the 14 d cultivation many parameters including alga cell count, dry biomass, pH, and metal content in TPW and alga cells were monitored. Algal dry biomass was established by filtering (1 μm pore size, Whatman GF/B) at

least 3 mL TPW containing *P. kessleri* cells, followed by drying for 24 h at 55 °C. Dried cells were weighed using a Mettler Toledo, AB204-S/Fact scale. The pH of aliquots from the experimental flasks was measured with a probe from Accumet® Research, AR50, Fisher Scientific. Since the results showed a pH increase in blanks, the actual pH increase as a result of algae growth was determine from the following calculations:

$$A = B - C, \qquad (1)$$

where A is pH increase due to abiotic characteristics of TPW, B is pH of TPW blank sample and C is initial pH of the TPW blank sample at the beginning of experiment. The actual pH increase due to algae growth was then calculated by subtracting pH increase due to abiotic characteristics of TPW (A) from pH observed in algae containing sample (F):

$$E = F - A, \qquad (2)$$

where E is the actual pH variation due to algae growth (without abiotic interference). Fig. 2 shows the E values (the actual pH increase) in both TPWs.

2.1. Metal partitioning analysis

Metal analysis was performed with an inductively coupled plasma-mass spectrometry (ICP-MS) (PerkinElmer SCIEX, ELAN 9000). The technical details of the ICP-MS method have been described by Mahdavi et al. (2012). In this research for the target metals which possess more than one isotope, the number of nucleons was reported at the upper left of the chemical symbol (e.g. ^{53}Cr). To measure metal removal percentage, TPW containing algae was centrifuged ($3700 \times g$ for 20 min) and the supernatant was utilized for metal removal confirmation (Mahdavi et al., 2012). The remaining algal pellet was resuspended in 0.2 M phosphate buffer (pH 7.5), which dissolves the precipitated metals (Matsunaga et al., 1999). This mixture was recentrifuged ($3700 \times g$ for 20 min) and the supernatant was analyzed by ICP-MS. The mass of the precipitated metals was determined by measuring metal concentrations in the phosphate buffer before and after contact with the algal pellet (Matsunaga et al., 1999). Afterward, the remaining algal pellet was resuspended in 5 mM EDTA/HEPES buffer, which stripped the metals attached to the extracellular functional groups (Hassler et al., 2005). ICP-MS analysis was completed on the supernatant to determine the extracellular bioaccumulated metals.

To determine intracellular bioaccumulated metal concentrations, the algal pellet was dissolved and digested in 30% HNO$_3$, left for 24 h at 40 °C and then analyzed for metal content. To validate the results of the above employed digestion method, a second microwave-assisted digestion method was used: 4 mL concentrated nitric acid (70%, trace-metal grade Fisher Scientific), 2 mL H$_2$O$_2$ (30%, Fisher Scientific) and 4 mL DI water (Moreda-Piñeiro et al.,

Table 1
Initial chemical composition of tailings pond water samples collected from Syncrude and Albian. (a) Metals (data represent the average of four replicates ± standard deviation), and (b) major anions and cations.

Sample	Initial trace metal concentrations (μg L^{-1})									
	^{53}Cr	Mn	Co	^{60}Ni	^{65}Cu	^{66}Zn	As	^{88}Sr	^{95}Mo	Ba
(a)										
Syncrude	6.7 ± 1.0	44.0 ± 3.66	2.13 ± 0.20	9.90 ± 0.89	15.7 ± 2.9	24.3 ± 9.73	5.11 ± 0.22	698 ± 9.60	123 ± 4.50	135 ± 6.5
Albian	3.8 ± 0.3	7.20 ± 0.65	1.43 ± 0.14	10.3 ± 0.93	20.5 ± 6.0	20.1 ± 2.06	3.57 ± 0.10	568 ± 5.46	53.1 ± 1.09	240 ± 3.4

	Cations (mg L^{-1})						Anions (mg L^{-1})					
	Li$^+$	Na$^+$	NH$_4^+$	K$^+$	Mg^{2+}	Ca^{2+}	F$^-$	Cl$^-$	NO$_2^-$	Br$^-$	NO$_3^-$	SO$_4^{2-}$
(b)												
Syncrude	0.21	842	30.2	17.4	10.6	18.7	2	635	5	<1	17	565
Albian	0.14	299	1.2	16.7	11.3	22.1	2	171	<1	<1	<1	159