THESIS

COMBINING CHEMICAL WITH BIOLOGICAL OXIDATION FOR EFFICIENT TREATMENT OF CHLORONITROBENZENE IN GROUNDWATER

Submitted by

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ABSTRACT

COMBINING CHEMICAL WITH BIOLOGICAL OXIDATION FOR EFFICIENT TREATMENT OF CHLORONITROBENZENE IN GROUNDWATER

Chloronitrobenzene (CNB) is a chloronitroaromatic compound widely used in the synthetic production of pharmaceuticals, pesticides, dyes, lumber preservatives, and many other industrial products. CNB has been recognized as a toxic organic contaminant to humans and is recalcitrant to microbial biodegradation in anoxic environments. When receptors are threatened by CNB-contaminated groundwater, regulators may demand immediate remedial approaches, such as advanced oxidation processes (AOPs). While AOPs are effective for the removal of many organic contaminants from water, these techniques are often costly, especially when complete mineralization is the goal.

In this study, it was hypothesized that chemical oxidation for the primary purpose of ring cleavage followed by biological oxidation of the generated intermediates is more cost-effective than relying on AOPs only for complete mineralization. Electrochemical oxidation via hydroxyl radicals was chosen as model AOP and performed at various applied potentials and for different treatment durations. Liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC/QToF-MS) revealed that the aromatic ring in CNB is rapidly hydroxylated and cleaved to form dicarboxylic products including muconic acid, succinic acid, malic acid, and maleic acid. Further electrochemical oxidation of these dicarboxylates was slower by about two orders of magnitude.

To evaluate the universal biodegradability of the generated intermediates, the electrochemically oxidized samples of CNB were then exposed to a microbial culture enriched from a rhizosphere soil. Results showed that the dicarboxylic ring opening products biodegraded under anoxic conditions within 7 days while aromatic species including CNB, chloronitrophenol, chlorohydroquinone and dihydroxybenzoquinone persisted over 28 days of biological treatment.

A comprehensive cost analysis considering both capital costs (electrodes) and operational costs (electric energy) revealed that the most efficient treatment strategy is to apply electrochemical oxidation at a low applied potential around 6 V until complete cleavage of the aromatic ring is achieved. Beyond that, advanced oxidation of the readily biodegradable ring cleavage products becomes uneconomical. Consequently, the coupling of chemical oxidation for persistent parent compounds with biodegradation of transformation intermediates is an efficient approach for the treatment of groundwater contaminated with CNB and likely other aromatic contaminants.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE. INTRODUCTION

1.1. Problem statement

Since the nineteenth century, a wide range of anthropogenic chemical compounds have been introduced to the environment from agricultural, industrial, and municipal sources. Among these chemicals, chloronitroaromatic compounds (CNACs) are widely used in the production of many industrial and consumer products such as dyes, pharmaceuticals, corrosion inhibitors, and pesticides (e.g. Shen et al., 2008; Arora et al., 2012) and are introduced to the environment as a result of application and inadvertent releases of these compounds (Tiwari et al., 2017). CNACs are persistent organic compounds; their chemical stability is associated with the symmetric π-electron system of the benzene ring, which makes them recalcitrant to microbial degradation (Vogt et al. 2011; De Voogt, 2015). Despite strict regulations, some countries including the United States and Canada still use CNACs as fungicide (Arora et al., 2014). Due to the highly toxic and potentially carcinogenic characteristics of CNACs, most of them have been listed as priority pollutants by the United States Environmental Protection Agency (USEPA) (Arora et al., 2014) and the European Economic Community (EEC) (Park et al., 1999).

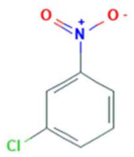
A growing concern about the effects of these compounds on the human health and the environment has led to a large body of literature on the fate of these pollutants as well as the chemical and biological alternatives to remove them from contaminated soil and water systems (Morrill et al., 1982; Heijman et al., 1993; Xu et al., 2009; Arora et al., 2012; Barndok et al., 2014; Bai et al., 2017; Speight, 2016; Ghattas et al., 2017; Li et al., 2018). *o*-Chloronitrobenzene (*o*-CNB) was chosen as a model compound for CNACs for the purpose of this study and described as follows.

1.2. Chloronitrobenzene (CNB)

CNB is one of the CNACs which is extensively used as intermediate materials in the production of dyes, pesticides and herbicides, antioxidants, pharmaceuticals, wood preservatives and many other industrial chemicals (Park et al., 1999; Jones et al., 2006; Wu et al., 2006; Shen et al., 2008; Pizarro et al., 2014).

Depending on the position of chloro- and nitro-groups on benzene rings, CNB has three isomers: *ortho*-chloronitrobenzene (*o*-CNB), *meta*-chloronitrobenzene (*m*-CNB), and *para*-chloronitrobenzene (*p*-CNB) (Li et al., 2018). The position of these substituents on benzene rings plays an important role in the degradability of CNB. CNACs with nitro-substituents at ortho or meta positions are more resistant to microbial degradation than compounds with nitro-groups at para positions (Arora et al., 2012). For example, 4-chloro-2-nitrophenol is more recalcitrant to biodegradation than 2-chloro-4-nitrophenol (Tabak et al. 1964). In addition, Arora et al. (2012) noted that the refractory nature of benzene rings can be more influenced by the position of nitro-substituent compared to chloro-groups. As stated earlier, chloro- and nitro-substituents have a strong electron-withdrawing effect which makes the degradation of CNB a difficult and expensive process (Huang et al., 2016). Furthermore, increasing the number of these substituents typically adds to the recalcitrant behavior of CNB (Tabak et al. 1964). Ring opening of CNB with multiple substituents, especially chlorine, is an endothermic reaction requiring extensive energy due to strong bonds between these substituents and carbon atoms (Arora et al., 2012). The structure of different species of CNB is presented in Figure 1.

***o*-Chloronitrobenzene (2-chloronitrobenzene)**



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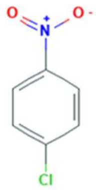

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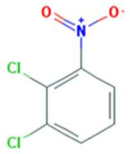
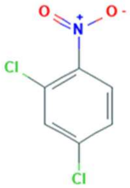


2,3-Dichloronitrobenzene



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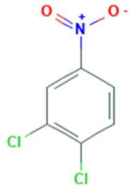

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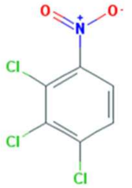

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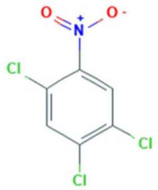
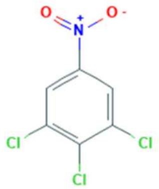


2,4,5-Trichloronitrobenzene

2,4,6-Trichloronitrobenzene



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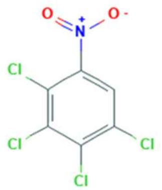
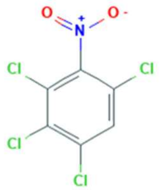
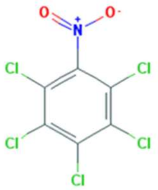


2,3,4,5-Tetrachloronitrobenzene

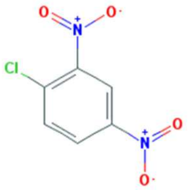
2,3,5,6-Tetrachloronitrobenzene



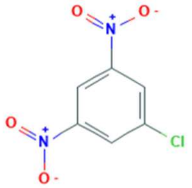
1,2,3,5-Tetrachloro-4-nitrobenzene



Pentachloronitrobenzene



1-Chloro-2,4-dinitrobenzene (2,4-Dinitrochlorobenzene)



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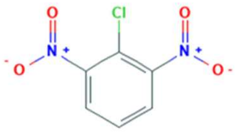

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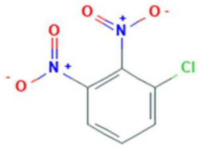

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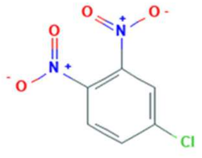
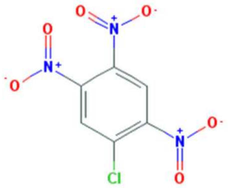
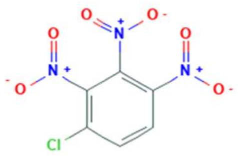


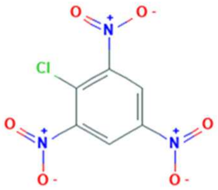
4-Chloro-1,2-dinitrobenzene



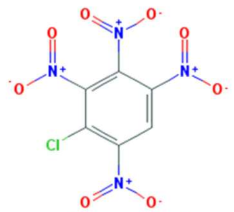
1-Chloro-2,4,5-trinitrobenzene



1-Chloro-2,3,4-trinitrobenzene



1-Chloro-2,4,6-trinitrobenzene



2-Chloro-1,3,4,5-tetranitrobenzene

Figure 1. Structure of different species of chloronitrobenzene (PubChem, 2018)

A summary of physical and chemical properties of different species of CNB is presented in Table 1. As an example, *p*-CNB has light yellow crystals with characteristic sweet odor, with a boiling point of 242 °C at 760 mm Hg, a melting point of 82-84 °C, and a negligible solubility in water. It is also very toxic if inhaled, ingested and absorbed via skin, and highly toxic fumes are generated if heated to decomposition (PubChem, 2018).

CNB has been recognized as a toxic organic contaminant to humans and many living beings. It may cause methaemoglobinaemia, which is dysfunctionality in red blood cells to carry oxygen throughout organs and tissues (Travlos et al., 1996; Prakash et al., 2011). If CNB is absorbed through skin or respiration, it is transformed in blood cells, bones, liver and kidney (Arora et al., 2012; Symons et al., 2006). Therefore, exposure to CNB can cause health problems including vomiting, weakness, unconsciousness, coma (Wang et al., 2014), fatigue, dizziness, eye and skin irritation, headache, splenomegaly, and cardiovascular diseases (Jones et al., 2006). Furthermore, CNB presence in the environment can cause a high biological toxicity to animals and plants over extended periods (Hong et al., 2002; Matsumoto et al., 2006; Arora et al., 2012).

The toxicity of CNBs stems mainly from the nitro- and chloro- substituents (Henschler, 1994) where among different isomers of CNB, *p*-CNB is the most toxic form (Davydova, 1967; Watanabe et al., 1976). The removal of these substituents during the degradation process, however, may produce daughter products or intermediates which are sometimes more toxic than the parent compound (Spain, 1995).

Table 1. Physicochemical properties for selected chloronitrobenzene species (source: PubChem, 2018

Compound	Molecular weight (g mol^{-1})	Color/Form	Boiling point (°C at 760 mm Hg)	Melting point (°C)	Solubility in water	Density (g cm^{-3})
o-Chloronitrobenzene	157.553	Yellow crystals	246	33	< 0.1 mg/mL at 20 ° C	1.4
m-Chloronitrobenzene	157.553	Pale yellow crystals	236	44	Insoluble	1.3
p-Chloronitrobenzene	157.553	Yellow crystals	242	82-84	< 0.1 mg/mL at 24° C	1.52
2,3-Dichloronitrobenzene	191.995	Light yellow crystals	257-258	61	< 0.1 mg/mL at 25° C	1.7
2,4-Dichloronitrobenzene	191.995	Yellow crystals	258.5	30-33	< 0.1 mg/mL at 16° C	1.54
3,4-Dichloronitrobenzene	191.995	Colorless crystals	255	39-41	< 0.1 mg/mL at 23.5° C	1.56 at 15°C
2,3,4-Trichloronitrobenzene	226.437	Light yellow fluffy solid	-	55-56	<1 mg/mL at 23° C	-
2,4,5-Trichloronitrobenzene	226.437	yellow powder	288	57	< 1 mg/mL at 16° C	1.79 at 23° C
2,4,6-Trichloronitrobenzene	226.437	light beige crystals	-	71	< 1 mg/mL at 20° C	-
3,4,5-Trichloronitrobenzene	226.437	Pale-yellow crystals	-	72.5	< 1 mg/mL at 15° C	1.807
2,3,4,5-Tetrachloronitrobenzene	260.879	Pale yellow crystals	304	65-67	In water, 7.31 mg/L at 20 °C	-
2,3,5,6-Tetrachloronitrobenzene	260.879	Colorless crystals	304	99	<0.1 mg/mL at 22° C	1.744
1,2,3,5-Tetrachloro-4-nitrobenzene	260.879	-	-	-	-	-
Pentachloronitrobenzene	295.321	Pale yellow crystals	328	146	< 1 mg/mL at 22° C	1.7
1-Chloro-2,4-dinitrobenzene (2,4-Dinitrochlorobenzene)	202.55	Pale yellow crystals	315	54	13.8 [µg/mL]	1.7
1-Chloro-3,5-dinitrobenzene (3,5-Dinitrochlorobenzene)	202.55	-	-	-	-	-
2-Chloro-1,3-dinitrobenzene (2,6-Dinitrochlorobenzene)	202.55	Yellow crystals	315	86-87	Practically insoluble in water	1.6867 g ml^{-1} at 16.5 ° C
1-Chloro-2,3-dinitrobenzene	202.55	yellow crystalline solid	-	-	Insoluble in water	-
4-Chloro-1,2-dinitrobenzene	202.55	Yellow crystals	-	40-41	-	1.687 (relative to water=1)
1-Chloro-2,4,5-trinitrobenzene	247.547	-	-	-	-	-
1-Chloro-2,3,4-trinitrobenzene	247.547	-	-	-	-	-
1-Chloro-2,4,6-trinitrobenzene	247.547	White needles or plates	-	83	< 1 mg/mL at 19° C	1.797 g/cu cm at 20 ° C
2-Chloro-1,3,4,5-tetranitrobenzene	292.544	-	-	-	-	-

1.3. Biodegradation of chloronitroaromatic compounds

Biodegradation is a process in which toxic chemicals (e.g., organic contaminants) are mineralized into harmless compounds (i.e. carbon dioxide, water) by microorganisms or plants (Speight, 2016; Karimi Askarani et al., 2018). Biodegradation is a relatively inexpensive, environmentally sound, operationally manageable, and publicly accepted approach (Rodgers & Bunce, 2001; Symons et al., 2006; Salles et al., 2010). The presence of microorganisms capable of biodegrading contaminants and the availability of electron donors and electron acceptors under the prevailing environmental conditions can determine the efficiency of the biodegradation approach (Speight, 2016; Karimi et al., 2019). Native or inoculated microorganisms can be added to the subsurface environment to improve the process of biodegrading organic contaminants to non-toxic products (Speight, 2016). However, biodegradation may not be an effective method to remove CNACs from the environment due to the low rates of microbial biodegradation (Yahiaoui et al., 2013), recalcitrant nature of organic contaminants (Martinez-Huitle & Ferro, 2006; Salles et al., 2010), absence of microbial metabolic pathways to degrade aromatic compounds, and toxicity of aromatic compounds to microbial community (Spain, 1995). Microorganisms can biodegrade pollutants with close chemical structures to existing natural compounds in the environment; however, CNACs are anthropogenic compounds which do not occur naturally (Speight, 2016). In addition, as mentioned previously, the strong electron-withdrawing effect makes them toxic and recalcitrant to biodegradation (Wu et al., 2006).

Many researchers have studied the use of microorganisms for the biodegradation of chloro- and nitro-aromatics (Symons and Bruce 2006; Ju & Parales, 2010; Zhang et al., 2013; De Voogt, 2015; Ghattas et al., 2017; Tiwari et al., 2017). Biodegradation of CNACs can occur in aerobic or anaerobic systems. The presence of an electron acceptor is essential under both conditions (De Voogt, 2015) and its availability and energy balance determine the type and metabolic pathway of the microorganisms (Philipp & Schink, 2012; Ghattas et al., 2017).

1.3.1. Biodegradation under aerobic conditions

So far, many biodegradation studies have implemented aerobic conditions for the removal of organic contaminants (e.g. Cafaro et al., 2004; Neilson & Allard, 2007; Arora et al. 2009). Aerobic bioremediation is effective in terms of energy because mineralization of organic compounds is enhanced in the presence of oxygen and less waste by-products are generated (Rodgers & Bunce, 2001; Ghattas et al., 2017). Under aerobic conditions, oxygen acts as electron acceptor to improve the biodegradation of aromatic compounds and, therefore, biodegradation of many organic contaminants is unfavorable under anoxic conditions (Ghattas et al., 2017).

Söhngen (1913) reported that aerobic microorganisms capable of biodegrading benzene are universal. Aerobic biodegradation of CNACs can start via four different pathways before aromatic ring opening occurs. In one pathway, nitro-substituents are first reduced to form amino-substituents through nitroso- and hydroxylamino- intermediates under anaerobic conditions (Spain, 1995). For example, Heijman et al. (1993) noted the transformation of CNB to chloroaniline in an iron reducing system. Oxygenation of the reduced products is the next step prior to the aromatic ring cleavage (Wu et al., 2009; Arora et al., 2012). In another pathway, the nitro-substituent is removed through a mechanism catalyzed via oxygenase in which the addition of oxygen to the benzene ring enhances removal of nitro -substituents on CNACs. Additional degradation of CNACs leads to chloro-substituent removal and ring cleavage (Bruhn et al., 1988; Ghosh et al., 2010). In the third pathway, reductive dechlorination proceeds via reductive dehalogenation before oxidative nitro-substituent removal occurs (Pandey et al., 2011). In the last pathway, both chloro- and nitro-substituents are released via oxygenation followed by ring cleavage (Prakash et al., 2011; Arora et al., 2012).

1.3.2. Biodegradation under anaerobic conditions

Under anaerobic conditions, a compound other than oxygen (e.g. nitrate or sulfate) serves as an electron acceptor to enhance biodegradation (Anderson & Lovley, 2000; Vogt et al. 2011). In contrast to a number of studies (Rodgers & Bunce, 2001; Cafaro et al., 2004; Neilson & Allard, 2007; Arora et al. 2009; Ghattas et al., 2017) that reported biodegradation is enhanced under aerobic conditions, several other studies reported that biodegradation of highly nitrated aromatic compounds such as trinitrotoluene (TNT) can be improved in the absence of oxygen (e.g. Angermaier & Simon, 1983; Rafil et al., 1991; Spain, 1995 or Symons et al., 2006). Spain (1995) stated that both bacteria and fungi are capable of biodegradation or transformation of nitroaromatics. These microorganisms consume nitroaromatic compounds as their carbon and energy source (Symons et al., 2006). Transformation of nitroaromatics can be catalyzed via redox enzymes, such as nitroreductases, or enzymes which let microorganisms consume nitroaromatics as carbon and nitrogen sources (Bryant & DeLuca, 1991; Spain, 1995).

Anaerobic microorganisms make a reducing environment which facilitates the reduction of nitro- groups (Heijman et al., 1993). Macalady et al. (1986) reported that nitro-substituent reduction is the initial step in transforming or mineralizing TNT in anaerobic systems. Therefore, anaerobic microbial reduction is one of major degradation mechanisms of TNT.

Although biodegradation of highly nitrated aromatic compounds is efficient under anoxic conditions, compounds with less nitro-substituents, such as CNB, are often persistent to microbial degradation in absence the of oxygen, a common condition at most contaminated sites (Ghattas et al., 2017). Previous research has reported that only in specific redox environments under anoxic conditions, CNB may be transformed biologically. For example, an initial reduction of the nitro-group in CNB to produce chloroaniline can occur under sulfate-reducing (Susarla et al., 1996) or Fe(III)-reducing (Hejman et al., 1993) conditions. Susarla et al. (1996) also observed no further transformation of monochloroaniline; however, for chloroaniline species with two or more chlorine atoms, reductive dechlorination was observed. Furthermore, Kuhn and Suflita, (1989) found that further removal of chlorine atoms in chloroaniline may occur only in methanogenic environments. However, these processes in the absence of oxygen do not lead to complete mineralization (on relevant time scales).

1.4. Chemical oxidation

1.4.1. Advanced oxidation processes (AOPs)

When receptors are threatened by groundwater contaminated with slowly biodegrading CNACs, regulators may demand rapid remedial action, for instance through advanced oxidation processes (AOPs). AOPs include wet air oxidation (Klwla et al., 1994; Dieckmann & Gray, 1996), photocatalytic oxidation (Egerton et al., 2005; Priya & Madras, 2006), Fenton's reagent (Goi & Trapido, 2002; Kavitha & Palanivelu, 2005), ozonation and O_3/UV (Latifoglu & Gurol, 2003), UV/H_2O_2 (Alnaizy & Akgerman, 2002), and electrochemical oxidation (Liu et al., 2008; Chen et al., 2014; Barndok et al., 2014). The main degradation mechanism of organic contaminants via AOPs is based on the generation of strong oxidants such as hydroxyl radicals ($\cdot OH$) capable of opening aromatic rings and/or full mineralizing CNACs (Rodgers & Bunce, 2001; Vilhunen & Sillanpaa, 2010). $\cdot OH$ is as a highly reactive oxidant compared to other oxidizing species such as ozone or hydrogen peroxide (Zazo et al., 2005; Oturan & Aaron, 2014).

AOPs have been studied extensively and shown to be effective in degrading toxic and persistent organic contaminants including pharmaceuticals (Belkheiri et al., 2011; Yahiaoui et al., 2013; Olvera-Vargas et al., 2016; Monteil et al., 2018), dyes (Torres et al., 2003; Babu et al., 2011; Yahiaoui et al., 2014), pesticides (Salles et al., 2010; Liu et al., 2010), herbicides (Salles et al., 2010; Fontmorin et al., 2012; Fontmorin et al., 2014), landfill leachate (Fernandes et al., 2014; Baiju et al., 2018), olive wastewater (Ganzenko et al., 2014), textile wastewater (Ganzenko et al., 2014), and hydrocarbons (Chen et al., 2016; Trellu et al., 2016). The toxic and recalcitrant properties of organic contaminants can be diminished or removed using AOPs (Shen et al., 2008). Furthermore, AOPs can effectively treat several contaminants simultaneously from a contaminated water due to non-selective oxidation of contaminants (Vilhunen & Sillanpaa, 2010). However, AOPs are not often economically efficient (e.g. Canizares et al., 2009; Sáez et al., 2010; Davezza et al., 2011; Huguenot et al., 2015; Oturan et al., 2015) since they are energy and/or chemical intensive. For example, Canizares et al. (2009) reported that the average operational costs may vary from 2.4 to 4 € kg^{-1} ($ 2.6 to $ 4.4 US kg^{-1}; conversion factor of 1.1) equivalent oxygen for electrochemical oxidation, from 0.7 to 3 € kg^{-1} ($ 0.77 to $ 3.3 US kg^{-1}) equivalent oxygen for Fenton-based oxidation, and from 8.5 to 10 € kg^{-1} equivalent oxygen for ozonation.

1.4.1.1. Formation of intermediates during advanced oxidation

The ultimate target of advanced oxidation of CNACs is the transformation or mineralization of these compounds to non-toxic compounds, such as water or carbon dioxide. However, during the degradation process of CNACs, some intermediates may form which are sometimes more toxic than the parent compound (e.g. Spain, 1995; Fontmorin et al., 2012). When the benzene ring on CNACs is attacked by $\cdot OH$, nitro- and chloro-groups can be substituted by $\cdot OH$ to form different species of phenols such as chloronitrophenol, chlorophenol, nitrophenol, phenol, and nitrocatechol (Shen et al., 2008). Further oxidation may lead to hydrogen abstraction of these phenolic compounds to produce benzoquinone (Alnaizy & Akgerman, 2002). Aromatic ring opening of these intermediates may then be followed by the formation of organic carboxylic acids, such as maleic, formic, muconic and fumaric acids (Zazo et al., 2005). Final products of CNAC mineralization are water, CO_2, and inorganic compounds (Martinez-Huitle & Ferro, 2006). However, ring cleavage of phenol species to organic acids or CO_2 can occur directly (Shen et al., 2008).

1.4.1.2. Electrochemical oxidation

Among different AOPs, a significant body of research has recently studied the electrochemical oxidation of groundwater and wastewater contaminated with organic compounds (e.g. Martínez-Huitle & Brillas, 2006; Garcia-Segura & Brillas, 2011; Sirés & Brillas, 2012; Chen et al., 2014; Barndok et al., 2014; Radjenovic & Sedlak, 2015; Barazesh et al., 2016). Electrochemical oxidation is a robust AOP due to its many advantages including but not limited to the capability of degrading a large number of organic pollutants (Radjenovic & Sedlak, 2015), adaptability to automation and operation at atmospheric pressure and temperature (Torres et al., 2003), and adjustability to changes in influent flow and mixture (Torres et al., 2003; Garcia-Segura & Brillas, 2011; Chen et al., 2014; Radjenovic & Sedlak, 2015; Radjenovic & Sedlak, 2015). Furthermore, electrochemical oxidation does not require additional oxidant chemicals and is thus a relatively environmentally friendly treatment method since it requires only electricity and supporting electrolytes (Martınez-Huitle et al., 2004; Canizares et al., 2007; Özcan at al., 2009; Panizza et al., 2009; Barndok et al., 2014) such as sodium, bicarbonate, phosphate, and nitrate. In addition, the presence of supporting electrolytes improves the conductivity of the electrooxidation and thus degradation of aromatic compounds (Barndok et al., 2014).

Barndok et al. (2014) compared electrochemical oxidation with O_3 and O_3/H_2O_2 based AOPs, and found that electrooxidation had the highest degradation rate in terms of the amount of oxidant consumption and reached the optimal biodegradation (highest Chemical Oxygen Demand (COD) removal and least energy consumption) after one hour of treatment. Furthermore, Barndok et al. (2014) demonstrated that considering energy usage and cost per amount of COD removal, electrochemical oxidation was economically more efficient than O_3 and O_3/H_2O_2.

1.4.1.3. Electrochemical oxidation mechanisms

Electrochemical oxidation can occur through direct or indirect mechanisms. In direct electrolysis, contaminants react at the electrode surface through direct electron transfer while in indirect electrolysis, a strong oxidizing species is generated at the electrode which facilitates the degradation of contaminants (Radjenovic & Sedlak, 2015). Diffusion, catalytic activity of the electrode material, and reaction kinetics influence direct chemical oxidation (Comninellis et al., 2010). On the other hand, the material and adsorptive characteristics of the electrode are contributing factors to the properties of generated oxidants in indirect chemical oxidation (Radjenovic & Sedlak, 2015).

Comninellis (1994) first developed a general scheme for the electrolysis mechanism of organic compounds on metal oxide anodes. In the first stage, adsorbed $^{\cdot}OH$ are generated ($MO_x(^{\cdot}OH)$) as a result of discharging H_2O in acidic media (or OH^- in alkaline media) on oxide anode (Eq. 1).

$$MO_x + H_2O \rightarrow MO_x(.OH) + H^+ + e^- \qquad (1)$$

In the next stage, adsorbed $^{\cdot}OH$ may react with existing oxygen in the anode to form a metal oxide layer (Eq. 2).

$$MO_x(.OH) \rightarrow MO_{x+1} + H^+ + e^- \qquad (2)$$

When an oxidizable organic compound is not available, molecular oxygen is generated (Eqs. 3 and 4).

$$MO_x(.OH) \rightarrow 0.5\,O_2 + MO_x + H^+ + e^- \tag{3}$$

$$MO_{x+1} \rightarrow 0.5\,O_2 + MO_x \tag{4}$$

However, when an oxidizable organic compound is present, full combustion of organic compounds (Eq. 5) or production of selective oxidation compounds (Eq. 6) may occur.

$$R + MO_x(.OH)_n \rightarrow CO_2 + MO_x + nH^+ + ne^- \tag{5}$$

$$R + MO_{x+1} \rightarrow MO_x + RO \tag{6}$$

1.4.1.4. Electrode material

Electrode characteristics are of great significance when the degradation of organic compounds using electrochemical oxidation is the objective. Electrode materials influence the degradation pathway of contaminants during electrolysis due to the reactions between electrode surface and organic contaminants (Torres et al., 2003). For instance, Huang et al. (2016) stated that the dissociation reaction of C-Cl is significantly impacted by the electrode material. Furthermore, anode characteristics determine the efficiency and potential production of toxic intermediates and by-products (Radjenovic & Sedlak, 2015; Butkovskyi et al., 2014; Wu et al., 2014). In order to prevent polymerization reactions during electrolysis and inactivation of electrodes, a sufficiently high potential should be used (Iniesta et al., 2007; Pacheco et al., 2007). When the applied potential is high enough, the degradation of organic compounds follows an indirect chemical oxidation via oxidizing species, such as hydroxyl radicals or hydrogen peroxide (Michaud et al., 2003; Marselli et al., 2003; Marselli, 2004; Brillas et al., 2006).

Among different electrode materials, dimensionally stable anodes (DSA), also known as mixed-metal oxide (MMO) anodes, have been widely used in the last three decades (Radjenovic & Sedlak, 2015). These electrodes consist of a metal base, e.g. titanium (Ti), coated with a metal oxide (i.e. RuO_2, IrO_2) or mixed metal oxide (MMO) (e.g. RuO_2/IrO_2). Ti-MMO electrodes are relatively cheap compared to boron-doped diamond (BDD) and have a low solubility; therefore, they exhibit a high durability under extreme conditions (Gilbert et al., 2010). Although MMOs have higher oxygen evolution potential (OEP) compared to other electrodes, their durability against corrosion, high current density, and availability and the cost of electrode materials play a significant role in their selection as an efficient electrode (Martinez-Huitle & Ferro, 2006; Wu et al., 2014).

MMO anodes have demonstrated to be effective in the treatment of many organic compounds (e.g. Simond et al., 1997; Bock & MacDougall, 2000; Lanza & Bertazzoli, 2002; Malpass et al., 2006; Comninellis & Chen, 2010; Butkovskyi et al., 2014; Radjenovic & Sedlak, 2015; Jasmann, 2016). Butkovskyi et al. (2014) were the first to use MMO anodes for the electrochemical oxidation of micro-contaminants in graywater effluent and concluded that Ir-Ru- based anodes have the highest efficiency in terms of organic compounds removal and lower generation of toxic-intermediates. Furthermore, Johnson et al. (1999) and Houk et al. (1998) applied MMO anodes with Ti, Ru, antimony (Sb), and tin (Sn) bases for the purpose of treatment of nitroaromatic compounds. They noted that despite highly time-consuming degradation of COD and Total Organic Carbon (TOC), MMO electrodes can be used for the oxidation of nitroaromatic contaminants due to their durability and active electrolysis. In addition, Ti-MMO electrodes have performed effectively in the mineralization of chlorinated solvents in situ and without formation of chlorinated organic intermediates in batch experiments (Petersen et al., 2007).

1.5. Combined treatment approaches for persistent organic contaminants

As stated before, the purpose of AOPs is to transform or mineralize organic contaminants to harmless products. Some of the studies mentioned earlier reported that applied AOPs were effective in removing the parent organic contaminants. However, many studies reported that generated intermediates during the electrochemical AOPs persist to further oxidation and full mineralization (e.g. Torres et al., 2003; Oturan et al., 2008; Garcia-Segura & Brillas, 2011; Fontmorin et al., 2012; Barndok et al., 2014; Trellu et al., 2016; Baiju et al., 2018; Monteil et al., 2018). These intermediates are slow to oxidize by generated •OH, causing a low oxidation rate at the later stage of degradation process (Garcia-Segura & Brillas, 2011; Ganzenko et al., 2014). Garcia-Segura and Brillas (2011) concluded that carboxylic acids, including oxalic and oxamic acids, are the final intermediates of the oxidation of aromatic compounds such as chlorophenol (Canizares et al., 2003). These organic acids are difficult to oxidize via anodic oxidation and thus considerably lower the effectiveness of oxidation processes (Oturan et al., 2008). Furthermore, Trellu et al. (2016) reported that electrochemical oxidation of phenanthrene, a polycyclic aromatic hydrocarbon, achieved only 27% of COD removal due to the generation of persistent by-products. In another study by Fontmorin et al. (2012), using electrochemical oxidation, only 34% of the dissolved organic carbon (DOC) and 41% of the initial COD of effluent contaminated with 2,4-dichlorophenoxyacetic acid were mineralized. Furthermore, Barndok et al. (2014) reported the COD removal kinetics of 1,4-dioxane leveled off with any increase in current density beyond 12 $mA \cdot cm^{-2}$ in anodic oxidation, indicating that further energy input did not bring about significant COD removal. Similarly, Baiju et al. (2018) found that Electro-Fenton (EF) processes may not be an efficient technique to oxidize landfill leachate and to lower COD to standard levels. Houk et al. (1998) found that succinic and acetic acids were formed after 8 hours and 64 hours of the electrochemical incineration of benzoquinone; indicating that electrochemical oxidation of the intermediates of organic compounds, is not energetically efficient. As a result, complete mineralization of aromatic contaminants using AOPs can be excessively time-consuming and costly due to high operational cost and/or chemical consumption. Therefore, there is a need for the development of more cost-efficient techniques for CNACs removal from contaminated sites.

Biological treatment techniques, on the contrary, are significantly cheaper than AOPs. An economic comparison between biodegradation and AOPs demonstrated that in terms of investment and treatment costs, biodegradation can be up to 20 times and 10 times more economical, respectively (Scott & Ollis, 1996; Marco et al., 1997; Mathews et al., 1991). Microorganisms can readily biodegrade compounds with similar structures to existing natural chemicals in the environment; however, CNACs are anthropogenic chemicals which do not occur naturally in the environment (Tiwari et al., 2017). Moreover, the presence of chloro- and/or nitro-groups in many CNACs gives them chemical stability and makes them bio-refractory to microbial metabolisms (e.g. Wu et al., 2006). At most contaminated sites with aromatic compounds, anoxic conditions are dominant which makes these pollutants more persistent to microbial degradation (Ghattas et al., 2017). As a result, biological treatment is often inefficient in biodegrading persistent organic compounds (e.g. Chiron et al., 2000; Badawyet al., 2006; Yahiaoui et al., 2013). Therefore, new strategies in biological treatment are required to improve the biodegradability efficiency of these technologies, in particular when oxygen is absent in the environment.

Combining AOPs with biological treatment can be a promising strategy to overcome the high costs associated with AOPs and low rates of microbial degradation. A large number of research work reported that coupling AOPs and biodegradation can be used effectively to enhance the degradation of recalcitrant compounds while minimizing the drawbacks of standalone processes (e.g. Ledakowicz & Gonera, 1999; Al-Momani, 2003; Salles et al., 2010; Babu et al., 2011; Fontmorin et al., 2012; Ganzenko et al., 2014; Chen et al., 2016; Olvera-Vargas et al., 2016; Trellu et al., 2016; Minière et al., 2017; Baiju et al., 2018).

Applying AOPs prior to biological treatment may improve the rate of microbial biodegradation (Al-Momani, 2003; Ganzenko et al., 2014; Mousset et al., 2014; Chen et al., 2016; Minière et al., 2017; Baiju et al., 2018) while AOPs after the biological treatment can be used when high quality effluent is desired (Scott & Ollis, 1995; Fernandes et al., 2016). Although the by-products of advanced oxidation of organic compounds are difficult to mineralize by AOPs, they may be more readily biodegradable than the compounds before ring opening (e.g. Katayama & Matsumura, 1991; Stockinger et al., 1995; Trellu et al., 2016; Baiju et al., 2018).

Monteil et al. (2018) demonstrated that an integrated EF-biological treatment significantly increased the economic efficiency and COD removal of wastewater effluents and pharmaceuticals. Similarly, Baiju et al. (2018) reported that EF processes followed by biological treatment using indigenous microorganisms was able to remove 97% of COD from the landfill leachate. Ganzenko et al. (2014) found that combining EF/ H_2O_2 with anaerobic biological treatment of olive processing wastewater was effective in 75% of COD removal while 50% of COD was removed via electrochemical treatment alone. The authors also stated that the integrated technique is potentially able to compensate the energy cost through the methane gas generation. Olvera-Vargas et al. (2016) found that metoprolol, a type of pharmaceuticals, was mineralized to 90% via one hour of EF pre-treatment combined with 4 days of biological post- treatment. In another study by Minière et al. (2017), phenol degradation was studied using a sequential wet air oxidation and packed-bed biofilm reactors and they reported 97% of TOC removal compared to 84% of TOC removal when standalone wet air oxidation was applied. In addition, Chen et al. (2016) found that the by-products of heavy petroleum hydrocarbons ozonation were quickly biodegraded using native bacteria in the field. Stockinger et al. (1995) reported full mineralization of chloro- and nitroaromatic contaminants from wastewater using integrated ozonation-biological treatment. Trellu et al. (2016) used electrochemical oxidation of phenanthrene followed by biotreatment and found that the combined technique improved COD removal from 27% via electrooxidation and 44% via biotreatment to 80% in the combined method.

1.6. Hypothesis and Objectives

Given recent successes in combining chemical with biological oxidation for the mineralization of persistent organic pollutants, it is hypothesized that an efficient approach to mineralizing CNACs is to apply AOPs for opening the aromatic ring, generating intermediates that can be readily biodegraded under naturally anoxic conditions. Electrochemical oxidation was chosen as the AOP, CNB as a model contaminant. The biodegradability of the generated intermediates from electrochemical oxidation was also tested using a microbial culture enriched from a rhizosphere soil.

The specific objectives of this study were (i) to determine the most efficient electrochemical treatment conditions with respect to applied potentials and treatment time, and (ii) to investigate the biodegradability of generated intermediates to achieving contaminant mineralization under naturally anoxic conditions.

CHAPTER TWO. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were used as received without further purifications. A list of these chemicals with their purity and vendors is provided in the table below.

Table 2. List of name, purity, and supplier of the chemicals used in this study

Chemical	Purity (%)	Supplier
o-Chloronitrobenzene	99	VWR
Chloroaniline	98	VWR
p-Benzoquinone	98	Sigma-Aldrich
Dihydroxybenzoquinone	98	Fisher Scientific
Chlorohydroquinone	99	VWR
Chloronitrophenol	99	Sigma-Aldrich
Fumaric acid	99	VWR
Muconic acid	98	VWR
Malic acid	99	VWR
Maleic acid	99	Fisher Scientific
Succinic acid	99.55	Fisher Scientific
Oxalic acid	98	Sigma-Aldrich
Sodium citrate dihydrate	99	Fisher Scientific

Sodium phosphate monobasic and dibasic, used as supporting electrolytes during the electrooxidation, were provided by Fisher Scientific. Solutions were prepared using deionized water from a deionized water system (Barnstead Nanopure-Diamond^{TM}). Dichloromethane (HPLC grade) was used for liquid-liquid extraction for gas chromatography-mass spectrometry (GC-MS) tests and supplied from MilliporeSigma, USA.

2.2. Experiments

2.2.1. Electrochemical oxidation treatment

Electrochemical experiments were carried out in an undivided rectangular batch reactor (polycarbonate, 14×10×10 cm^3) at ambient temperature (Figure 2). Titanium mixed metal oxides (Ti-MMO: $Ti/IrO_2-Ta_2O_5$) mesh electrodes of 100 cm^2 were used as cathodes and anodes (Corrpro Companies, Inc.). Electrodes were positioned vertically parallel to each other using poly(methyl methacrylate) frames at a distance of 1.5 cm (0.6 in). Ti-MMO electrodes were selected for this study because of their high stability and capability to remove organic contaminants (e.g. Petersen et al., 2007; Gilbert et al., 2010). Additional details on the reactor setup can be found in Cottrell (2018). A synthetic solution of 50 mg L^{-1} CNB containing of 2000 mg L^{-1} sodium phosphate electrolytes was added to the reactor and the electrodes were fully immersed. Electrochemical experiments were performed under potentiostatic conditions using a bench-top DC power supply (GW Instek® GPS-3030D) at 6 volt (V), 10 V, and 14 V. The pH of the solution was not controlled during the experiment. Samples were collected at 0, 5, 10, 15, 30, 45 minutes and 1, 1.5, 2, 3, 5, 7 hours during electrolysis from the middle of the reactor and extracted immediately.

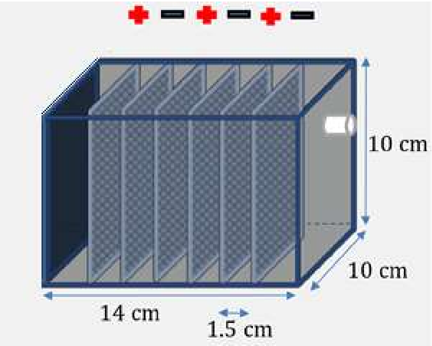


Figure 2. Batch reactor diagram (modified from Cottrell, 2018)

Electric energy per order (E_{EO}) is a good parameter to evaluate the energy efficiency of electrochemical oxidation process when the concentration of pollutants are low. E_{EO} (kWh m^{-3}) is defined as the amount of energy needed to remove one order of magnitude of a contaminant concentration in a unit volume of solution (Bolton et al., 2001) (Eq. 7).

$$E_{EO} = \frac{Pt}{V \log(\frac{C_0}{C})} \tag{7}$$

where P: power (kW), t: treatment time for the removal of one order of magnitude of the contaminant (hr), V: volume of solution (m^3), C_0, C: influent and effluent concentrations of the target contaminant.

A higher efficiency per mesh electrode during the electrochemical process can be evaluated by apparent anode surface area per order of removed contaminant (ASA_O). ASA_O (hr. m^2 m^{-3}) is defined as the area of electrodes (m^2) required to reduce one order of magnitude of a contaminant in a unit volume of contaminated solution. A higher efficiency of an electrooxidation process in terms of electrode area is expressed by a lower ASA_O. (Eq. 8):

$$ASA_O = \frac{\mathrm{A\,t}}{\mathrm{V} \log(\frac{C_0}{C})} \tag{8}$$

where A: anode surface area (m^2), t, V, C_0, and C are the same variables defined in equation 7. In this study, the ASA_O results are demonstrated in the unit of m^2 gpm^{-1}.

The kinetics of electrochemical oxidation process was evaluated using the best regression model fitted to the normalized concentrations of CNB (CNB/CNB_0) and COD (COD/COD_0) plots. Once the reaction kinetics for the CNB degradation was determined, using the first-order regression equation, a model for reaction kinetics was generated.

In order to estimate the contribution of physical losses, such as volatilization, to CNB removal during electrochemical oxidation, two control experiments were performed: one for CNB and another one for the intermediates of CNB oxidation. In the CNB control experiment, the undivided batch reactor (Figure 2) was filled with a 50 mg L^{-1} CNB solution and using two ceramic sparge bulbs. N_2 was sparged into the solution for 300 minutes visually similar to the rate of bubble gas observed on the electrodes during the electrochemical oxidation of CNB at 14 V as worst case scenario. Samples were taken in the beginning and every 30 minutes, and analyzed for Chemical Oxygen Demand (COD), CNB concentration, and UV absorbance. In the intermediate control experiment, a 50 mg L^{-1} CNB solution was initially electrochemically oxidized for 300 minutes at 14 V. After 300 minutes, electrolysis was stopped and N_2 gas was sparged into the solution for another 300 minutes similar to the rate of bubble gas generation on the electrodes at 14 V. Samples were taken in the beginning and every 60 minutes and analyzed for COD and absorbance.

In order to extend the results of this study to other AOPs, two additional electrochemical oxidation experiments were performed in a divided rectangular batch reactor: one with 50 mg L^{-1} CNB solution only and the second test with a mixture solution of 50 mgL^{-1} of CNB and 200 mgL^{-1} of benzoquinone (BQ) added to the anode side of the batch reactor. In both experiments, 50 mgL^{-1} of CNB was added to the cathode side of the reactor and the CNB solution contained 2000 mgL^{-1} of sodium phosphate electrolytes. A 100 cm^2 Ti-MMO anode and one 100 cm^2 Ti-MMO cathode were set parallel to each other with a distance of 6 cm (2.4 in). Anodic chamber was separated from the cathodic chamber using a proton exchange membrane; therefore, BQ was oxidized only at the anode. The membrane prevents transfer of liquid, salts, and contaminants between the anode and the cathode except the protons. Electrochemical oxidation experiments were performed using an electric potential of 10 V. Samples were collected every 15 minutes for 3 hours from the anodic compartment after initiation of electrochemical oxidation.

2.3. Analytical methods

The concentrations of CNB, chloroaniline, and benzoquinone were quantitatively determined using peak areas identified by GC/MS with an Agilent Technologies 6890N GC System equipped with an Agilent 5973 Mass Selective Detector. One mL of each electrolyzed sample was mixed with one mL of dichloromethane (DCM) for liquid-liquid extraction and 0.3 mL of the extract was transferred into a GC vial and injected to the GC/MS. The oven temperature was initially set at 80 °C for 1.5 minutes and then increased to 270 °C at a rate of 20 °C min^{-1}. Peak areas for analytes were then corrected based on the response factor for the internal standard nitrobenzene-d_5 (20 mg L^{-1}) as internal standard. Helium (He) was used as the auxiliary carrier gas at a total flow of 13.7 mL min^{-1} and 10.1 mL min^{-1} at the front- and back-inlet, respectively.

Since reaction intermediates were not identifiable by GC/MS, they were determined using Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (LC/QToF-MS). Samples for non-targeted intermediate analysis were analyzed on an Agilent 1290 LC paired with an Agilent 6530 QToF-MS. Liquid chromatography was performed with a 2.1 x 150 mm, 5 μm Atlantis dC18 column (Waters), a 2.1 mm x 5 mm, 5 μm Atlantis dC18 VanGuard guard column (Waters), and a mobile phase consisting of 60% water (A) and 40% acetonitrile (B). An isocratic method was applied for 5 minutes at a flow rate of 0.5 mL/min with an injection volume of 10 μL. Electrospray ionization was operated in negative mode with a capillary voltage of 1600 V, a fragmentor voltage of 110, and a gas temperature of 350 °C. Nitrogen (>99.999% purity, Airgas) was used as the nebulizer and drying gas with flow rates of 9 and 11 L/min, respectively. High-resolution accurate mass spectra were recorded across the range *m/z* 88 - 195. Species were identified using the personal compound database library.

Mineralization was evaluated using COD, UV absorbance, and average carbon oxidation state. To quantify the oxygen requirement for the oxidation of organic content in the samples, COD was measured according to the USEPA Reactor Digestion Method for waters and wastewaters (Jirka & Carter, 1975) using Hanna COD Low Range (LR) vials. Two mL of each sample were added to the COD vials and then heated at 150 °C for two hours. in a Hanna HI 839800 COD reactor. Two mL of DI water served as the blank. At the end of digestion, the reactor was turned off and samples were left in the reactor for another 20 minutes until the temperature cooled down to approximately 120 °C. These samples were then inverted a few times and COD reading was performed in a Hanna HI83399 Multiparameter Photometer with COD instrument once the vials had cooled down to the room temperature.

UV absorbance measurements were performed on a GENESYS^{TM} 10uv scanning spectrophotometer at the wavelength of 254 nm. DI was used as the blank for the absorbance measurements. Average carbon oxidation state for mineralization of CNB to CO_2 was calculated using the CNB concentration and the number of electrons required for CNB oxidation to CO_2.

2.4. Biodegradability assessment

2.4.1. Enrichment culture

To test whether the products of ring opening from electrochemical oxidation are readily biodegradable, a microbial enrichment culture was prepared. A rhizosphere soil sample was used as inoculum, provided by the High Plains Environmental Center in Loveland, Colorado. Approximately 10 g of the sieved soil was weighed in three 60-mL VOA vials and then 45 mL of M9 minimal salts medium were added to the vials. The M9 minimal salts media for the enrichment culture consisted of 7.5 g L^{-1} of Na_2HPO_4, 3 g L^{-1} of KH_2PO_4, 0.5 g L^{-1} of NaCl, 0.1 g L^{-1} of $MgSO_4 \cdot 7H_2O$, 5.5 mg L^{-1} of $CaCl_2$, 0.5 g L^{-1} of NH_4Cl, and 1 mL L^{-1} of trace metals. The M9 media was filter-sterilized using 0.22 μm PES filter (Thermo Scientific) before addition to the enrichment culture. To avoid electron acceptor deficiency, 200 mgL^{-1} of nitrate was also added to the culture. Furthermore, to provide the carbon source for growth of microorganisms in the enrichment culture, the following electron donors were added: 30 mg L^{-1} BQ, 30 mg L^{-1} oxalic acid, 30 mg L^{-1} sodium citrate, and 50 mg L^{-1} maleic acid. The mixture was shaken several times until all the chemicals were well mixed. To simulate in situ anoxic conditions, the enrichment culture and its headspace were purged with N_2 gas for 20 minutes and 5 minutes, respectively. The cap was tightened and the vials were transferred to an anoxic chamber (Coy Laboratory Products, Inc.). A starting sample was taken right after transferring the vials to the chamber and analyzed immediately for COD. To keep track of the degradation of organics in the enrichment culture through time, samples were taken every 3-5 days and filter-sterilized using a 0.2 μm Nylon filter (Thermo Scientific) and analyzed for COD and organic intermediates. The steps for COD and QToF analyses were the same as stated earlier. The vials were periodically kept in the shaker between samplings (Thermo Scientific). When the COD removal for an enrichment culture reached 50 to 75% of the initial COD, the culture was transferred to a fresh media by decanting the previous culture to a fresh medium. The fresh medium contained the aforementioned concentrations of M9 media, nitrate, benzoquinone, oxalic acid, sodium citrate, and maleic acid. When transferring to a new culture, it was attempted to transfer the least soil from the previous culture to the new one. Depending on the target volume of the new enrichment culture, the dilution factor of the old enrichment culture to the next culture varied from 1:1 to 1:10. The process of transferring the enrichment culture to a fresh medium was repeated 3 times before the enrichment culture was added to the electrolyzed samples to evaluate the biodegradability of the generated intermediates.

2.4.2. Intermediate generation

In order to oxidize CNB to different stages for efficiency evaluation, 7 additional electrochemical oxidation experiments were carried out. A summary of the electric potentials and the electrolysis times used in these experiments are shown in Table 3. In each electrochemical experiment, two sets of triplicate 46-mL samples were collected in 60-mL autoclaved VOA vials before and after the electrochemical oxidation started for each biotic and abiotic microcosms. These samples were then filter-sterilized with a 0.2-µm Nylon filter and after tightening the cap, the vials were stored at -20 °C until use.

Table 3. Applied potentials, current density, and electrolysis time for the generation of intermediates at different extents

Experiment #	Potential (V)	Current density (mA cm^{-2})	Electrolysis time (min)
1	6	4.2	0
2	6	4.2	15
3	6	4.2	45
4	6	4.2	120
5	6	4.2	420
6	10	9.82	420
7	14	14.45	420

Simultaneous with the 46-mL samples, a separate 5-mL sample was taken and analyzed.

2.4.3. Microcosm experiment

Before starting the microcosm experiment, the frozen electrolyzed samples were thawed at ambient temperature and then purged with N_2 for 20 minutes. The vials were then transferred to the anoxic chamber. In addition, 0.5 mL of 100x concentrated M9 media salts were added to reach the previous concentration of M9 in the enrichment culture.

For microcosm inoculation, 3.5 mL of the enrichment culture were transferred directly to the electrolyzed samples oxidized to different stages. In order to minimize microbial activities in the abiotic control vials, 3.5 mL of 50 mg L^{-1} sodium azide dissolved in the enrichment culture was added to the electrolyzed samples. Extreme caution was taken while adding highly toxic sodium azide to the vials. In addition, to minimize the microbial biomass from the enrichment culture in the abiotic samples prior to inoculation, the enrichment culture was filtered. Samples (5-7 mL) were taken every 1-14 days and analyzed for COD, CG/MS, and QToF analyses.

3.1. Electrochemical treatment of CNB

Electrochemical oxidation of CNB with time at applied potentials of 6 V, 10 V, and 14 V is demonstrated in Figure 3. At the beginning of the electrochemical oxidation, CNB concentrations decreased at a high rate at all applied potentials with a slightly faster rate at increasing potential. Almost all the CNB was oxidized after 180 minutes of electrolysis with CNB removal of 98.0 %, 98.7 %, and 99.6 % observed at 6 V, 10 V, and 14 V, respectively.

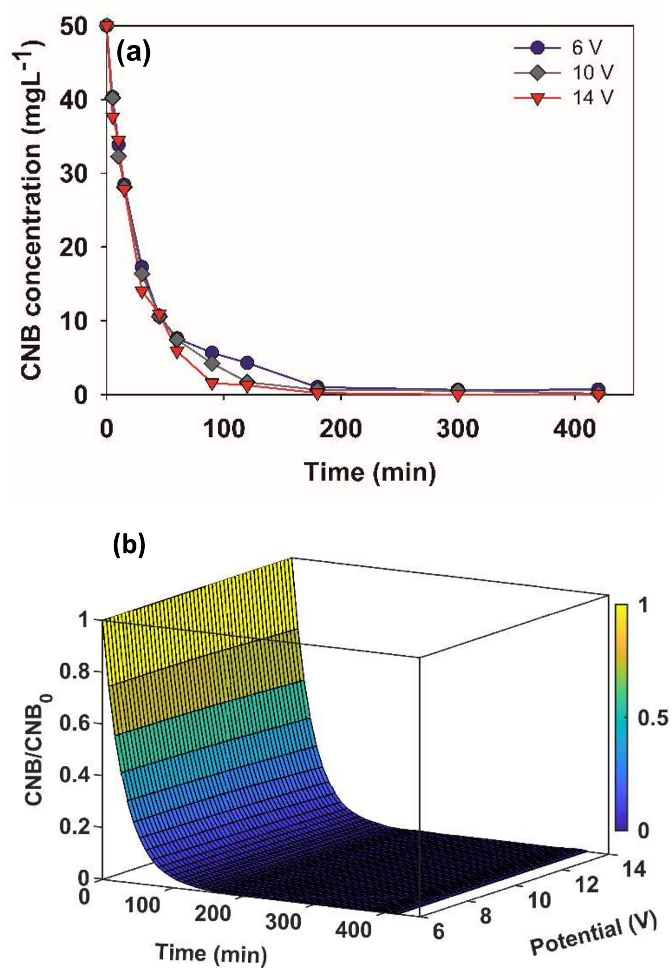


Figure 3. 2D (a) and 3D (b) degradation of CNB as a function of electrolysis time at different applied potentials

Figure 4 shows the kinetics of CNB degradation for the initial 60 minutes of electrolysis at different applied potentials. The regression analysis of CNB removal with electrolysis time (t=0 min to t=60 min) showed that CNB degredation followed pseudo first-order kinetics (Table 4), indicating a mass-transfer limited mechanism (Li et al., 2008). The observed rate constants (k) were determined to be 0.0332 min^{-1}, 0.0337 min^{-1}, and 0.0362 min^{-1} at 6 V, 10 V, and 14 V, respectively.

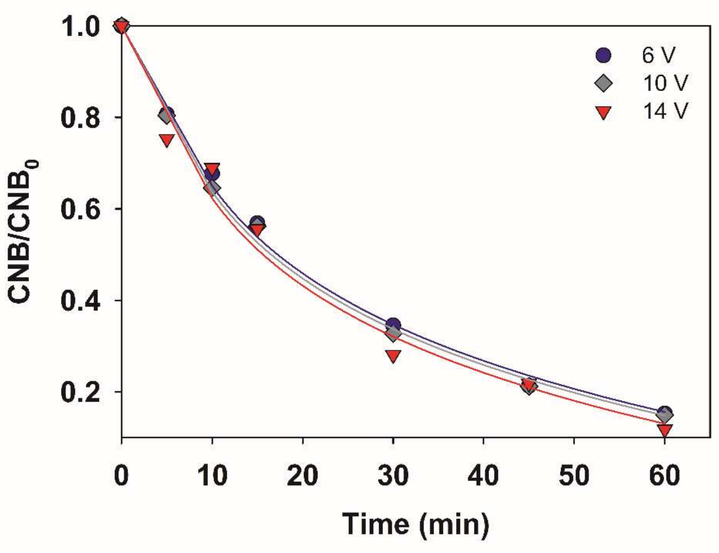


Figure 4. Pseudo-first order kinetics of CNB removal with electrolysis time at different applied potentials

Table 4. Regression coefficients and pseudo-first-order rate constants of the CNB removal kinetics at different applied potentials

Time (min)	Potential (V)	Current density (mA cm^{-2})	R^2	k (min^{-1})
0-60	6	4.2	0.99	0.0332
0-60	10	9.8	0.99	0.0337
0-60	14	14.5	0.98	0.0362

3.1.1. CNB and intermediate control experiments

Control experiments were performed to test whether the observed changes in the concentrations of CNB and its oxidation intermediates were mainly due to electrochemical oxidation, or if physical processes such as gas bubble-induced stripping or volatilization were also contributing to the removal of CNB and its products during electrochemical oxidation. Results showed that 6% of parent CNB removal was due to volatilization, sorption, or other non-electrochemical processes. However, physical processes did not contribute to any measurable changes in COD and UV abosption for the CNB oxidation intermediates. The changes in CNB, COD, and UV absorption normalized to initial values in the control experiments and during electrochemical oxidation at 14 V are demonstrated in Figures A1 and A2 in the Appendix.

3.1.2. Fate of COD during electrochemical oxidation

The results of COD removal during the electrochemical oxidation at different electric potentials are depicted in Figure 5. It was observed that COD decreased initially at a faster rate at higher applied potential. For example, the extent of COD removal was 32.3%, 40.9%, and 59.7% after 180 minutes of electrooxidation at 6 V, 10 V, and 14 V, respectively. This was followed by a plateau trend with a substantially slower decrease in the COD at the later stages of electrolysis. COD removal was only 4.8%, 8.8%, and 10.5% at 6 V, 10 V, and 14 V during the last 4 hours of electrooxidation of CNB, respectively. After 420 minutes (7 hours) of electrolysis, COD was not fully removed at all applied potentials.

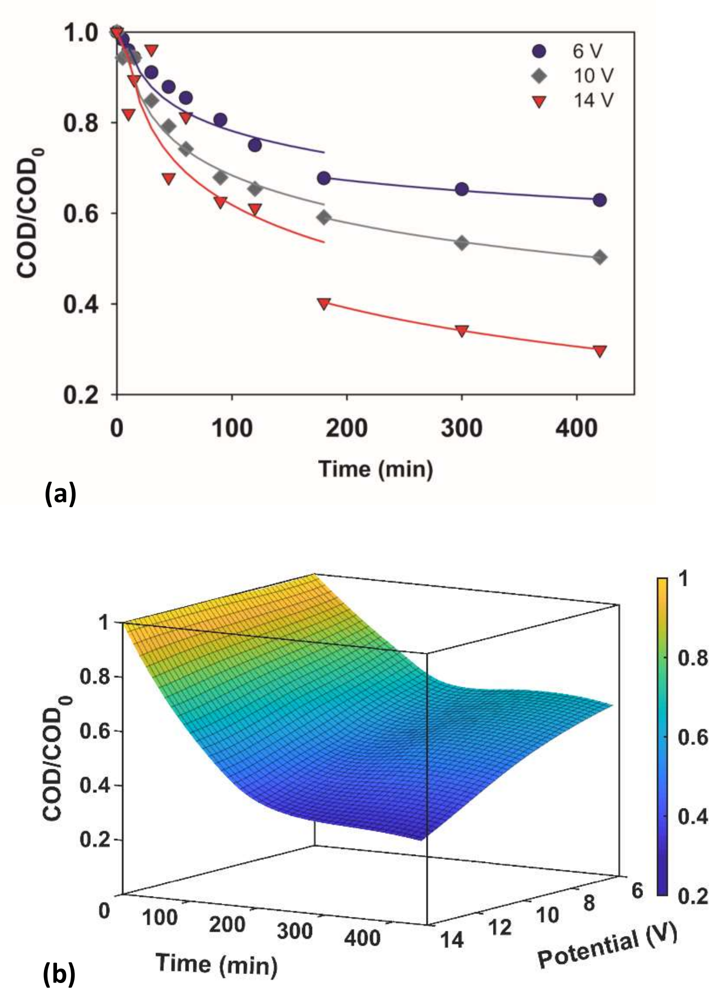


Figure 5. 2D (a) and 3D (b) behavior of COD/COD_0 degradation during the electrochemical oxidation of CNB at different applied potentials

The regression analysis of COD/COD_0 indicated that the COD removal followed a two-step pseudo-first-order kinetic with a higher pseudo-first-order constant (k) at the beginning of the electrooxidation and at higher applied potentials (Table 5). The k values for the first kinetic step were 0.00232, 0.00349, and 0.00484 at 6 V, 10 V, and 14 V, respectively ($R^2_{6V}=0.98$, $R^2_{10V}=0.88$, $R^2_{14V}=0.89$). Furthermore, the k values corresponding to 6 V, 10 V, and 14 V were 0.00015 min^{-1}, 0.00032 min^{-1}, and 0.00056 min^{-1}, respectively, at the second stage of the first-order reaction of COD removal ($R^2_{6V} = 0.72$, $R^2_{10V} = 0.88$, $R^2_{14V} = 0.66$).

Table 5. The regression coefficient and first-order rate constant of the COD removal kinetics during the electrochemical oxidation at different applied potentials

Time (min)	Potential (V)	R^2	k (min^{-1})
0-180	6	0.98	0.00232
180-420	6	0.72	0.00015
0-180	10	0.88	0.00349
180-420	10	0.69	0.00032
0-180	14	0.89	0.00484
180-420	14	0.66	0.00056

3.1.3. Average carbon oxidation state during electrochemical treatment

As mentioned earlier, average carbon oxidation state was determined based on the number of electrons consumed to oxidize CNB to CO_2. Figure 6 represents the changes in the average carbon oxidation state with electrolysis time during the electrochemical oxidation at 6 V, 10 V, and 14 V. Carbon oxidation state initially increased at a higher rate at higher electric potential until around 180 minutes of electrochemical oxidation. For example, it increased from -0.33 to +1.06 at 6 V, from -0.33 to +1.43 at 10 V, and from -0.33 to +2.25 at 14 V after 180 minutes of electrooxidation. After 180 minutes of electrolysis, carbon oxidation state reached a plateau at all applied potentials. For full mineralization of CNB, the average carbon oxidation state should reach +4.00. However, the maximum carbon oxidation state at 6 V, 10 V, and 14 V were +1.27, +1.82, and +2.71 at 420 minutes of electrolysis, respectively.

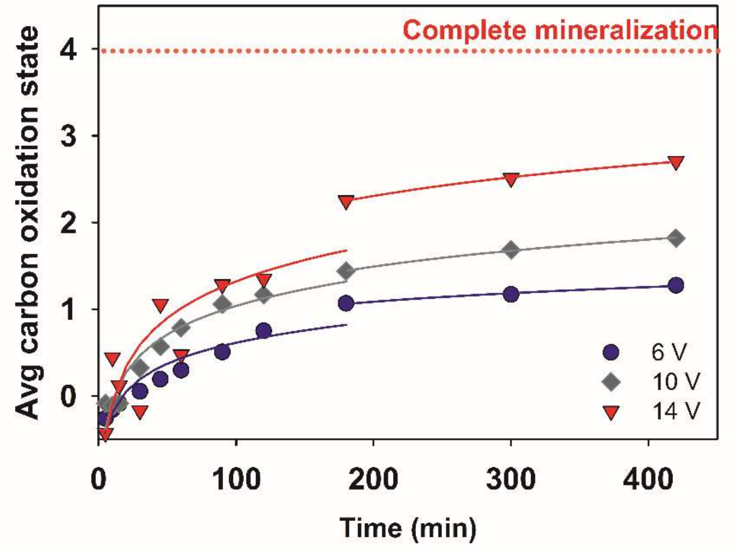


Figure 6. Changes in average carbon oxidation state as a function of time during the electrochemical oxidation of CNB at different applied potentials

3.1.4. UV absorbance during the electrochemical oxidation

In order to test whether UV absorption is a rapid and cost-effective approach to track ring opening, the absorbance of the electrolyzed samples was measured with time. In this study, the compounds' absorption maxima were determined to be around 254 nm. This is a wavelength at which aromatic compounds are known to absorb light (Weishaar et al. 2003). The variations in UV absorbance with time during the electrochemical oxidation at the three electric potentials are presented in Figure 7. As the reaction proceeded, the absorbance decreased until around 60 minutes of electrolysis at all applied potentials. For example, the absorbance decreased from 1.05 to 0.421 at 6 V, from 1.17 to 0.453 at 10 V, and from 1.17 to 0.549 at 14 V at 60 minutes of electrochemical oxidation. This was followed by a substantially slower trend with temporary increases, likely due to analytical variations. Analytical variability of UV absorption is even higher when working with environmental matrices, for example when groundwater is too turbid or natural organic matter such as humic substances is present at elevated concentrations.

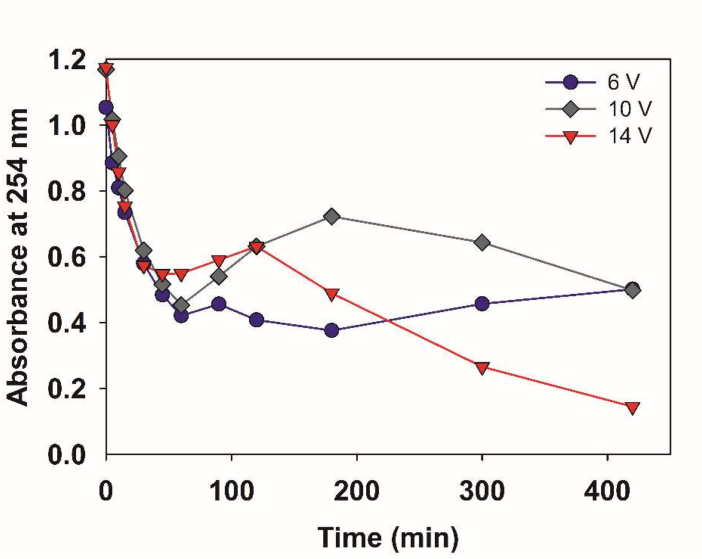


Figure 7. Changes in absorbance at 254 nm with electrolysis time at different applied potentials

3.1.5. Transferability of electrochemical CNB oxidation to other AOPs

BQ is a hydroxyl radical (•OH) scavenger, therefore, it was added to probe whether CNB was oxidized through indirect electrolysis by •OH (Bejan et al., 2012). Figure 8 shows degradation of CNB with and without the BQ during the anodic oxidation at 10 V. As can be seen in the figure, CNB degradation was slower in the presence of BQ. For instance, at 180 minutes of electrooxidation, CNB was reduced from 50 mg L^{-1} to 38.5 mg L^{-1} in the solution containing BQ and from 50 mg L^{-1} to 30.2 mgL^{-1} in the solution that had no added BQ. The observed rate constants (k) were determined to be 0.00152 min^{-1}, and 0.00306 min^{-1}, for with and without BQ tests, respectively (Table 6).

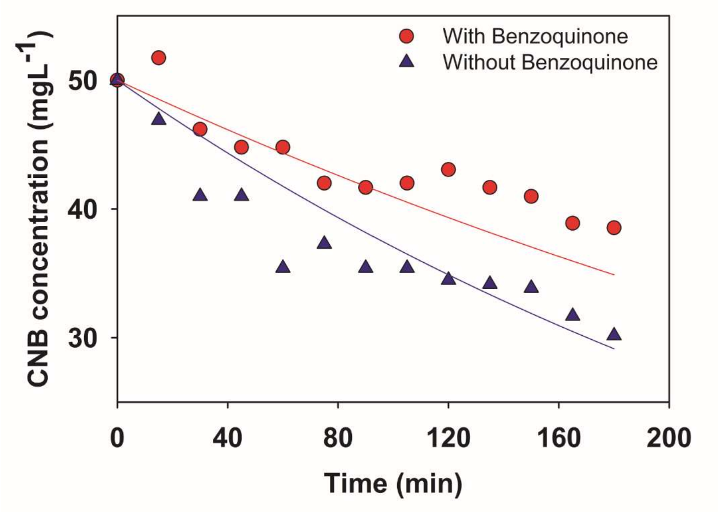


Figure 8. CNB oxidation with and without added benzoquinone (BQ) during electrochemical oxidation at 10 V in a divided cell reactor.

Table 6. Regression coefficients and pseudo-first-order rate constants of the CNB removal kinetics with and without BQ at 10 V

Test	Time (min)	Potential (V)	Current density (mA cm^{-2})	R^2	k (min^{-1})
With BQ	0-180	10	9.8	0.84	0.00152
Without BQ	0-180	10	9.8	0.98	0.00306

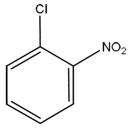
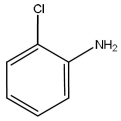
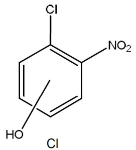
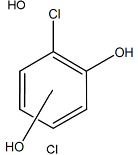
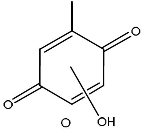
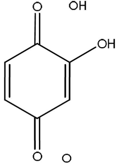
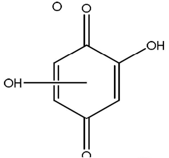
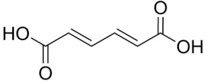
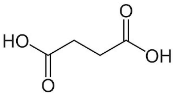
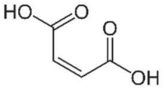
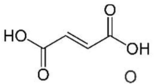
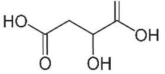
3.2. Degradation intermediates and CNB reaction pathway

In order to identify the degradation intermediates of CNB and the reaction pathway, LC-QToF-MS was used. The results for the identified intermediates and the possible pathway for CNB degradation during the electrochemical oxidation are shown in Table 7 and Figure 9, respectively. The oxidation pathway of CNB can be classified in two major steps. First, the substitution of chloro- and/or nitro-substituents by •OH and/ or the addition of •OH to the aromatic ring of CNB forms ring addition products. Second, further attack of the initial oxidation products by •OH leads to the cleavage of the benzene ring forming dicarboxylic acids (Figure 9 and Table 7).

During the electrochemical oxidation, •OH attacks the CNB and may lead to addition of a hydroxyl group to CNB to form isomers of chloronitrophenol including *o*-chloronitrophenol, *m*-chloronitrophenol, and *p*-chloronitrophenol. Simultaneously, •OH attack on CNB may cause the release of the nitro-group and substitution by •OH, generating isomers of dihydroxychlorobenzene, including chloronitrocatechol, chloronitroresorcinol, and chloronitrohydroquinone. As a result of further oxidation, chloronitrohydroquinone is subsequently transformed to chlorohydroxybenzoquinone and/or hydroxyl-benzoquinone. Further attack of the two aforementioned intermediates by •OH may be followed by the formation of dihydroxybenzoquinone. As the electrochemical oxidation proceeds, the aromatic ring was cleaved to form dicarboxylic acids as oxidation intermediates including muconic acid, succinic acid, malic acid, maleic acid, and/or fumaric acid.

As a transient side reaction, the nitro-group on CNB was reduced to an amino-group at the cathode to form chloroaniline. Chloroaniline was formed after 5 minutes of electrochemical oxidation at 6 V, 10 V, and 14 V and was completely removed after 420 minutes of electrolysis at 10 V and 14 V.

Table 7. Identified intermediates of CNB oxidation on Ti-MMO electrodes using LC-QToF-MS

Intermediate	Molecular structure	Molecular formula	Base peak	Observed *m/z*	Theoretical *m/z*	Error (ppm)
o-Chloronitrobenzene		$C_6H_4O_2NCl$	$[M-H]^-$	155.9849	155.9858	-5.8
o-Chloroaniline		C_6H_6NCl	$[M-H]^-$	126.0113	126.0116	-2.4
Chloronitrophenol		$C_6H_4O_3NCl$	$[M-H]^-$	171.9815	171.9807	4.7
Chlorohydroquinone		$C_6H_5O_2Cl$	$[M-H]^-$	142.9897	142.9905	-5.6
Chlorohydroxy-benzoquinone		$C_6H_3O_3Cl$	$[M-H]^-$	156.9685	156.9698	-8.3
Hydroxybenzoquinone		$C_6H_4O_3$	$[M-H]^-$	123.0098	123.0088	8.1
Dihydroxybenzoquinone		$C_6H_4O_4$	$[M-H]^-$	139.0037	139.0037	0.0
Muconic acid		$C_6H_6O_4$	$[M-H]^-$	141.0187	141.0193	-4.3
Succinic acid		$C_4H_6O_4$	$[M-H]^-$	117.0190	117.0193	-2.6
Maleic acid		$C_4H_4O_4$	$[M-H]^-$	115.0040	115.0037	2.6
Fumaric acid		$C_4H_4O_4$	$[M-H]^-$	115.0040	115.0037	2.6
Malic acid		$C_4H_6O_5$	$[M-H]^-$	133.0145	133.0142	2.3

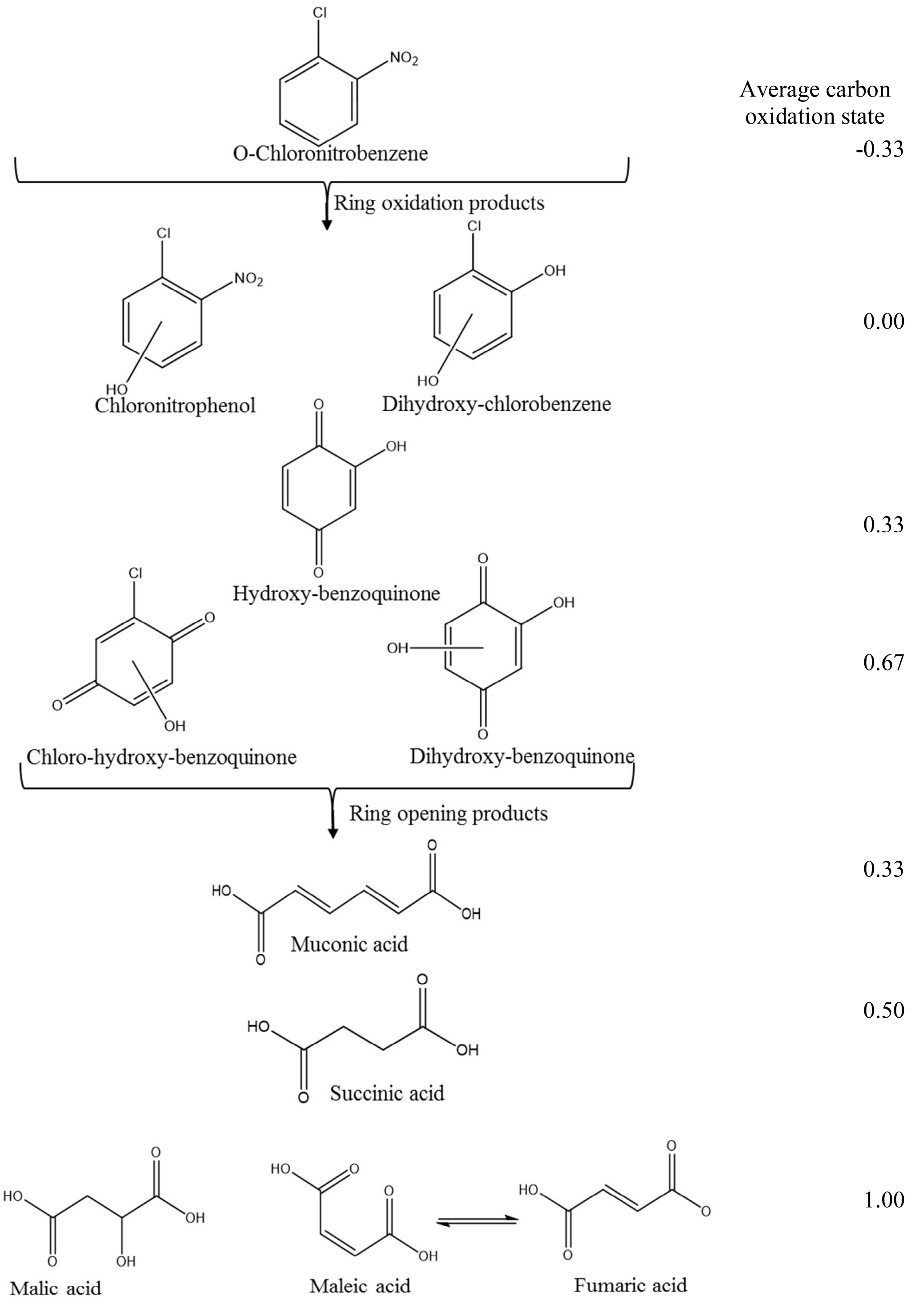


Figure 9. Proposed pathway for the oxidation of CNB by the electrochemical oxidation on Ti-MMO electrodes

3.3. Apparent anode surface area per order (ASA_O), electric energy per order (E_{EO}) and cost analyses of electrochemical oxidation

Figure 10 shows the apparent anode surface area per order (ASA_O) and electric energy per order (E_{EO}) at different electric potentials. ASA_O was 5.10 m^2 gpm^{-1} at 6 V, 5.03 m^2 gpm^{-1} at 10 V, and 4.65 m^2 gpm^{-1} at 14 V. In other words, 5.10 m^2, 5.03 m^2, and 4.65 m^2 of electrodes were required at 6 V, 10 V and 14 V, respectively, to remove one order of magnitude of CNB per 1 gpm of solution treated in the reactor. The E_{EO}, on the other hand, was determined to be 5.82 kWh m^{-3} $order^{-1}$, 22.5 kWh m^{-3} $order^{-1}$, and 42.7 kWh m^{-3} $order^{-1}$ at 6 V, 10 V, and 14 V, respectively. Therefore, in order to remove 1 order of magnitude of CNB in 1 m^3 synthetic solution, 5.82 kWh, 22.5 kWh, and 42.7 kWh energy is required at applied potentials of 6 V, 10 V, and 14 V, respectively.

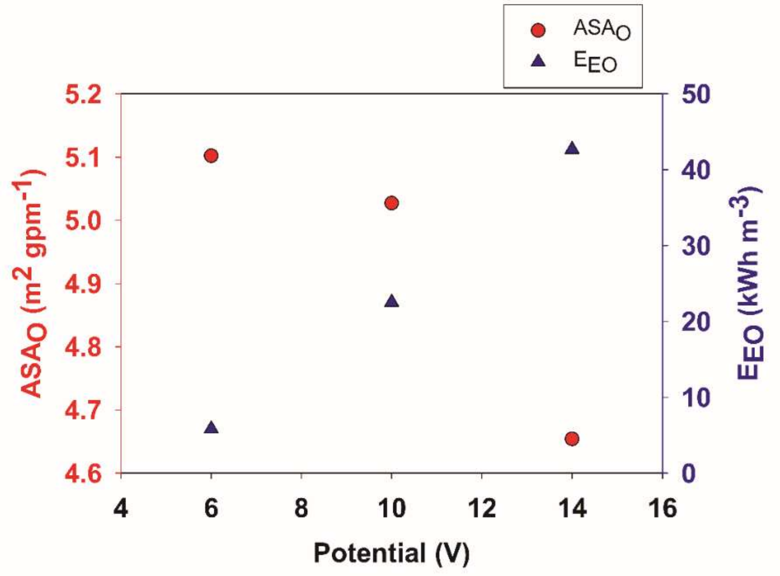


Figure 10. Apparent anode surface area per order (ASA_O) and electric energy per odder (E_{EO}) for electrochemical oxidation of CNB at different electric potential

The cost analyses for the electrodes and electricity costs and total cost during the anodic oxidation of CNB are shown in Figure 11 and Figure 12, respectively. For the electrochemical oxidation process, the cost analysis was performed estimating \$400 per m^2 mesh electrode and \$0.10 per kWh electricity. It was also assumed that the electrodes do not have to be replaced. In order to make the results comparable to different flow rates and electric usage, the electrode costs were normalized to 1 gpm and electricity costs were normalized per 1 kWh. The electrode costs included both cathodes and anodes.

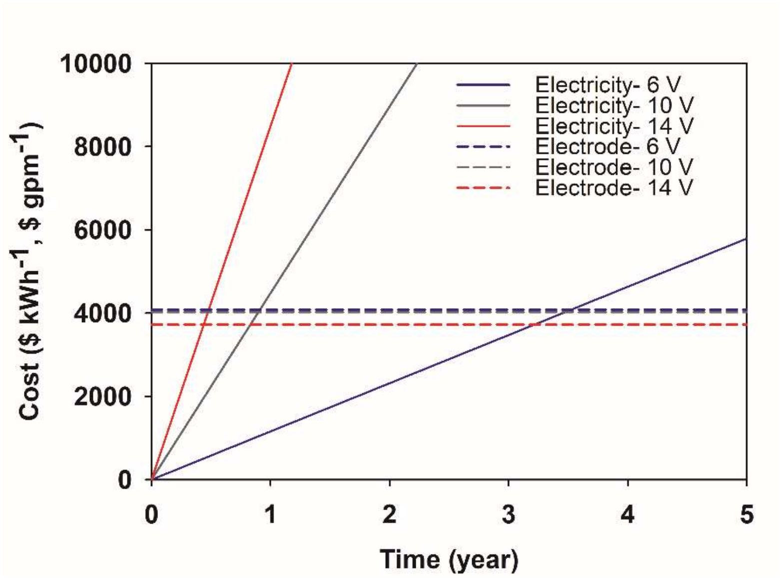


Figure 11. Cumulative cost for electrodes and energy through time for the electrochemical oxidation of CNB on Ti-MMO electrodes at different electric potentials

The capital cost for the electrodes were estimated to be \$4,080, \$4,020, and \$3,720 per 1 gpm per order of magnitude of CNB removal at 6 V, 10 V, and 14 V, respectively. The annual energy cost was \$1,160, \$4480, and \$8490 at the applied potentials of 6 V, 10 V, and 14 V, respectively. At 6 V, electrodes are the major technology cost driver until 3.5 years (1277 days), after which energy consumption exceeds electrode cost. In addition, it takes 0.9 years (328 days) and 0.4 years

(146 days) until the cost for energy consumption overrides the electrodes cost at 10 V and 14 V, respectively (Figure 11).

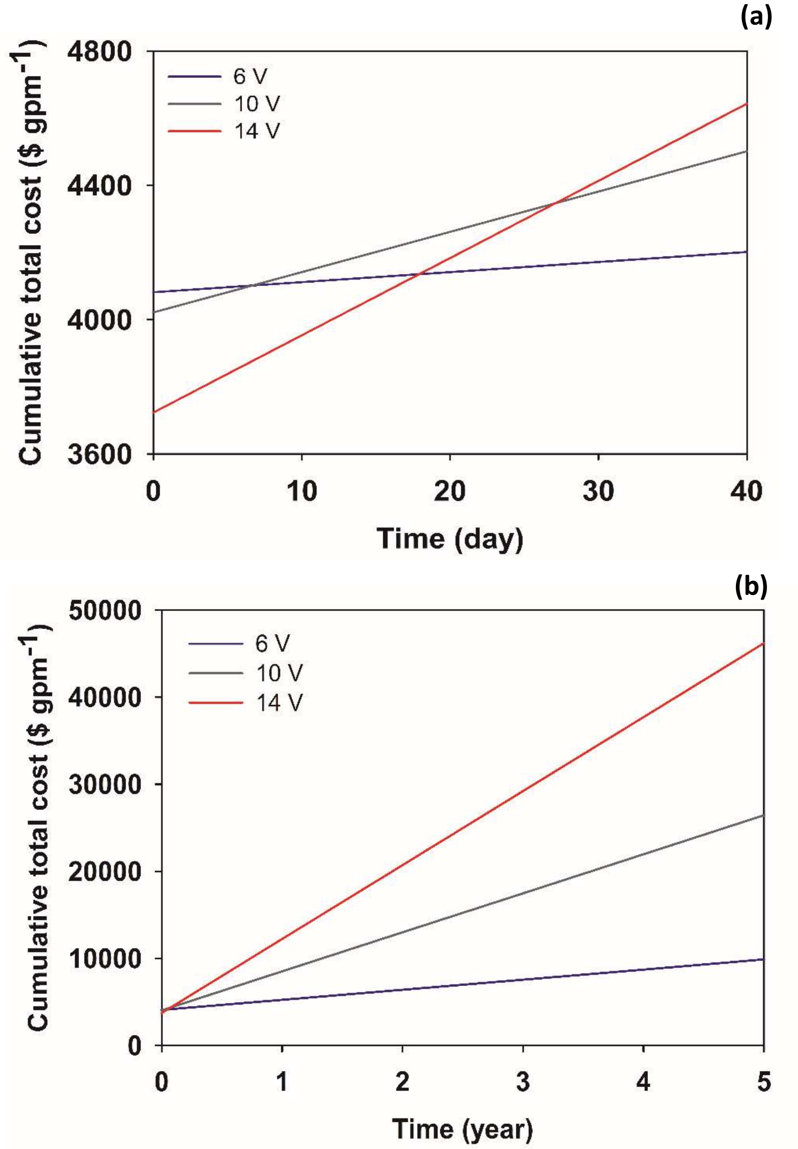


Figure 12. Cumulative total cost with time during the electrochemical oxidation of CNB on Ti-MMO electrodes at different applied potentials (a): early stage, (b): long-term

As Figure 12 shows, the cumulative total cost of 6 V was initially higher than 10 V and 14 V; however, after 7 days, the cumulative total cost for 10 V exceeded the total cost of 6 V. In addition, 18 days after initiation of electrochemical oxidation, cumulative total cost at 14 V starts to exceed the total cost at 6 V.

3.4. Biodegradability experiment

3.4.1. Degradation of COD during biological treatment

Figure 13 demonstrates the degradation of COD during biological treatment of pretreated CNB samples. Two days after biodegradation initiated, COD decreased rapidly in the CNB samples that had been electrochemically treated for relatively short durations of 0 minute, 15 min, 45 min, and two hours at 6 V. For instance, COD removal in the 0min-6V-biotic, 15min-6V-biotic, 45min-6V-biotic, 2hr-6V-biotic samples were 26%, 24%, 19%, and 19% respectively. Similarly, COD decreased in the corresponding abiotic samples. An overall decreasing trend in COD was observed for all the biotic and abiotic samples from two to 42 days of biological treatment, however, COD did not reach zero during 42 days of biodegradation.

In order to test the impact of filtration on COD measurement, COD was analyzed for filtered DI water passed through the 0.2-µm Nylon filter. In this test, unfiltered DI was used as blank and COD for filtered DI was found to be 11 ± 3 mg L^{-1}. Thus, some of the background COD and variability in these measurements may have stemmed from the filters.

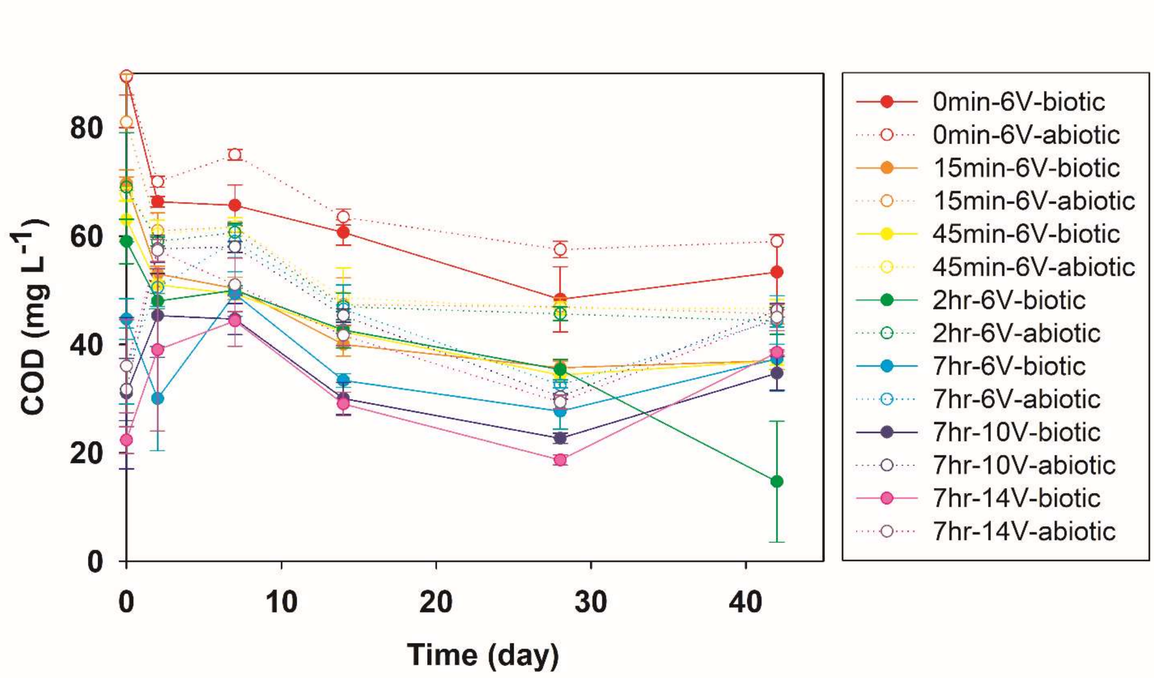


Figure 13. Evolution of COD during biological treatment of the electrolyzed CNB at different levels

To unravel how much of COD removal was due to biological activities, the biotic COD was subtracted from abiotic COD. The differences in COD between biotic and abiotic COD normalized to the initial difference during biological treatment is illustrated in Figure 14. In general, a higher extent of biological COD removal with increasing treatment time was observed, except for the 7hr-14V microcosms.

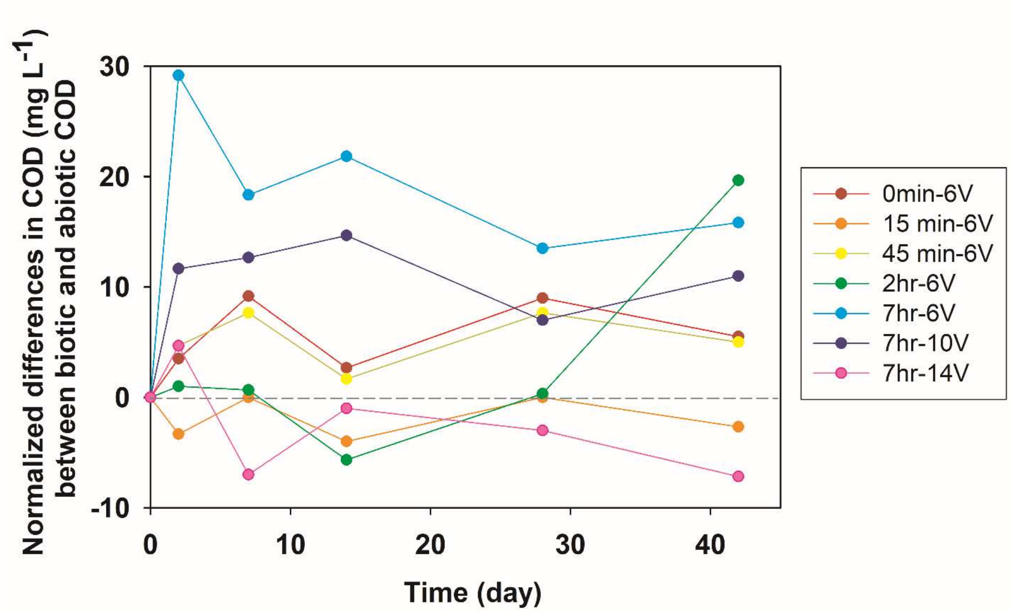


Figure 14. Normalized differences in COD between biotic and abiotic COD during biological treatment of electrolyzed samples

3.4.2. Changes of CNB concentration during biological treatment

Figure 15 shows the changes of CNB concentrations during biological treatment of pretreated CNB samples. Similar to the COD trend in Figure 13, CNB concentrations decreased in the first two days after biodegradation started. The initial decline in CNB concentrations was followed by a period of relatively stable concentrations. These rapid initial declines in COD and CNB suggest sorption losses inside the microcosms. No CNB was detected in the samples that had been treated for 7 hours at all applied potentials.

Changes of chloroaniline concentration during biodegradation of pretreated CNB samples is demonstrated in Figure A3 in Appendix. An initial sorption of chloroaniline was followed by up to 25% removal of chloroaniline in the biotic microcosms (2h-6V-biotic) and up to 36% in the abiotic microcosms (45min-6V-abiotic) during 2-28 days of biological treatment.

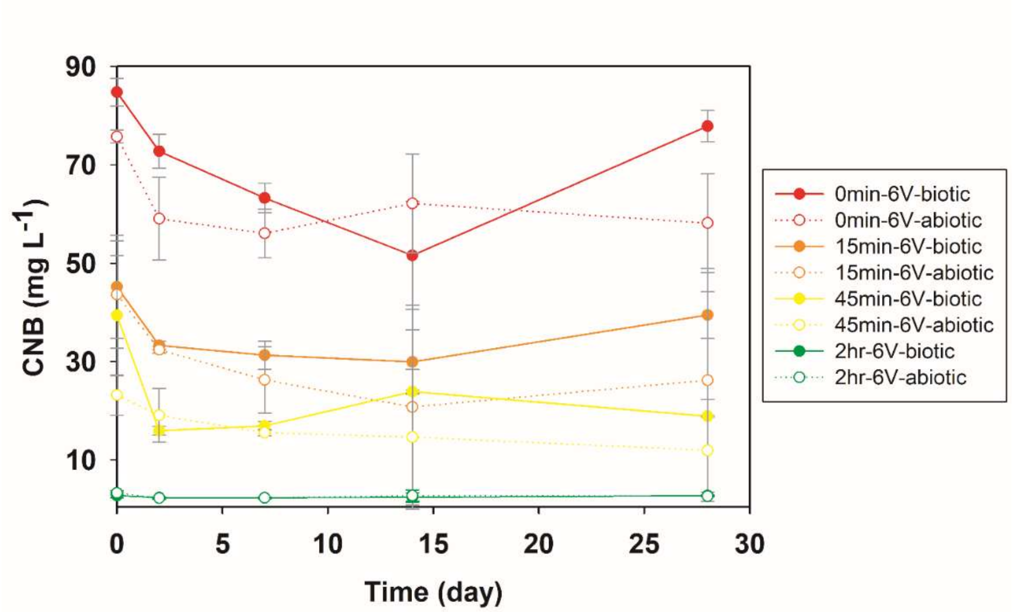


Figure 15. Changes of CNB concentration during biological treatment of pre-treated CNB samples

3.4.3. Biodegradation intermediates during biological treatment

Since the COD measurements were potentially biased by sample filtration, LC-QToF-MS was used to further identify and quantify the biodegradation intermediates of pretreated CNB. The results for removal of the biodegradation intermediates during 7 days and 28 days of biological treatment are presented in Table 8 and 9, respectively.

According to the results of the CNB and COD analyses, physical removal of ring compounds can occur initially due to processes such as volatilization and sorption. Therefore, to remove the contribution of initial sorption, the biological removal rates for chloronitrobenzene and chloroaniline (Figure A3 in the Appendix) were calculated for 2-7 days (Table 8) and for 2-28 days (Table 9). The intermediate control experiment, on the other hand, showed that ring opening products are hydrophilic and do not tend to be removed through physical processes. Therefore, the removal rates for the intermediates were calculated for 0-7 days (Table 8) and 0-28 days (Table 9) of biodegradation.

As seen in Tables 8 and 9, no CNB removal was detected during 28 days of biological treatment in any of the microcosms. Chloroaniline was detected for pre-treated CNB samples at 15 minutes and disappeared after 7 hours of electrolysis. The removal rates for chloroaniline were <10 % during 7 days of biological treatment and were determined to be up to 27 % during 28 days of biodegradation. Furthermore, chloronitrophenol was detected for 0min-6V-biotic sample. The presence of chloronitrophenol in the aforementioned sample was possibly due to aerobic degradation of CNB while freezing the sample before inoculation. Chloronitrophenol did not appear to biodegrade during 7 days and 28 days of biodegradation. Other ring intermediate compounds including chlorohydroquinone and dihydroxybenzoquinone were more biodegradable in higher oxidized microcosms. For instance, 40% of chlorohydroquinone and 86% of dihydroxybenzoquinone was removed in the 2h-6V-biotic microcosm while in the 7h-10V-biotic microcosm, 80% of chlorohydroquinone and 90% of dihydroxybenzoquinone was removed during 7 days of biodegradation (Table 8). Maleic acid was also detected in initially oxidized samples, likely transferred from the initial enrichments. All ring cleavage products (i.e. dicarboxylic acids including malic, succinic, and maleic/fumaric acids) were 100% removed after 7 days and 28 days of biodegradation.

Table 8. The removal rates of CNB and intermediates during 7 days of biological treatment

Sample	CNB*	Chloro-aniline*	Chloronitro-phenol	Chlorohydro-quinone	Dihydroxy-benzoquinone	Malic acid	Succinic acid	Maleic/Fumaric acid
0 min-6 V-biotic	13%	n.d.	16%	n.d.	n.d.	n.d.	n.d.	80%
15 min-6 V-biotic	6%	7%	0%	n.d.	n.d.	n.d.	n.d.	100%
45 min-6 V-biotic	0%	0%	0%	n.d.	n.d.	n.d.	n.d.	100%
2 h-6 V-biotic	15%	2%	0%	40%	86%	n.d.	n.d.	100%
7 h-6V-biotic	n.d.	0%	0%	37%	85%	100%	100%	100%
7 h-10 V-biotic	n.d.	n.d.	0%	80%	92%	100%	100%	100%
7 h-14 V-biotic	n.d.	n.d.	0%	n.d.	n.d.	100%	100%	100%
Average carbon oxidation state	-0.33	-0.33	0.00	0.00	0.67	1.00	0.50	1.00

* To exclude initial sorption, CNB and chloroaniline removal rates were calculated for 2-7 days

n.d. = not detected

Table 9. The removal rates of CNB and intermediates during 28 days of biological treatment.

Sample	CNB*	Chloro-aniline*	Chloronitro-phenol	Chlorohydro-quinone	Dihydroxy-benzoquinone	Malic acid	Succini acid	Maleic/Fumari acid
0 min-6 V-biotic	0%	n.d.	11%	n.d.	n.d.	n.d.	n.d.	100%
15 min-6 V-biotic	0%	18%	0	n.d.	n.d.	n.d.	n.d.	100%
45 min-6 V-biotic	0%	20%	28%	n.d.	n.d.	n.d.	n.d.	100%
2 h-6 V-biotic	0%	27%	0	8%	16%	n.d.	n.d.	100%
7 h-6V-biotic	n.d.	14%	0	26%	60%	100%	100%	100%
7 h-10 V-biotic	n.d.	n.d.	0	83%	100%	100%	100%	100%
7 h-14 V-biotic	n.d.	n.d.	0	n.d.	n.d.	100%	100%	100%
Average carbon oxidation state	-0.33	-0.33	0.00	0.00	0.67	1.00	0.50	1.00

* To exclude initial sorption, CNB and chloroaniline removal rates were calculated for 2-28 days
n.d. = not detected

CHAPTER FOUR. DISCUSSION

4.1. Electrochemical treatment of CNB

To test the viability and efficiency of electrochemical CNB oxidation, three potentials of 6 V, 10 V, and 14 V were applied. In general, a higher oxidation rate at higher applied potential (and current density) is explained by the higher formation of hydroxyl radicals (•OH) capable of opening the aromatic ring (Rodgers & Bunce, 2001; Vilhunen & Sillanpaa, 2010). For instance, Garcia-Segura and Brillas (2011) observed an increasing oxidation rate of oxamic acid on boron-doped diamond anodes by raising current densities from 16.6 mA cm^{-2} to 100 mA cm^{-2}. However, in this study the differences in observed rate constants were minor, indicating that •OH radical formation was not the limiting process. Rather, pseudo-first-order reaction kinetics in the electrochemical oxidation of organic compounds are indicative of mass transfer limitations (Li et al., 2008; Liu, 2009; Garcia-Segura & Brillas, 2011; Barndok et al., 2014, and Cottrell; 2018). Thus, a higher applied potential of 10 V or even 14 V did not improve CNB removal, but decreased treatment efficiency due to excessive energy consumption.

4.1.1. Fate of COD during electrochemical oxidation

A COD decrease with increasing electrolysis time and applied potential is in agreement with the findings of Babu et al. (2011), Barndok et al. (2014), and Chen et al. (2016) and due to oxidation of reducible species in the organic contaminant, such as carbon. COD was initially decreasing readily due to rapid oxidation of the aromatic ring; however, beyond 180 minutes of electrooxidation, COD did not decline substantially as removal rates dropped by about one order of magnitude. The residual COD beyond 180 minutes of electrooxidation was inconsistent with the initially rapid removal rate of CNB, indicating that the complete mineralization of CNB was not achieved after 420 minutes of electrolysis even at the highest potential. Partial mineralization of aromatic compounds is attributed to the generation of persistent short-chain carboxylic acids, such as glycolic acid (Fontmorin et al., 2012), oxalic and oxamic acids (Garcia-Segura & Brillas, 2011), glyoxylic, formic, acetic and malonic acids (Trellu et al., 2016). These carboxylic acids are slower to be oxidized by •OH than the ring compounds (Katayama & Matsumura, 1991; Stockinger et al., 1995; Garcia-Segura & Brillas, 2011; Ganzenko et al., 2014; Monteil et al., 2018), leading to a slower degradation rate at the later stage of electrochemical oxidation. Therefore, electrochemical oxidation was not able to completely mineralize CNB during the 420 minutes experiments.

As for parent CNB removal, COD removal rates increased only slightly with increasing applied potential, but have to be assessed in the context of increased energy consumption. For instance, while the COD removal rate increased by factors of 2.1 for initial oxidation and 3.7 for late-stage oxidation going from 6 V to 14 V applied, respectively (Table 5), power consumption increased by a factor of 8.1 (Table 4).

4.1.2. Average carbon oxidation state during electrochemical treatment

In agreement with CNB and COD measurements, the rapid initial increase in the average carbon oxidation state is attributed to initial oxidation of the aromatic ring. After around 180 minutes of electrolysis, the carbon oxidation state only slightly increased further at all applied potentials. The plateau of carbon oxidation state toward the end of electrochemical oxidation indicated that further advanced oxidation does not bring about significant changes in the chemical oxidation of the degradation intermediates. These intermediates are hardly oxidizable by •OH species and thus partial mineralization of CNB was attained after 420 minutes of electrochemical oxidation.

4.1.3. UV absorbance during electrochemical oxidation

Similar to COD and carbon oxidation state trends, oxidation of CNB during the initial stage of electrooxidation led to a decrease in UV absorption due to oxidation of the aromatic ring, which generally has an absorption maximum around 254 nm (Weishaar et al. 2003). The plateau in absorbance at the later stage of electrochemical oxidation was due to the generation of intermediates which persist to complete mineralization of CNB, and indicates that these intermediates still absorb light at 254 nm, although to a smaller extent. The results indicate that UV absorption at 254 nm may be a rapid, cost-effective tool to assess ring oxidation of aromatic contaminants in waters with low turbidity or natural organic matter content.

In summary, although parent CNB was rapidly removed via electrochemical oxidation, COD, average carbon oxidation state, and absorbance confirm that partial mineralization of CNB was achieved due to the formation of intermediates which are not easily oxidizable by •OH.

4.1.4. Transferability of electrochemical CNB oxidation to other AOPs

BQ acts as a chemical scavenger for •OH formed during anodic oxidation of water. The slower rate of CNB degradation in the presence of BQ indicates that CNB was oxidized by •OH during electrochemical treatment. Since the mechanism of organic contaminant degradation in most other AOPs is based on Fenton's chemistry (•OH), these results demonstrate that the findings of the electrochemical oxidation study are widely applicable to other AOPs (Jing & Chaplin, 2017).

4.2. Degradation intermediates and reaction pathway

Hydroxyl radicals are the major species involved in the oxidation of CNB while applying AOPs (Rodgers & Bunce, 2001; Vilhunen & Sillanpaa, 2010). Chloroaniline formed during the pre-reduction of CNB and was eventually oxidized. This is in agreement with (Rodgers and Bunce,

2001) who stated that the products of nitro-group reduction of nitrophenols are readily oxidizable. Formation of phenol as a result of the release of nitro-group and replacement of the substituents by •OH on the aromatic ring of nitrobenzene has also been reported by Chen et al. (2014). Furthermore, Torres et al. (2003) found catechol and hydroquinone as a result of further oxidation of phenols at the ortho- and para- positions. Benzoquinone was also detected as an oxidized form of dehydrogenated hydroquinone by Chen et al. (2014) and Torres et al. (2003). In this study, denitration of the benzene ring of CNB occurred more readily than dechlorination which is in agreement with Zhu et al. (2007), who stated that denitration during the electrochemical oxidation of nitrophenol occurred easily due to the strong electrophilic properties of •OH. As confirmed by Shen et al. (2008), the reaction pathway of aromatic compound degradation may not follow one major pathway but rather several parallel pathways due to the strong redox potential and the electrophilic characteristics of •OH.

The dicarboxylic acids identified in this study are in accordance with Shen et al. (2008) who applied ozonation for *p*-CNB oxidation. Additional studies also found acetic, formic, oxalic, malonic acids which were not identified in this study (Zazo et al., 2005). Scheck and Frimmel (1995) stated that the formation of dihydroxybenzene to muconic acid can occur readily and further oxidation by •OH and oxygen on the double bond of muconic acid can form the two isomers of maleic and fumaric acid. Furthermore, the authors mentioned that maleic acid can be formed via •OH addition to the dihydroxybenze leading to the ring fission and then formation of maleic acid. Formation of dihydroxysuccinic acid from degradation intermediates with double bond, for instance maleic acid, has been reported by Milas et al. (1937). Malic acid can be formed by hydroxylation to the fumaric or maleic acid with a subsequent dehydrogenation (Scheck & Frimmel, 1995). Eventually, prolonged oxidation of these dicarboxylic acids can mineralize them to water and carbon dioxide. Since these dicarboxylic acids are difficult to mineralize through AOPs, the degradation process at the later stage of electrochemical oxidation is slow as represented in the plateau curve of COD, UV absorbance, and carbon oxidation state. The slow degradation rate toward the end of advanced oxidation of organic contaminants is in agreement with findings by Oturan et al. (2008), Garcia-Segura & Brillas (2011) and Ganzenko et al. (2014). It is important to note that despite the persistent properties of these dicarboxylic acids, they are readily biodegradable by microorganisms compared to the initial oxidation products (Katayama & Matsumura, 1991; Stockinger et al., 1995; Liu et al., 2010).

4.3. Apparent anode surface area per order (ASA_O), electric energy per order (E_{EO}) and cost analyses of electrochemical oxidation

Figure 10 implies that at higher applied potentials less electrode mesh was needed to degrade one order of CNB per 1 gpm flow of contaminated solution. Since a higher efficiency of an electrooxidation process was determined by less mesh electrode area, 14 V was more efficient than 10 V and 6 V. However, these differences were rather minor.

In contrast, at higher applied potentials more electric energy was required to degrade 1 order of CNB contaminant in 1 m^3 of water. The lower the E_{EO}, the more efficient the electrochemical oxidation is with respect to electric energy. Therefore, 6 V has a higher electrical energy efficiency compared to 10 V and 14 V. In summary, at a specific applied potential, electric energy and electrode area were inversely correlated.

Even though the capital costs for this remediation technology may be lower at higher applied potentials, energy consumption and thus operating cost have to be considered for overall efficiency evaluation. Figure 12 demonstrates that after only 17 days of operation, a low applied potential such as 6 V becomes overall more cost-efficient regarding parent CNB removal than higher potentials. To confirm this conclusion, the biodegradability of the generated intermediates was assessed and discussed in the following section.

4.4. Biodegradability experiment

4.4.1. Degradation of COD during biological treatment

A rapid decrease in COD in the first two days of biological treatment for the initially electrolyzed samples (0 minute to 2 hours), in which CNB and ring intermediates were still present, is likely attributed to the sorption of the ring compounds to the container and possibly microbial biomass. These results are in agreement with findings by Yahiaoui et al. (2013) who reported that biosorption to activated sludge contributed to a declining trend in DOC of electrochemically oxidized tetracycline hydrochloride.

For the later electrolyzed samples, for instance 7hr-6V-biotic, 7hr-10V-biotic, 7hr-14V-biotic, the COD trend in the first two days were expected to be stable due to the generation of polar dicarboxylic acids which do not tend to sorb. A decrease in COD for 7hr-6V-biotic, and an increase in 7hr-10V-biotic and 7hr-14V-biotic samples can be related to the experimental and analytical variabilities of COD measurement in complex microbial systems.

A general decreasing trend in COD during biological treatment is related to the oxidation of reduced species, including organic acids, but possibly also of NH_4^+ from the M9 media. Furthermore, a COD decrease in abiotic microcosms indicated that sodium azide was only able to minimize, but not fully inhibit the microbial activities. Additional abiotic removal processes cannot be fully excluded.

The COD results for filtered DI showed that apparently some organics come off the filter which is possibly one reason for the observed variability in COD analyses. Furthermore, in order to remove the microbial biomass from the enrichment culture in the abiotic samples prior to inoculation, the enrichment culture was filtered. An initial filtration in the abiotic samples may explain occasional increases in abiotic COD compared to biotic COD. As stated earlier, for COD analyses all the biotic and abiotic samples were filtered using 0.2 µm Nylon filters. In addition to an extra source of COD from filters, there may have been some intermediates formed during biological treatment, which may be another reason for residual COD after 42 days of biodegradation. Residual COD at the end of biodegradation may be also attributed to partial biodegradation of the intermediates formed during electrochemical oxidation (Trellu et al., 2016).

A positive normalized difference between biotic and abiotic COD indicated that microbial processes were the likely cause of COD removal in most samples. Overall, the longer the CNB-containing solutions were electrochemically oxidized, the more biodegradable the generated intermediates were. These results are consistent with findings by Trellu et al. (2016) who reported an improvement in BOD_u/COD biodegradability of phenanthrene at extended electrochemical treatment. The normalized differences in COD for the 7hr-14 V sample, the only outlier in the observed trend, were not consistent with higher COD removal at higher extent of oxidation. This is may be due to a low starting COD value and analytical variabilities which can have a significant impact on the trend of COD. Furthermore, COD variability can be related to heterogeneity in distribution of cell mass between the samples.

4.4.2. Changes of CNB concentration during biological treatment

The initial decrease in CNB concentrations was likely attributed to losses from sorption to the container. Furthermore, photolysis may be another contributing factor to the initial losses of CNB. Slight changes in CNB concentrations after 2 days of biodegradation are related to analytical and experimental variabilities. A period of relatively stable trend in CNB concentration supports the hypothesis of this study that CNB is not biodegradable under anoxic conditions (Ghattas et al., 2017).

4.4.3. Biodegradation of intermediates during biological treatment

Similar to CNB, chloroaniline and chloronitrophenol showed low to no biodegradability over the 28-day period, while the ring opening products were readily biodegradable. Chloroaniline biodegradation may be possibly enhanced over a longer period of biological treatment. Furthermore, chloronitrophenol was persistent to biological treatment even at the highest electrochemically oxidized samples. Chlorohydroquinone and dihydroxybenzoquinone were partially biodegraded during 7 and 28 days of biological treatment.

According to these results, electrochemical oxidation drastically improved the biodegradability of the generated intermediates during biological treatment. More importantly, using a combined AOP-biological treatment approach, dicarboxylic acids were completely biodegraded under anoxic conditions. Trellu et al. (2016) also reported the removal of dicarboxylic acids during biological treatment of previously oxidized phenanthrene. In conclusion, these results confirm that over a longer time period the ring opening products are readily biodegradable while the ring compounds are substantially more persistent. Therefore, combining AOPs for initial oxidation with subsequent biodegradation of the transformation intermediates is an efficient approach for the treatment of CNB-contaminated groundwater in anoxic environments.

As observed in Figure 14, there is no significant impact of applying higher electric potential on enhancing biodegradation of ring compounds. Furthermore, as initial cost evaluation of the electrochemical oxidation of CNB at applied potentials of 6 V, 10 V, and 14 V demonstrated that 6 V was more cost efficient than 10 V and 14 V. Consequently, in light of biodegradation rates of

CNB and its ring intermediates, the most cost-efficient strategy is to apply advanced oxidation at a low potential until the aromatic ring is completely cleaved.

CHAPTER FIVE. SUMMARY AND CONCLUSIONS

In the present study, it was hypothesized that the coupling of advanced chemical with biological oxidation is more cost-efficient than using advanced oxidation only for the mineralization of chloronitrobenzene. Electrochemical oxidation was used to remove CNB in a synthetic groundwater in a batch reactor. The results showed that CNB was rapidly oxidized at all applied potentials with an extent of degradation of above 98% after 3 hours of electrolysis. The results also demonstrated that the removal rate did not substantially increase when increasing the applied potential from 6 V to 10 V and 14 V as the process was limited by mass transfer rather than •OH radical generation.

In order to track the generated intermediates during the electrochemical oxidation, COD, absorbance, and the average carbon oxidation state of the electrolyzed samples were determined. COD decreased initially up to 180 minutes of electrolysis, then reached plateau at the later stage of electrolysis. Likewise, average carbon oxidation state increased up to 3 hours after electrochemical oxidation had been initiated, then almost leveled off at all applied potentials. For complete mineralization of CNB, the average carbon oxidation state should have reached +4; however, even at the highest potential, full mineralization was not achieved. Additionally, the UV absorbance trend was decreasing in the beginning of electrooxidation, then likewise leveled off. A rapid change in COD, carbon oxidation state, and absorbance at the beginning of electrochemical oxidation is attributed to the initial ring oxidation of CNB. However, the residual COD, carbon oxidation state, and absorbance at the later stage of electrolysis was not consistent with the rapid removal of parent CNB indicating the formation of more persistent intermediates. These intermediates are hardly oxidizable by •OH contributing to a slow degradation at the latter step of electrooxidation and thus partial mineralization of CNB. The COD removal at the latter step of electrochemical oxidation slows substantially due to the generation of persistent intermediates.

A potential pathway for CNB degradation was proposed. CNB oxidation proceeds via two major steps. First, •OH attack on the aromatic ring led to addition and/or substitution reactions on the aromatic ring forming chloroaniline, isomers of chloronitrophenol, isomers of dihydroxychlorobenzene, chlorohydroxybenzoquinone, hydroxybenzoquinone, and dihydroxybenzoquinone. Second, further attack by •OH led to ring cleavage of the aromatic intermediates forming dicarboxylic acids such as muconic, succinic, maleic, fumaric, and malic acids.

In this study, in order to assess the feasibility of extending the results of CNB degradation via electrooxidation to other AOPs, electrochemical oxidation processes were carried out with- and without BQ added to the CNB-contaminated water. BQ is as a chemical scavanger which only reacts with •OH formed during electrochemical oxidation. It was found that CNB degradation was slower when BQ was present due to the consumption of •OH by BQ. Since the degradation mechanism of organic contaminants degradation in other AOPs is also based on •OH, the results of CNB oxidation via electrooxidation are widely applicable to other AOPs.

Apparent anode surface area per order (ASA_O) and electric energy per order (E_{EO}) were used to evaluate the efficiency of the electrochemical oxidation with respect to area of mesh electrodes and energy requirements, respectively. At higher applied potential, less electrode surface was needed to remove one order of magnitude of CNB in the contaminated solution indicating that higher electric potential was more efficient in terms of electrode area. However, differences were minor. In contrast to ASA_O, increasing the electric potential from 6 V to 14 V, more electric energy was required to reduce one order of CNB in the contained water illustrating that a lower applied potential is more efficient with respect to energy consumption. Therefore, at a designated potential, electrode area and energy consumption were inversely correlated.

Cost analyses for the electrochemical oxidation processes were performed with an estimate of \$400 per m^2 electrodes and \$0.10 per kWh electricity. The results showed that the total costs were initially higher at the lower potential attributing to the high capital cost of the electrodes. However, a lower energy consumption at the lower potential resulted in substantially decreased electric energy consumption, making low potentials the most total cost-efficient option after only a few weeks of remedial operation.

The biodegradability tests showed that the dicarboxylate ring opening products were readily biodegradable while the ring compounds including CNB, chloronitrophenol, chlorohydroquinone and dihydroxybenzoquinone were persistent during 7 and 28 days of biological treatment. Furthermore, due to the observed low biodegradation rate of CNB and other ring compounds, the most cost efficient treatment strategy is to apply advanced oxidation at a low potential to cleave the aromatic ring. The generated ring opening products are then easily biodegradable even under anoxic conditions.

In conclusion, the combination of AOPs for initial oxidation with subsequent biodegradation of the transformation intermediates is an efficient approach for the treatment of CNB-contaminated groundwater and may potentially be extended to other aromatic contaminants in anoxic environments.

CHAPTER SIX. RECOMMENDATIONS AND FUTURE WORK

This study was performed in a batch reactor at the laboratory scale under controlled conditions. However, further research is needed to assess the efficiency of the treatment approach under real-world conditions. Testing should be performed first at a pilot scale, and the possibility of implementing the combined treatment under continuous mode should be evaluated. The pilot design should be optimized based on mass transfer, hydrogeological characteristics, and side reactions during the electrochemical treatment (Monteil et al., 2018). Furthermore, the results of this study should be validated using real site waterwater. Unknowns to investigate further also include the effect of contaminant concentration, presence of metal ions, and biogeochemical conditions (Ghattas et al., 2017) on the overall effectiveness of contaminant removal via AOPs.

This study proved that advanced oxidation of CNB followed by biological treatment was effective in removing CNB under idealized conditions at a laboratory scale. Considering the complexity of the natural environment, the efficiency of the combined AOP-biological treatment in removing the persistent organic contaminants may be lower when applying the technique in the field. For instance, hydroxyl radicals generated during the electrochemical oxidation are very reactive and may react with other species at the site, such as natural organic matter or reduced iron, and lower the efficiency of the process. In addition, as the microorganisms are enriched in the lab under optimal growth conditions, the metabolic/cometabolic processes for the degradation of the contaminants may differ under real environmental conditions. Further work is needed to include/assess the environmental variability when implementing the combined approach in the site.

In this study, it was found that energy consumption was the major technology cost while applying electrochemical oxidation for the treatment of CNB. Of key significance for future research is to investigate the feasibility of implementing in situ systems, or solar cells to minimize the cost and improve the sustainability of the process associated with energy usage. Furthermore, in this work, Ti-MMO electrodes were examined for the anodic oxidation of CNB. Further research is needed to evaluate the removal efficiency of organic contaminants using more durable and economical options for electrode materials. Finally, to estimate more accurate treatment cost, a site-specific, comprehensive cost analysis needs to take into account the costs associated with labor, equipment amortization, land leveling, and required substances/machineries during the treatment procedure (Canizares et al., 2009).

In this work, CNB was efficiently minimized through a combined electrochemical-biological strategy. Although the results of this research can be extended to many organic contaminants, the process optimization will be the subject of future research. Similar to this study, the kinetics of contaminant removal and the transformation pathway under environmental conditions for various pollutants should be determined. Furthermore, developing models to predict the removal of various pollutants via the integrated process is an effective tool in economic consideration and efficiency evaluation are the subjects for future studies.

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APPENDIX

CNB control experiment supplemental information

Control experiments were performed to evaluate whether the dectected changes in the concentrations of CNB and its oxidation intermediates were solely due to electrochemical oxidation or if volatilization was also one of the contributing factors to CNB removal. As can be seen in Figure A1, a declining trend of CNB and COD/COD_0 concentrations as well as UV absorption during gas bubble-induced stripping indicates that a portion of CNB removal during the electrochemical oxidation was due to volatilization. The k values for CNB removal corresponding to the electrochemical oxidation and control experiment during 180 minutes were 0.031 min^{-1} and 0.0019 min^{-1}, respectively indicating that 6% of CNB loss is attributed to volatilization and some other mechanisms. Other potential contributing factors to the CNB removal are reduction of CNB to chloroaniline, sorption, and polymerization of intermedites. These results are consistent with findings by Cottrell (2018).

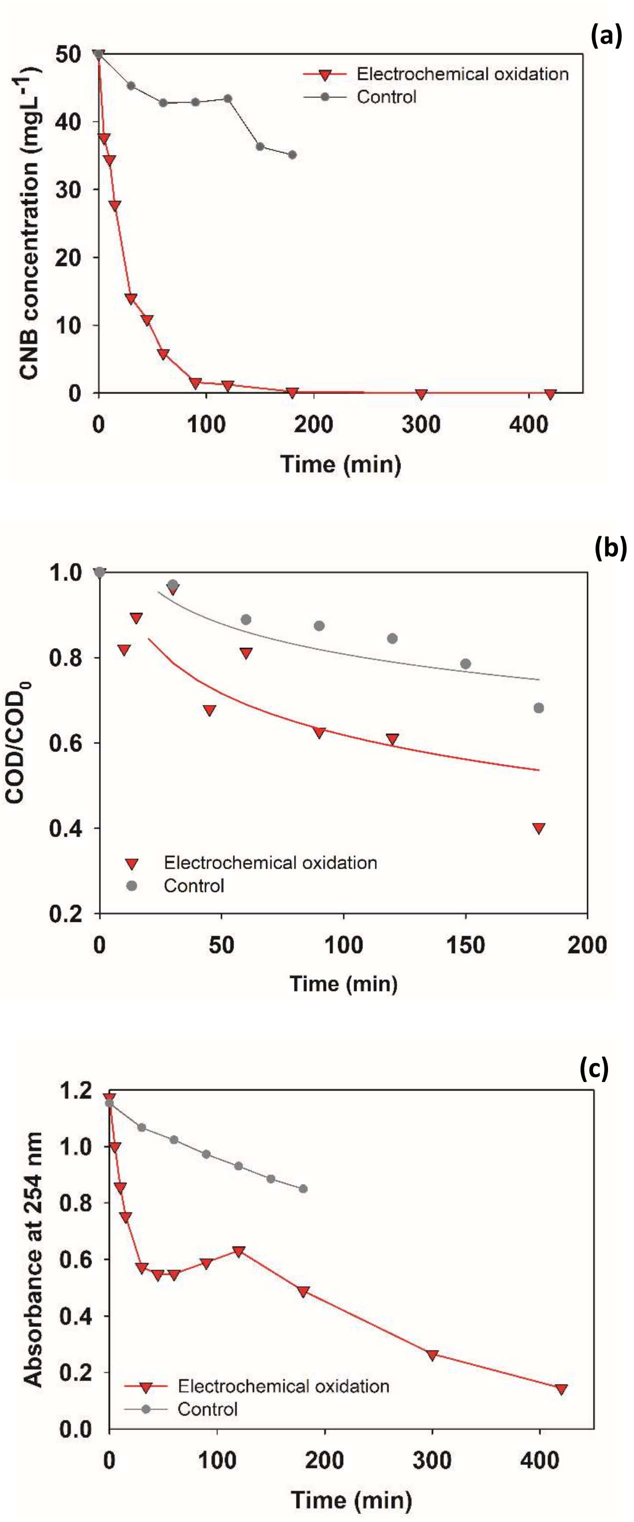

Figure A1. Changes of (a) CNB concentration, (b) COD/COD$_0$, and (c) UV absorption due to gas bubble-induced stripping in CNB control experiment and electrochemical oxidation at 14 V

Intermediate control experiment supplemental information

The control experiment for the CNB oxidation products showed that the removal of COD and UV absorption normalized to starting values for the ring opening compounds were almost negligible after 240 minutes of spagring gas (Figures A2).

A relatively stable trend of COD and absorbance for the intermediates of CNB oxidation during 240 minutes of sparging gas confimed that due to hydrophilic characteristics of ring opening dicarboxylic acids, gas bubble-induced stripping did not contribute to COD reomval and absorbance decrease of the intermediates, therefore, elecrochemical oxidation was the main mechanism for the removal of the ring opening products.

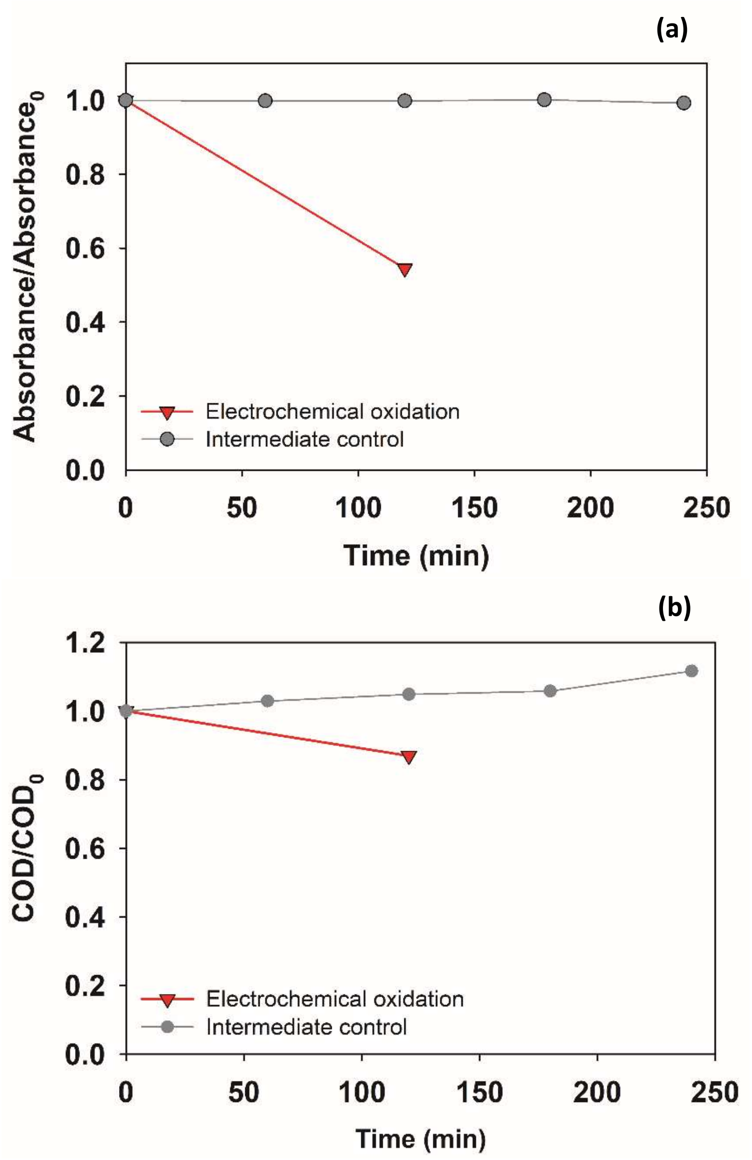

Figure A2. Changes of (a) COD/COD$_0$ degradation and (b) UV absorption/absorption$_0$ due to gas bubble-induced stripping (intermediate control experiment) and electrochemical oxidation at 14 V

Chloroaniline concentration during biological treatment of oxidized CNB supplemental information

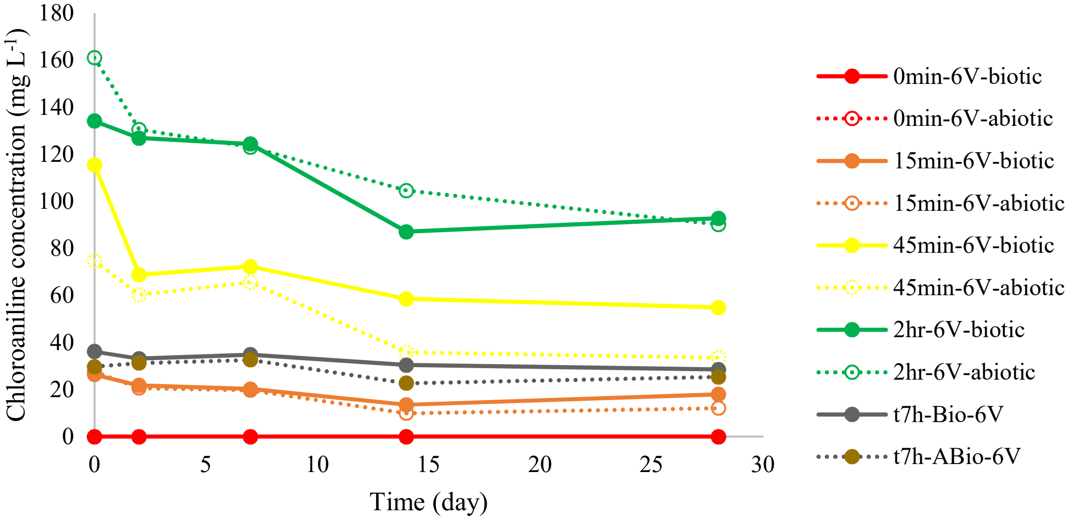

Figure A3. Changes of chloroaniline concentrations during biodegradation of pretreated CNB