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The impact of various ozone pretreatment doses on the performance of endogenous microbial communities for the remediation of oil sands process-affected water

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ABSTRACT

The effects of different ozone pretreatment doses on the performance of endogenous microbial populations in degrading naphthenic acids (NAs) for the treatment of oil sands process-affected water (OSPW) were evaluated in this study. Ozone pretreatment enhanced OSPW biodegradability and accelerated the growth of microbial populations in bioreactors. After ozone pretreatment, the maximum chemical oxygen demand (COD) removal through biodegradation occurred at an ozone dose of 50 mg/L. Although OSPW pretreated with a higher ozone concentration removed a higher COD concentration and acid extractable fraction (AEF), organics removal through biodegradation was not further improved. After pretreatment with an ozone dose of 200 mg/L and bioreactor operation for 73 days, the batch bioreactor removed more than 80% of the COD and more than 95% of AEF. High-resolution mass spectrometry analysis showed complete degradation of NAs with specific degrees of cyclization ($Z = -2$ and -4) after combined treatments of ozonation and biodegradation. At high pretreatment doses of ozone (116 and 200 mg/L), biodegradation completely eliminated the toxicity of OSPW toward *Vibrio fischeri* ($IC_{20} > 100\%$ v/v).

Introduction

With the decline in conventional light oil reserves worldwide, the development of alternative resources for oil production is becoming more attractive. One of the world's largest single accumulations of oil sands was discovered in the shallow reservoirs of Canada. It is estimated that there are 173.2 billion barrels of recoverable bitumen within the oil sands of the Athabasca Basin (Frank et al., 2008). However, most oil sands refineries use large volumes of hot, alkaline water when extracting the bitumen (Schramm et al., 2000; Hadwin et al., 2006) which generates large amounts of wastewater, known as oil sands process-affected water (OSPW) (Hadwin et al., 2006), that may lead to acute and chronic aquatic toxicity if the untreated OSPW is released into the environment. As a result, OSPW is currently retained in large tailings ponds. On a very conservative estimate, there will be 1 billion m^3 of tailings pond water accumulated over the next 15–20 years (MacKinnon, 1989; Herman et al., 1994; Lo et al., 2003; Del Rio et al., 2006).

Much of the toxicity of OSPW is attributed to its organic acids, known as naphthenic acids (NAs) (Seifert and Teeter, 1970; Hsu et al., 2000). To date, many different kinds of treatment processes have been investigated for their ability to remove NAs from OSPW, some of which are physical treatments, such as membrane filtration and physical adsorption (Peng et al., 2004; Mohamed et al., 2008), while others are physico-chemical, such as coagulation and flocculation (Kim et al., 2011; Pourrezaei et al., 2011). Many of these physical and physico-chemical approaches have shown some success in OSPW detoxification and the removal of recalcitrant OSPW organic compounds such NAs. These previously mentioned treatment processes together with their high costs, preclude their acceptability for use in large-scale industrial applications (Kannel and Gan, 2012).

Biological treatment, which exploits the degradation of organic compounds through reactions mediated by microorganisms, has been used successfully for wastewater reclamation in various

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industries. Biological treatment could allow for the oxidation of a wide variety of organic compounds, to lead to true destruction instead of only having a phase separation as in the case of the adsorption processes. However, previous work has demonstrated recalcitrance of oil sands' NAs after biological treatment, indicating that pretreatment is required to improve the biodegradability of OSPW (Scott et al., 2005). Ozone is one of the strongest oxidants available and it can attack organic contaminants either directly or indirectly, through generation of free radical intermediates (Hoigne and Bader, 1983). Ozone can preferentially break down complex organic compounds into simpler compounds that are easier to degrade within subsequent biological processes. Ozonation has shown the capability of breaking the highly branched and cyclic carboxylic fraction of NAs and of mitigating the toxicity of OSPW (Han et al., 2008; Pérez-Estrada et al., 2011; Gamal El-Din et al., 2011; Anderson et al., 2012). However, ozonation alone is not economically feasible for fully mineralizing organic pollutants present in OSPW due to its heavy demand of energy. To reduce the treatment costs, ozonation is usually used in combination with biological treatment (Oller et al., 2011). Compared with other advanced oxidation processes which have potential to decontaminate and detoxify OSPW, ozonation does not leave behind chemicals in the water and the high amount of oxygen released by the ozonation process could greatly benefit the microbes to enhance their aerobic degradation capability.

Combining ozonation with biological treatment has been successfully applied for OSPW and other industrial wastewater treatment (Martin et al., 2010; Gamal El-Din et al., 2011; Oller et al., 2011). Gamal El-Din et al. (2011) and Wang (2011) have demonstrated that ozonation can decrease the amount of NAs in OSPW while increasing its biodegradability. It was found that ozonation, followed by biological degradation using a native microbe inoculation, significantly accelerated the detoxification and biodegradation of NAs in batch reactors (Martin et al., 2010; Gamal El-Din et al., 2011; Wang et al., 2013). More recently, Hwang et al. (2013) observed the removal of over 99.9% of parent NAs when pretreating the feed OSPW into biofilm reactors with ozonation. These studies have indicated that ozonation followed by biological treatment has strong potential for OSPW treatment.

To our knowledge, little research has been undertaken to determine the effect of differing ozone doses on the degradation of parent NAs and on the subsequent biologically mediated NAs degradation using endogenous microorganisms in OSPW. For instance, a utilized ozone dose that is too low may not adequately break down the recalcitrant NAs, while a utilized ozone dose that is too high would be unfeasible economically. Previous study showed that prolonged, five-hour ozonation of oil-contaminated sand and peat did not substantially improve its biodegradability (Goi et al., 2006). A high dose of utilized ozone could actually be disadvantageous to biodegradation, as seen in one study where an increase in ozone dose led to a sharp rise followed by a slow decrease in the biochemical oxygen demand (BOD) during the treatment of phenolic wastewater (Amat et al., 2003). This finding indicated that ozonation before biotreatment must be carefully controlled, to avoid redundant ozonation which generates carboxylic acids of low molecular weight that do not show enhanced biodegradability (Amat et al., 2003). Moreover, by-products of ozonation can be even more toxic than the parent compounds, such as when an ozonated naphthalene sulphonic acid derivative (<1 kDa) was shown to exhibit increased toxicity toward the marine algae *Phaeodactylum tricornutum* (Germirli Babuna et al., 2009). Previous study investigating ozonated resin acid solutions showed a limit of 0.3–0.5 mg O_3/mg COD before toxicity increased (Ledakowicz et al., 2006). Another study related to the degradation of an azo dye, observed the increase in toxicity after 150 of the total 360 min ozone treatment (Wang et al., 2003). An increase of bacterial inhibition towards *Vibrio fischeri* has been also reported during the ozonation of non-ionic surfactants while improved biodegradability of surfactants was observed (Ledakowicz et al., 2005). In addition, Shang et al. (2006) revealed that oxidized chlorophenol intermediates induced more toxicity to pure bacteria and mixed microorganisms than parent compounds in the early stage of ozonation (<1 min) and then the inhibition gradually decreased until it disappeared after 6 min of reaction. For the treatment of OSPW, where biodegradability and toxicity are both of paramount concern, there is still need for more work to determine the optimal utilized dose of ozone for pretreatment prior to biodegradation. The objective of this study was to evaluate the effect of utilized ozone dose on the detoxification and biodegradation of OSPW. The role of ozonation and biodegradation in the removal of organic compounds, the changes in microbial community structure and composition, and the reduction of microbial toxicity were also investigated.

Materials and methods

Source of OSPW

Raw OSPW was received in a polyvinyl chloride barrel from the water pumping station at one of the OSPW recycle ponds in Fort McMurray, Alberta, Canada. The raw OSPW samples were stored at 4 °C before treatment.

Batch experiment overview

Six Erlenmeyer flasks of 2000 mL capacity each, were designed and operated as bioreactors to degrade and remove NAs from OSPW. Each reactor containing 1000 mL of OSPW was autoclaved to inactivate endogenous microorganisms. Ozone was then injected into five different reactors for varying lengths of time and the final utilized ozone concentrations in different reactors were calculated following the procedure described by Gamal El-Din et al. (2011). To achieve standardized levels of endogenous bacterial strains in each bioreactor, separate 1000 mL batches of raw OSPW were centrifuged at 4000 × g for 20 min and the pellets were collected and inoculated into the test bioreactors. The reactors were operated at room temperature (20 ± 1.0 °C) and were rotated at 150 rpm on a shaker. Water samples were collected periodically for the analysis of the residual organic compounds, using measurement of the chemical oxygen demand (COD), acid-extractable fraction (AEF), and NAs using methods provided in Section "Water chemistry analysis". The biomass in the reactors was also collected to identify the structure and population of the microbial communities using methods provided in Section "Microbiological analysis". All batch experiments were conducted in duplicate.

Ozonation of OSPW

To evaluate the effect of ozone dose on OSPW biodegradation, each batch reactor except for one (0 concentration for "raw OSPW" as a control), was ozonated for different times in order to reach the desired utilized ozone doses. An ozone generator (GSO-40, Herford, Germany) was used to produce ozone gas using extra dry, high purity oxygen. Each 2000 mL-glass reactor was equipped at the bottom with a ceramic fine-bubble gas diffuser, enabling the feed gas to be sparged into the liquid phase with a flow rate of 1 L/min. The ozone concentrations in the feed and off-gas lines were continuously monitored during the process by two identical ozone monitors (model HC-500, PCI-WEDECO, USA), while the ozone residual in the reactor was measured using the Indigo method (APHA, 2005).