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Ozonation and biodegradation of oil sands process water

by

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ABSTRACT

To ensure oil sands process water (OSPW) is suitable for discharge into the environment, advanced water treatment technologies are required. In this study, integrated ozonation-biodegradation was investigated as a potential treatment option for OSPW. The treatment efficiency was evaluated in terms of naphthenic acid (NA) degradation, chemical oxygen demand (COD), carbonaceous Biological oxygen demand (CBOD), and acute toxicity reduction. Degradation of NAs of more than 99% was achieved using a semi-batch ozonation system at a utilized ozone dose of 80 mg/L combined with subsequent biodegradation. The results also show that ozone decreased the amount of COD while increasing the biodegradability of COD. It was noted that the carbon number and number of NA rings influenced the level of NA oxidation. With a utilized ozone dose of approximately 100 mg/L, the ozonated and biodegraded treated OSPW showed no toxic effect towards bacterium *Vibrio fischeri*. The results of this study indicate that integrated ozonation-biodegradation is a promising treatment technology for OSPW.

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List of Abbreviations and Symbols

OSPW	Oil sands process water
TS	Total solids
TSS	Total suspended solids
TDS	Total dissolved solids
NAs	Naphthenic acids
PAHs	Polycyclic aromatic hydrocarbons
DOM	Dissolved organic matter
BOD	Biological oxygen demand
BOD_5	Biological oxygen demand in five days
$CBOD_5$	Carbonaceous Biological oxygen demand in five days
COD	Chemical oxygen demand
FTIR	Fourier Transform Infrared Spectroscopy
MS	Mass spectrometer
UPLC	Ultra Performance Liquid Chromatography
HRMS	High resolution Mass spectrometer
$C_{G,in}$	Ozone concentration in the feed gas (mg/L)
$C_{G,out}$	Ozone concentration in the off gas (mg/L)
C_L	Residue ozone concentration in the liquid phase (mg/ L)
V_L	Effective reactor volume (litre)
$Q_{G,in}$	Feed-gas flow rate (L/min)
$Q_{G,out}$	Off-gas flow rate (L/min)
Ψ	Gas absorption rate (mg/L/S)

KL	Local liquid mass transfer coefficient (1/ms)
a	Gas bubbles' specific interfacial area (1/m)
CL*	The concentration of dissolved ozone gas in equilibrium with bulk ozone gas (mg/L)
CL	The concentration of dissolved ozone gas in bulk liquid (mg/L).
DO	Dissolved oxygen
TOC	Total organic carbon

1. INTRODUCTION

1.1 Oil sands process water

The Northern Alberta oil sands region contains approximately 174 billion barrels of oil in the form of bitumen, which represents the second-largest oil deposit in the world, after Saudi Arabia (Alberta Energy, 2008). Different from conventional oil, oil sands exist in a solid phase as a mixture of bitumen, sands, and clays. The process of extracting bitumen from oil sands is more costly and complicated than that of conventional oil. In past decades, due to the high costs of extraction and low price of crude oil, development of the oil sands was not economically attractive. Recently, with an increased demand for oil and the price of oil soaring, the oil sands industry has grown rapidly and will keep growing in the future.

In order to extract the bitumen from oil sands, fresh water has to be introduced into mines from a local supply. On average, the extraction process consumes three barrels of fresh water to produce one barrel of oil (Allen, 2008). This water-based extraction process produces a tailings mixture of wastewater, fine tailings (water and solids), and non-recovered bitumen (50:50:1). Because of a zero discharge policy, all of the oil sands tailings and process water have to be stored in tailings ponds. As a result, an estimated one billion m^3 of oil sands process water (OSPW) will have accumulated in the Athabasca oil sands region by 2025 (Herman *et al.*, 1994). When the mines are closed, all the OSPW and tailings will have to be reintegrated into the landscape through different methods without affecting the local aquatic and terrestrial ecosystem (Allen, 2008).

While the efficiency of water usage has been improved and less fresh water is removed from the Athabasca River per barrel produced, operators still face some problems. These problems include the impact of recycling process water, which contributes to the decline of water quality within the system, threatening the extraction process by disrupting extraction chemistry and increasing infrastructure scaling and corrosion. As a result, the rate of bitumen extraction is limited by the supply of fresh water from the river (Allen, 2008).

OSPW is moderately hard with Ca^{2+} and Mg^{2+} present and alkaline with a pH value usually above 8. It contains a high concentration (>2000 mg/L) of total dissolved solids (TDS) which mainly includes sodium, bicarbonate, chloride, and sulphate. Ammonia concentration is variable. Besides inorganic contaminants, many organic compounds are also found in OSPW. These organic compounds include bitumen, naphthenic acids (NAs), asphaltenes, benzene, creosols, humic and fulvic acids, phenols, phthalates, polycyclic aromatic hydrocarbons (PAHs), and toluene. The concentration of dissolved organic matter (DOM) ranges from 50 to 100 mg/L, which is mostly composed of organic acids of which 80% are NAs.

Table 1: Main organic compounds in OSPW and treatment objectives for discharge into the environment (Data from Allen, 2008)

Variable (mg/L)	OSPW in tailings pond	Environmental guideline (mg/L)	
		CEQG[1]	EPEA[2]
Benzene	<0.01-6.3	0.37	
BOD	<10-70		25
COD	86-973		200
Cyanide	0.01-0.5	0.005	
oil and grease	9-92	No visible or odour	5-10
Phenols	0.02-1.5	0.004	1
Toluene	0.01-3	0.002	
PAHs	0.01	0.00001-0.00006	
Naphthenic Acid	50-70		

1. CEQG: Canadian Environmental Quality Guidelines; surface water guidelines for the protection of aquatic life (Canadian Council of Ministers of Environment, 2005)
2. EPEA: Environmental Protection and Enhancement Act; maximum discharge limits for various Alberta industries (Alberta Environment, 1999)

Table 1 shows that the concentrations of all the main organics in the tailings pond water exceed the acceptable limits for environmental discharge. Thus, OSPW stored in tailings ponds cannot be discharged unless treated to meet regulated standards. As a result, effective water treatment technologies need to be developed and applied in order to enhance water quality for recycling without compromising bitumen extraction efficiency and to remove pollutants which contribute to acute and chronic toxicity in aquatic biota.

1.2 Naphthenic acids

Currently, bitumen extraction is based on a caustic hot water digestion, which results in low molecular weight carboxylic acids known as naphthenic acids (NA). NAs are natural components of petroleum and are released into OSPW at concentrations ranging from 40 to 120 mg/L as measured by the Fourier Transform Infrared Spectroscopy (FTIR) method. The NA concentration depends on the age of the OSPW, ore quality, and extraction process (Quagraine *et al.*, 2005). NAs are non-volatile, chemically stable organic compounds and work as surfactants during the extraction process.

The components of NAs vary for different OSPW sources and include a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n-Z}O_2$. In the formula, *n* indicates the carbon (C) number, and *Z* specifies the hydrogen deficiency resulting from the ring formation. In fresh OSPW, dominant NAs have a C number between 13 and 16, while in aged OSPW, the C number of the dominant NAs shifts to higher values (*i.e.*, C_{22}). Examples of NA structures are shown in Figure 1 (Clemente and Fedorak, 2005).

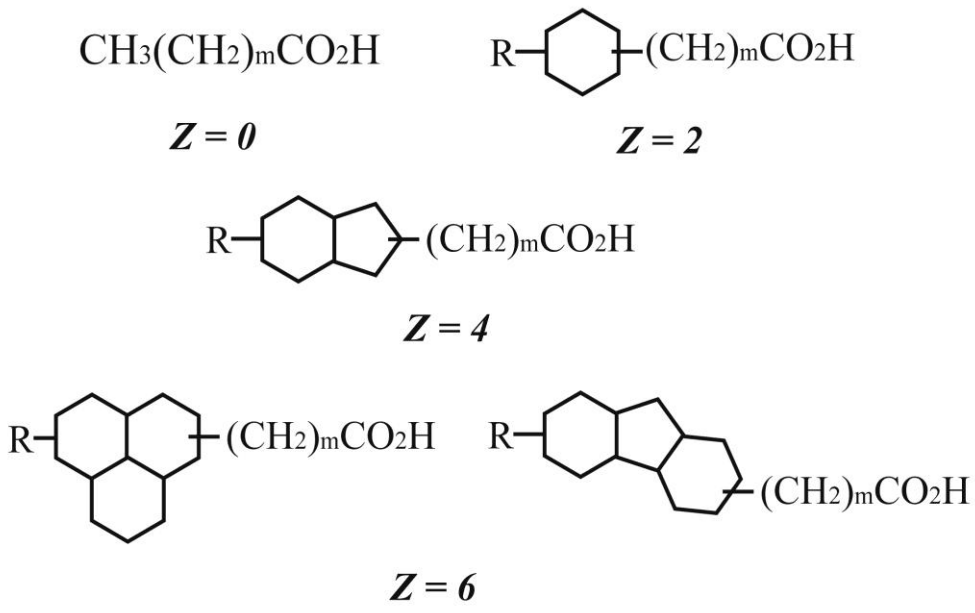


Figure 1: Sample NA structures, where R is an alkyl chain, *Z* describes the hydrogen deficiency, and m is the number of CH_2 units

Because of the complexity of NAs, there is currently no method that can identify or quantify individual acids in detail. Thus, all current analytical methods treat these acids as a group or as sub-groups based on C and *Z* numbers. The former standard for quantifying NAs in the oil sands industry was the FTIR spectroscopy method. Briefly, the method can be described as NA extraction by dichloromethane and subsequent concentration. The concentrated sample is then analyzed by FTIR. The absorbance of the monomeric and dimeric forms of the carboxylic groups at 1743 and 1706 cm^{-1} are measured and compared to the NA standard (Scott *et al.*, 2008). However, this method can only give the total mass of NAs, and the accuracy of the results is easily affected by the extraction process. In order to explain the toxic effect or characteristics of NAs, the total NA concentration is not sufficient. The molecular structures and compositions of NAs

are needed to completely understand these effects. To date, mass spectrometry (MS), especially high-resolution MS (HRMS), has been used to provide detailed information about NAs. In MS, the distribution and relative abundance of components in NAs are presented in 3D plots which provide direct and detailed information of these compounds. Thus, the structures of many components can be presented in a meaningful way which sorts the C number along the X-axis and the Z number along the Y-axis. This sorting also allows the reader to note the weights of different components in NAs (Scott *et al.*, 2008).

Of all the dissolved contaminants, NAs are believed to be the main source of acute toxicity (Clemente and Fedorak, 2005). NAs are quite soluble in neutral or slightly alkaline waters such as OSPW, which have a pH usually higher than 8 (Clemente and Fedorak, 2005). If NAs are discharged into surface water, aquatic organisms are exposed to the toxic effects of NAs. Adjustment of pH to 2.5 followed by centrifugation was shown to remove acute toxicity from tailings water taken from the Mildred Lake settling basin (MacKinnon and Boerger, 1986). Research shows that the acute lethality of OSPW to rainbow trout and water fleas was significantly reduced when NAs in OSPW were removed (MacKinnon and Boerger, 1986). Different reports have shown NAs have inhibitory or toxic effects on a variety of organisms including plants, fish zooplankton, rats, and luminescent bacteria (Clemente and Fedorak, 2005). In this study, to effectively detoxify OSPW, NAs will be targeted for removal.

The biodegradation of NAs in OSPW occurs naturally during the degradation process, and the acute toxicity of OSPW decreases as the relative abundance of smaller NAs (C number 13-16) decreases (Holowenko *et al*., 2002). Holowenko *et al.* used the Microtox system to analyze several oil sands tailings water of various ages and found that lowering the overall NA concentration and having abundant C_{22+} NA clusters resulted in less toxic tailings pond water. This decrease in NA concentration and the change to their compositions with age were attributed to natural biodegradation (Holowenko *et al.*, 2002). Among all of the wastewater treatment industry strategies, biodegradation is generally the most cost-effective way to mitigate toxicity and other undesirable characteristics of wastewater. The biodegradation of commercially available NAs and NAs extracted from oil sands tailings water was studied by different groups (Clemente and Fedorak, 2005; Han *et al*., 2008; Herman *et al.*, 1994). In those studies, biodegradation was reported to have degraded approximately 50% of commercial NAs in different times. Although commercial NAs have a different structure than natural NAs (Clemente and Fedorak, 2005; Han *et al.*, 2008), biodegradation may be counted as a potential method to remove NAs from OSPW in practice (Scott *et al.*, 2008). Much research has focused on understanding the biodegradation and detoxification of NAs in OSPW (Han *et al.*, 2008; Martin *et al.*, 2010). NAs in OSPW showed much more resistance to biodegradation: the reason for such resistance was attributed to the high branch structure of these NAs (Han *et al.*, 2008). This study of the ozonation of OSPW and its effects on subsequent

biodegradation will provide insights into the mechanisms of NA degradation in different processes.

1.3 Ozonation and biological treatment

As the strongest commercially available oxidant, ozone can effectively oxidize organic pollutants in conventional wastewater (El-Din *et al.*, 2006; Ikehata and El-Din, 2005; Zhou and Smith, 2001). Similar to other advanced oxidation processes, ozonation can degrade different pollutants through a series of radical reactions. There are two pathways for an ozone reaction: 1) Ozone reacts with other chemicals in water to form hydroxyl radicals. The hydroxyl radicals can react non-selectively with almost all of the organic compounds in wastewater; or 2) Ozone molecules can directly react with organic compounds which have high electronic density sites. In contrast to the hydroxyl radicals, these direct reactions are usually very selective (Zhou and Smith, 2001).

The hydroxyl radical formed in the ozonation process is highly reactive and can change the molecular structure of chemical compounds, which is especially advantageous for non-biodegradable or refractory organics (Zhou and Smith, 2001). This method of oxidation can potentially be applied to OSPW to degrade refractory contaminants. However, we have to note that, similar to other advanced oxidation processes, ozonation is not economically feasible for fully mineralizing those pollutants because of the large amounts of energy and chemicals that are necessary for this treatment. Ozonation is usually used in combination with other

remediation methods such as biological treatment to reduce costs (Bijan and Mohseni, 2005). Generally, ozonation can be applied first at a relatively low level which can break recalcitrant organic compounds.

If not fully mineralized, some of the by-products of ozonation may be still toxic or even more toxic than the parent compounds. At this point, the subsequent biological process has a chance to remove some of the residual organic compounds formed during the ozonation process. This integrated ozonation and biological treatment may provide a viable means to remove contaminants from OSPW economically and effectively.

Ozone is effective over a wide pH range and can react rapidly. The ozonation process does not add chemicals to the water or leave any residual because ozone quickly self-decomposes into oxygen. The high amount of oxygen residual in ozonated water greatly benefits the subsequent biological process. Integrated ozone and biological treatment of other wastewater, such as pulp mill effluent, has been studied by Bijan and Mohseni (2005). They found that a small dosage of ozone combined with a biological process yielded approximately 30% higher total organic carbon (TOC) mineralization compared to individual ozonation or biological treatment. The pulp mill effluent's biodegradability and ratio of low molecular weight compound improved during ozonation, which consequently increased the efficiency of the biological treatment (Bijan and Mohseni, 2005).

An ozone contactor with bubble diffusers is the most common method of ozonation employed at water and wastewater treatment facilities. When ozone gas

is introduced into the water, several processes occur simultaneously. These processes include: the convection and mixing of liquid and gas phases; the ozone gas transfer process between the liquid and gas phase; the ozone self-decomposition process; and the different reactions between the constituents in the water and the dissolved ozone. Among these processes, the gas diffusion between the liquid and gas phase is the rate-limiting step; thus, it is considered to be the controlling process that dictates the overall performance of an ozone contactor (Zhou and Smith, 2001). During the ozonation process, the gas absorption rate can be defined as:

$$\Psi=K_La(C_L^*-C_L) \quad (1)$$

Where Ψ is the gas absorption rate (mg/L/s), K_L is the local liquid mass transfer coefficient (m/s), a is the gas bubbles' specific interfacial area (1/m), C_L^* is the concentration of dissolved ozone gas in equilibrium with bulk ozone gas (mg/L), and C_L is the concentration of dissolved ozone gas in bulk liquid (mg/L).

This equation represents mass transfer without a chemical reaction. During the ozonation of OSPW, a chemical reaction happened. However, according to Beltran *et al.* (1997), the effect of the chemical reaction between ozone and water with organic substances on the local mass transfer coefficient can be negligible. Therefore, this physical mass transfer equation can also be used to describe the ozonation process for OSPW.

1.4 Toxicity assay

OSPW toxicity was first measured by conducting whole-animal testing in rainbow trout during the early stages of oil sands development. The drainage water from the oil sands industry was acutely toxic with lethal concentrations (LC50) of less than 20% (Hrudey, 1975). Whole-animal toxicity testing is time consuming and difficult to use. Recently, research regarding the toxicity of OSPW was conducted using the Microtox system (Holowenko *et al*., 2002; Lo *et al.*, 2006). Microtox is a commercialized system and a standard method for measuring the toxicity of samples. Compared to other toxicity tests, Microtox has some advantages. For example, Microtox uses standard single-line bacteria for testing, which make the results of Microtox inter-laboratory comparable. Microtox is also a fast and convenient way to compare toxicity between different samples (Elnabarawy *et al.*, 1988). Compared to traditional, complex, and expensive whole-animal testing with invertebrates and fish, Microtox is rapid, simple, cost-effective, and sensitive with large sample throughput capabilities (Blaise and Férard, 2005). The Microtox toxicity assay, which uses luminescent bacterium *Vibrio fischeri* as an indicator, is a quicker, easier, less expensive test than whole-animal testing, but has high collinearity with other toxicity assays such as the Fathead minnow fish assay (Kaiser and Esterby, 1991).

The reagent used in the Microtox test is a freeze-dried preparation of a specially selected strain of the marine bacterium *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*, NRRL number B-11177). The Microtox test system measures the light output of the luminescent bacteria after they have been

exposed in a liquid sample and compares it to the light output of a control that contains only a pure water saline solution. The difference in light output between the sample and the control is attributed to the effect of the sample on the organisms (Microtox User Guide, AZUR Environmental, 1998).

As a widely used toxicity test, the sensitivity of Microtox is an important issue to clarify. Relative sensitivity and correlations between the Microtox test and three commonly used traditional acute lethality bioassays (Rainbow trout, Fathead minnow, and Duphnia) were compared (Munkittrick *et al.*, 1991). In that study, the authors found that Microtox was as sensitive as or even more sensitive than the acute lethality tests for pure individual organics, but was less sensitive to most inorganics. They also concluded that the sensitivity of Microtox increased with increasing complexity and toxicity of industrial effluents. As a result, Microtox could be used for screening the relative toxicity of highly toxic complex industry effluents.

1.5 Objective

The objective of this project is to find the most suitable ozone treatment dose which yields the most effective detoxification efficiency when combined with subsequent biological treatment. In this work, three different sizes (4, 20, 200 L) of semi-batch ozonation reactors with different diffusers and mixing devices were applied to Syncrude Canada, Ltd. OSPW. The residuals of NAs, COD, toxic effect, and $CBOD_5$ were measured to evaluate the ozone treatments, and the relationship between ozone dose and the residuals was assessed. After ozonation,

the biodegradability of OSPW was tested by shaking treated OSPW with inoculums cultured from oil sands tailing sludge for a period of 28 days. The potential effects of biological treatment were compared between untreated and different levels of ozonated OSPW.

The biodegradation of NAs in untreated and ozonated OSPW was analyzed by Microtox toxicity assay and HRMS. Basic parameters of water such as CBOD and COD were also measured.

2. METHODS

2.1 Material and chemicals

Three different batches of OSPW were collected in December 2009, January 2010, and May 2010 from Syncrude's West In-pit Pond (WIP). These water samples were used for ozone dose-related experiments. Tailings pond sludge, which was used to prepare bacterial seed, was collected in July 2009 from the WIP by Syncrude. All samples were stored in plastic barrels at 4°C.

Extra dry pure oxygen (99.6%) and pure nitrogen (99.995%) was purchased from Praxair Specialty Gas, Inc. (Edmonton, AB, Canada).

A nitrification inhibitor for the BOD test (Formula 2533) was purchased from HACH (Mississauga, ON, Canada).

Tetradecanoic acid-1-13C ($C_{14}H_{28}O_2$; $Z = 0$) was used as the internal standard for NA analysis and was purchased from Sigma-Aldrich (Oakville, ON, Canada).

All of the general chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2 Ozonation process

Figure 2 shows the schematic of the semi-batch ozonation system constructed in the lab for the ozonation of OSPW. An ozone generator (WEDECO, GSO-40, Herford, Germany) was used to produce ozone gas using extra dry, high purity oxygen. To obtain a stable ozone concentration in the feed gas, the ozone generator was allowed to stabilize for 10 min before ozonation. The feed gas, containing 6.2 to 7.7% w/w ozone (*i.e.*, 83.7 to 96.1 mg/L), was sparged into the liquid phase though different gas diffusers. Three different sizes of reactors were

adopted: the size, material, and related gas diffusers are listed in Table 2. Two ozone monitors, model HC-500, were also purchased from WEDECO. The ozone monitors were calibrated using KI periodically according to Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2005).

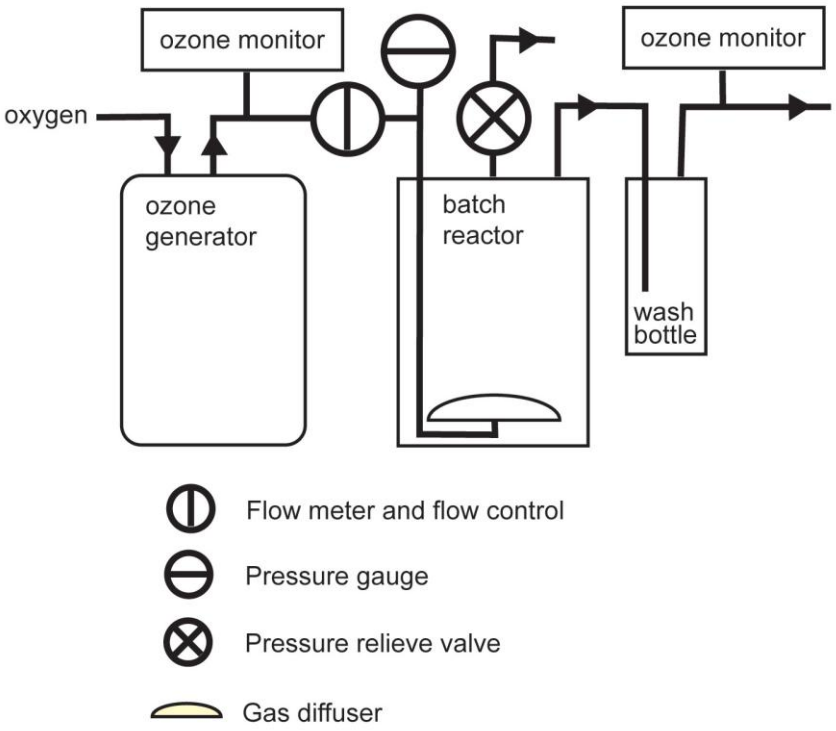


Figure 2: The schematic diagram of a semi-batch ozonation system (No pressure relief valve for the 4 L and 20 L reactors.)

The ozone residual in the reactor was measured using the Indigo method (APHA-AWWA-WEF, 2005). The gas flow rate was measured by a calibrated flow meter (4 to 20L/min and 0.5 to 2L/min). The utilized ozone dose for this system was calculated by using the following equation (El-Din and Smith, 2002):

$$\Delta O_3 = \int_0^t \frac{(Q_{G,in}C_{G,in} - Q_{G,out}C_{G,out})}{V_L} dt - C_L \quad (2)$$

Where ΔO_3 is the amount of the utilized ozone (mg/L), $C_{G,in}$ is the ozone concentration in the feed gas (mg/L), $C_{G,out}$ is the ozone concentration in the off gas (mg/L), C_L is the residue ozone concentration in the liquid phase (mg/ L), V_L is the effective reactor volume (L), $Q_{G,in}$ is the feed gas flow rate (L/min), $Q_{G,out}$ is the off-gas flow rate (L/min), and t is the ozone contact time (min). Details are listed in Appendix A.

After ozonation, the OSPW was purged by a pure nitrogen flow for 10 min to strip the ozone residual and oxygen from the reactor.

Table 2: Reactor and diffuser types

	Reactor		
	4L	20L	200L
Material	Glass	PVC	HDPE
Reactor volume (L)	4	20	200
Reactor aspect ratio	2	1.3	2
Diffuser type	Coarse	Fine	Fine
Flow rate (L/min)	2	4	12

The ozone doses mentioned in this thesis are actually utilized ozone doses. Doses equal to zero mean that the sample was not ozonated, or in other words, it is an untreated sample.

2.3 Biodegradation

A Bushnell-Haas Broth (BHB) medium was used to grow a biomass from tailings pond sludge and to evaluate the ability of grown microorganisms to decompose hydrocarbons. The formula for this basic medium is (per litre): K_2HPO_4 1 g, KH_2PO_4 1 g, NH_4NO_3 1 g, $MgSO_4$ 0.2 g, $CaCl_2$ 0.02 g, $FeCl_3$ 0.05 g. The process

of selective enrichment first introduced 10 ml of sludge into 90ml BHB medium with 10 mg glucose. The concentration of glucose was 100 mg/L in the final culture. Aeration continued with a small air pump for one week. Then, 15 ml of cultured solution was transferred into another flask with 90ml BHB medium and 45 ml OSPW. The concentration for glucose and OSPW was 66.7 mg/L and 30%, respectively. Aeration continued for one week then 15 ml of culture was diluted into the same BHB/OSPW mixture again. After another week, the grown inoculums were ready for use in the BOD and biodegradability tests. The inoculums were prepared weekly for different tests. All the processes were performed at room temperature (20°C). Details are listed in Appendix B.

The biodegradation was performed according to a modified version of the Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals – 301 Ready Biodegradability (OECD, 1992). COD, $CBOD_5$, and NA concentration were chosen as indicators of water quality. COD samples were collected every week from the shaken flasks, and other samples were collected before and after treatment. Four kinds of stock solutions (Phosphate buffer solution (PBS), $MgSO_4$, $CaCl_2$, and $FeCl_3$) were used for the biodegradability test and the BOD test. Samples were treated in duplicate, and all the glass wares were sterile. Details are listed in Appendix B.

2.4 Analysis of water quality and Microtox toxicity

COD and $CBOD_5$ were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2005). Because the volume of biodegraded OSPW was limited, the results of the $CBOD_5$ test were based on one test which included samples with and without biodegradation to make a comparison.

The TOC of samples were sent to Maxxam Analytic, Inc. for analysis. To measure TOC in the instrument, the sample was introduced into an autoanalyzer where acidification and sparging were performed to remove inorganic carbon. Then, the organic material in the sparged sample was oxidized with a UV digester in an acid-persulphate mixture. The resulting carbon dioxide passes through a semi-permeable membrane, where it reacted with a phenolphthalein reagent. The intensity of the phenolphthalein colour change was measured at a wavelength of 550 nm.

The toxicity of untreated and some of the ozonated water samples was tested using a Microtox 500 Analyzer (AZUR Environmental, Carlsbad, USA). The exposure time of 5 min was chosen for the 81.9% basic test procedure (provided by analytic software that came with the Microtox analyzer). The highest sample concentration yield is 81.9% in the Microtox test because the addition of reagent and osmotic adjustment of the solution is necessary for testing. For the biodegraded water samples which had very low toxicity, the EC_{20} value was higher than 100% or could not be measured at all. Therefore, the 81.9% screen

test was adapted to compare the inhibition effect of luminance emission on Microtox reagent *Vibrio fischeri*. All the samples were tested in triplicate, and a Student-T test was used to compare the toxic effects between different doses and treatment.

2.5 Analysis of NAs by UPLC/HRMS

The concentrations and profiles of NAs were analyzed by UPLC and HRMS (Martin *et al.*, 2010). With this method, detailed information including the concentration of different components of NAs in OSPW can be measured. For example, the individual concentration of NA with C numbers from 7 to 22 and *Z* numbers from 0 to 12 were measured separately. The summation of all these individual concentrations is the total concentration of NAs in the OSPW sample. With this method, not only can the total concentration of NAs be analyzed, but also the character of different components of NAs.

Before measurement, a 1 mL portion of each sample was filtered through a 0.2 μm nylon syringe filter, and 50 μL of 4 mg/L internal standard (tetradecanoic acid-1-13C) in methanol was added to 950 μL of each filtered sample. Waters ACQUITY UPLC® System (Waters, MA, USA) chromatographic separation of the NAs and their oxidized products was performed. Chromatographic separations were run on a Waters UPLC Phenyl BEH column (150 × 1 mm, 1.7 μm) using a gradient mobile phase of (A) 10mM ammonium acetate and (B) 10 mM ammonium methanol in 50% acetonitrile. Gradient elution was as follows: 1% B

for the first 2 min, ramped to 60% B by 3 min, ramped to 70% B by 7 min, ramped to 95% B by 13 min, held until 14 min, and finally returned to 1% B, followed by a further 5.8 min of equilibration time. The flow was constant at 0.11 mL min^{-1}, and column temperature was 50°C.

Detection was performed using a high resolution (~10,000 m/Δm) QSTAR® Pulsar i mass spectrometer equipped with a TurboIon Spray source (Applied Biosystems/MDS Sciex, Concord, ON, Canada) operating in negative ion mode. Analyst QS 1.1 and Multiquant 1.1 software (Applied Biosystems, Foster City, CA) were used for data analysis, and the relative ratio of each analyte's chromatographic peak (for each isomer class corresponding to each *n* and *Z* combination) area to the internal standard was calculated for subsequent kinetic analysis. Total NA degradation was estimated by the decrease in the sum response of all the UPLC-HRMS peak areas with exact masses corresponding to NAs ($C_nH_{2n-Z}O_2$).

3. RESULTS

3.1 Wastewater chemistry

The OSPW samples collected at different times varied in physical and chemical characteristics. Table 3 shows some characteristics of the original WIP-OSPW used in this work. According to Table 3, the chemical characteristics of OSPW collected at different times varied significantly. Batch 2 OSPW had the highest TS value, but the lowest TSS value, meaning it is more clear, but brackish. Batch 2 OSPW also had the lowest COD content and the highest toxicity among the three batches of WIP water. The TOC did not differ significantly between WIP water samples, varying only from 45 to 51 mg/L. BOD_5 significantly varied, measuring between less than 10 and close to 40 mg/L. The $CBOD_5$ of all samples was less than 5 mg/L and was relatively constant.

Table 3: The characteristics of OSPW collected at different times at the WIP

	WIP OSPW		
Batch	1	2	3
Collection date	Oct-2009	Jan-2010	May-2010
TS (mg/L)	2612	2794	2494
TSS (mg/L)	125	4	123
pH*	9.02	9.13	9.12
TOC (mg/L)	48	50.5	45.1
EC_{20}* (%)	16.7	11.5	23.7
COD (mg/L)	310.5	232.7	300.3
BOD_5 (mg/L)	38.1	38.8	7.8
$CBOD_5$ (mg/L)	4.2	<2	<2

*: pH values and EC_{20} were measured after equilibration for two days at room temperature and atmosphere.

The pH of different batches of OSPW in this table was measured after shaking for two days because the pH of OSPW was not stable after being taken out from the storage barrel. For example, the pH of Batch 2 OSPW was 7.95 after being taken out of the storage barrel, and then the pH increased to 9.13 after being shaken for two days. The pH of the sample that was bubbled with air for two days increased from 7.95 to 9.26; bubbling samples with pure nitrogen even increased the pH to 10. Figure 3 shows the titration curve of Batch 2 OSPW, which indicates the pH is not stable when pH is around 8. The alkalinity of this batch of OSPW is close to 600 mg/L $CaCO_3$.

The Microtox measurements of OSPW toxicity showed that toxicity was greatly affected by pH. Figure 4 shows the EC_{20} of Batch 3 OSPW at different pH in a plot. The plot indicates when the pH equalled 8, the OSPW sample had the lowest toxicity. However, the OSPW pH was not stable at 8 and only stabilized at 9, according to Figure 3. In order to make the results comparable, all the Microtox screen tests were performed at pH 9 to eliminate the error induced by different pH.

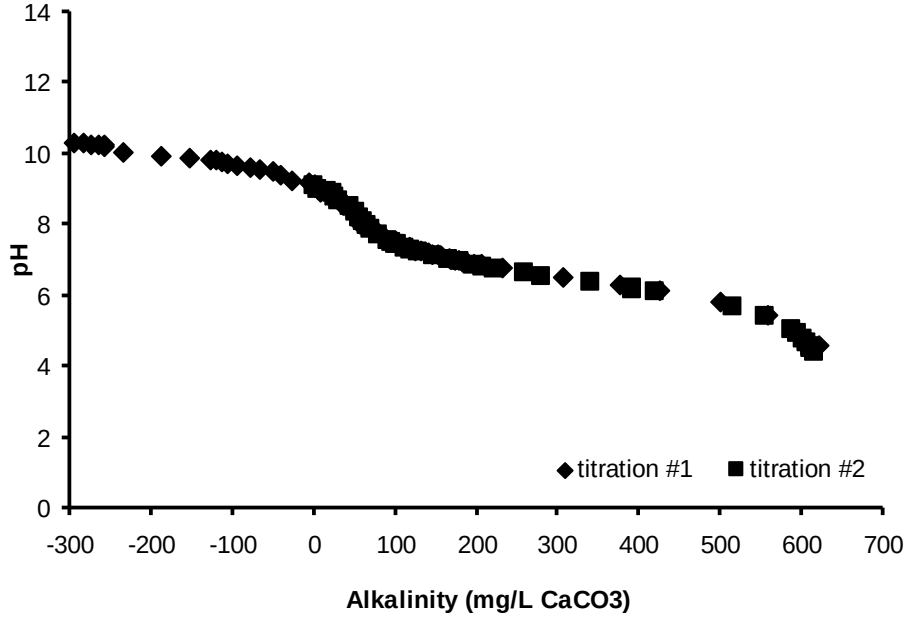


Figure 3: Titration curve of Batch 2 OSPW

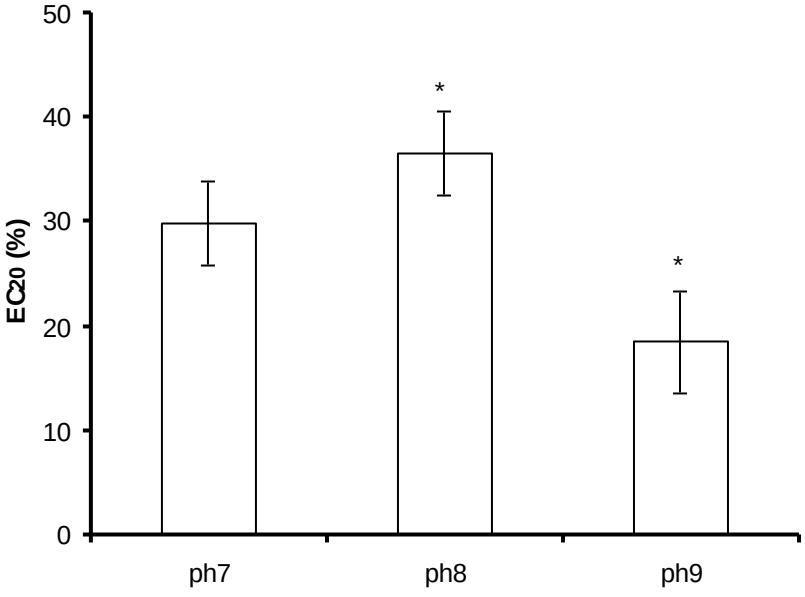


Figure 4: EC_{20} of Batch 3 OSPW at different pH (n=3, *: $p<0.05$)

For this experiment, the OSPW from WIP Batch 1 was ozonated in the 4 L reactor, WIP Batch 2 was ozonated in the 200 L reactor, and all other samples were ozonated in the 20 L reactor.

3.2 Ozonation process

Different dosages of ozonation were applied in the different reactors. The details of each reactor's treatment are listed in Table 4. The ozone dosages utilized in different reactors and the related ozone efficiencies are listed in Table 5 and Figure 5.

$$\text{Efficiency} = \frac{\text{Utilized ozone}}{\text{Applied ozone}} \times 100\,\% \quad (3)$$

Table 4: Parameters of reactors and processes

	Reactor		
	4 L	20 L	200 L
Material	Glass	PVC	HDPE
mean efficiency %	15.7	25.2	49.5
Reactor volume (L)	4	20	200
Reactor aspect ratio	2.0	1.3	2.0
Diffuser type	Coarse	Fine	Fine
Flow rate (L/min)	2	4	12
Time for approx 30mg/L ozonation (min)	3	6	10

Table 5: Efficiencies and dosages of ozonation processes

Reactor	Dosage (mg/L*)	CT# (min)	Efficiency (%)
4 L	30	3	15.6
	50	7	15.4
	80	10	15.9
	115	15	16.1
	180	25	15.3
	360	35	22.0
20 L	10	1	42.0
	20	3	27.3
	30	6	25.8
	50	10	22.5
	90	20	22.0
200 L	30	10	51.2
	80	30	47.9
	130	60	37.6

*: Doses are approximate
#: Contact time.

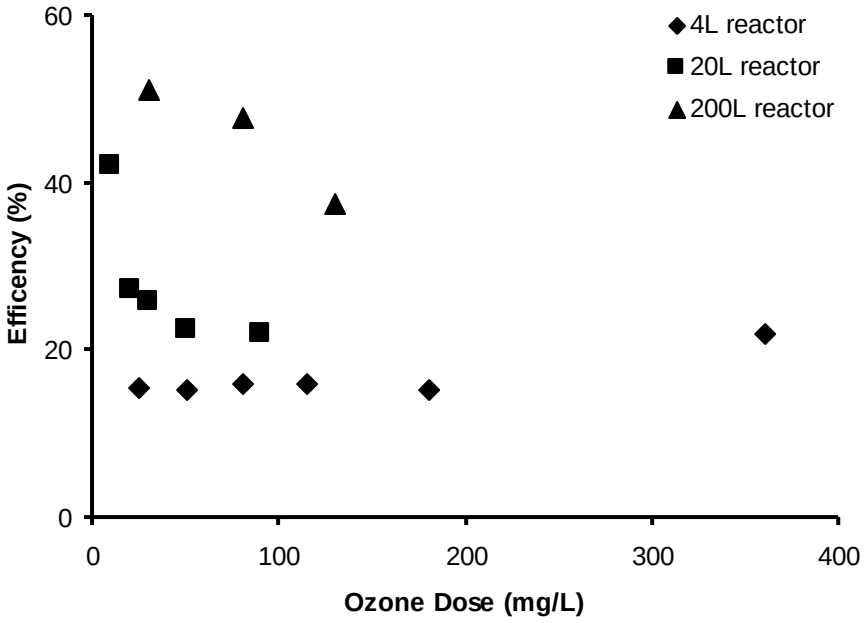


Figure 5: Efficiency for different reactors at different doses

The plot shows that the 200 L reactor has the highest efficiency. For the 20 and 200 L reactor, low-dose treatments are more efficient than high-dose treatments. In general, the bigger the reactor, the higher ozone efficiency it had.

3.3 Water quality of OSPW ozonated in a 4 L reactor

3.3.1 COD results

After ozonation, the COD of Batch 1 OSPW decreased with increasing ozone dose. The change in the COD of OSPW ozonated in the 4 L reactor after ozonation and subsequent biodegradation is shown in Figure 6. At a 360 mg/L dose, ozonation and biodegradation decreased 50% of original COD. The COD of the OSPW samples kept decreasing throughout the biodegradability test which lasted 28 days. The decreasing amount of COD during biodegradation over the course of 28 days is shown in Figure 7.

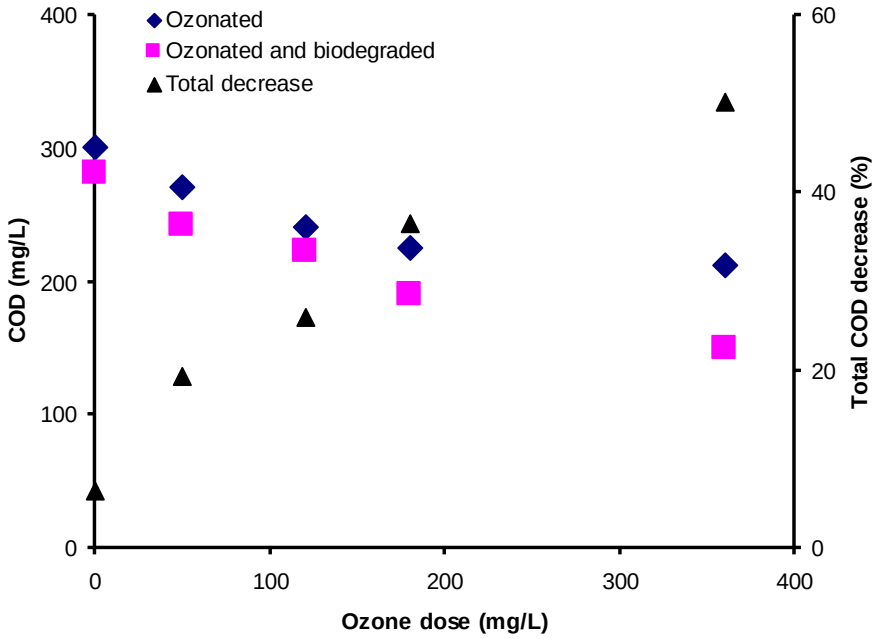


Figure 6: COD change after ozonation in the 4 L reactor with and without subsequent biodegradation

Figure 7 shows COD depletion during biodegradation over 28 days. The initial COD was normalized to 100% in the plots. The COD depletion continued increasing as ozone dose increased; especially when ozone doses exceeded 180 mg/L, COD depletion was more significant. The amount of COD depleted by 50% after 360 mg/L ozonation with biodegradation.

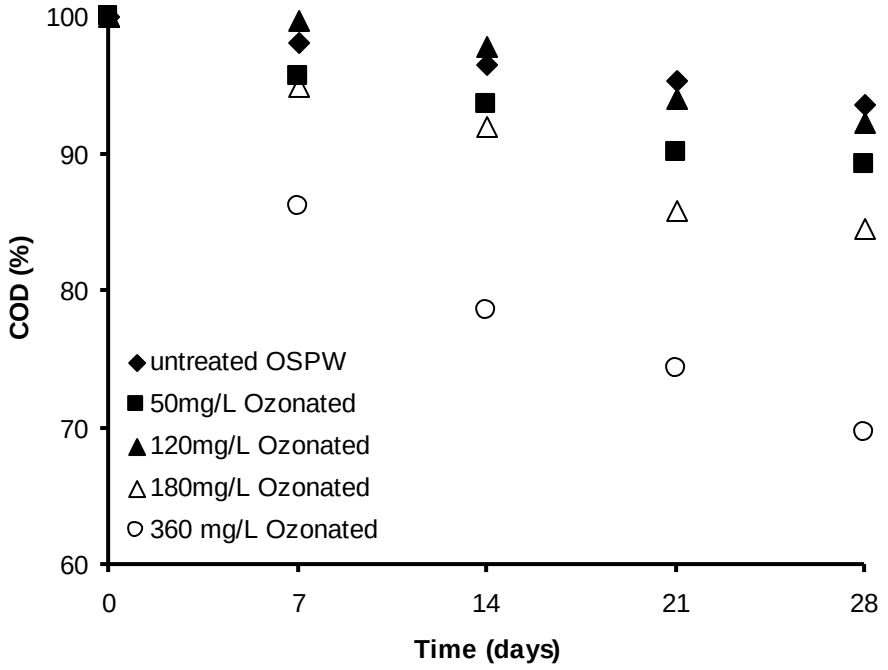


Figure 7: COD of Batch 1 OSPW during biodegradation

COD biodegraded more easily with higher doses of ozone treatment. Compared to other doses, the fastest depletion curve is found in 360 mg/L ozonated OSPW. Approximately 30% of COD was removed after 28 days in the 360 mg/L ozone-treated sample. Only approximately 5% of COD was removed in the untreated raw OSPW sample.

3.3.2 CBOD

For this batch of OSPW, the difference of BOD_5 (Table 3) and $CBOD_5$ in untreated OSPW (ozone dose 0 mg/L, Figure 8) represented oxygen used in the nitrification process. It is deduced that much more oxygen was consumed for nitrification in this batch of OSPW because BOD_5 is four times higher than $CBOD_5$.

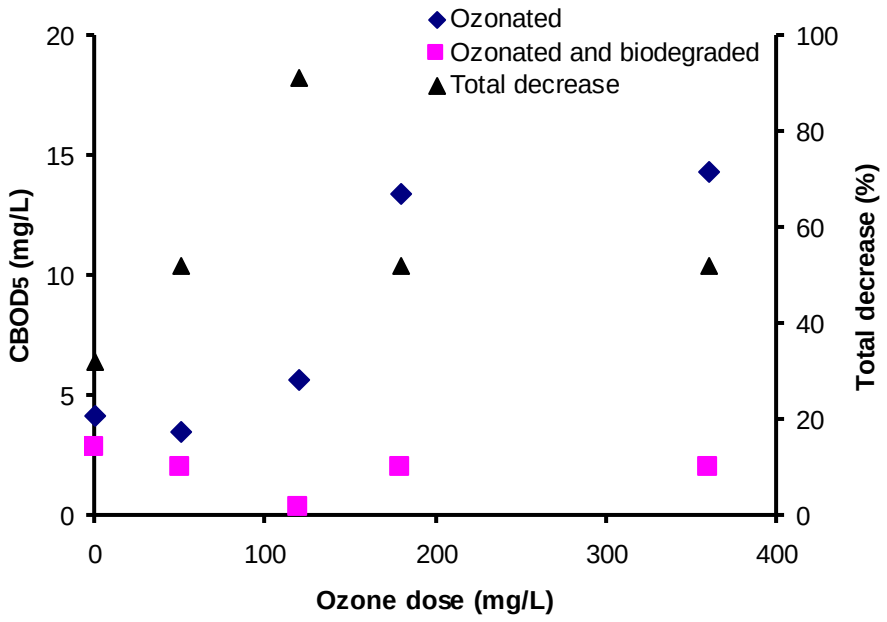


Figure 8: $CBOD_5$ after ozonation in the 4 L reactor with and without subsequent biodegradation

The change in $CBOD_5$ after ozonation is shown in Figure 8. $CBOD_5$ shows an increase after ozonation. The $CBOD_5$ value changed from approximately 4 to 15 mg/L after 360 mg/L ozone treatment. After biodegradation, all $CBOD_5$ values decreased to approximately 2 mg/L, and the total depletion rate was close to 50% when the ozone dose was higher than 100 mg/L. Because ozonation increased the

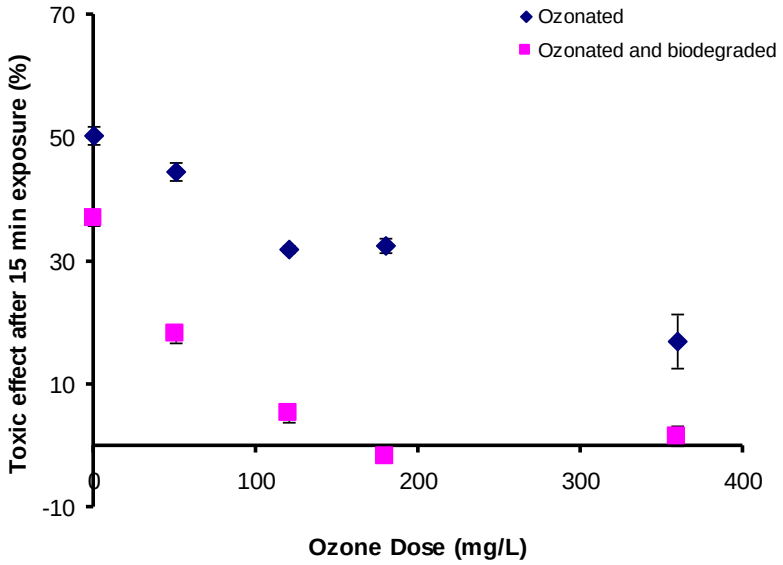
$CBOD_5$ of OSPW, depletion of $CBOD_5$ during biodegradation was as high as 90% in the 120 mg/L ozonated OSPW samples.

3.3.3 Toxic effect

For the triplicate 81.9% screen test, Figure 9 shows the toxic effect on the bacteria after 15- and 60-min exposures in ozonated and biodegraded OSPW. For OSPW treated with doses of 120 and 180 mg/L ozone, the toxic effect after exposure of 15 and 60 min did not show a significant difference ($P>0.05$). Samples treated with more than 120 mg/L ozone also did not show any differences after biodegradation in the 60-min screen test ($P>0.05$). All of the other treatments showed significant differences.

According to the plots, the toxic effect of OSPW decreased with increasing ozone dose. A high dose of 360 mg/L ozone removed 60% of the original toxic effect after 15 min of exposure. However, when biodegradation was also applied, an ozonation dose of approximately 50 mg/L gave a similar effect as 360 mg/L ozone treatment alone. After 60-min of exposure, the level of toxic effect was similar to the sample after 15-min of exposure. While ozone doses were higher than 120 mg/L, increasing the ozone dose did not significantly decrease the toxic effect of OSPW after biodegradation ($P>0.05$).

A:



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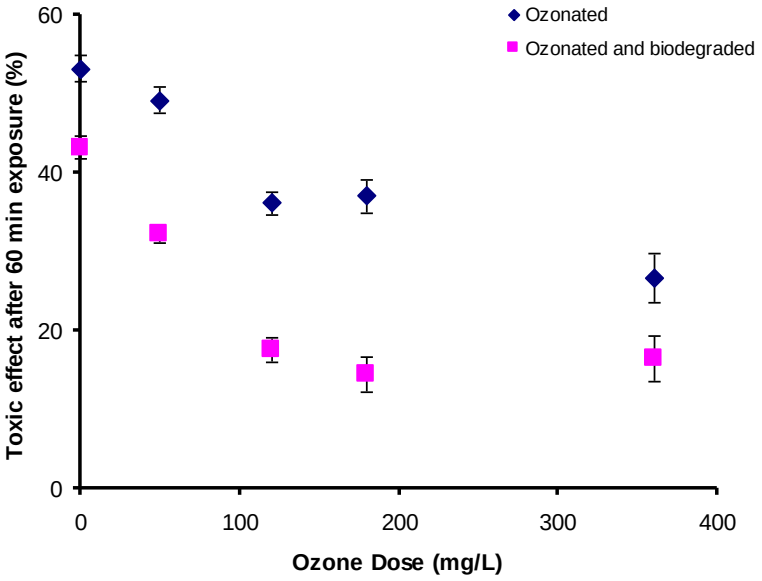


Figure 9: Toxic effect change after 15 (A) and 60 (B) minutes exposure to samples ozonated in the 4 L reactor with and without subsequent biodegradation

3.4 Water quality of OSPW ozonated in a 20 L reactor

Similar to the treatment applied in the 4 L reactor, Batch 3 of OSPW was treated in a 20 L reactor. This batch of water was treated with a lower dose range than the 4 L reactor.

3.4.1 COD

Similar to the results from the 4 L reactor, the COD of OSPW decreased after ozonation. The COD of OSPW ozonated in a 20 L reactor and the amount of COD after biodegradation is shown in Figure 10. The COD of ozonated OSPW did not change with small ozonation doses (<30 mg/L). At 90 mg/L ozone, ozonated and biodegraded OSPW showed a 38% COD decrease. The depletion of COD during 28 days of biodegradation is shown in Figure 11.

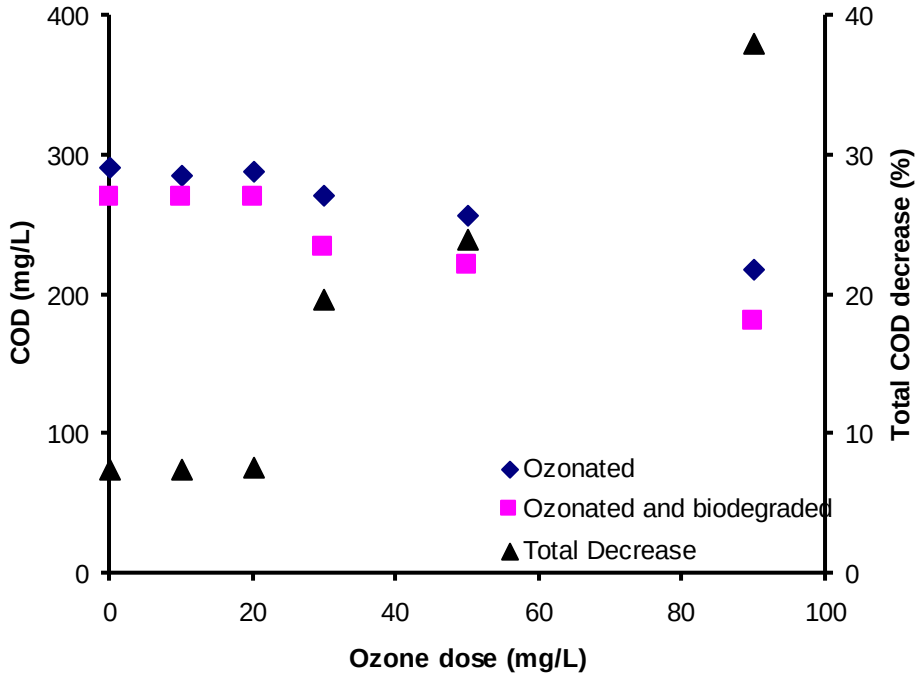


Figure 10: COD change after ozonation in the 20 L reactor with and without subsequent biodegradation

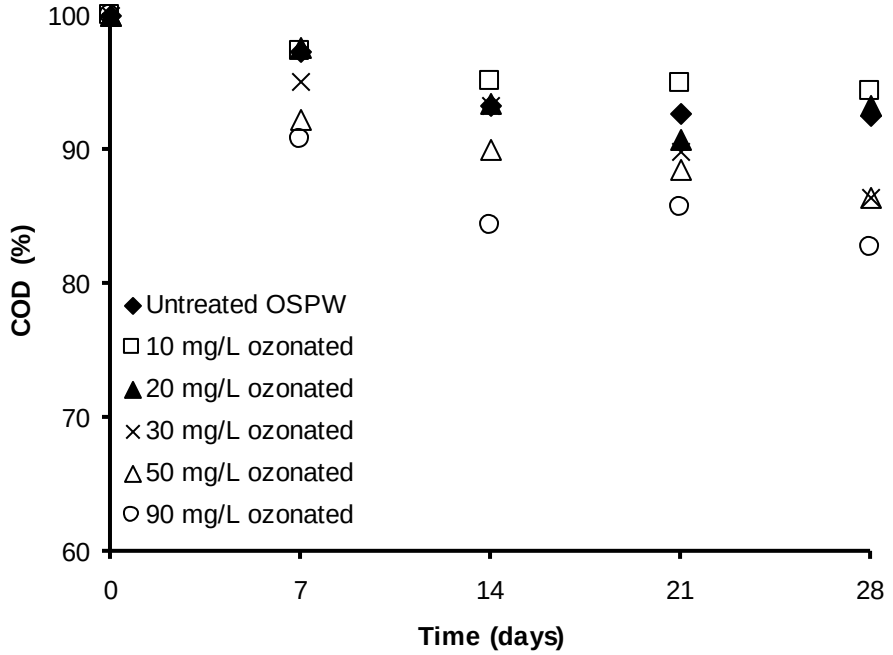


Figure 11: COD of Batch 3 OSPW during biodegradation

Figure 11 shows that COD can be biodegraded more easily at higher ozone doses. While the ozone dose was less than 30 mg/L, the biodegradability of COD did not significantly increase compared to untreated OSPW. COD depletion due to biodegradation was more than 10% only when ozone doses were more than 30 mg/L. With 90 mg/L ozonation, COD decreased 15% with biodegradation.

3.4.2 CBOD

The OSPW used for treatment in the 20 L reactor had lower BOD_5 than the OSPW used in the 4 L reactor. The $CBOD_5$ of this OSPW batch was also low (<2 mg/L). The $CBOD_5$ of OSPW after ozonation and biodegradation are shown in Figure 12.

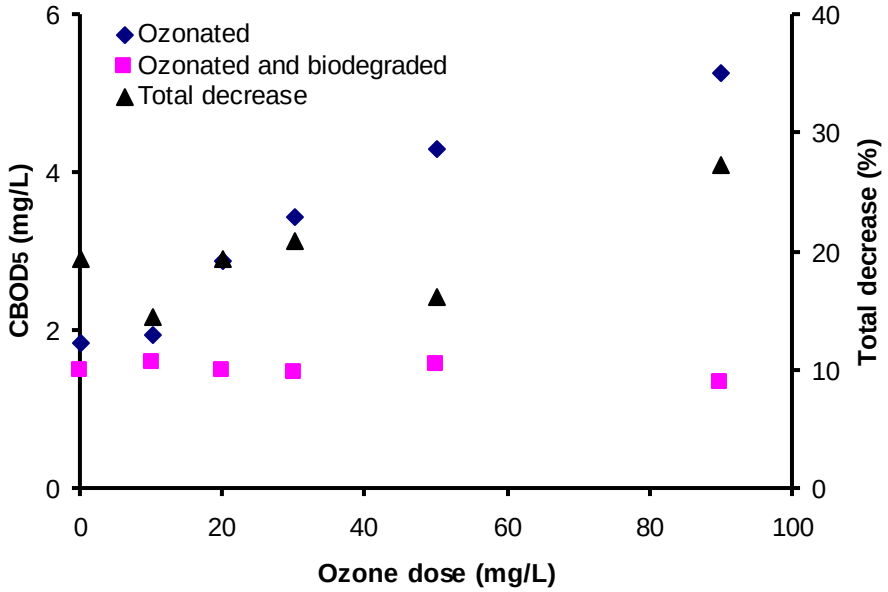
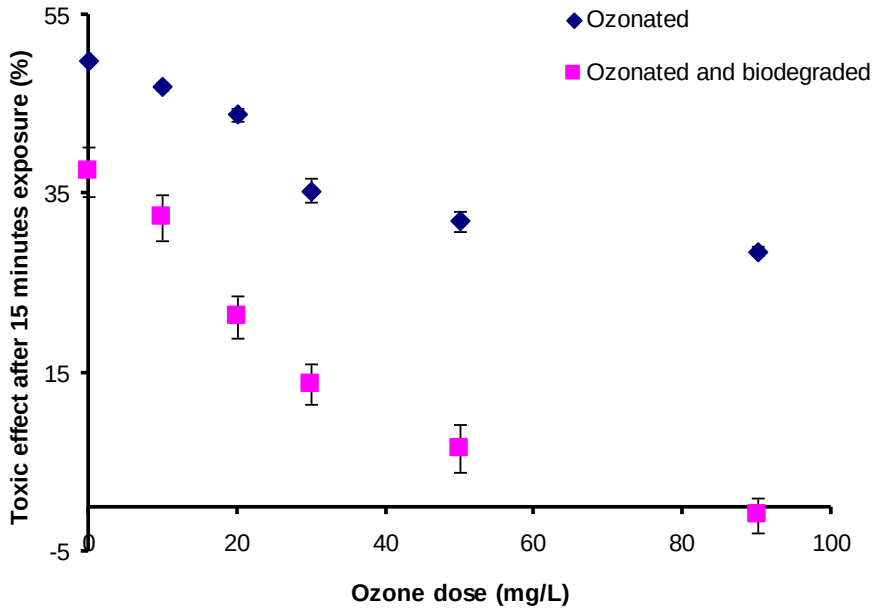


Figure 12: $CBOD_5$ change after ozonation in the 20 L reactor with and without subsequent biodegradation

As shown in Figure 12, the $CBOD_5$ of this OSPW batch changed in a similar manner as Batch 1 OSPW: the $CBOD_5$ continued to increase after ozonation. After biodegradation, $CBOD_5$ decreased to less than 2 mg/L. Compared to untreated OSPW, the total decrease was not significant because $CBOD_5$ increased after ozonation. The actual depletion through biological treatment was as high as 70% in 90 mg/L ozone-treated OSPW.

3.4.3 Toxic effect

The toxicity change in this OSPW batch was similar to the toxicity change in Batch 1 OSPW ozonated in the 4 L reactor. Figure 13 shows the decreasing toxic effect after ozonation and biodegradation.



A:

B:

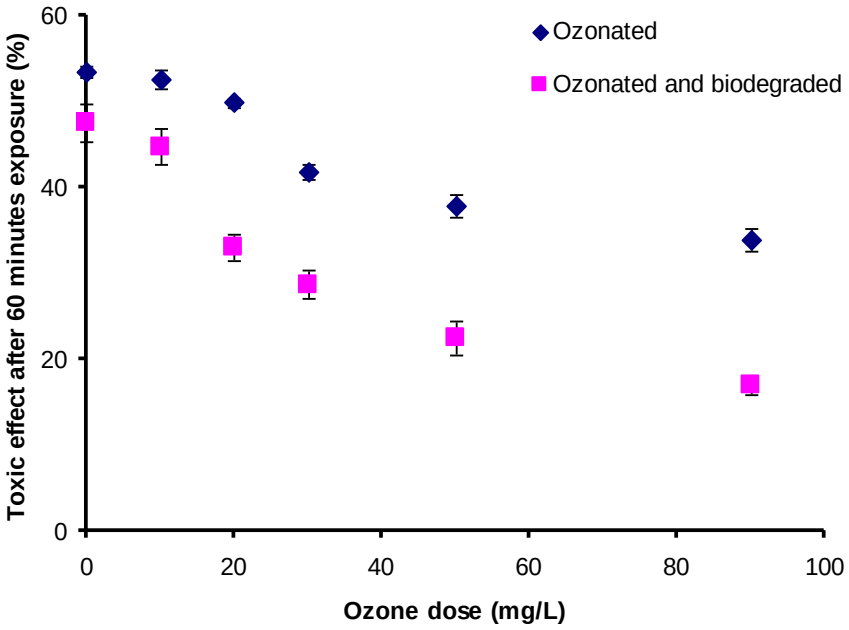


Figure 13: Toxic effect change after 15 (A) and 60 (B) minutes exposure to samples ozonated in the 20 L reactor with and without subsequent biodegradation

According to Figure 13, the toxic effect of OSPW decreased with increased ozone dose. A dose of 90 mg/L ozone treatment can remove the original toxic effect from 50% to 30% after 15 min of exposure. For OSPW treated with 50 and 90 mg/L ozone doses, the toxic effect after a 15-min exposure did not show a significant difference ($P>0.05$). For OSPW treated with 0 and 10 mg/L ozone doses, the toxic effect after a 60-min exposure did not show a significant difference ($P>0.05$). All other ozonation doses showed significant differences. When biodegradation was also applied subsequent to ozonation, a dose of approximately 10 mg/L ozonation produced the same detoxification effect as OSPW only treated with 50 mg/L ozone. A combination of 90 mg/L ozone and biodegradation resulted in the removal of all the toxic effects after 15 min of exposure. In the 60-min exposures, biodegradation did not effectively decrease the toxic effects as much as the 15-min exposures. A dose of 90 mg/L ozonation plus biodegradation depleted toxic effects from 50% to 20%.

3.5 Water quality of OSPW ozonated in a 200 L reactor

The Batch 2 OSPW was treated in a 200 L reactor equipped with a ceramic fine bubble gas diffuser. The ozone doses for treatment ranged from 30 to 130 mg/L.

3.5.1 COD

Similar to the results from the other reactors, the OSPW COD decreased after ozonation. The OSPW COD samples ozonated in the 200 L reactor and subsequent biodegradations are shown in Figure 14. This OSPW batch's COD

still decreased with increasing ozone doses. With subsequent biodegradation, 130 mg/L ozone removed 42% of the original COD. The COD over 28 days of biodegradation is shown in Figure 15.

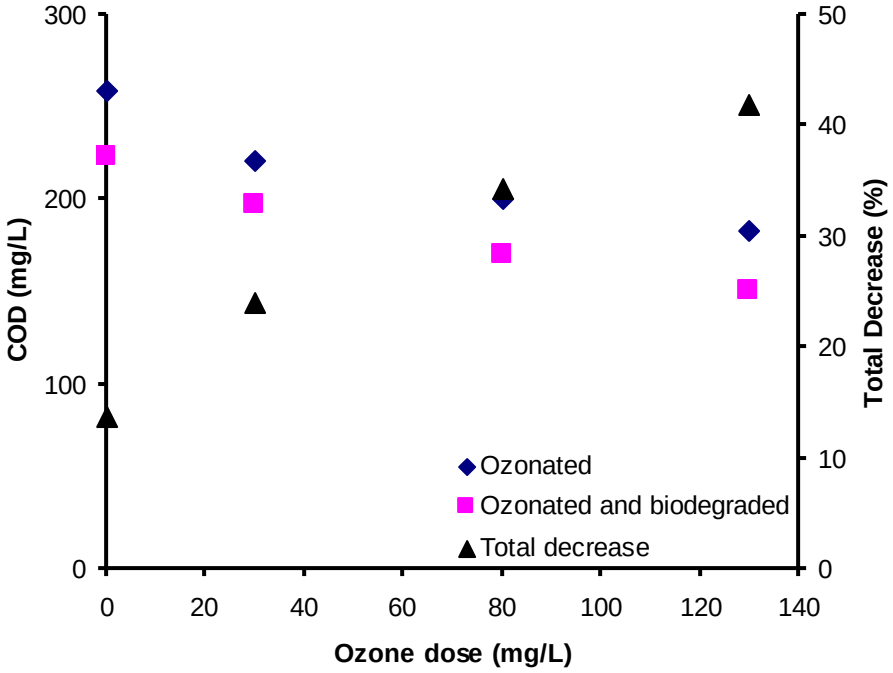


Figure 14: COD change after ozonation in the 200 L reactor with and without subsequent biodegradation

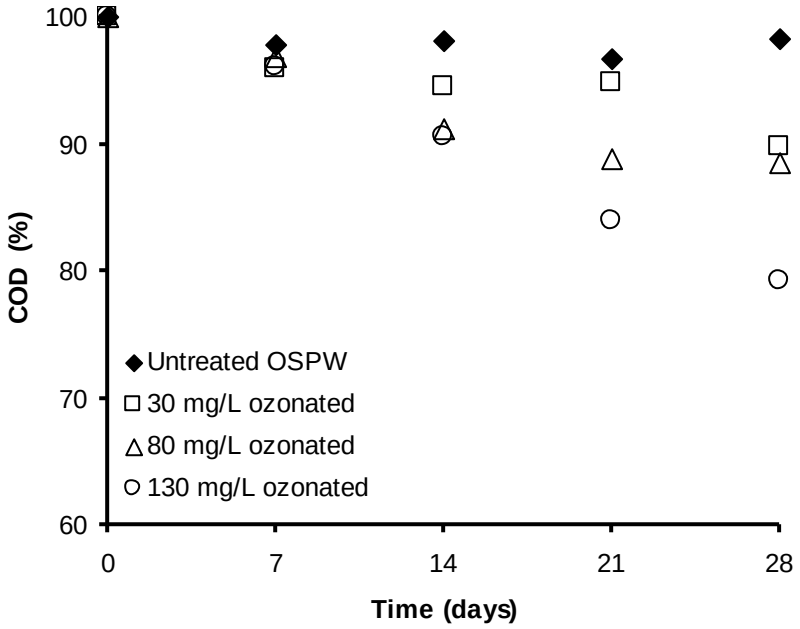


Figure 15: COD of Batch 2 OSPW during biodegradation

Similar to other sample batches, over 28 days of biodegradation, the OSPW COD biodegraded more easily in samples treated with higher ozone doses. When the ozonation dose was 130 mg/L, 20% of COD decreased after biodegradation.

3.5.2 CBOD

Like Batch 1 OSPW, $CBOD_5$ of Batch 2 OSPW was much lower than the BOD_5 (38.8 mg/L, Table 3). The $CBOD_5$ of OSPW samples ozonated and biodegraded in the 200 L reactor are shown in Figure 16.

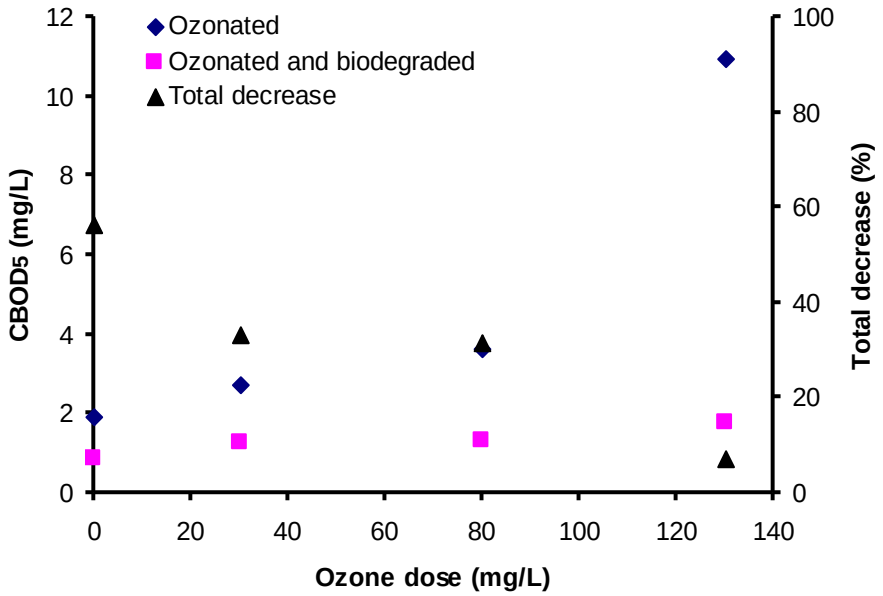


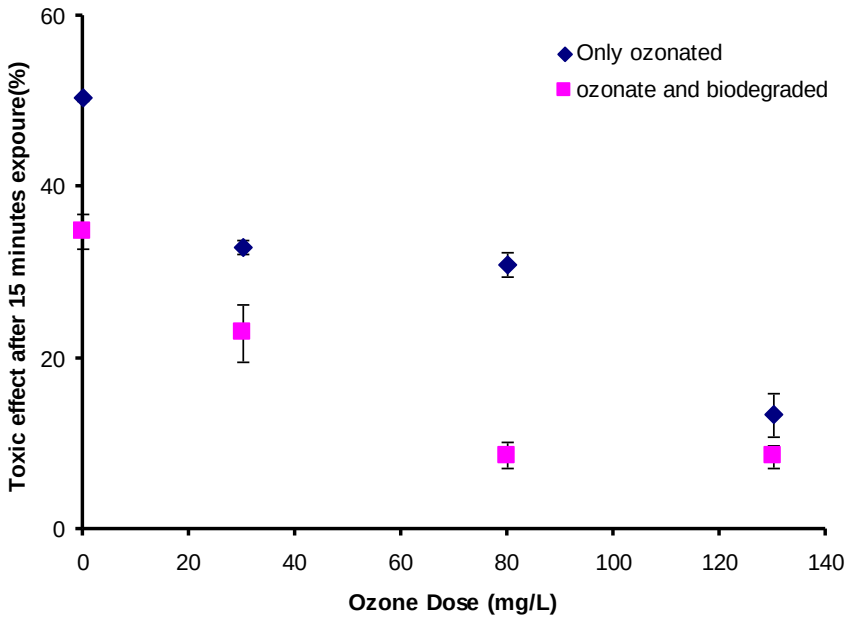
Figure 16: $CBOD_5$ change after ozonation in the 200 L reactor with and without subsequent biodegradation

The $CBOD_5$ of OSPW slowly increased as the applied ozone dose increased. At 130 mg/L ozone, the $CBOD_5$ of treated OSPW rose to 11 mg/L, which was five times larger than that of untreated OSPW. After biodegradation, most of the $CBOD_5$ was removed, and all of the samples had similar final $CBOD_5$ values of less than 2 mg/L.

3.5.3 Toxic effect

The change of toxic effect in this OSPW batch was similar to that in other reactors. Figure 17 shows the decrease in toxic effect after ozonation and biodegradation.

A:



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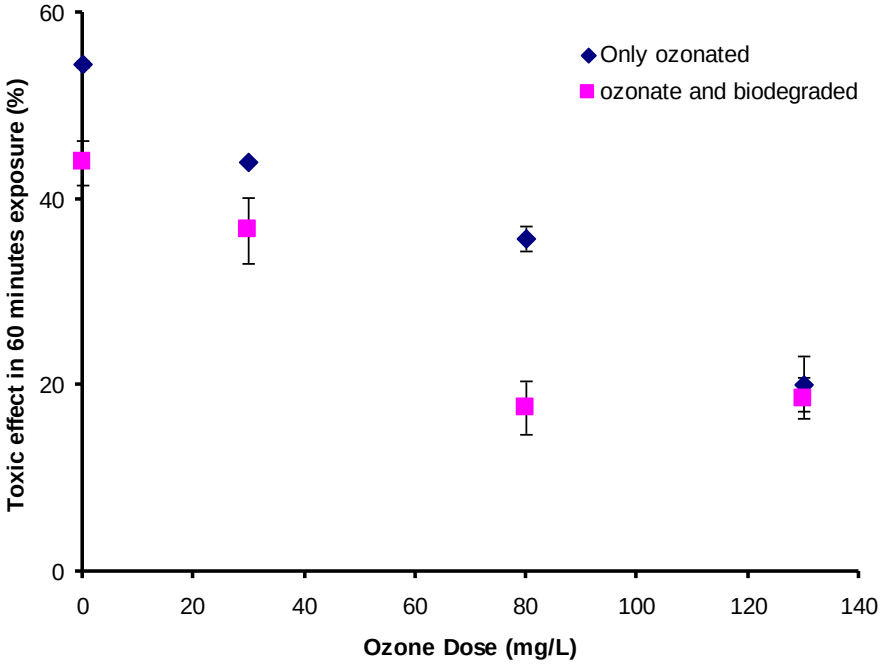


Figure 17: Toxic effect change after 15 (A) and 60 (B) minutes exposure to samples ozonated in the 200 L reactor with and without subsequent biodegradation

According to the plots, the toxic effect of the OSPW decreased with increasing ozone doses. In 15 min exposure test, 130 mg/L ozonation decreased toxic effect significantly from approximately 52% to 15%. After biodegradation was applied, 80 and 130 mg/L ozonated OSPW did not show significant inhibition effects ($P>0.05$). A combination of 130 mg/L ozonation and biodegradation removed more than 80% of the toxic effect after 15 min of exposure. Biodegradation effectively detoxified all of the samples in the 60-min exposure test, except the 130 mg/L ozone-treated OSPW whose 20% toxicity did not change much after biodegradation ($P>0.05$).

3.6 Comparison between reactors and batches of OSPW

3.6.1 Change of COD

Although the contact time varied for the different sizes of reactors, COD consistently decreased as ozone doses increased. Given the different chemical characteristics of OSPW batches, the effect of ozonation on decreasing COD was quite close in all reactors. When the ozone dose was less than 30 mg/L, which was only done in the 20 L reactor, the COD change was undetectable. In the 4 and 200 L reactor, when the ozone dose was high (> 100 mg/L), the decrease in COD was not as large as when the ozone dose was low (<100 mg/L) according to Figure 18.

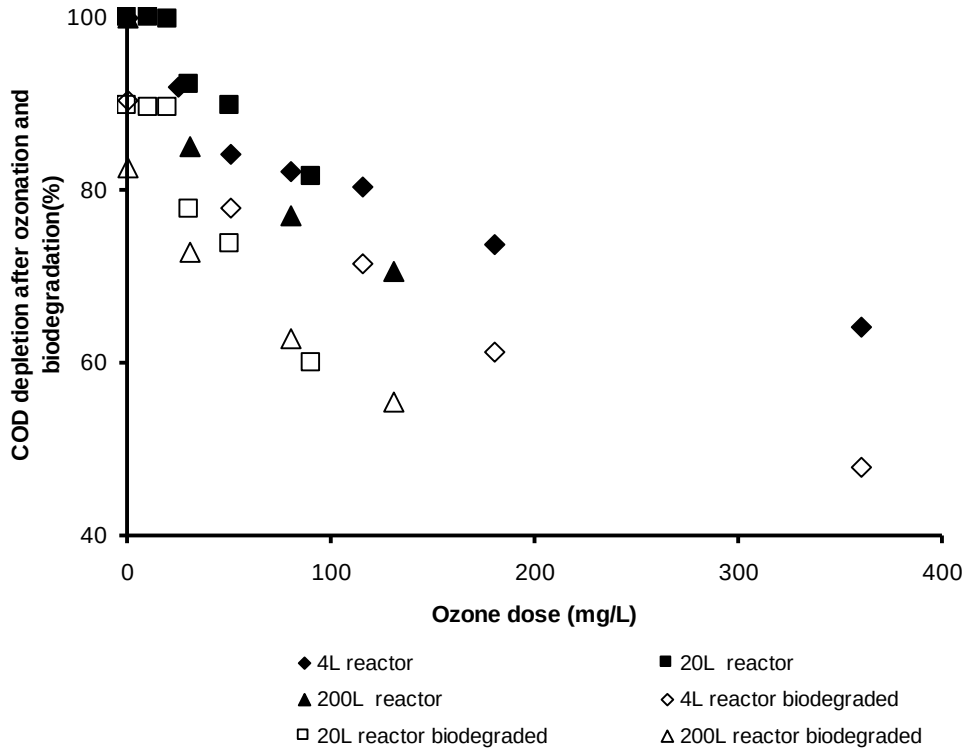


Figure 18: COD change in different reactors with and without subsequent biodegradation

Biodegradation consistently decreased the COD in all treated and untreated OSPW samples. However, the percentage of depletion varied between samples. Figure 18 shows the COD of OSPW ozonated in the 20 and 200 L reactors was biodegraded more easily than OSPW ozonated in the 4 L reactor. The COD of 100 mg/L ozone-treated OSPW decreased 40% in the 20 and 200 L reactors, but decreased less than 30% for OSPW ozonated in the 4 L reactor.

3.6.2 Change of CBOD

$CBOD_5$ was compared in different reactors because BOD_5 of OSPW samples collected at different times varied substantially. $CBOD_5$ of all the OSPW batches

showed relatively constant values, and most of the hydrocarbons including some NAs belonged to the $CBOD_5$ of this water.

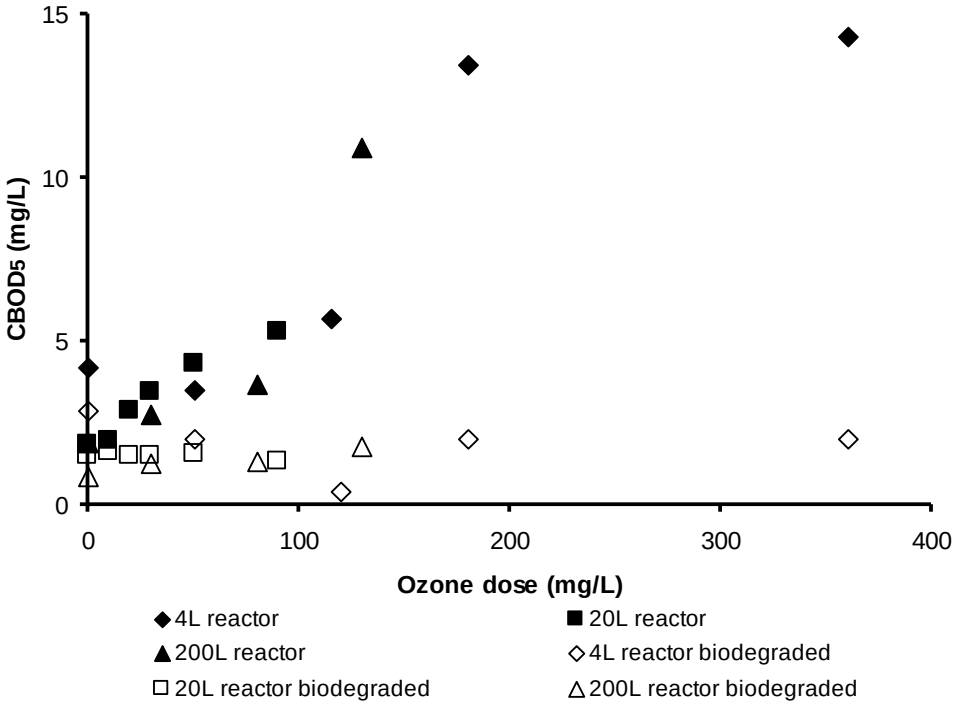


Figure 19: $CBOD_5$ change in different reactors after ozonation with and without subsequent biodegradation

According to Figure 19, the $CBOD_5$ of OSPW in the different reactors increased in a relatively constant manner after ozonation. The increase of $CBOD_5$ in different samples was also fairly close. The $CBOD_5$ increased from less than 5 to 15 mg/L after 360 mg/L ozone treatment. After biodegradation, $CBOD_5$ of OSPW ozonated in different reactors decreased to the same level of approximately 2 mg/L despite the amount of ozone utilized.

3.6.3 Change in Toxicity

The EC_{20} of Batch 3 OSPW was measured after ozonation. Figure 20 shows the EC_{20} increased from 20% to 60% after 90 mg/L ozonation at pH 9. At pH 8, EC_{20} of 90 mg/L ozone-treated OSPW could not be measured and compared. High doses of ozone- and biodegradation-treated OSPW samples were then compared with the Microtox screen test procedure at pH 9.

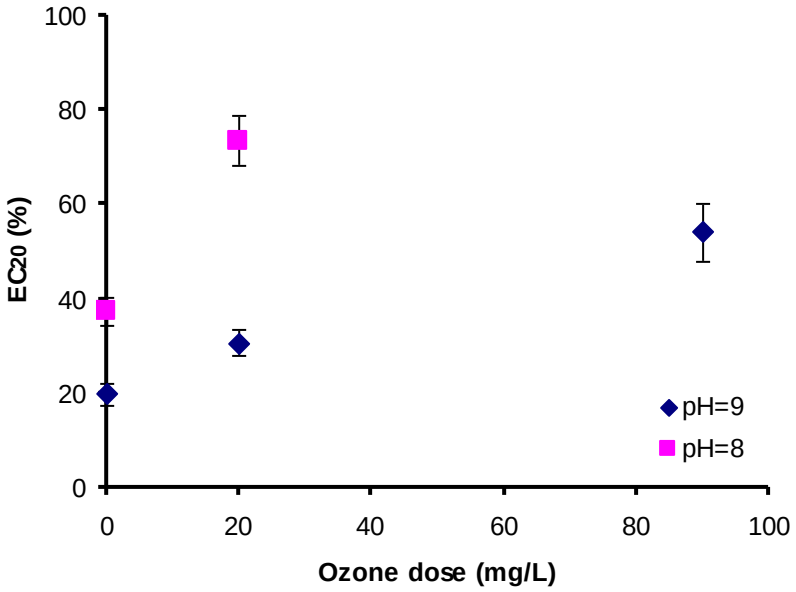


Figure 20: EC_{20} of Batch 3 OSPW after ozonation at different pH (n=6, $p<0.05$ for all ozonated samples. For 90 mg/L of ozone-treated OSPW at pH 8, toxicity is too low to measure EC_{20}.)

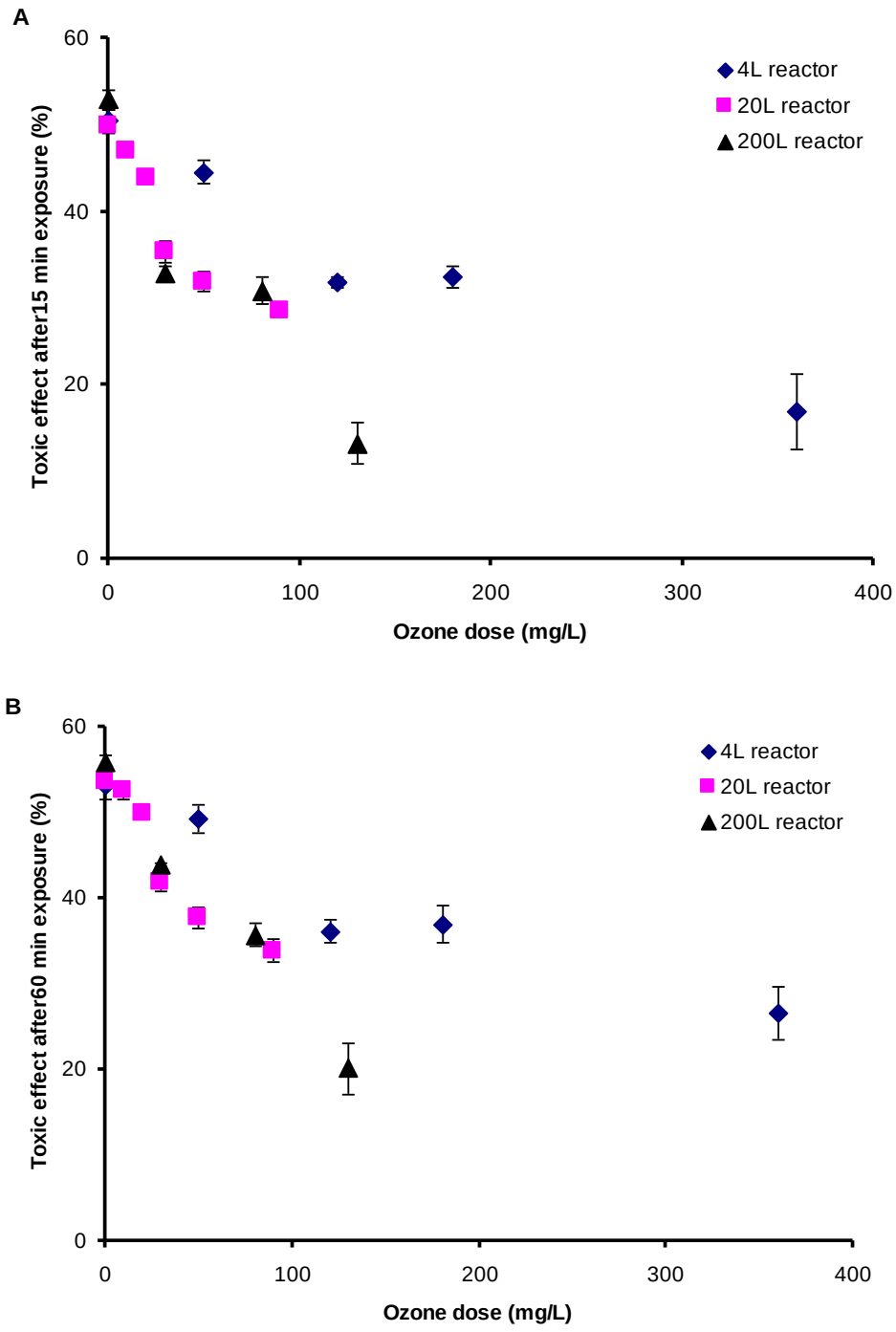


Figure 21: Toxic effect change after 15- (A) and 60- (B) min exposure for ozonated OSPW (n=3)

The changes in toxicity after ozonation in different reactors are compared in Figure 21. Unlike COD and $CBOD_5$, the toxicity of treated OSPW differed among the reactors. Although all the treated samples showed a decrease in toxic effect after ozonation, OSPW samples treated in the 20 and 200 L reactors were detoxified more effectively than samples treated in the 4 L reactor. For example, the toxic effect of Batch 2 OSPW after a 15-min exposure decreased from 50% to 13% after 130 mg/L ozone treatment. However, the toxic effect of Batch 2 OSPW only decreased from 50% to 17% after 360 mg/L ozone treatment.

After biodegradation, the toxic effect of untreated and ozonated OSPW was further depleted. The toxic effect significantly decreased ($P<0.05$) in all the samples after 28 days of biodegradation. Figure 22 shows the toxic effect after 15 min of exposure was almost negligible after biodegradation for Batch 3 OSPW treated with 90 mg/L ozone and for Batch 1 OSPW treated with 150 mg/L ozone. Batch 2 OSPW had about 10% toxicity leftover after 130 mg/L ozonation and biodegradation. Despite using high doses of ozone, 15% of the toxic effect after 60 min of exposure could not be removed.

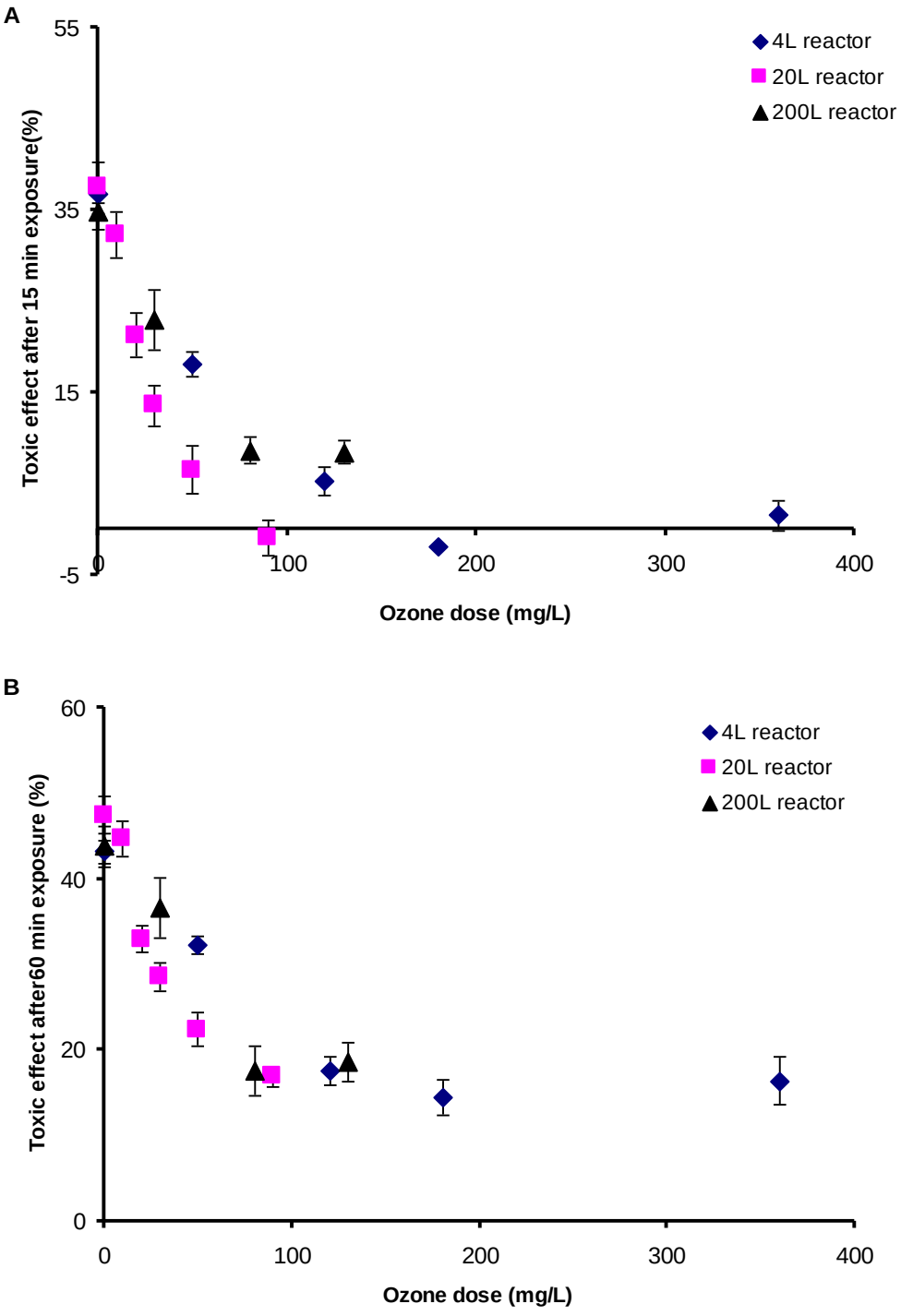


Figure 22: Toxic effect change after 15- (A) and 60- (B) min exposure for ozonated and biodegraded OSPW (n=3)

3.6.4 Change of NAs

The total NA concentrations of Batch 1, 2, and 3 OSPW are 23.6, 19.7, and 21.8 mg/L, respectively. The changes in NA concentration after ozonation in the three reactors are plotted in Figure. 23. The decrease in concentration was similar in all of the samples. After 100 mg/L ozonation, concentrations of NAs in all of the samples were less than 2 mg/L. The depletion of NAs was more effective in Batch 3 OSPW ozonated in the 20 L reactor compared to other OSPW batches. After 90 mg/L ozonation, only 0.3 mg/L NAs were left in the OSPW samples.

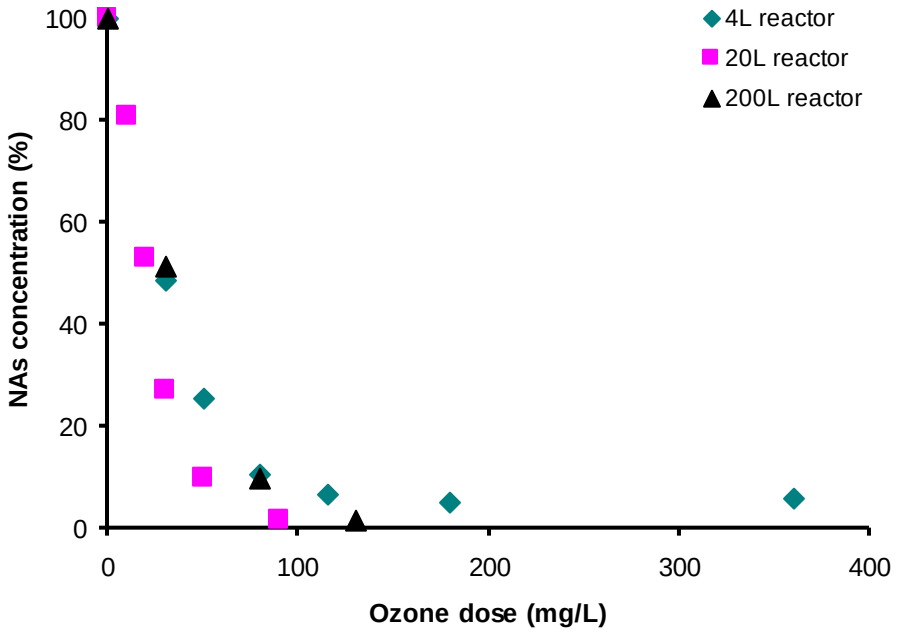


Figure 23: Total concentrations of NAs in untreated and ozonated OSPW

NA concentrations after ozonation and subsequent biodegradation were also measured. Figure 24 indicates that, with biodegradation after the ozonation process, all of the NAs in the water were removed after 80 mg/L ozonation with biodegradation, and 80% of NAs were removed by 30 mg/L ozonation with

biodegradation. The overall efficiency of both processes combined was higher than that by ozonation alone. The biodegradable NAs increased slightly with the increasing ozone dose, from approximately 50% to 70%.

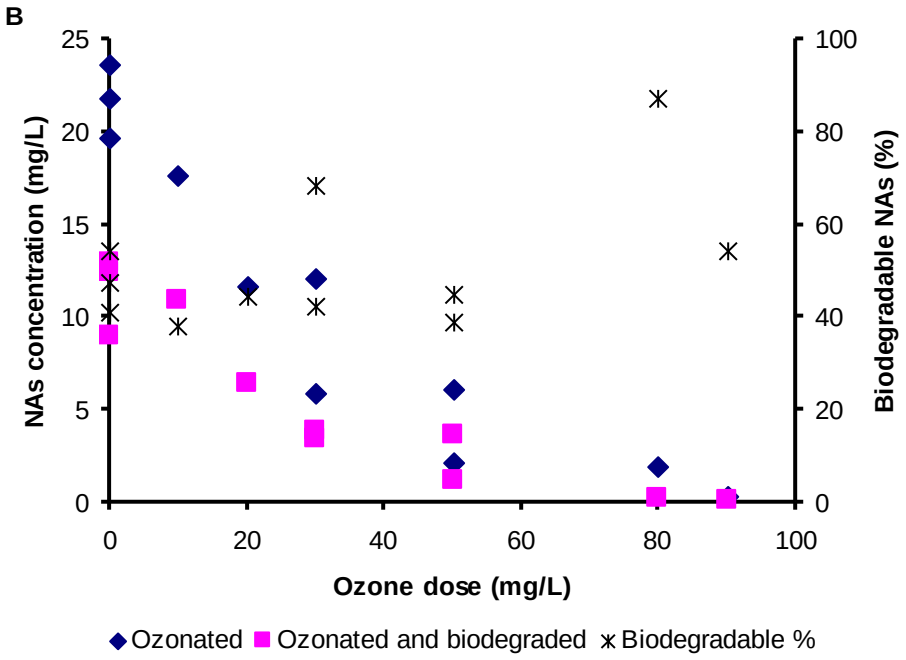


Figure 24: Total concentration of NAs after ozonation and biodegradation

Besides the total NA concentration, the concentration of NAs with different *Z* and C numbers were also analyzed and compared in all reactors. In Figure 25 A, the concentrations of three NAs with different *Z* numbers all decreased after ozonation. Figure 26 A shows that NAs with small *Z* numbers decreased slower than NAs with large *Z* numbers with increasing ozone dose. NAs with different C numbers also decreased with increasing ozone dose (Figure 25 B). The NAs with small C numbers decreased slower than NAs with large C numbers with increasing ozone dose except for one data point at 50 mg/L ozone (Figure 26 B).

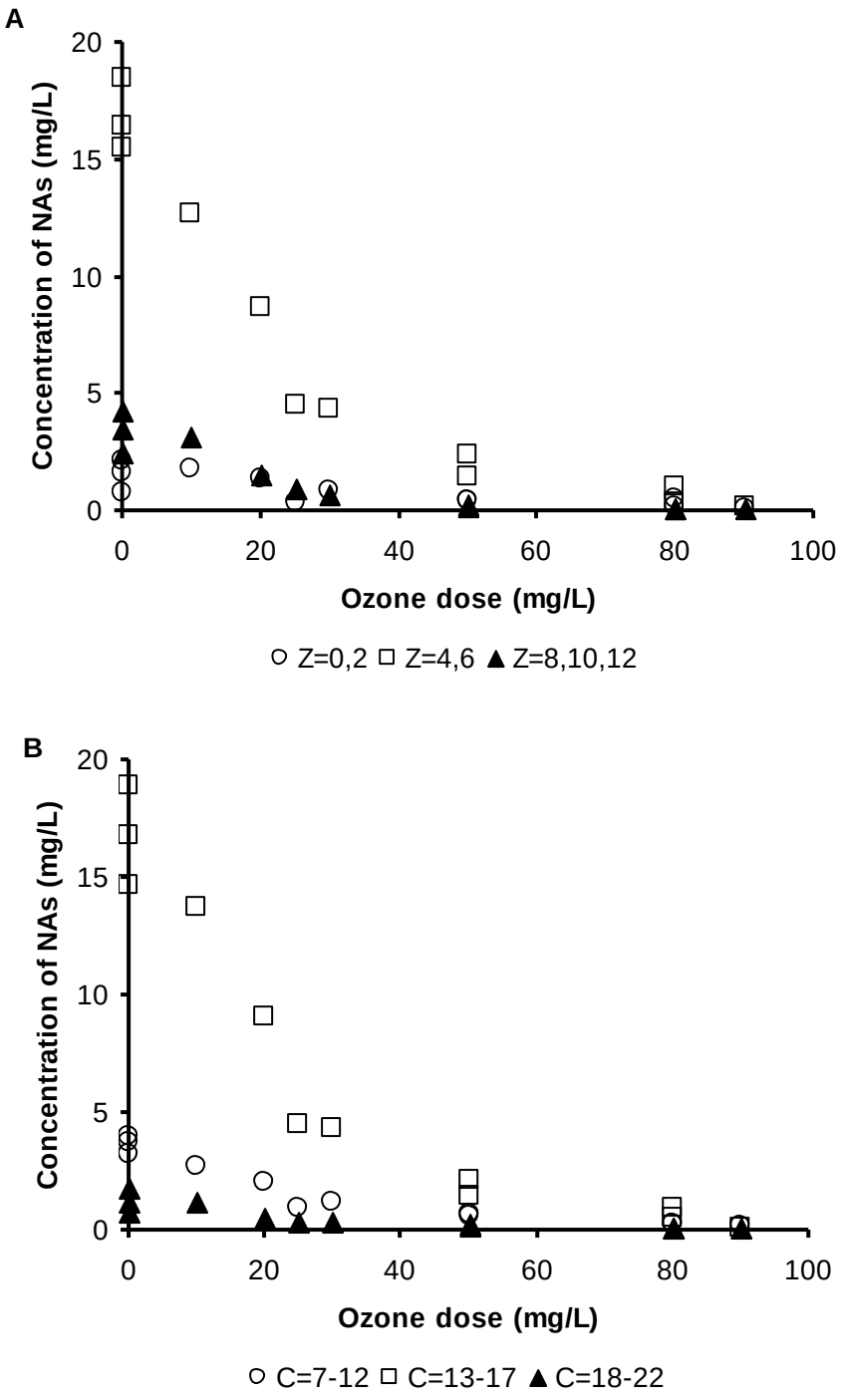


Figure 25: Concentration of NAs with different Z (A) and C (B) numbers after ozonation

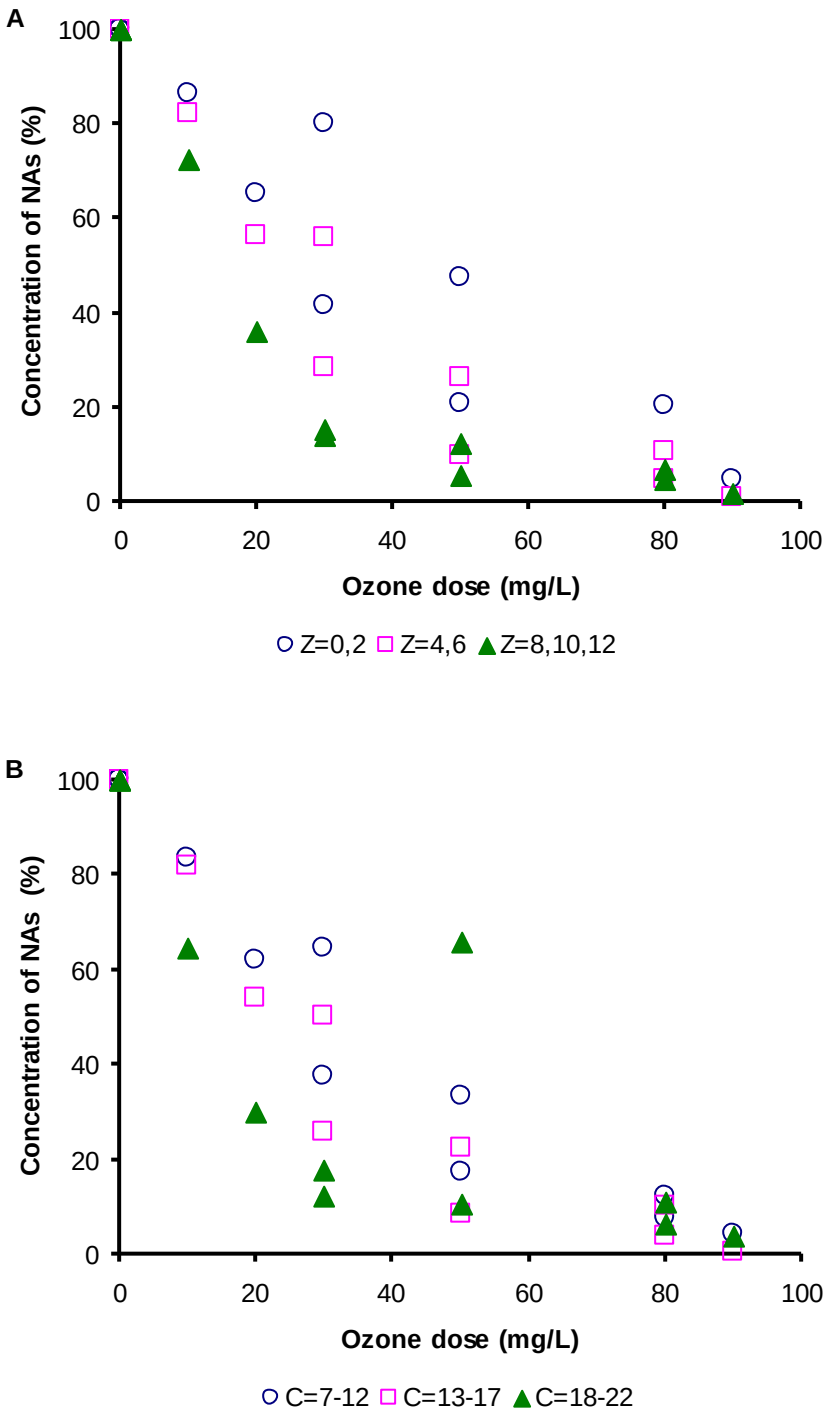


Figure 26: Concentration of NAs with different Z (A) and C (B) numbers after ozonation (normalized to 100% original concentration)

After biodegradation, the concentrations of different NAs further decreased. Figure 29 shows the change in concentrations after biodegradation for different NA groups. For NAs with different *Z* numbers (Figure 27 A-C), biodegradation did not decrease the concentration of NAs with large Z numbers (Figure 27 C) as effectively as those with small Z numbers (Figure 27 A). Similarly, biodegradation did not decrease the concentration of NAs with large C numbers (Figure 27 F) as effectively as those with small C numbers (Figure 27 D).

The percentages of biodegradable NAs before and after high-dose (30 mg/L) ozone treatment are shown in Figure 28. Before high-dose ozone treatment, NAs with smaller *Z* and C numbers are more biodegradable than those with large *Z* and C numbers. For different *Z* number groups, the biodegradability of NAs in low-dose ozone-treated and untreated OSPW was 67, 42, and 31%, respectively, which are statistically different. For different C number groups, biodegradability was 57, 40, and 23%, respectively, which are also significantly different according to the one-way ANOVA test. However, in high-dose ozonated OSPW, these variations all disappeared because the biodegradability of NAs with large C and *Z* numbers increased more than those with small *Z* and C numbers.

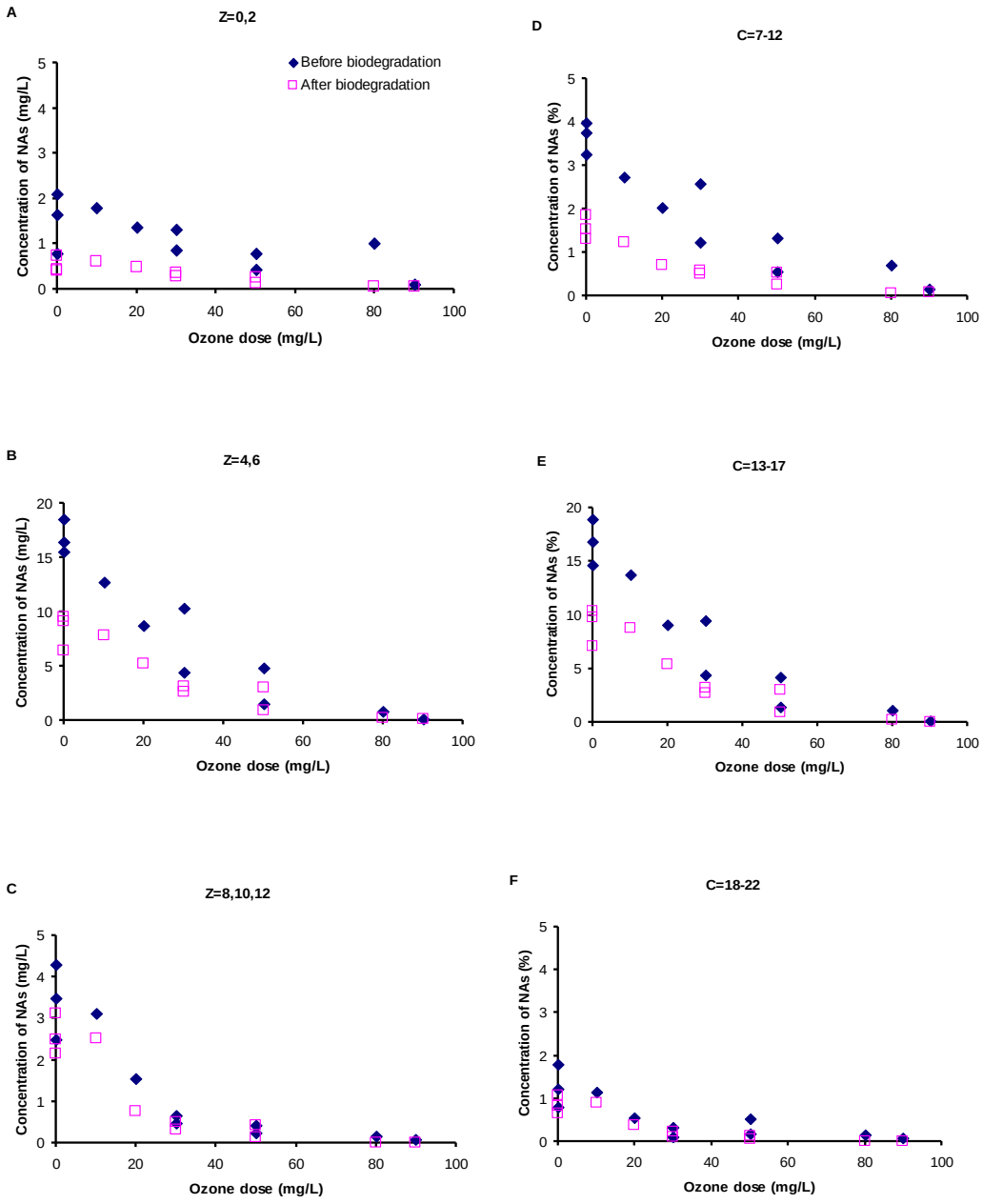


Figure 27: Concentration of NAs with different Z (A, B, C) and C (D, E, F) numbers before and after biodegradation

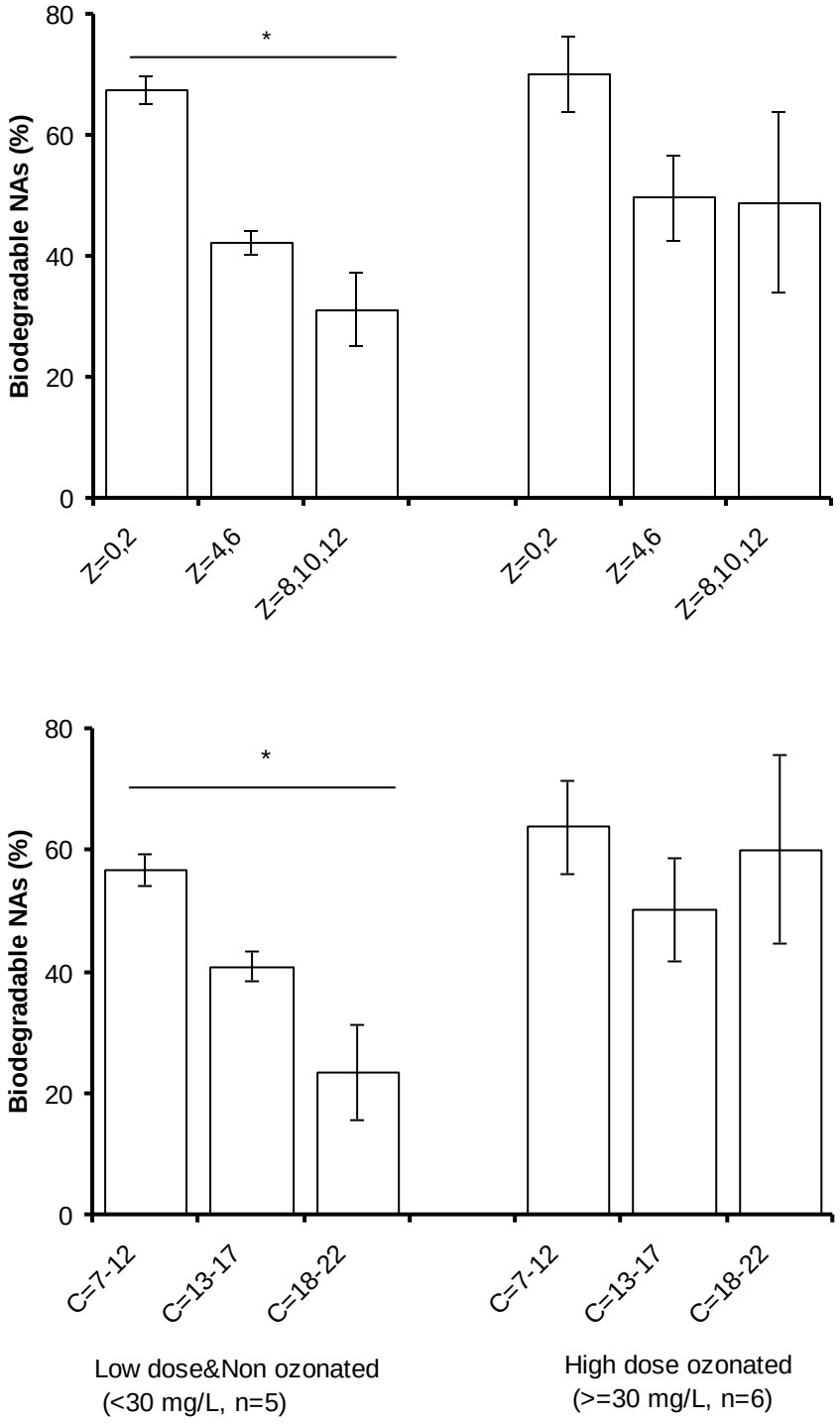
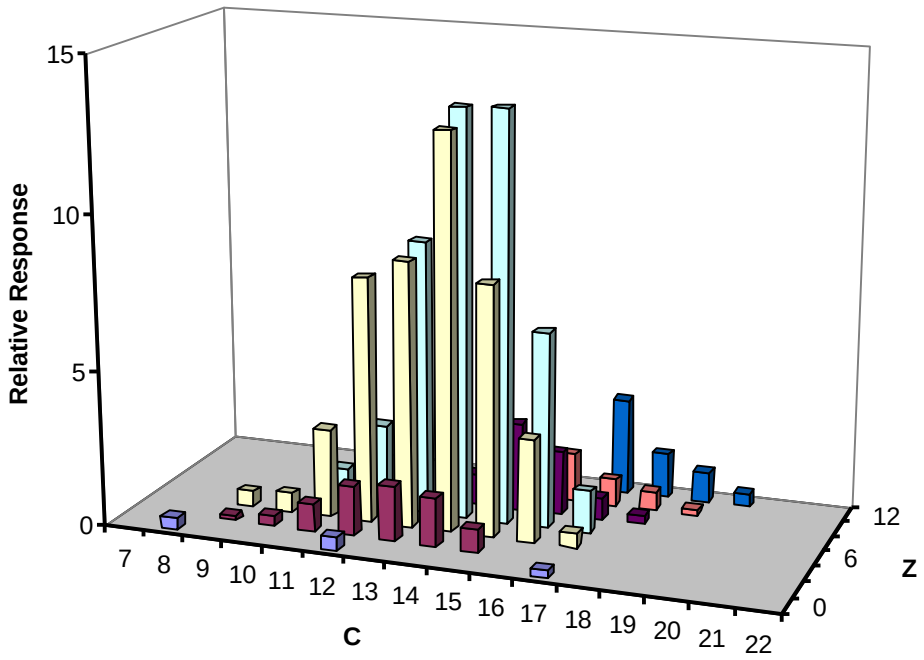
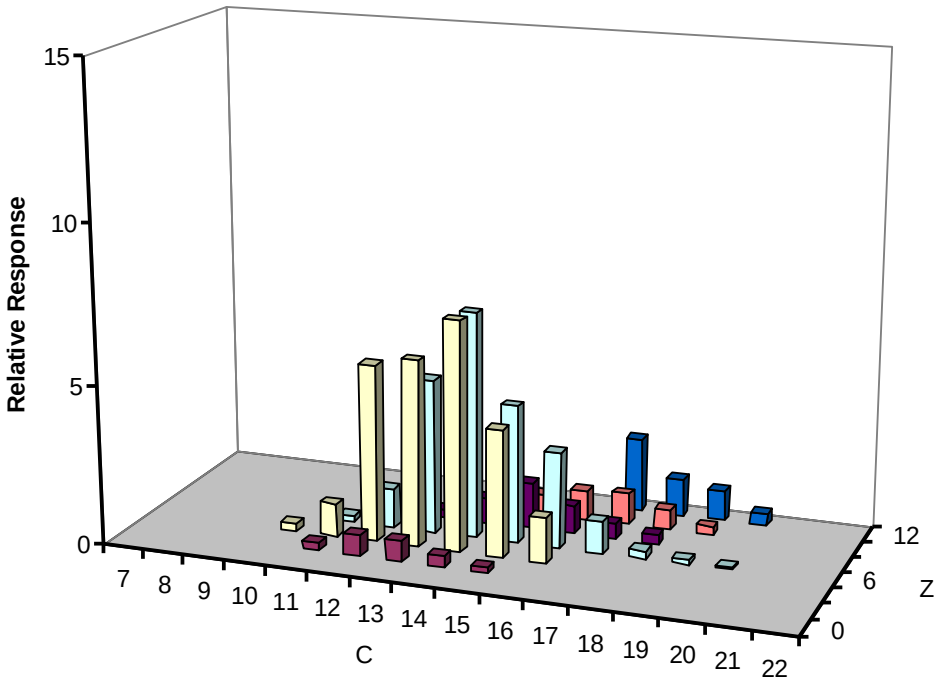


Figure 28: Biodegradable NAs with different Z (A) and C (B) numbers before and after high-dose ozonation (*: $P<0.05$)

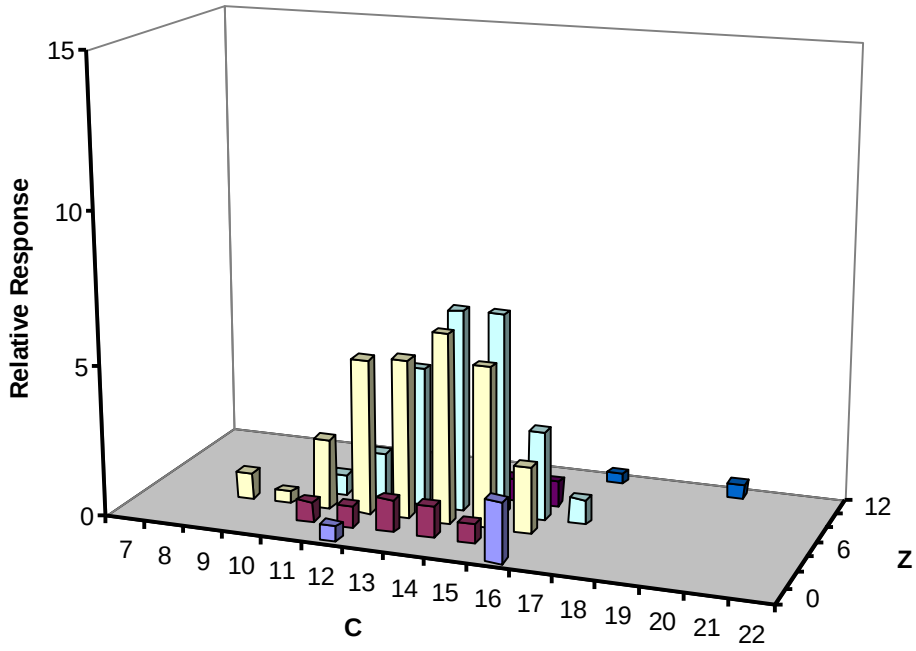
Profiles for different C and *Z* numbers are listed in Figure 29. The relative response represents the relative concentration of NA (%) compared to the internal standard tetradecanoic acid. These pictures show how the concentration of each individual component changed after treatment.



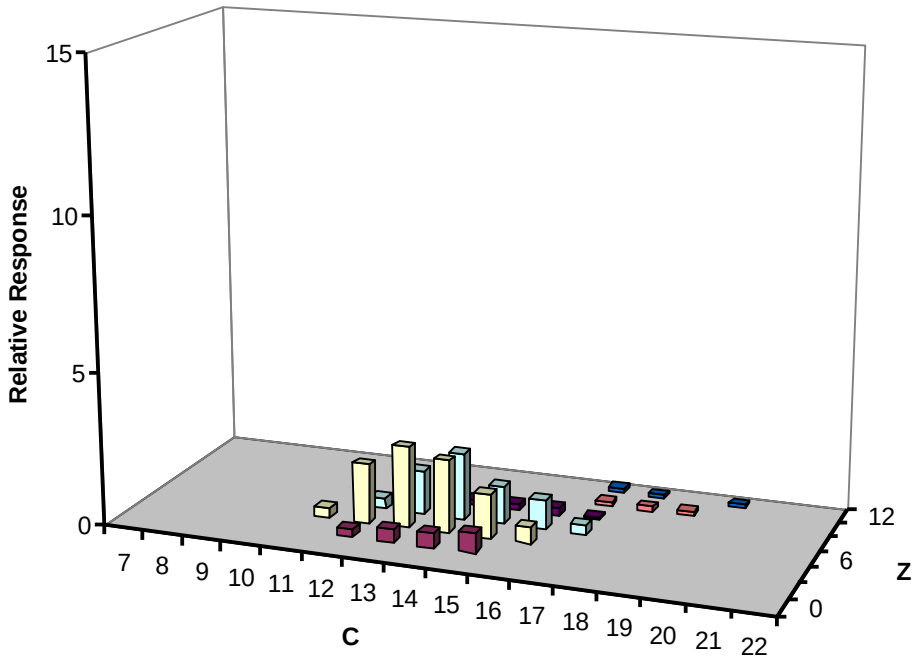
A. NAs in untreated OSPW



B. NAs in biodegraded raw OSPW



C. NAs in 30 mg/L ozone-treated OSPW



D. NAs in 30 mg/L ozone-treated and biodegraded OSPW

Figure 29: Profiles of untreated and treated Batch 1 OSPW

In this experiment, the concentration of NAs in the 30 mg/L ozone-treated Batch 1 OSPW had a very similar result as the sample that was only biodegraded (12.5 and 12.1 mg/L NAs compared to the original concentration of 23.6 mg/L NAs). Figure 29 B and C show that, although the total concentration was the same, the concentrations of NAs with low and high Z numbers are different. Biodegraded untreated OSPW showed relatively unchanged NA concentration when $Z > 8$, but a large reduction occurred when $Z < 6$. For OSPW treated only with ozonation, NAs with $Z = 0$ increased in concentration, $Z = 2$ was almost unchanged, and NAs with $Z > 10$ were greatly depleted compared to the untreated OSPW.

4. DISCUSSION

4.1 Characteristics of OSPW

The OSPW collected from the same pond at different times showed variability in chemical parameters. The TSS value largely depended on the season or tailings pond condition. Re-suspended settlement during collection can add more TSS to the water sample. The water collected in January 2010 had the lowest turbidity and highest TDS concentration. For water collected in May 2010, thawed snow and precipitation might have increased the turbidity and decreased the concentration of dissolved solids. This seasonal variation may be an important factor that can affect the OSPW water chemistry.

Compared to the reported data (Allen, 2008), the pH of the tested OSPW was much higher in this study because all of the water samples were allowed to equilibrate at room temperature and ambient atmosphere by using the platform shaker before measurement. Shaking was done to stabilize the pH of OSPW which otherwise kept changing after being taken out of the storage barrel. The titration curve of OSPW shown in Figure 32 was generated by measuring the amount of 0.02N HCl solution that was added to the OSPW. This titration curve is a typical curve of the carbonate-bicarbonate buffer system (Sawyer *et al.*, 2003). According to the titration curve, the pH changed rapidly in two pH ranges: 7.5 to 9 and 4 to 5. It is postulated that the water in the sealed barrels was in an anaerobic state and appeared to have an artificially high carbonate level and therefore an artificially low pH around 8. The high carbonate concentration might come from the biodegradation of hydrocarbon by microorganism existing in the

storage barrels. Once the water was exposed to the atmosphere, the pH increased until it reached between 8.9 and 9.1. During any given experiment, many factors can affect the pH including temperature and the amount of air present in the container by changing the dissolved CO_2 levels in the OSPW. CO_2 affects pH levels: bubbling OSPW with air for two days made the pH rise from 7.95 to 9.26; but bubbling OSPW with pure nitrogen can make pH rise to 10. Therefore, CO_2 must play an important role in the OSPW pH level.

For most of the tests, such as BOD and COD, the variability of pH in small ranges did not matter because a buffer solution or strong acid was added in these tests. However, for the Microtox toxicity assay, which is based on the light emission of living bacteria, pH is critical.

Based on the results of toxicity measured at different pH, it can be argued that all of the Microtox tests should be conducted at the same pH in order to remove the effect of pH. After shaking the sample at room temperature for two days, all of the pH levels of treated or untreated OSPW increased and stabilized at 9. In this study, all of the Microtox experiments were performed at this pH. The water used for the *Chironomid* exposure assay was also aerated between one and two weeks, making the pH about 9. Different dilutions made the pH slightly different from 8.5 to 9.

4.2 Utilization of ozone in different reactors

The efficiency of ozone usage shown in Figure 2 shows that 20 and 200 L reactors equipped with the fine bubble diffuser had the highest utilization

efficiency. However, it is still far less than the average 90% transfer rate of the fine bubble contactor used in practice (Zhou and Smith, 2001). The main reason is that the 200 L reactor was not deep enough to allow the ozone gas transfer between the gas and liquid phase.

According to Formula 1, the reasons the 200 L reactor has the highest efficiency include the following: 1) the high-performance gas diffuser in the 200 L reactor produced a large amount of fine gas bubbles which increased the effective surface area; 2) high gas-flow rates and fine bubbles made liquid flow in the reactor more turbulent, producing large shear force, which made the liquid film thinner; and 3) deep column-made gas bubbles have a long time to exchange with bulk liquid. All of these factors increased ozone diffusion into liquid in the large reactor during the ozonation process.

In Table 5, although the 4 L reactor has the lowest utilization rate, it also took the least amount of time to consume the same amount of ozone per unit volume (L). The 4 L reactor consumed the same amount of ozone per unit volume in a shorter time because there was a higher ozone:liquid volume ratio. In practice, a high rate of utilization is preferred due to the cost of producing ozone.

In the 20 L reactor, a small dose of ozonation (10 or 20 mg/L) had higher utilization efficiency than high doses of ozonation (>30 mg/L). There are several possible explanations for this observation. One possibility is that, at the beginning of ozonation, the ozone concentration in the liquid phase was zero, which made diffusion effective and fast. After a short period of time, ozone concentration in the liquid phase increased and diffusion efficiency decreased. Another possibility

may be that only a small amount of ozone was introduced into the system: due to the relatively large inert space in the entire system, such as air space in the reactor and all of the tubes, the small amount of unreacted ozone can not be purged out immediately and stays in these spaces for a long time until they decompose or react with other air molecules. As a result, monitored effluent ozone concentration was underestimated, making the utilization efficiency falsely high. For short-time ozonation with small amounts of ozone gas, the falsely high utilization efficiency value is more obvious than long-time ozonation.

4.3 Change of COD and CBOD after ozonation and biodegradation

The OSPW COD tended to decrease with increasing ozone dose in all of the reactors. The nature of the COD change was similar between different samples, which means the amount of ozone reacted is the main factor which affected COD depletion. The decrease of COD is attributed to the progressive oxidation of oxidable compounds. The reaction of ozone or oxidizing radicals with organics enhances the oxidation state of the carbon in organic compounds by breaking their chemical structure and adding oxygen (or ozone) to the molecules (Bailey, 1982). A 40% COD decrease in OSPW was reported after 130 min of ozonation (Scott *et al.*, 2008).

Besides increasing the oxidation state, ozone can also break complex molecules to smaller ones by adding oxygen to the chemical structure in the form of a hydroxyl or carboxyl group which is more amendable to biodegradation (Alvares et al., 2001). This is why higher ozone doses produced higher COD biodegradability in

different batches of OSPW in all of the reactors. This phenomenon is similar to what was found in pulp mill effluent: ozone treatment decreased COD and increased biodegradability by converting high molecular weight compounds to low molecular weight ones (Bijan and Mohseni, 2005).

Biodegradation of treated OSPW COD varied among the reactors. COD of Batch 2 and 3 OSPW decreased more than COD of Batch 1 OSPW after biodegradation. If we assume that the components of these OSPW from the same pond are similar, there are two possibilities that may cause this variance. One possibility is that the bacterial community had different activity in different OSPW because of different levels of toxicity or biodegradable hydrocarbon. Another possibility is that different reaction times made the OSPW COD have different levels of biodegradability. For example, in order to achieve the same ozone dose, the contact time for the 20 or 200 L reactors almost doubled that for the 4 L reactor. Longer contact times may increase the biodegradability of chemicals in OSPW.

As a component of OSPW, ammonia and related molecules can increase the BOD test result by nitrification (Metcalf and Eddy, 2002). Comparing the results between the BOD and CBOD tests, different batches of OSPW had different ammonia levels. For example, the CBOD/BOD tests showed that most of the BOD for Batch 1 OSPW was nitrogenous biochemical oxygen demand (NBOD). For Batch 3 OSPW, CBOD and NBOD were at a similar level. In this study, the main concern was the carbonaceous pollutants including NAs. In order to avoid

the effect of ammonia on the analysis, a relatively constant parameter, CBOD, was chosen to compare the different reactors and OSPW.

The nitrification process could be totally suspended during the CBOD test because only small amounts of organic carbon existed in the OSPW (Metcalf and Eddy, 2002). As a result, the oxygen consumed by the bacterial community was entirely for the oxidation of organic carbon. Similar to the COD results, CBOD of OSPW increased with increasing ozone dose in different reactors. After biodegradation, CBOD decreased to a very low level (<2 mg/L). Compared to OSPW treated with lower doses of ozone or untreated OSPW, the increased CBOD of OSPW samples after high-dose ozonation were all biodegraded.

The increase of BOD in OSPW after ozonation has been reported by Scott *et al.* (2008). In that study, the BOD of OSPW increased from less than 5 to nearly 20 mg/L after 130 min of ozonation. This change is similar to what we measured after 360 mg/L ozone-treated OSPW. However, that study did not report any utilized ozone doses, making it difficult to compare the decreasing COD trend between these studies.

4.4 Degradation of NAs by ozonation and biodegradation

Compared to HRMS, FTIR consistently gave much higher NA concentration values based on the data measured in this lab. According to Scott *et al.* (2008), the differences between the results from the gas chromatography-mass spectrometry (GC-MS) and the FTIR are most likely due to hydroxy-NAs, which cannot be separated using FTIR. Thus, the NA concentration measured by FTIR is falsely

high. In this study, UPLC-HRMS was applied, and it is more accurate than the low-resolution GC-MS method. Thus, the NA concentrations measured in this study (19 to 24 mg/L) are much lower than the measured FTIR value (approximately 70 mg/L) (data measured in this lab). Furthermore, with HRMS, the structure persistence of NAs after ozonation and biodegradation can be studied specifically. The quantitative changes of NAs with different C and *Z* numbers were also compared after treatment.

In all of the OSPW samples, the total concentration of NAs rapidly decrease after ozonation. The decrease was very similar to the decrease of NA when ozonation was applied in a 1 L reactor and measured using FTIR previously (Unpublished data by Helen Fu, 2008). Compared to the other two reactors, the 4 L reactor had a significant residual NA concentration (approximately 1 mg/L) after a long period of ozonation. The reason for the residual might be due to the configuration of the reactor, in which the position of the gas diffuser was 5 cm off the bottom of the reactor, creating dead space.

Scott *et al.* (2008) reported that ozone can react with NAs. In that study, 130 min of ozonation decreased 95% of NAs in OSPW, which was measured by GC-MS. In this study, the longest contact time is 60 min in the 200 L reactor, which used 130 mg/L ozone and resulted in 98.5% depletion of total NAs. The OSPW used for ozonation in this work was not pre-treated, unlike the filtered OSPW in Scott's report. The depletion of NAs was much faster than COD, which means the NAs easily reacted with ozone compared to other oxidable compounds in OSPW.

The analysis of NAs with different structures showed that molecules which had a large *Z* number were less resistant to ozone oxidation than those with a small *Z* number (Figure 26). This means the ring structure was easily attacked by ozone molecules or radicals, which resulted in the loss of carbon rings. The molecules with a different C number also show a similar trend: NAs with a large C number depleted faster than those with a small C number. In Scott *et al.*'s (2008) paper which measured NAs with GC-MS, the proportion of NAs with high C numbers decreased after 130 min of ozonation, and those with a small C number increased in concentration more than three times. However, it is hard to tell whether these small C number NAs were original or generated from the ozonation of NAs molecules with a large C number.

With regards to the biodegradability of NAs, it was reported that under aerobic conditions biodegradation of NAs in OSPW was slower than commercial NAs, even when the NAs had the same *n* and *Z* classification (Han *et al.*, 2008; Scott *et al.*, 2005). Han *et al.* (2008) reported approximately 50% depletion of total NAs after 92 days of biodegradation. In this study, different batches of untreated OSPW had approximately 50% biodegradable NAs after 28 days of biodegradation. Another recently published paper found different kinds of NAs in untreated OSPW had different half-life values, most of which were longer than 28 days (Martin *et al.*, 2010). The difference between these studies and this work indicated that the biodegradability of the NAs in OSPW might vary under different conditions for different samples. After ozonation, the biodegradability of total NAs in OSPW increased only slightly, which decreased the residual

concentration of NAs to almost 0 mg/L after 80 mg/L ozonation with biodegradation. The ozone may change the complex structure of these NAs during the ozonation process to make them readily biodegradable (Alvares *et al.*, 2001). The increase of biodegradability may also be due to the decreased toxicity of OSPW by ozonation, which increases activity in the bacterial community more than untreated OSPW.

It was reported that the structure of NAs affected the biodegradability of NAs (Han *et al.,* 2008). Han *et al.* proved that cyclization is a major factor contributing to persistence in biodegradation. In this study, the effect of structure on the biodegradation of NAs in OSPW was similar to what has been reported (Han *et al.*, 2008), but only in untreated or slightly ozonated (<30 mg/L) samples. In untreated or slightly ozonated OSPW, NAs with larger *Z* numbers were difficult to biodegrade in 28 days. Further, we observed that NAs in untreated and slightly treated OSPW with large C numbers were also difficult to degrade by biological processes (Figures 27 and 28).

Published research proves that the high degree of alkyl branching was the principal factor affecting the biodegradability of different NAs with the same C number (Johnson *et al.*, 2010; Smith *et al.*, 2008). After the ozonation process, these branches might have been attacked and destroyed, and NAs became more biodegradable (Han *et al*., 2008). A recently published paper (Martin et al., 2010) reported that the ozonation process accelerated the biodegradation of NAs with different structures. In this study, the significant variation between the

biodegradability of NAs with different structures was removed in high-dose (>30 mg/L) ozonated OSPW. That means the increased level of biodegradability was more obvious in NAs having large C and *Z* numbers. The reason for this phenomenon may be due to ozone destroying the branching structures that exist in large, complicated NA molecules. For NAs with small C numbers, there are less branch structures than NAs with larger C numbers. Therefore, the phenomenon of increased biodegradability by ozonation was not obvious in small NA molecules.

Similarly, increased biodegradability after ozonation was clearer in NAs with larger *Z* numbers (Figure 28, Z = 8, 10, 12). This result is contrary to Martin *et al.*'s (2010) results which showed that decreased half-life by ozonation was more obvious in NAs with small *Z* numbers (*Z* = 2). This difference is due to the different biodegradation processes since the analysis of NAs was performed with the same procedure and instrument. For this study, the result was based on more than 10 samples of different ozonation and biodegradation processes.

The different effects on NAs by ozonation and biodegradation process suggested different mechanisms were employed in the degradation of NAs. The combination of these two treatment processes will increase the overall efficiency of degradation, which has been observed in pulp mill wastewater treatment (Bijan and Mohseni, 2005).

4.6 Change of toxicity measured by Microtox

In this study, because of the low toxicity of treated OSPW and large number of samples, the 81.9% screen test was applied instead of the basic test to compare

toxicity among untreated and treated samples. The advantage of the screen test is that it allowed the comparison of many samples in one run, which reduced system error caused by the varying sensitivities of the bacteria. This procedure was also appropriate for samples with low toxicity such as OSPW treated with ozone and biodegradation. Among the three batches of OSPW, Batch 2 has the highest acute toxicity and TDS levels, but the lowest NA concentration, which means the source of acute toxicity comes from other dissolved chemicals, not only NAs.

EC_{20} of untreated Batch 3 OSPW and 20 and 90 mg/L ozone-dose treated Batch 3 OSPW was checked in this study: the two treated samples had 50% and 99% of NAs removed, respectively. The untreated control sample had a significantly lower EC_{20} value compared to the 20 and 90 mg/L ozonated samples, starting at 20% and reaching 30 and 54% at pH 9, respectively. According to Scott *et al.* (2008), the OSPW EC_{20} increased from 23% to 31% after 10 min of ozonation. Although no pH or ozone dose was reported, the OSPW EC_{20} increased after ozonation. However, Martin *et al.* (2010) reported that the EC_{20} of ozonated OSPW did not change. In that report, the EC_{20} of the ozonated OSPW sample did not change significantly compared to untreated samples even after 50% and 72% of NAs were degraded. These two articles did not report the pH value of the OSPW samples. Due to the effect of pH on Microtox results, it is difficult to compare the results between these studies.

After ozonation, the toxic effect of all the OSPW samples decreased as ozone dose increased. OSPW ozonated in the 20 L and 200 L reactors were more efficiently detoxified with the same ozone dose compared to the 4 L reactor. Since the 200 L reactor needed longer contact time to obtain the same ozone dose, this reactor may have detoxified OSPW more efficiently because some of the toxic chemicals needed a longer reaction time to decompose. The different water chemicals in different OSPW batches may also contribute to this variation.

Comparing the change in the toxic effect to the concentration of NAs, it was found that 100 mg/L ozone removed more than 90% of the NAs from OSPW, which is much more effective than the decrease of only 20-50% of the toxic effect. There are two possible reasons for this phenomenon: 1) the ozone destroyed NAs, but did not fully mineralize them, forming oxidized NAs (Martin *et al.*, 2010). Many oxidized products are still toxic, but were not measured in this study; or 2) other contaminants which were not effectively removed by ozonation also contributed to the acute toxic effect of OSPW.

As a result, toxicity was not removed as effectively as NAs were. The toxic effects after 60 min of exposure were similar to those after 15 min of exposure. This shows that the contaminants in these samples had similar effects in the 15- and 60-min ranges.

After biodegradation, all of the samples were detoxified effectively ($P<0.05$) in the 15 min exposure test. For Batch 2 OSPW treated in the 200 L reactor, 80 mg/L ozonation with biodegradation resulted in 99% of NAs being removed and approximately 20% of acute toxic effects remained. The 20% toxic effect was

definitely not from NAs, but from other toxic chemicals such as refractory organics or heavy metals which were not removed by the two treatment processes. To remove this toxicity, other water treatment methods may need to be applied. The detoxification effects of biodegradation were also quite different between the three batches of OSPW. A comparison of Figures 21 and 22 shows that ozonation detoxified the Batch 3 OSPW treated in the 20 L reactor as effectively as the Batch 2 OSPW treated in the 200 L reactor. However, after biodegradation, Batch 3 OSPW showed the least inhibition effect among all three batches of samples. The reason for the different detoxification efficiencies during biodegradation might be because the bacteria community had the highest activity in the Batch 3 OSPW. After biodegradation, the toxic effects after 60 min of exposure were higher than those after 15 min of exposure, which indicates that biodegradation primarily degraded contaminants contributed to the acute toxic effects.

Holownko *et al.* (2002) reported the decrease in toxicity of OSPW might be due to an increase in the proportion of NAs with large C numbers. In this study, OSPW ozonated in the 200 L reactor with 30 mg/L ozone had the same total NA concentration with the sample that was only biodegraded. The two samples' NA components were different as shown on Figure 31. In Figure 17, the toxic effects of these two samples are not significantly different ($P<0.05$). That means the proportion of NAs with large C numbers may not be an important factor affecting the toxicity of the OSPW in this case.

4.7 Conclusion

Ozonation did not decrease COD effectively in OSPW. With a high dose of 360 mg/L ozone, only approximately 30% of COD in Batch 1 OSPW was depleted. The biodegradability of COD in the 20 L and 200 L reactors is higher than the 4 L reactor. Although ozonation increased the biodegradability of OSPW, the highest depletion rate by ozonation with biodegradation was only 50%. In practice, this high dose is not feasible.

After ozonation, OSPW $CBOD_5$ increased with increasing ozone doses. After biodegradation, the CBOD level was less than 2 mg/L and difficult to detect.

Different batches of OSPW from the same pond had different chemical characteristics and toxicities. After ozonation, the acute toxicity was depleted effectively. With biodegradation, approximately 80 mg/L of ozonation depleted all of the toxic effects of OSPW after 15 min of exposure as measured by Microtox. The depletion of the toxic effect during ozonation in different reactors was not similar.

NA concentration was sensitive to ozone treatment. In general, 100 mg/L ozone removed more than 90% of NAs in Batch 1 OSPW and 100% of NAs in Batch 3 OSPW. With biodegradation, all of the NAs were decomposed at this dose level. However, this sample with zero NA concentration still had approximately a 20% toxic effect, which means that some of the treated by-products were still toxic.

NAs with large C and *Z* numbers were less resistant to ozone than those with small C and *Z* numbers. In untreated or slightly ozonated OSPW, NAs with smaller *Z* and C numbers were easier to biodegrade. However, in high-dose (>30

mg/L) treated OSPW, this difference in biodegradability disappeared, which means the two treatment processes have different mechanisms to degrade NAs.

For practical applications, if NAs and the toxicity of OSPW are the main targets for removal, ozonation at 80 mg/L with biological treatment would produce a very good result. If COD and BOD are also of concern, then increasing the ozone dose to approximately 200 mg/L is optimal.

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6. APPENDICES

Appendix A. The ozonation process

Procedure of calibrating ozone monitor HC-500:

1. Dissolve 10g KI in 400 mL water. Put the solution into a 500 mL wash bottle.

2. Connect two HC-500 ozone monitors in series and by-pass the flow meter.

3. Connect the wet meter to the effluent port of the wash bottle. Prepare the connection between the monitor and wash bottle influent port.

4. Open the ozone generator, then adjust the ozone concentration knob to full.

5. After monitors are stabilized for 10 min, connect the tube between the wash bottle and ozone monitor. Disconnect the connection when approximately 3 L gas passes the wet meter (1 cycle). While the gas passes the wet meter, record monitor readings from the two monitors every 5 seconds.

6. Transfer all of the solution from the wash bottle into a 500 mL volumetric flask. Wash the wash bottle with water several times, and transfer all of the washed solution into the volumetric flask. Use water to fill the solution to 500 mL and mix thoroughly.

7. Repeat Steps 1 through 5 three times and change the ozone concentration knob to three different positions (to change the ozone concentration in the gas) to produce three volumetric flasks of solution in total.

8. Pour 50 mL solution from each volumetric flask into a 100 mL flask, then add 2 mL 2 M H_2SO_4, and titrate this solution with 0.1 N $Na_2S_2O_3$. Record the volume of $Na_2S_2O_3$ used.

9. Calibrate 0.1 N $Na_2S_2O_3$ solution according to the standard method (APHA-AWWA-WEF, 2005). Then, prepare another solution by adding 1 mL concentrated H_2SO_4, 10 mL 0.1000N $KH(IO_3)_2$, and 1 g KI to 80 mL water. Titrate this new solution with 0.1 N Na_2SO_3. Record the volume of solution used in the titration. The normality of $Na_2S_2O_3$ is equal to:

$$\text{Normality } Na_2S_2O_3 = \frac{1}{mL Na_2S_2O_3 Consumed}$$

10. Input the measurements into a Microsoft Excel file to deduce the formula for converting the monitor readings into ozone concentration (W/W%). The relationship between the monitor readings and real weight percentages was calculated by linear regression. The linear regression relationship is:

Monitor reading = Weight percentage * a + b

The calculated a and b values are listed in the following table:

Table A1. Transformation constant for two ozone monitors

	Monitor 1		Monitor 2	
	a	b	a	b
Jan 2010	1.11	-0.3301	1.0872	1.3404
April 2010	1.1157	-0.2833	1.0735	1.4191
Aug 2010	1.095	-0.3268	1.1236	-0.2796
Nov 2010	1.0912	-0.2289	1.1427	0.3864

Use the following formula to convert W/W% concentration into mg/L:

O_3 concentration (mg/L) = O_3 concentration (W/W %) *1308/(100-1308*0.000255* O_3 concentration (W/W %))

Procedure of Ozonation:

1. Load the predetermined volume of OSPW sample into the reactor. Connect all of the system components according to Figure 2. Check leakage carefully and ensure the vacuum hood works properly.

2. Turn on the ozone generator, but do not begin generating ozone. Check the meter to ensure there is ample oxygen in the cylinder for the entire ozonation process. Let the oxygen pass through the entire system, and adjust the flow rate to the required value according to the reactor being used. Then switch the gas flow to by-pass mode.

3. Start ozone generation by switching the knob from "purge" to "on". Wait about 5 to 10 min to allow the ozone concentration to stabilize.

4. Record the ozone concentration readings on both ozone monitors, and switch the gas flow from by-pass mode to reactor mode. Use a stop watch to measure the time.

5. Record the ozone concentration readings on both ozone monitors every minute, and check the flow rate to ensure accuracy.

6. Once the ozonation is complete, stop ozone generation by switching the knob from "on" to "purge". Wait approximately 5 min until the ozone concentration reading in the effluent monitor decreases to zero. During this step, readings in the two monitors still need to be recorded.

7. Conduct the Indigo test on the sample. Record the concentration of ozone residue.

8. Stop the oxygen supply, and purge the reactor with pure nitrogen to remove any ozone residue. Move the ozonated sample to a cold room (-5°C) and store the

sample for at least three days before any usage to ensure all of the ozone has decomposed.

Calculation of Ozone dose:

Using the 30 mg/L ozonation in the 20 L reactor as an example, the process of ozone dose calculation is as follows:

1. List and plot the ozone concentrations (mg/L) versus time (min). Calculate the area under each curve, which represents the amount of cumulative ozone flowing in and out of the reactor.

2. Calculate how much ozone was utilized in the reactor per volume of sample. Subtract the cumulative ozone amount in the effluent gas from the influent gas; divide the amount by the volume of the sample (e.g., 18.5 L sample for a 20 L reactor).

3. Subtract the residue ozone concentration (always less than 0.1 mg/L and may be negligible) from the result obtained from the previous step. The result represents the utilized ozone dose (mg/L).

Table A2. Example of ozone dose calculation

Time (min)	Ozone con. (mg/L)		Flow rate	Cumulative O_3 (mg)	
	in	out	L/min	in	out
0	89.00282	0	4.074		
1	90.59748	0	4.074	365.8458	0
2	90.10668	43.49927	4.074	368.0944	88.608
3	89.86133	62.39785	4.074	366.5948	215.7124
4	89.73866	65.92204	4.074	365.8452	261.3876
5	89.37071	66.55202	4.074	547.2687	404.7745
6	3.558989	66.42601	4.074	94.64889	135.4381
7	0	55.86942	4.074	7.249661	249.1158
8	0	19.60358	4.074	0	153.7385
9	0	0	4.074	0	39.9325
			Sum=	2115.547	1548.707

Utilized ozone dose = (2115.5-1548.7)/18.5-0.06 = 30.6 mg/L

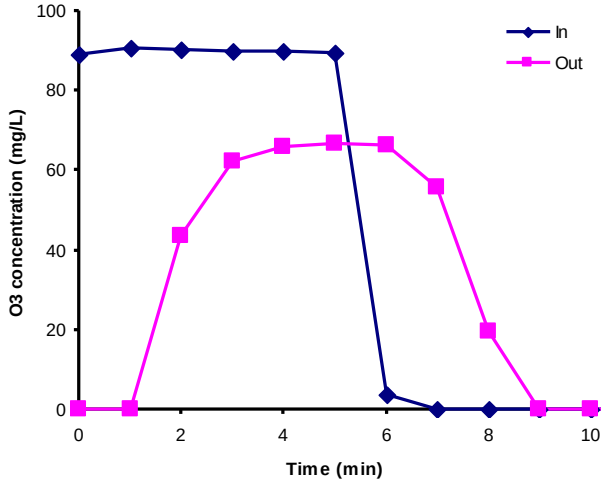

Figure A. Ozone concentration in influent and effluent gas for 30 mg/L ozonation of Batch 3 OSPW in a 20 L reactor

Appendix B. Procedures for growing biomass in tailings pond sludge and for biodegradation

Procedure for growing biomass in tailings pond sludge:

1. Prepare the Bushnell-Haas Broth (BHB) medium. The formula for this basic medium is (per L): 1 g K_2HPO_4, 1 g KH_2PO_4, 1 g NH_4NO_3, 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 0.05 g $FeCl_3$.

2. Place 10 mL tailings pond sludge into 90 mL BHB medium with 10 mg glucose (100 mg/L). Aerate with a small air pump and gas diffuser for one week.

3. Put 90 mL BHB medium, 10 mg glucose, and 45 mL OSPW into a 250 mL flask. Transfer 15 mL of the cultured solution from Step 2 into the 250 mL flask. The final concentration of OSPW and glucose should be 30% (V/V) and 66.7

mg/L, respectively, in the final solution. Aerate with a small air pump and gas diffuser for one week. Repeat this step.

4. After three weeks of total incubation time, the inoculums are ready to use for the BOD test and biodegradation. The entire process should be performed at room temperature (20°C).

Procedure of biodegradation:

1. Prepare four stock solutions (phosphate buffer solution (PBS), $MgSO_4$, $CaCl_2$, and $FeCl_3$) according to the standard BOD test method (APHA-AWWA-WEF, 2005).

2. Place 350 mL OSPW sample into a 500 mL flask with 3.5 mL PBS and 0.35 mL of the other three stock solutions. Seed the mixture with 5 mL cultured inoculums and shake the flask constantly at 200 rpm for 28 days.

3. Take COD samples every seven days including day zero. After the samples are collected, add concentrated H_2SO_4 to the samples until the pH is less than 2. Keep the samples refrigerated before measuring COD.

4. After 28 days, filter 10 mL samples before and after biodegradation with 0.2 μm nylon syringe filters for NA analysis. Acidify the samples for the CBOD test with concentrated H_2SO_4 to a pH of less than 2, and keep them refrigerated. Adjust CBOD samples' pH back to 7 before performing the CBOD test. Conduct the Microtox test without treating the samples. Conduct the Microtox test as soon as possible to avoid further degradation.

Appendix C. Raw COD and CBOD data for all of the samples

COD data were the mean values of samples from two duplicate flasks. Each sample was measured in triplicate. CBOD data were based on a controlled experiment which included all of the samples in one batch to omit random error. Due to the limited volume of the biodegraded sample, an experiment with duplicate bottles was performed.

COD of OSPW before/after biodegradation:

Table C1. COD results of untreated (dose = 0) and treated OSPW

O_3 dose (mg/L)	Ozonated			Ozonated and biodegraded		
	COD (mg/L)		mean	COD (mg/L)		mean
Batch 1 OSPW						
0	298.6	302.1	300.4	267.2	294.9	281.1
50	264.2	278.9	271.6	235.8	248.5	242.2
120	240.9	241.3	241.1	225.7	218.9	222.3
180	210.5	241.3	225.9	180.2	201.4	190.8
360	200.4	225.3	212.9	124.5	174.2	149.3
Batch 2 OSPW						
0	270.2	247.9	259.0	223.6	223.3	223.5
30	220.5	220.9	220.7	199.5	194.0	196.8
80	193.1	206.8	200.0	168.1	172.1	170.1
130	177.4	188.6	183.0	159.1	141.7	150.4
Batch 3 OSPW						
0	291.4	290.7	291.0	270.6	268.1	269.3
10	284.7	285.8	285.3	268.9	269.7	269.3
20	293.0	283.4	288.2	272.2	265.6	268.9
30	284.7	256.7	270.7	238.1	229.3	233.7
50	263.1	249.5	256.3	225.6	217.1	221.4
90	225.6	210.7	218.1	191.5	169.4	180.5

COD of OSPW during biodegradation:

Table C2. COD results after the biodegradation of ozonated and untreated OSPW

O_3 dose	Day 0			Day 7			Day 14			Day 21			Day 28		
(mg/L)	COD (mg/L)		mean	COD (mg/L)		mean	COD (mg/L)		mean	COD (mg/L)		mean	COD (mg/L)		mean
Batch 1 OSPW															
0	298.6	302.1	300.4	287.5	302.1	294.8	281.4	298.1	289.8	276.3	296.5	286.4	267.2	294.9	281.1
50	264.2	278.9	271.6	258.1	260.5	259.3	247.0	261.3	254.2	231.8	258.1	245.0	235.8	248.5	242.2
120	240.9	241.3	241.1	243.9	236.5	240.2	233.8	238.1	236.0	227.7	225.3	226.5	225.7	218.9	222.3
180	210.5	241.3	225.9	204.5	223.7	214.1	197.4	217.3	207.4	184.2	203.0	193.6	180.2	201.4	190.8
360	200.4	225.3	212.9	168.0	199.0	183.5	144.7	191.0	167.8	128.5	190.2	159.3	124.5	174.2	149.3
Batch 2 OSPW															
0	228.1	226.7	227.4	218.6	225.9	222.2	220.1	225.9	223.0	217.8	222.0	219.9	223.3	223.6	223.5
30	210.6	228.3	219.4	190.69	191.1	190.9	215.4	198.0	206.7	213.0	201.9	207.4	194.0	199.5	196.8
80	206.8	200.0	203.4	176.6	170.0	173.3	205.2	189.5	197.4	185.0	179.4	182.2	172.1	170.1	171.1
130	183.7	195.6	189.7	177.4	187.1	182.2	167.8	175.4	171.6	153.6	164.6	159.1	141.7	159.1	150.4
Batch 3 OSPW															
0	290.7	291.4	291.0	277.0	289.7	283.3	267.3	275.6	271.4	267.3	272.3	269.8	268.1	270.6	269.3
10	285.8	284.7	285.3	273.7	281.4	277.6	271.3	271.4	271.3	275.3	266.4	270.9	269.7	268.9	269.3
20	283.4	293.0	288.2	283.4	278.9	281.2	267.3	271.4	269.3	260.8	262.2	261.5	265.6	272.2	268.9
30	256.7	284.7	270.7	249.5	264.7	257.1	249.5	254.8	252.1	243.8	241.4	242.6	229.3	238.1	233.7
50	249.5	263.1	256.3	227.6	245.6	236.6	222.8	238.9	230.9	220.4	233.1	226.7	217.1	225.6	221.4
90	210.7	225.6	218.1	184.0	212.3	198.1	175.1	193.1	184.1	175.1	199.0	187.0	169.4	191.5	180.5

Table C3. CBOD results before and after the biodegradation of ozonated and untreated OSPW

O_3 dose (mg/L)	CBOD (mg/L)	
	Before biodegradation	After biodegradation
Batch 1 OSPW		
0	4.21	2.85
50	3.49	2.03
120	5.70	0.37
180	13.43	2.03
360	14.30	2.03
Batch 2 OSPW		
0	1.86	1.5
10	1.95	1.59
20	2.88	1.5
30	3.45	1.47
50	4.31	1.56
90	5.28	1.35
Batch 3 OSPW		
0	1.91	0.84
30	2.73	1.28
80	3.65	1.32
130	10.92	1.77

Appendix D. Toxicity data for all of the samples

For the inhibition test, all of the samples from one batch of OSPW were tested in one run to omit random errors induced by different bacteria because the sensitivity of bacteria varied from bottle to bottle and continually changed after being reconstituted. The inhibition of luminance emitted from the Microtox regent *Vibrio fischeri* was compared between the samples. A student-t test was performed to identify the significance of the treatment in each batch of OSPW.

Table D1. Results of the Microtox screen test (S.E.: standard error)

O_3 dose (mg/L)	Ozonated		Ozonated and biodegraded	
	mean (%)	S.E.	mean (%)	S.E.
Batch 1 OSPW				
0	50.41	1.40	36.84	1.02
50	44.55	1.37	18.12	1.39
120	31.90	0.68	5.28	1.47
180	32.46	1.19	-1.91	0.20
360	17.01	4.33	1.56	1.68
Batch 2 OSPW				
0	50.37	0.33	34.85	2.04
30	32.97	0.74	22.97	3.34
80	30.90	1.49	8.63	1.45
130	13.33	2.46	8.46	1.29
Batch 3 OSPW				
0	49.80	0.20	37.46	2.76
10	46.91	0.43	32.31	2.57
20	43.84	0.69	21.26	2.39
30	35.37	1.29	13.61	2.26
50	31.89	1.11	6.49	2.59
90	28.55	0.49	-0.96	2.00

To obtain the EC_{20} result, the Microtox 81.9% basic test was performed for untreated and 20 and 90 mg/L ozonated Batch 3 OSPW. Untreated Batch 3 OSPW samples with different pH were also tested. A student-t test was performed to identify the significance of ozonation and pH on EC_{20}. The EC_{20} results are shown in Tables D2 and D3.

Table D2. EC_{20} of untreated and ozonated Batch 3 OSPW at pH 8 and 9

O_3 dose (mg/L)	EC_{20} (%)			
	pH 9		pH 8	
	mean	S.E.	mean	S.E.
0	19.72	2.42	37.16	2.94
20	30.64	2.72	73.49	5.27
90	54.03	6.33	-	-

Table D3. EC_{20} of untreated Batch 3 OSPW at pH 7, 8, and 9

	EC_{20} (%)	
pH	mean	S.E.
7	29.86	4.04
8	36.55	4.07
9	18.52	4.85

Appendix E. NA data for all of the samples

NA analysis was done using a UPLC-HRMS system, and profiles of all of the samples were analyzed using Microsoft Excel software. Total NA concentrations of each sample are listed in Table E1.

Table E1. Total concentration of NAs after ozonation and biodegradation (mg/L)

O_3 dose (mg/L)	Ozonated	Ozonated and biodegraded	Biodegradable (%)
Batch 1 OSPW			
0	23.60	12.44	47.29
30	12.10	3.81	68.50
50	6.04	3.68	39.07
80	2.49		
115	1.56		
180	1.23		
360	1.43		
Batch 2 OSPW			
0	19.65	9.00	54.40
30	12.10		
80	1.94	0.25	87.25
130	0.30		
Batch 3 OSPW			
0	21.83	12.92	40.82
10	17.61	10.88	38.22
20	11.60	6.42	44.63
30	5.89	3.40	42.23
50	2.15	1.19	44.72
90	0.31	0.14	54.26

In order to analyze the effect of ozonation and biodegradation on different NAs, concentrations of different groups of OSPW were calculated in Microsoft Excel. The concentration of different NAs in OSPW after ozonation and biodegradation are listed in Tables E2 and E3, respectively.

Table E2. Concentration of NAs from different structure groups after ozonation

O_3 dose (mg/L)	Concentration of NAs (mg/L)					
	Z=0,2	Z=4,6	Z=8,10,12	C=7-12	C=13-17	C=18-22
Batch 1 OSPW						
0	1.65	18.52	3.48	3.97	18.88	0.80
30	1.32	10.32	0.48	2.57	9.46	0.10
50	0.78	4.83	0.42	1.32	4.19	0.53
80	0.33	2.00	0.16	0.49	1.90	0.09
115	0.57	0.80	0.20	0.56	0.83	0.17
180	0.59	0.54	0.10	0.63	0.48	0.12
360	0.93	0.42	0.09	0.39	0.92	0.13
Batch 2 OSPW						
0	0.77	16.39	2.49	3.76	14.68	1.21
80	0.51	0.38	0.08	0.29	0.54	0.08
130	0.00	0.06	0.09	0.04	0.08	0.03
Batch 3 OSPW						
0	2.09	15.46	4.28	3.26	16.77	1.79
10	1.81	12.70	3.11	2.73	13.73	1.15
20	1.36	8.69	1.54	2.02	9.04	0.54
30	0.87	4.36	0.66	1.23	4.35	0.32
50	0.43	1.47	0.24	0.56	1.41	0.19
90	0.10	0.13	0.07	0.14	0.09	0.07

Table E3. Concentration of NAs from different structure groups after ozonation and biodegradation

O_3 dose (mg/L)	Concentration of NAs (mg/L)					
	Z=0,2	Z=4,6	Z=8,10,12	C=7-12	C=13-17	C=18-22
Batch 1 OSPW						
0	0.21	4.76	1.25	0.92	4.88	0.42
30	0.18	1.57	0.16	0.29	1.57	0.05
50	0.13	1.50	0.20	0.26	1.52	0.06
Batch 2 OSPW						
0	0.39	6.42	2.14	1.30	7.03	0.64
80	0.02	0.10	0.00	0.03	0.09	0.00
Batch 3 OSPW						
0	0.73	9.08	3.11	1.51	10.34	1.06
10	0.60	7.75	2.52	1.23	8.75	0.90
20	0.49	5.17	0.76	0.70	5.35	0.37
30	0.27	2.63	0.50	0.51	2.67	0.23
50	0.13	0.92	0.13	0.25	0.88	0.06
90	0.05	0.08	0.01	0.08	0.05	0.01

Table E4. Biodegradable NAs in each structure group before and after high-dose (30 mg/L) ozone treatment

	Low-dose ozonated and untreated OSPW (<30 mg/L, n=5)			High-dose ozonated (>=30 mg/L, n=6) OSPW		
Group	Z=0,2	Z=4,6	Z=8,10,12	Z=0,2	Z=4,6	Z=8,10,12
Mean (%)	67.54	42.33	31.30	70.11	49.81	49.01
S.E.	2.15	1.91	6.07	6.23	7.05	15.01
Group	C=7-12	C=13-17	C=18-22	C=7-12	C=13-17	C=18-22
Mean (%)	56.83	40.93	23.47	63.87	50.34	60.13
S.E.	2.51	2.35	7.80	7.56	8.45	15.49