Treatment of Municipal Wastewater Reverse Osmosis Concentrate using Biological Activated Carbon based Processes

A thesis submitted in fulfilment of the requirement for the degree of

Doctor of Philosophy (PhD)

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Declaration

I hereby declare that:

- the work presented in this thesis is my own work except where due acknowledgement has been made;
- the work has not been submitted previously, in whole or in part, to qualify for any other academic award;
- the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program.

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(Shovana Pradhan)

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Nomenclature

A	Average surface area in the UV reactor (463.8/cm^2)
C_o	Initial concentration of substrate (mg/L)
C_e	Final concentration of substrate (mg/L)
*E*ED	Electrical energy dose (kWh/m^3)
H’	Shannon diversity index
H	Hectare
K	Reaction rate constant
$HO^{\bullet}$	Hydroxyl Radical
NH_4^+-N	Ammonium nitrogen (mg/L)
NO_3^--N	Nitrate Nitrogen (mg/L)
NO_2^--N	Nitrite Nitrogen (mg/L)
P	Lamp operating power (kW)
t	Time (hours or minutes or seconds)
Em	Fluorescence Emission wavelength (X-axis of EEM) (nm)
Ex	Fluorescence Excitation wavelength (Y-axis of EEM) (nm)
α	Alpha
β	Beta
γ	Gamma
μ	Specific growth rate of biomass (1/d)
μ_{max}	Maximum growth rate of biomass (1/d)
K_s	Saturation coefficient
λ	Wavelength (nm)
Y	Yield coefficient (g/g)
S	Concentration of the limiting substrate or nutrient (g/m^3)

List of abbreviations

AOC	Assimilable organic carbon
AOP	Advanced Oxidation Process
APHA	American Public Health Association
AP I	Aromatic Protein I (from EEM spectra)
AP II	Aromatic Protein II (from EEM spectra)
BAC	Biologically Activated Carbon
BAF	Biological aerated filter
BDD	Boron-doped Diamond
BDOC	Biodegradable Dissolved Organic Carbon
BET	Brunauer –Emmett-Teller
BOD	Biochemical Oxygen Demand
CDI	Capacitive Deionization
COD	Chemical Oxygen Demand
Da	Dalton
DBP	Disinfection By-product
DO	Dissolved oxygen
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
EBCT	Empty Bed Contact Time
EC	Electrical conductivity
EDC	Endocrine Disrupting Compounds
EEM	Excitation Emission Matrix
EfOM	Effluent Organic Matter
EPA	Environmental Protection Agency
FA	Fulvic Acid
FAC	Free Available Chlorine
FRI	Fluorescence Regional Integration
GAC	Granular Activated Carbon
GC	Gas Chromatography
HA	Humic Acid
HAA	Haloacetic Acid
HAN	Haloacetonitriles
HMW	High Molecular Weight

HPLC	High Performance Liquid Chromatography
HS	Humic Substances
H_2O_2	Hydrogen Peroxide
ICP-MS	Inductively coupled plasma mass spectrometry
IDEA	Intermittently Decanted Extended Aeration
LC-OCD	Liquid Chromatography – Organic Carbon Detection
LMW	Low Molecular Weight
MF	Microfiltration
MIEX	Magnetic Ion Exchange Resin
MilliQ	MilliQ water (Organic-free water)
MW	Molecular Weight
MWD	Molecular Weight Distribution
NDMA	N-Nitrosodimethylamine
NF	Nanofiltration
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Unit
O_3	Ozone
PAC	Powdered Activated Carbon
PAH	Polyaromatic hydrocarbon
PAOs	Polyphosphate accumulating organisms
PCA	Principal component analysis
pCBA	ρ-Chlorobenzoic acid
PPCP	Pharmaceuticals and Personal Care Products
RDOC	Refractory Dissolved Organic Carbon
RO	Reverse Osmosis
ROC	Reverse Osmosis Concentrate
SBS	Sodium Bisulphite
SUVA	Specific UV Absorbance
SMP	Soluble Microbial Products
SS	Suspended Solids
TDS	Total Dissolved Solids
THM	Trihalomethane
THMFP	Trihalomethane Formation Potential
TAN	Total Ammonium Nitrogen

TKN	Total Kjeldhal Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TPH	Total petroleum hydrocarbon
UF	Ultrafiltration
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVA$_{254}$	Absorbance measured at 254 nm
UVT	Ultraviolet transmittance
VUV	Vacuum Ultraviolet (radiation at 253.7 and 185 nm)
WHO	World Health Organisation
WW	Wastewater
WWTP	Wastewater Treatment Plant

Summary

Reclamation of municipal wastewater has become an increasingly attractive alternative to supplement the limited fresh water supply all over the world. For this purpose, reverse osmosis (RO) based wastewater reclamation processes are being increasingly used to produce high quality recycled water that is suitable for a wide range of beneficial uses. However, the RO treatment processes generate the waste streams known as RO concentrate (ROC) which contain almost all contaminants present in the RO influent (usually the biologically treated secondary effluent) at elevated concentrations (4-6 times). As these contaminants include many harmful micropollutants and nutrient in addition to the organics recalcitrant to biological treatment, the ROC can pose significant risks to environment and human health if discharged to receiving water environments without proper treatment. The organics present in the ROC are refractory to further biodegradation because these organics are originated from the secondary effluent that has been subjected to extensive secondary treatment.

Biological treatment such as biological activated carbon (BAC) is considered as a potentially cost-effective and environmentally benign option for removing organic matter and nutrients from the ROC via adsorption and biodegradation. Some preliminary studies have investigated the potential of BAC treatment in removing organic matter only from the relatively low salinity ROC (TDS <5 g/L). However, there is generally a lack of study on removing both organic matter and nutrients from different types of ROC (e.g., with high salinity, containing industrial process wastewater etc.), and the microbial communities contributing to the ROC remediation. Therefore, effectiveness of BAC was investigated for organic matter and nutrient removal from different types of ROC using different pre-treatment options in this study. The different types of ROC used in this study vary greatly in salinity levels, ionic concentrations, initial organic and nutrient concentrations. The UV/H_2O_2 was used as pre-treatment of ROC as it improves its biodegradability by degrading recalcitrant organic compounds via oxidation

by hydroxyl radicals and making the ROC more amenable for biodegradation by microorganisms in the BAC system.

The combined UV/H_2O_2-BAC treatment of a ROC, which had extremely high salinity (TDS ~ 16 g/L and initial DOC ~36 mg/L) led to effective reductions in organic matter (57% DOC removal) and nitrogen species at the empty bed contact time of 60 minutes. This was attributed mainly to the generation of simpler organic molecules during the oxidative treatment, which were readily removed by the microorganisms embedded in the BAC column. High total nitrogen removal (60%) was achieved with complete nitrification and partial denitrification taking place in the BAC system without supplementing additional carbon source or aeration. However, total phosphorus removal was very low (15%) due to the high salinity of the ROC (>5 g/L), at which plasmolysis of phosphorus removing bacteria would occur. The treated ROC had similar characteristics to the secondary effluent, which was used as the influent for the reclamation process, in terms of DOC, COD and TN. Moreover, the treated ROC was markedly lower in colour and UVA_{254} compared with the RO influent, confirming that the BAC process could be acclimated to treat the very high salinity municipal wastewater ROC.

The BAC treatment system was found to be robust as the organic matter removal was not greatly affected by varied ROC salinity (TDS 7- 16 g/L). However, total nitrogen removal was higher for the ROC at high salinity (TDS 16 g/L) compared with low (7 g/L) and medium (10 g/L) salinity ROC as a result of the considerably higher denitrification at high salinity (39% cf. 23% and 27% at low and medium salinity, respectively). This was attributed to prevalence of diversified halotolerant bacteria which were mostly responsible for denitrification in the BAC treatment system. The major bacterial communities identified in the BAC treatment system were *Bacillus* sp., *Pseudomonas* sp. and *Rhodococcus* sp., as revealed by PCR-DGGE and sequencing, which were able to remove organic matter and the nitrogen species.

The combined UV/H_2O_2-BAC treatment of another type of ROC (TDS 4.5 g/L and initial DOC ~52 mg/L) which was derived from a municipal wastewater containing a significant proportion of petrochemical wastes led to overall 58% DOC removal. The combined treatment of this ROC led to higher phosphorus removal (60%) and low (15%) total nitrogen removal, implying that nutrient removal could be greatly dependent on salinity level of ROC and the groups of bacteria present in the BAC system. The presence of *Micrococcus* sp. *Ralstonia* sp., *Agrobacterium* sp., S*phingopyxis* sp. and *Pseudomonas* sp, which were closely related to phosphorus accumulating organisms (PAOs) in BAC treatment system, were considered to be responsible for phosphorus removal. Furthermore, the BAC treatment system effectively removed the total petroleum hydrocarbon (TPH) from the ROC, thus indicating its good potential for removing petrochemical compounds of interest.

For the convenience of comparison, aforementioned two types of ROC were denoted as ROC A (TDS 16 g/L and initial DOC 36 mg/L) and ROC B (TDS 4.5 g/L and initial DOC 52 mg/L). These two ROC types were different in terms of inorganics (as indicated by the TDS concentration) and organics as indicated by DOC concentration and Liquid Chromatography-Organic Carbon Detection (LC-OCD) analysis. The UV/H_2O_2-BAC treatment of two types of ROC led to comparable DOC reduction (58%) due to considerable reduction of high molecular weight compounds (HA-like) and generating low molecular weight compounds during oxidation, which were more amenable to biodegradation in the BAC treatment. The COD removal was higher (59%) for the ROC B compared with ROC A. It was found that nitrification was consistently higher as more than 90% ammonium nitrogen removal was achieved for both ROC regardless of different inorganic and organic compositions of both

ROC. Total nitrogen and phosphorus removals were mainly dependent on the existence of different bacterial communities in the two BAC systems treating different ROC streams.

The impact of other pre-treatments including coagulation and sequential coagulation-UV/H_2O_2 were also evaluated for their capabilities in organic matter and nutrient removal from the aforementioned two types of ROC (ROC A and ROC B). Coagulation pre-treatment achieved > 90% phosphorus removal regardless of the type of the ROC. For the coagulation-BAC treatment, organic matter removal was greater for the ROC A compared with ROC B. This was attributed to the significant removal of higher molecular weight organic compounds due to the formation of more rigid flocs in the higher salinity water environment, which led to better settleability of organic matter. The sequential coagulation-UV/H_2O_2-BAC treatment on the two types of ROC markedly improved the overall organic matter removal, with a comparable reduction in DOC (62-67%) due to reduced organic load by individual pre-treatment and better oxidation of the remaining organics in coagulated ROC, and consequently more effective biodegradation occurred in the BAC treatment. Ammonium nitrogen removal (90%) was consistently higher for the two types of ROC when using coagulation-BAC and sequential coagulation-UV/H_2O_2-BAC combinations.

This study demonstrated that the BAC based processes are effective and resilient in removing organic matter and nutrients from the municipal wastewater ROC of significantly different natures and water quality characteristics. Since the BAC treatment could lead to significant reductions in chemical consumption (such as H_2O_2 and coagulant) and energy cost (such as UV light), it is potentially a feasible option for reducing the environmental and health risks associated with the ROC on disposal or reuse. However, the technological feasibility of the processes should be assessed further with larger scale trials, and more detailed cost analyses should be conducted to justify their full-scale applications.

Chapter 1 - Introduction

Wastewater reuse is increasing day by day for conservation of limited fresh water resources and to overcome the water scarcity around the world (Shon et al., 2006). According to Schouppe (2010), by 2030 worldwide water supply to demand gap will be 40% and by 2050, around 60% of the world's population could experience severe water shortages, with 33% thought to be already under water shortage stress. In recent decades, membrane filtration processes have emerged as one of the most promising technologies for reclaiming domestic and industrial wastewater to meet the increasing demands on water supply.

Traditionally, water or wastewater treatment processes include physical separation techniques for particle removal; biological and chemical treatments to remove suspended solids, organic matter and dissolved pollutants including toxins; and evaporative techniques and other physical and mechanical methods. Membrane filtration processes replace or supplement these techniques through selectively permeable barriers, with pores sized to permit the passage of water molecules but small enough to retain a wide ranges of particulate and dissolved compounds depending on their nature (Schouppe, 2010).

Membrane filtration processes can be classified according to the membrane pore size. Microfiltration (MF) membranes have pores ranging from 0.1-2 μm and operate at pressures below 5 bar for removing suspended solids, bacteria, protozoa from feed streams (Jacangelo and Buckley, 1996). Ultrafiltration (UF) has been used to remove suspended particles, turbidity, microorganisms, colloids, and compounds with a molecular weight of 5–100 kDa at the operating pressure up to 8 bar (Khan et al., 2009). High pressure membranes such as nanofiltration (NF) and reverse osmosis (RO) (operating pressure up to 100 bar) have been used to remove trace organics such as emerging pollutants and dissolved solids including ions present in water and wastewater for reclamation purposes (Stephenson et al., 2000). Of the pressure driven membrane processes, RO is being increasingly used for desalting brackish water and seawater, and the treatment of drinking water and wastewater (Fritzmann et al., 2007). Besides the removal of salts and ions, RO membranes can effectively remove wide varieties of organic compounds in feedwater, such as micropollutants and biological materials (bacteria, viruses, oocysts, cell fragments). A study by Foussereau et al. (2003) found that out of the 100 full scale wastewater reclamation facilities using membrane technology worldwide, 97% of those plants used RO based processes to produce high purity water from wastewater for drinking water production, groundwater replenishment, irrigation or industrial applications.

The RO-based wastewater reclamation processes generate clean water that passes through the membranes, known as permeate and a waste stream, known as RO concentrate (ROC) (also known as membrane reject or brine). In municipal wastewater reclamation, the RO-based plants commonly generate the ROC accounting for 15-25% of the volume of the secondary effluent (as RO influent), resulting in high concentrations of organic and inorganic contaminants and nutrients in the waste streams (Shon et al., 2006). The characteristics of the ROC depend on feedwater characteristics, pre-treatment efficiency, membrane system employed, water recovery and chemical additives used (Van der Bruggen et al., 2003). Currently, the most common practice for managing municipal wastewater ROC is to discharge it into water ways (e.g., ocean, rivers) with or without dilution. Great concerns have been raised by the general public and environment authorities about the long term environmental and health risks associated with this management option, particularly for the confined receiving water environment such as bays. The organic contaminants in municipal wastewater ROC commonly include recalcitrant chemicals such as some pharmaceuticals and personal care products (PPCPs), pesticides and herbicides, endocrine disrupting compounds (EDCs), disinfection by products (DBPs) and other organic species, many of which are toxic and bio-accumulative (Dialynas et al., 2008, Krasner et al., 2009). Depending on nutrient removal at the wastewater reclamation treatment plant, the concentrate may contain considerable amounts of nutrients including phosphorus and nitrogen. Some nitrogen is likely to be present in the form of ammonia, which is toxic to many aquatic organisms (Khan et al., 2009). The continuous discharge of ROC to receiving water environment can contribute to massive algal growth leading to subsequent deoxygenation with devastating consequences to sensitive water bodies (Davis and Koop, 2006). As such, there is a growing need to explore cost-effective treatment options for the ROC for reducing its environmental and health risks on disposal or reuse.

Several treatment options including UV-based advanced oxidation processes (AOPs) such as UV/H_2O_2, ozonation, Fenton reaction, coagulation, biological process and the combination of these techniques have been investigated for the removal of organic matter from the ROC. The hydroxyl radical ($HO^•$) produced by AOPs is non–selective in oxidising organic molecules to CO_2 and water (Westerhoff et al., 2009). The UV/H_2O_2 process has been widely used for water and wastewater purification (Parsons, 2004). The first full scale UV/H_2O_2 process for drinking water treatment was commissioned in 1998 in Salt Lake City, USA (Sarathy and Mohseni, 2006), and since then the UV/H_2O_2 process has found more commercial applications for drinking water treatment and water reuse compared with other UV-based AOPs such as UV/TiO_2 and UV/O_3 (Figure 1.1). It has been reported that there were 20 full scale UV/H_2O_2 installations for municipal drinking water production or municipal water reuse worldwide in 2012 (Audenaert, 2012). The number of UV/H_2O_2 installations is expected to grow rapidly worldwide as a part of advanced wastewater treatment plants for the removal of endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) (Malley, 2010).

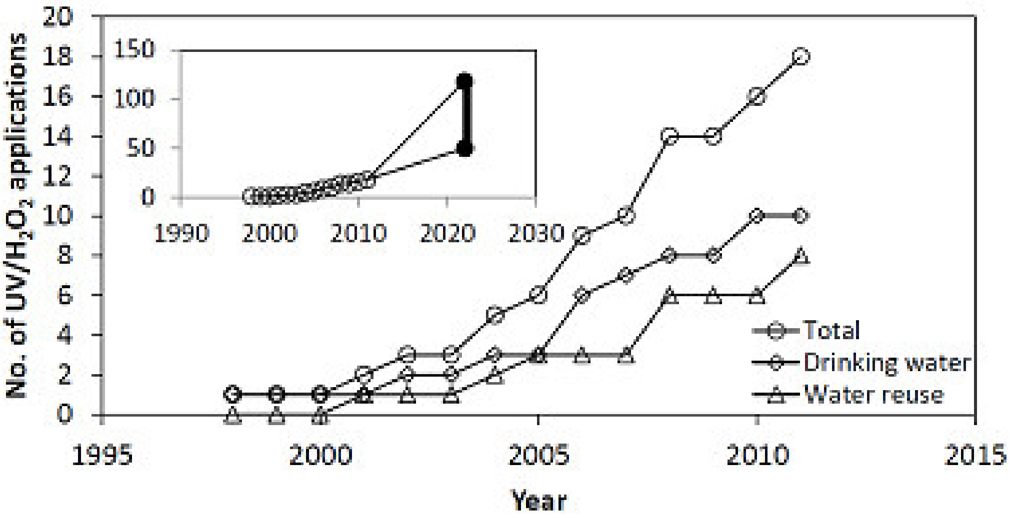


Figure 1.1 Number of UV/H_2O_2 installations worldwide for water treatment and water reuse since 2000. Inset: prediction for next decade (between 50 and 100 new installations) based on Malley (2010) adapted from Audenaert (2012).

Coagulation processes using aluminium (Al) and iron (Fe) based coagulants have also been widely used in water and wastewater treatment for the removal of suspended, colloidal and dissolved matter. Coagulation is commonly employed for the removal of natural organic matter (NOM) from drinking water due to its cost-effectiveness (USEPA, 1998).

Similarly, several biological treatment processes such as activated sludge, membrane bioreactor (MBR), trickling filter, upflow anaerobic sludge blanket (UASB), biological activated carbon, etc., have been used to treat wastewater to remove organic carbon as well as nutrients, especially, nitrogen and phosphorus. Of the several biological treatment processes, biological activated carbon (BAC) filtration has been increasingly used to treat wastewater because of its simplicity in operation and cost effectiveness. BAC process was developed on the basis of activated carbon technology, which uses the synergistic effect of adsorption by activated carbon and biodegradation of organic matter by microbes attached on the carbon media (Ying and Weber Jr, 1979). The activated carbon has a high specific surface area and porous structure for physico-chemical adsorption of organic matter. With time, microorganisms grow and reproduce on the activated carbon and finally form the BAC (Weber et al., 1978), and the biofilm containing bacteria in the macropores and their exoenzymes in the micropores enable biodegradation (Scholz and Martin, 1997). In general, BAC is a cost effective and environmentally friendly treatment process in terms of small footprint, low energy consumption and no addition of chemicals (Walker and Weatherley, 1999).

BAC filters have been used for a long time for drinking water treatment to remove organic compounds (Urfer et al., 1997), as well as micropollutants, halogenated hydrocarbons and taste and odour compounds (Velten et al., 2007). BAC process was firstly proposed in 1978 by G.W. Miller from US and R.G.Rice from Switzerland for potable water treatment as reported by Jin et al. (2013). Since, 1988, BAC process has become a major process in advanced water and wastewater treatment in most developed countries such as USA, Japan, Holland, Switzerland, etc. BAC processes, usually following oxidation treatment, have been proven as effective in removing natural organic matter, oxidation transformation products, disinfection by-products, taste and odour causing compounds such as geosmin and 2-methylisoborneol from water and wastewater (Simpson, 2008).

In recent years, BAC has been increasingly investigated for treating municipal wastewater ROC. The BAC is usually coupled with pre-treatment(s) such as UV/H_2O_2, ozonation, coagulation using aluminium and iron based salts and their combinations (Westerhoff et al., 2009, Lee et al., 2009a, Bagastyo et al., 2011, Lu et al., 2013). Due to the recalcitrant nature of the organic matter in the ROC, several oxidative treatment methods and coagulation have been studied as a means of removing the organic content and/or enhancing the biodegradability of the organic content. Lee et al. (2009a) found that the coupling of a BAC column with ozone pre-oxidation enhanced the organic removal efficiency by 3 times that of BAC alone at an empty bed contact time (EBCT) of 60 min, with 70% DOC removed from the ROC (TDS 1.2 g/L, EC 2 mS/cm). In a more recent study, Lu et al. (2013) observed that the integrated treatment of UV/H_2O_2 followed by BAC removed 60% DOC, 64% colour and 78% UV absorbance at 254 nm (UVA_{254}) from a ROC (TDS 10 g/L, EC 13.5 mS/cm). However, the removal efficiencies for nutrients such as total nitrogen (TN) and total phosphorus (TP) were fairly low, with only 23% of TN and 7% of TP removed. In an earlier study, Ng et al. (2008) found that BAC treatment following capacitive deionization could achieve a higher TN removal (91%) from a ROC with relatively low salinity (EC 2 mS/cm). The biological removal of nitrogen at elevated salt concentration can be challenging due to the sensitivity of nitrogen-removing microorganisms to high salt conditions, along with other environmental conditions including temperature, DO concentration, pH, ammonia concentration, heavy metals and C/N ratio (Okabe et al., 1996). However, it is possible for the microorganisms to be acclimated to high salinity environments. To date, most of the published work has been on ROC with a TDS lower than 5 g/L. The potential of BAC treatment process for removing organic content and nutrients from ROC from different sources and different salinity with regard to the existence of different microbial communities has not been reported. In this thesis, UV/H_2O_2 coupled with biological treatment has been investigated for two different types of ROC which were significantly different in physical-chemical characteristics (e.g., organic compositions, salinity) for reducing organic content and nutrients, along with microbial community studies. Also, potentials of coagulation and sequential coagulation, UV/H_2O_2 and biological treatment for the two different ROC streams have also been investigated.

1.1 Aim of the study and research questions:

This study was aimed to gain better understandings about the BAC based treatment processes in treating the ROC from various sources and with different physical-chemical properties (e.g., salinity), with a view to enhancing the treatment efficiency for organic matter and nitrogen removal. This study was conducted to address the following research questions:

a) What are the effects of operating conditions such as empty bed contact time (EBCT) on the treatment efficiency of BAC in terms of organic matter and nitrogen for higher salinity ROC?

b) After UV/H_2O_2 pre-treatment, how does residual H_2O_2 impact on organic matter and nutrient removals for a high salinity municipal ROC by BAC?

c) How and why does salinity of municipal ROC (low, medium and high) affect organic matter and nutrient removals by BAC system, and the microbial communities in BAC media?

d) How does the presence of a significant amount of petroleum process waste in a ROC influence the BAC performance and the major bacterial communities present in the BAC system?

e) What are the effects of different treatment options such as UV/H_2O_2, coagulation and sequential coagulation-UV/H_2O_2 followed by BAC on organic matter and nutrient removal using two different types of ROC different salinity and origin?

1.2 Thesis Structure

Chapter 2 is Literature Review which provides a comprehensive and in depth review of the various treatment strategies reported in the previous studies for treating municipal wastewater ROC, and identifies the research gaps and suggestions to address them.

Chapter 3 covers the materials and methodologies used to carry out this research.

Chapter 4 reports on the investigation of the effect of UV/H_2O_2-BAC process for removing organic matter and nutrient content from a high salinity municipal wastewater ROC. This chapter provides information on impact of contact time and residual H_2O_2 on the BAC treatment performance. Some of the findings reported in this chapter were published in *Chemosphere* as a research paper entitled 'Removing organic and nitrogen content from a highly saline municipal wastewater reverse osmosis concentrate by UV/H_2O_2-BAC treatment' (Chemosphere, 136, 198-203).

The impact of varied salinity (low, medium and high) of municipal ROC on the UV/H_2O_2-BAC treatment for organic matter and nitrogen removal was then investigated and reported in Chapter 5. In this chapter, the characteristics of the microbial communities at different salinity are also provided, with a view to providing more insights into the BAC treatment. A paper based on this study was published in *Water Research*, entitled 'Impact of salinity on organic matter and nitrogen removal from a municipal wastewater RO concentrate using biologically activated carbon coupled with UV/H_2O_2' (Water Research, 94, 103-110).

Chapter 6 includes the details of an investigation of the potential of using BAC to treat a ROC with low salinity and containing petroleum organic contaminants. In this chapter, the study of the bacterial communities within the BAC system was also reported.

A comparison of the UV/H_2O_2 -BAC treatment of the two types of ROC used in the studies reported in Chapters 4 and 6 can be found in Chapter 7. The process efficiency was compared by taking into account the impact of the organic and inorganic characteristics of the two different ROC streams.

In Chapter 8, the impacts of coagulation and sequential coagulation-UV/H_2O_2 treatments on the BAC systems were studied for the two types of ROC reported in Chapters 4 and 6. In this chapter, the most cost effective treatment combination for safe reuse and disposal of the ROC is discussed.

The overall concluding remarks for this study and the recommendations for further work are made in Chapter 9.

Chapter 2 - Literature Review

This chapter provides an overview of the existing literature on the management of municipal wastewater ROC from wastewater reclamation facilities, and the various treatment options for safe reuse and disposal of the ROC. The focus of this review is on the potentially cost-effective biological activated carbon (BAC) based treatment processes.

2.1 Introduction

Membrane based separation processes are becoming popular in the treatment and reclamation of municipal wastewater as they are reliable and can produce high quality effluent (Wintgens et al., 2005, Comerton et al., 2005). Reverse Osmosis (RO) is a membrane technique which can be used to produce high quality recycled water from wastewater due to its high capability in reducing total dissolved solids, heavy metals, organic pollutants, viruses, bacteria, and other dissolved contaminants. The reclaimed water has a wide range of applications such as irrigation, groundwater replenishment, indirect potable use and industrial purposes.

In 2003, Foussereau et al. reported that more than 100 full scale wastewater reclamation facilities worldwide use membrane technology for tertiary treatment. Some of the major municipal wastewater reclamation facilities using RO-based processes include the Sulabaiya reclamation facility in Kuwait (375 megalitres per day (MLD)), Orange County, USA (328 MLD), Changi (232 MLD) and Ulu Pandan in Singapore (197 MLD) (Raffin et al., 2013). In Australia, the Western Water Recycled Water Scheme consisting of three advanced water treatment plants has recycling capacity of 232 MLD (Solley et al., 2010). According to Cumming (2014), the global market of RO technology continues to grow and is predicted to reach $ 8.1 billion by 2018 in the production of useable and potable water.

RO is a pressure-driven process to produce clean water through physical separation of the contaminants in the feed stream using thin-film membranes. In municipal wastewater reclamation, it is impractical to achieve 100% water recovery due to the limiting factors such as membrane fouling and scaling. Practical water recovery is commonly in the range of 70-90%, which means 10-30% of input stream will be generated as membrane reject streams known as RO concentrate (ROC) or brine. Solley et al. (2010) reported that the contaminants present in ROC could be 6-7 times more concentrated than those in the RO feed. The characteristics of the ROC may depend on the membrane used, feed water and permeate quality, pre-treatment method, and membrane cleaning and storage procedures (Ahmed et al., 2000, Chelme-Ayala et al., 2009).

2.2 ROC disposal options

The most common practice for disposal of municipal ROC is direct discharge to surface water with or without dilution. However, deep well injection, land application, evaporation ponds, and wastewater evaporators are also used. The different disposal options and their advantages and disadvantages are summarised in Table 2.1 (Ahmed et al., 2001, Arnal et al., 2005, Chelme-Ayala et al., 2009, Khan et al., 2009, Pérez-González et al., 2012).

The conventional ways to dispose municipal wastewater ROC are direct surface discharge and sewer discharge (Khan et al., 2009). Sewer disposal is mostly only suitable for small plants discharging into large capacity sewage treatment plants due to the detrimental effects of high TDS concentration of ROC on the biological treatment processes (Voutchkov, 2005). In order to reduce the volume of ROC concentrate and disposal costs, several zero liquid discharge (ZLD) technologies such as wastewater evaporators have been used in the past, mostly for industrial wastewater and concentrate from desalination plants but these technologies have been uneconomical options at large scale (Pérez-González et al., 2012). The conventional ROC disposal options have their own limitations as discussed in Table 2.1, emphasizing the need for new technical approaches that could minimise the environmental impacts and economically profitable reuse (Khan et al., 2009).

Table 2.1 Disposal options for municipal wastewater ROC

Options	**Process**	**Requirement**	**Advantages**	**Disadvantages**
Surface water discharge	Discharge to seawater, ocean, lakes, lagoons	• Permit required • Treatment of the concentrate may be required prior to disposal	Easy method for brine disposal	Eutrophication, pH value variations, accumulation of heavy metals as well as sterilizing properties of disinfectants (Meerganz von Medeazza, 2005, Voutchkov, 2005)
Disposal into sewers	Discharge concentrate to local wastewater treatment plant via sewer system or direct pipeline	Local permit required	Easy and less expensive method for brine disposal	• Wastewater effluent quality changes after addition of concentrate • Sewer capacity and wastewater treatment plant capacity in future needs to be addressed • Addition of concentrate into the wastewater effluent may reduce temperature (Chelme-Ayala et al., 2009).
Deep well injection	ROC is injected into a porous subsurface rock formation, ranging in depth from 300-2400m	Requires a permit for well operation and underground injection with the appropriate state agency (Kenna and Zander, 2001)	Ability to prevent the movement of concentrates into or between underground aquifer and /or drinking water sources	• Expensive, especially for drilling and well development • Thorough study of hydrogeological condition of the aquifer is required to isolate the saline concentrate from fresh water of other aquifers.

Land application	Spray irrigation mode is used on salt tolerant crops, spray on lawns, parks, or golf courses	Usually requires blending to decrease the salinity to an acceptable limit	• Cheap and easy option for waste conservation, preservation and enlargement of green belts and open spaces • No permits required (Kenna and Zander, 2001)	• Possibly impair the soil's physical and chemical properties • Increase secondary salinity • Reduction of soil permeability • Reduction of crop yield
Evaporation ponds	Usually man made shallow ponds are used to evaporate the concentrate leaving salts to accumulate; Lining is provided to prevent the leakage	-----	• Suitable for inland RO plants as well as for arid and semi-arid areas. • No marine impacts	• Land intensive • Lining of evaporation ponds are very expensive • Possible leakage of lining and pollute the underground aquifer • Periodic removal of sludge is required • Feasible in dry climate

2.3 General characteristics of ROC, contaminants and potential environmental impacts

As mentioned previously, the characteristics of ROC vary significantly depending on influent sources, type of pre-treatments, effluent water quality, and the nature of chemicals used. In RO membrane process, antiscalants such as polyacrylic acid, polymaleic acid and sodium hexametaphosphate are used to prevent the formation of scale; lime and sulfuric acid used are for pH corrections; and in some cases biocides are used to avoid the formation of biofilm on membrane surface (Chelme-Ayala et al., 2009, Khan et al., 2009). The addition of antiscalants, acids or bases and biocides may influence the chemical equilibrium of the dissolved constituents present in ROC, thereby affecting overall characteristics of ROC (Ahmed et al., 2000). The characteristics of municipal ROC reported in various studies are compared in Table 2.2. The wastewater concentrates generally contain hardness, heavy metals, high molecular weight organics, microorganisms and often sulphide gas (Tchobanoglous et al., 2003). Depending on the degree of nutrient removal at the sewage treatment plant, the ROC can contain nutrients including nitrogen and phosphorus.

As can be seen from Table 2.2, the characteristics of ROC used in various studies varied hugely in terms of salinity and organic matter. The salinity of municipal ROC ranged from low (1,276 mg /L) to high (17,400 mg /L) in terms of TDS. The study conducted by Umar et al. (2014) & (2016) used extremely high salinity municipal ROC (salinity > 10,000 mg /L) compared to ROC used in other studies. Due to its high salt content, the electrical conductivity of ROC is also generally high, and any pH adjustment will increase it (Lee et al., 2009b). According to Voutchkov (2005), high TDS of ROC exceeding 3 g/L has detrimental effects of the biological treatment processes. In some studies, even though TDS level of ROC was fairly low, DOC level was high (Radjenovic et al., 2011), indicating that ROC differs in concentration of contaminants in feed water.

Table 2.2 Characteristics of ROC used in previous studies

pH	**DOC (mg/L)**	**COD (mg/L)**	**TOC (mg/L)**	**TN (mg/L)**	**NH_4^+-N (mg/L)**	**TP (mg/L)**	**Colour (Pt.Co)**	**UVA_{254} (1/cm)**	**TDS (mg/L)**	**Conductivity (µS/cm)**	**Reference**
7.4	32	101	--	--	--		157	0.63	16,587	23,000	Umar et al., 2016
6.9 ±0.5	23.7 ±3.8	61.5±7.9	--	49±22.2	--	---	----	40.5 ±7.1	---	----	Justo et al., 2015
7.4-8.3	32.5-37.5	105-155	--	--	--		137-158	0.6-0.68	16,140-17,400	22,300-27,500	Umar et al., 2014
--	18-46	--	--	53.7-84.8	5.5-14	11.2-24.1	---	0.36-0.45	3210-4980	5500	Sun et al., 2014
8.2	47.5	220		24.5	--	33.1	216	0.80	10,020	--	Lu et al., 2013
8.3	--	77	27.6	--	--	--	--	0.595	--	5960	Vendramel et al., 2013
8.5	~21	65, 67	---	--	--	--	55, 88	0.43	1685	4445-11,160	Liu et al., 2012
7.8-8.1	42-62	147-168	--	6.2-13 as TKN	--	---	101-228	--	---	7300-12,760	Bagastyo et al., 2011
7.5-7.7	57	--	--	--	--	--	--	0.91-1.32	--	3970-4250	Radjenovic et al., 2011
6.9 ± 0.2	--	60±5	18 ± 2	10 ± 3 as TKN	--	--	144 ± 10	---	1129 ± 40	1705 ± 21	Zhou et al., 2011a
7.15	--	64.6	18.4	--	--	--	89	0.44	1218	1972	Lee et al., 2009a
7.5 ± 0.2			24.5 ± 5.0						1276 ± 166	1990 ± 259	Lee et al., 2009b
7	40	138	--	--	--	---	--	--	5560	10,000	Westerhoff et al., 2009
7.72 ± 0.53	--	--	31.1 ± 3.4	--	--	--	109 ± 1	--	--	2025 ± 151	Ng et al., 2008
7.91,8.21,8.74	--	151, 171, 218	--	34 as TKN	--	--	--	--	--	3990, 5060, 5290	Van Hege et al., 2004

The municipal ROC contains salts, dissolved nutrients and effluent organic matter (EfOM) (Al-Rifai et al., 2007, Westerhoff et al., 2009). EfOM is a combination of natural organic matter (NOM), soluble microbial products (SMPs), synthetic organic compounds used during domestic use and disinfection by-products generated during disinfection processes of wastewater treatment and trace harmful chemicals (Shon et al., 2006). EfOM is a complex heterogeneous mixture of organic compounds from the chemical and biological degradation of plant and animal residues, comprising of compounds with different properties and molecular sizes ranging from small molecules to macromolecules and large particles (Levine et al., 1985). EfOM usually contains emerging contaminants that can be classified as pharmaceuticals and personal care products (e.g., drugs, sunscreens, cosmetics), persistent organic contaminants (e.g., pesticides), endocrine disrupting compounds (EDCs) (e.g., estrogens), disinfection by-products and nanomaterials (e.g., nano-scale titania) (Meffe and de Bustamante, 2014). Some disinfection by-products including trihalomethanes (THMs), haloacetic acids, haloacetonitriles and N-nitrosodimethylamine (NDMA) may be formed during pre-chlorination of RO feed and are likely rejected and appear in ROC (Megan et al., 2008, Agus et al., 2009, Linge et al., 2013). Some organics in the EfOM such as humic acids and fulvic acids are of high molecular weight ranging from 10^3 to 10^6 Da and these compounds are recalcitrant to biological treatment. EfOM affects chemicals and biological processes as they are the precursor for disinfection by-product formation, increase the potential for microbial re-growth in the distribution system, increase coagulant and oxidant demands (Shon et al., 2006). Hence, the removal of EfOM is very important to avoid the potential risks to environment.

ROC is rich in several inorganic cations and anions, certain metals and heavy metals. Depending on the nutrient removal in the secondary treatment, ROC can also be rich in nutrients (nitrogen and phosphorus). The concentration of phosphate in ROC can be as high as 40 mg/L for a feed phosphate concentration of 5 mg/L (Kumar et al., 2007). These anthropogenic pollutants, metals and potentially carcinogenic volatile organic compounds may be present at levels several times higher than those in the wastewater treatment plant influents (Khan et al., 2009).

The disposal of untreated ROC can potentially cause severe damages to marine, freshwater and terrestrial environments. The untreated ROC adversely affects the water and sediment quality of receiving water bodies, impairs marine life as well as functioning and intactness of coastal ecosystems (Lattemann and Höpner, 2008). Many marine organisms are highly sensitive to variations in salinity, as cell dehydration occurs with increased salinity and as salinity rises, the number and diversity of species falls (Khan et al., 2009). High salinity also cause ion imbalance –triggered toxicity to aquatic flora and fauna (Khan et al., 2009).

It can also increase hardness of the underground water and the risk of soil salinization (accumulation of sodium chloride) (Mohamed et al., 2005). High sodium concentrations in soil can affect physical condition of the soil such as water logging, formation of crusts and reduce soil permeability causing great reduction in infiltration rate, thereby preventing plants or crops from accessing enough water for good growth (Ayers and Westcot, 1985). ROC containing considerable amounts of nitrogen and phosphorus can lead to eutrophication in receiving water bodies and hence algal blooms (Davis and Koop, 2006). The continuous discharge of ROC containing high concentration of nitrogen in the form of ammonium causes toxicity to many aquatic organisms (Walker et al., 2007).

There is an increasing concern from the general public and environmental authorities with the negative effects of ROC on its disposal to receiving environment, particularly confined water bodies such as bays and lakes (USBR, 2003). As such, several treatment schemes aimed at reducing organic and nutrient content of the ROC have been investigated in order to develop cost-beneficial and environment friendly strategies for reducing the environmental and health risks on the disposal or reuse of the ROC.

2.4 Treatment of municipal ROC

The treatment target for the ROC would depend on the end use. Different treatment options that have been investigated in previous studies for the treatment of municipal ROC are discussed in the following sections.

2.4.1 Physico-chemical treatment of municipal wastewater ROC

2.4.1.1 Coagulation

Coagulation is a common treatment option in water and wastewater industries to remove high molecular weight organic compounds. The coagulation process is comprised of four mechanisms: (1) compression of the diffuse layer (van der Waals interaction); (2) adsorption to produce charge neutralization (destabilization), (3) enmeshment in a precipitate when a high dosage of coagulant is used, leading to sweep coagulation; and (4) adsorption to permit inter-particle bridging (complex between particle and polymer with synthetic organic coagulant) (Vigneswaran and Visvanathan, 1995). Aluminium based and iron based coagulants are widely used for coagulation processes, and commonly perform most effectively at pH 5-6 (Sharp et al., 2006, Duan et al., 2003).

Dialynas et al. (2008) used alum (2 mM) and ferric chloride (0.4 mM) to treat a municipal wastewater ROC generated from RO processing of the effluent from a membrane bioreactor. In their study, DOC removal (initial DOC 12.3 mg/L) was 42% and 52% with the respective coagulants. Coagulation using alum (1.5 mM) and ferric chloride (1.48 mM) was also studied by Bagastyo et al. (2011) for treating two ROC samples obtained from two full-scale MF/RO plants in South East Queensland, Australia. The study found that ferric chloride gave better removal efficiencies in colour (74-79%) and COD (41-49%) on both types of ROC, whereas alum was found to be more effective in removing DOC and DON (52% and 30%) compared with ferric chloride (34% and 28%). The examination of molecular weight distribution showed that the coagulation by ferric chloride or alum is capable of removing high to medium MW colour causing compounds (3-10 kDa), which are mostly contributed by hydrophobic humic acids. However, a bench scale study by Zhou et al. (2011a) achieved much less removal of DOC from ROC (26%) with ferric chloride (1.0 mM), which was likely due to the different properties of the studied ROC. It was suggested that the lower removal could be attributed to the presence of higher amounts of soluble organics of low MW in the ROC (Bagastyo et al., 2011, Westerhoff et al., 2009). Recently, Umar et al. (2016a) used two aluminium based coagulants (alum and aluminium chlorohydrate (ACH)) and two ferric based coagulants (ferric chloride and ferric sulphate) to treat a high salinity municipal ROC (TDS 16,500 mg/L and initial DOC 32 mg/L) at pH 5. Alum reduced 23%-32% DOC, 69% colour and 42% UVA_{254} at 1 mM Al^{3+}. The reduction of organic matter was lesser for ACH. The iron based coagulants (1 mM Fe^{3+}) improved the organic matter removal significantly, with 40-42% DOC, 80% colour and 53% UVA_{254} removed. The study demonstrated that iron based coagulants, especially ferric chloride, removed a greater proportion of humics than the aluminium based coagulants.

2.4.1.2 Coagulation as pre-treatment

Some studies have been carried out to use coagulation as a pre-treatment followed by advanced oxidation processes to treat municipal wastewater ROC, however, the combined treatment has not been studied extensively. Zhou et al. (2011b) obtained 41-54% DOC removal (initial TOC 18 mg/L) with the combinations of $FeCl_3$ followed by UVC/TiO_2; $FeCl_3$ followed by UVA/TiO_2, and $FeCl_3$ followed by UVA/H_2O_2. Recently, Umar et al. (2016a) also used coagulation as pre-treatment of the high salinity ROC with alum, ACH, ferric chloride and ferric sulphate prior to UV/H_2O_2 treatment. When the coagulated ROC was subjected to

UV/H_2O_2 treatment (3 mM H_2O_2), it led to additional 14% reduction in DOC for the ROC pre-treated using ferric sulphate, whereas ferric chloride led to additional 7% removal in DOC at 60 min irradiation time (UV fluence 8.91 mW/cm^2). The coagulation increased the UV transmittance (UVT) markedly and primarily removed large molecular weight compounds (>10 KDa), with minimal removal of the low to medium molecular weight organic compounds. As a result, biological processes were suggested as an appropriate treatment for removing the remaining organic matter.

2.4.2 Advanced Oxidation Processes (AOPs)

Advanced oxidation processes are based on the principle that these processes produce hydroxyl radicals ($HO^\bullet$) which are very reactive and non-selective in oxidising almost all electron rich organic matter and eventually convert them to CO_2 and water (Parsons, 2004). The hydroxyl radicals also oxidise any emerging contaminants in the ROC and so potentially decrease the ecotoxicity (Westerhoff et al., 2009). The following reactions are suggested to take place during oxidation process (Legrini et al., 1993).

$RH^\bullet + O_2 \rightarrow RHOO^\bullet$ Equation 2.1

$RH + HO^\bullet \rightarrow R^\bullet + H_2O$ Equation 2.2

where R refers to the reacting organic compound.

The AOPs, including UV/H_2O_2, ozonation (O_3), electrochemical oxidation, photo catalysis, sonolysis and Fenton processes were reported to be effective in treating municipal wastewater ROC (Dialynas et al., 2008, Westerhoff et al., 2009, Chaplin et al., 2010, Bagastyo et al., 2011, Liu et al., 2012). The following section provides an overview of these applications. DOC removal efficiency for the various AOPs (single or combined) studied previously is presented in Table 2.3.

Table 2.3 DOC removal efficiency for the various AOPs

Treatment process	**Reaction time (min)**	**Oxidant dose**	**DOC removal (%)**	**References**
UVC/H_2O_2	60	3 mM	26-38%	Umar et al. (2013)
UVC/H_2O_2	61.7	0.54 mg/mgTOC	9.6% TOC removal	Vendramel et al. (2013)
UVC/H_2O_2	120	3 mM	40-60%	Liu et al. (2012)
UVC/H_2O_2	120	11.8 mM	38-40%	Bagastyo et al. (2011)
UVA/ H_2O_2	60	5 mM	43.5 %	Zhou et al. (2011b)
UVA/TiO_2	360	-	72%	Zhou et al. (2011b)
UVC/TiO_2	360	-	95%	Zhou et al. (2011b)
O_3	60	1 L/min	41%	Zhou et al. (2011b)
US/O_3	60	-	43.6%	Zhou et al. (2011b)
UV/TiO_2/O_3	60	-	68.1%	Zhou et al. (2011b)
UVC/H_2O_2	60	2-6 mM	49-76%	Liu et al. (2011)
UV/ H_2O_2	-	10 mM	40%	Westerhoff et al. (2009)
UV/TiO_2	-	-	80%	Westerhoff et al. (2009)
O_3	20	-	23.4%	Lee et al. (2009 a,b)
UVA/TiO_2	-	-	41-49%	Dialynas et al. (2008)

2.4.2.1 UV/H_2O_2 treatment

UV/H_2O_2 is one of the most common and promising treatment processes for water and wastewater. In recent years, use of UV/H_2O_2 in treating municipal ROC is increasing due to its ability to treat recalcitrant compounds present in it (Westerhoff et al., 2009; Zhou et al., 2011b) and its capability of removing a wide range of organic compounds of different molecular weight (Dwyer and Lant, 2008)

UV/H_2O_2 generates hydroxyl radicals through photolysis of H_2O_2 under UV irradiation. The reaction mechanism is given by the following equations (2.3-2.8) (Baxendale and Wilson, 1957).

$H_2O_2 + hv \rightarrow 2HO^{\bullet}$ Equation 2.3

Due to its weak acidity, H_2O_2 can dissociate to H^+ and HO_2^- (Equation 2.4):

$H_2O_2 \rightarrow H^+ + HO_2^-$ Equation 2.4

HO_2^- can be a source of $HO^{\bullet}$ under UV irradiation (Legrini et al., 1993) (Equation 2.5):

$HO_2^- + hv \rightarrow HO^{\bullet}$ Equation 2.5

Decomposition of H_2O_2 through dismutation is another way of $HO^{\bullet}$ generation (Legrini et al., 1993) as described in Equation 2.6:

$H_2O_2 + HO_2^- \rightarrow H_2O + O_2 + HO^{\bullet}$ Equation 2.6

At high local $HO^{\bullet}$ concentration, recombination of $HO^{\bullet}$ occurs to form H_2O_2 (Legrini et al., 1993) as given in Equation 2.7:

$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2$ Equation 2.7

Formation of hydroperoxyl radicals ($HO_2^{\bullet}$) which possess markedly lower oxidizing activity than $HO^{\bullet}$ takes place in the presence of excess H_2O_2 via the following reaction (Equation 2.8):

$H_2O_2 + HO^{\bullet} \rightarrow HO_2^{\bullet} + H_2O$ Equation 2.8

The use of UV/H_2O_2 in treating municipal ROC is given in Table 2.3. Westerhoff et al. (2009) found that using 10 mM H_2O_2 at pH 4, only 40% DOC removal (initial DOC 40 mg/L) was achieved (UV fluence not given). Zhou et al. (2011b) reported very low DOC reduction of 2.3 ± 2.8% with UVA/H_2O_2 treatment of ROC at irradiation intensity of 7.7 mW/cm^2, 60 min irradiation time and 5 mM oxidant dose. The low DOC reduction in that study could be due to the higher molar absorption coefficient of H_2O_2 at 253.7 nm for UVC than that at 360 nm for UVA (Liu et al., 2011). In the same study, when the ROC was pre-coagulated with 1 mM $FeCl_3$ and subjected to UVA/H_2O_2 treatment, DOC removal efficiency of the ROC was improved to 43.5 ± 5.7%. The study showed that the combined scheme significantly improved the water quality in terms of biodegradability, compositions of organic molecules and ecotoxicity as well. Liu et al. (2012) studied the treatment of a municipal wastewater ROC using UVC/H_2O_2 at 30 minutes irradiation time and 3 mM H_2O_2 dose under different pH, salinity and initial DOC conditions. They found that DOC and COD were more efficiently removed under acidic conditions (pH 4) and ROC salinity had only minimum impact on the removal of organics even when the salinity was varied by 4-fold. The biodegradability of the ROC as indicated by biodegradable dissolved organic carbon (BDOC) level also increased (24% removal of DOC) by increasing the irradiation time to 2 h. Overall, more than 80% of DOC removal was achieved with 2 h UVC/H_2O_2 treatment followed by the BDOC test. The study also showed that with the UVC/H_2O_2 treatment followed by BDOC greatly reduced high molecular weight (MW) compounds such as biopolymers, humics and lower MW compounds including building blocks (breakdown products of high MW humics), low MW neutrals. The study suggested that the DOC mineralised after 30 minute UV irradiation would be mainly biodegradable intermediates. Umar et al. (2013) demonstrated that UV/H_2O_2 treatment of municipal ROC samples of widely varying composition (DOC 47, 53 and 34 mg/L and conductivity 23, 8.3 and 22.8 mS/cm, respectively) could remove 26-38% DOC and 25-37% COD using UVC/H_2O_2 (UV fluence 12.89 mJ/s/cm^2; 3mM oxidant dose). Compared with the DOC and COD reductions discussed above, much greater reduction in A_{254} (75-80%) and colour (>90%) was reported (Umar et al., 2013). These large reductions were attributed to the breakdown of the conjugated and chromophoric organic compounds present in ROC.

It should be noted that stand alone UVC/H_2O_2 treatment can increase the removal of organic compounds by reducing pH as low as pH 4 (Liu et al., 2012). Low organic content reduction at high pH values was due to the presence of bicarbonate/carbonate species which are strong $HO^{\bullet}$ scavengers (Weeks and Rabani, 1966). However, then the pH of the treated water has to be raised to neutral for its reuse involving addition of chemicals, limiting its economic feasibility.

Hence, various studies on treatment of ROC using UV/H_2O_2 suggested the potential of using downstream biological treatment processes for enhanced organic matter removal, since the UV/H_2O_2 increased the biodegradability of the ROC by the effective cleavage of the bonds of large organic molecules, producing smaller molecules that can be readily consumed by microorganisms. Apart from enhanced organic matter removal, the combined process is suitable for removing nutrients present in the municipal ROC.

2.4.2.2 UV/TiO_2 photolysis

UV/TiO_2 is a promising technique in which UV irradiation in the presence of titanium dioxide (TiO_2) is applied to degrade the organic matter in ROC (Hofstadler et al., 1994, Baird, 1997). The UV/TiO_2 process is based on the absorption of photons with energy higher than 3.2 eV (wavelength lower than~390 nm). The electrons and hydroxyl radicals are generated according to Equations 2.9 to 2.11 (Hofstadler et al., 1994, Baird, 1997)

$$TiO_2 + hv \rightarrow e_{CB}^{-} + h_{VB}^{+} \qquad \text{Equation 2.9}$$

The generation of $HO^{\bullet}$ occurs when the hole produced by irradiation reacts with water or surface-bound hydroxyl ion (Baird, 1997) as given in Eqs. 2.10 and 2.11.

$H_2O_{(ads)} + h_{VB}^+ \rightarrow HO^\bullet + H^+$ Equation 2.10

$OH_{(ads)}^- + h_{VB}^+ \rightarrow HO^\bullet$ Equation 2.11

A list of studies conducted on the treatment of municipal ROC using UV/TiO_2 (single or combined) is provided in Table 2.3.

Westerhoff et al. (2009) studied the UVA/TiO_2 system followed by simple biological treatment using biological sand filter to treat a ROC from wastewater reclamation facilities. The study showed that the combined treatment could remove 91% of DOC (initial DOC 40 mg/L) at the highest applied UV dose (10 kWh/m^3) and titaniumdioxide dose between 1 and 5 g/L. The study suggested that the combination of AOPs and a simple biological treatment system could remove greater amounts of organic matter. The authors also reported that UV/TiO_2 process was effective in removing trace organics present in the ROC. They also reported that UV/TiO_2 process can perform better at lower pH (pH 5) than pH 7 as at higher pH the carbonate/bicarbonate species reduced the steady state of $HO^\cdot$ concentration, thereby reducing the oxidation efficiency of the process.

Similarly, Dialynas et al. (2008) found that UV/TiO_2 was able to remove 49% and 41% of DOC from a municipal wastewater ROC at high (1 g/L) and low (0.5 g/L) catalyst level, respectively, at the reaction time of 50-60 min.

In another study using the combinations of different types of AOPs including sonolysis (US), photocatalytic oxidation (PCO) and ozonation (O_3) to treat the ROC, it was shown lower DOC removal ranging from 4.9%-21.7% was obtained at reaction time of 60 min. The removal efficiency for the organics present in ROC was found in the order of US<UVA/TiO_2<UVC/TiO_2<O_3 (Zhou et al., 2011b). The experimental investigation showed the best result was obtained with the combination of coagulation ($FeCl_3$) followed by UVC/TiO_2, which could achieve 95% reduction in organic matter within an extended treatment time (6 h).

The UV/TiO_2 system has been proven to be efficient for degrading organic compounds at lab scale. However, the process may be inconvenient, time consuming and expensive because when the UV/TiO_2 system is used with suspended catalyst particle, the solution will be less penetrable by UV radiation (i.e., have low UV transmissivity (Ray and Beenackers, 1998)). Therefore, more studies are required in relation to optimise the process conditions including dosage of catalyst, pH and other variables to maximise its efficiency.

2.4.2.3 Ozonation

Ozonation is a chemical oxidation process which can break down the organics into simpler forms by splitting organic bonds and dissociating aromatic rings in the recalcitrant organic compounds and so increase the biodegradability (Zouboulis et al., 2007). Organic contaminants are oxidized through direct reaction with molecular ozone or through indirect reactions with free radicals ($HO^•$)(Broséus et al., 2009)

Ozonation of ROC has been reported either alone or in combination with other AOPs (Table 2.3). Zhou et al. (2011b) reported reductions in DOC, COD and colour of 22%, 14% and 90%, respectively, with ozonation alone (1 L/min; 17.6 ± 8.3 mg/h). Several combinations of ozonation with other AOPs have also been investigated such as UVA/O_3, ultrasound (US)/O_3, UVA/H_2O_2/O_3 and US/H_2O_2/O_3, UVA/TiO_2/O_3 (Zhou et al., 2011b). The bi- or tri - combinations of these AOPs did not show any significant improvement in DOC reduction compared with ozonation alone treatment, which might suggest the selective oxidation by molecular O_3 proved more efficient than the non-selective $HO^•$ oxidation in the degradation of the organic compounds present in the ROC.

In a batch experiment, Lee et al. (2009a) showed the reduction in TOC for a ROC was 25% after 20 min ozonation (10 mg O_3/L) and the improvement was <2% when the ozone dosage was increased from 6 to 10 mg/L, which was due to the remaining organic compounds being recalcitrant to ozone. As direct ozone attack is the prevailing mechanism at neutral pH, and given that the authors performed ozonation at pH 7.1, the organics were believed to be converted to carboxylic acids which are recalcitrant to molecular ozone (Lee et al., 2009a, 2009b).

Ozone (with a very high dose of 1000 mg/L) in combination with H_2O_2 (0.7 mol H_2O_2 per mol of O_3 dosage) was used by Westerhoff et al. (2009) to treat a ROC, and the DOC reduction of 75% was achieved.

Benner et al. (2008) studied the degradation of specific pharmaceuticals such as acebutolol, atenolol, metoprolol and propranolol, collectively known as beta blockers, present in ROC using ozonation rather than simply following the reactions by bulk water quality parameters. The study reported that ozonation at a dosage of 5 mg/L was able to remove propranolol in 0.8 s and a dosage of 10 mg could oxidise 70% of metoprolol in 1.2 s.

Collectively, these studies demonstrated the potential of ozonation for removing organic content from the ROC, particularly as a pre-treatment prior to biological processes which are readily able to remove the resultant smaller organic molecules.

2.4.2.4 Electrochemical oxidation

Electrochemical oxidation treatment is based on the bulk oxidation through generation of hypochlorite as given by equations 2.12 and 2.13.

$$2Cl^- \rightarrow Cl_2 + 2e^- \qquad \text{Equation 2.12}$$

$$Cl_2 + H_2O \rightarrow HClO + H^+ + Cl^- \qquad \text{Equation 2.13}$$

The process is effective in the treatment of wastewater containing recalcitrant organics, colour as well as ammonia nitrogen (Van Hege et al., 2004). Different types of electrode materials such as, SnO_2, PbO_2, RuO_2 boron-doped diamond (BDD) electrodes were studied for the use in electrochemical treatment of ROC to reduce COD and TAN (Van Hege et al., 2004). The study reported that PbO_2 and SnO_2 anodes were not suitable for the studied ROC (COD 151-

218 mg/L) due to the scaling caused by $Ca(OH)_2$ and $Mg(OH)_2$ on the anode surface and PbO_2 anodes were corroded due to a drastic rise in the pH in the one–compartment electrolytic cell. Among these four electrodes, BDD achieved better reductions of COD (74.1%) and TAN (56.2%) with complete colour removal at current density of 200 A/m^2.

Zhou et al. (2011a) used three different types of electrodes: BDD, Ti/IrO_2-RuO_2 and Ti/IrO_2-Ta_2O_5 to treat highly saline (TDS 14,745 mg/L) ROC from second stage RO treated wastewater from a process steel plant in China. The COD removal at the same current density was in the order of BDD >Ti/IrO_2-RuO_2 >Ti/IrO_2-Ta_2O_5. However, the energy consumption for BDD was higher than that of the other two anodes at the same current density (BDD: 0.203, Ti/IrO_2-RuO_2: 0.066, Ti/IrO_2-Ta_2O_5:0.130 kWh/g COD at 50 mA current). The work also showed COD removal was affected by high chlorine content of the ROC.

Dialynas et al. (2008) observed the overall DOC removal was 30% for 3.6 A and 36% for 17.8 A, suggesting the application of higher current had little effect for improving DOC removal by electrochemical oxidation.

Pérez et al. (2010) used BDD electrodes to find the treatability of emerging pollutants present in a ROC in tertiary water treatment at different current density and concluded that complete COD removal could be achieved at high current density and less time. The process also removed ammonium and emerging micropollutants (except ibuprofen) present in the ROC by over 90% after 2 h oxidation. However, during the process, trihalomethanes (THMs, a form of disinfection by products (DBPs)) formed and the concentration was high in first hours of experiment and at high current density. THMs have carcinogenic effects to humans as suggested by the International Agency for Research on Cancer (Matamoros et al., 2007). The process needs more optimisation with the current density. The electrolysis process also caused subsequent precipitation and electrode scaling, requires very high current density (50-200 A/m^2). Moreover, the process is commonly regarded as energy intensive and expensive, limiting its wider applications at large scale (Van Hege et al., 2004, Pérez et al., 2010, Chaplin et al., 2010, Radjenovic et al., 2011).

2.4.2.5 Capacitive deionization (CDI)

Capacitive deionization (CDI) is an emerging technology for treating ROC. It is a low pressure electrochemical process which is capable of removing dissolved ions from ROC (Lee et al., 2009b). Ng et al. (2008) used BAC as a pre-treatment to the CDI process and achieved the reductions of 78% TOC, 91% TN and 92% electrical conductivity. In general, CDI has a lower energy requirement than its counterparts. For example, it requires 3 times less energy than the electrodialysis reversal process (AWWA, 1999). However, selection of an appropriate pre-treatment method is important with regard to fouling of the CDI cells and final water quality. The fouling can be reduced by pre-treatment and reducing pH (Lee et al., 2009b), periodically switching the potential of the electrodes (Kerwick et al., 2005), and by applying a pulsed field to the electrodes (Perez-Roa et al., 2006).

2.4.2.6 Sonolysis

Sonolysis is an innovative AOP using high level of energy ultrasound at low to intermediate frequency (20-1000 kHz) (Dialynas et al., 2008). Dialynas et al. (2008) reported sonolysis led to 29% DOC removal at 67.5 W and 34% DOC removal at 135 W after 60 min treatment of municipal ROC (initial DOC 10.1 mg/L). The process was higher in energy consumption (810 kJ/mg DOC oxidized) compared with photocatalysis or electrochemical oxidation which were 9.3 kJ/mg of DOC oxidized and 16.7 kJ/mg of DOC oxidized respectively, thereby limiting its application in ROC treatment.

2.4.2.7 Other treatment options

Adsorption with granular activated carbon (GAC) has also been studied for treating ROC. The DOC removal was initially fast and slowed down gradually as observed by Dialynas et al.

(2008). The highest removal for DOC was 91% at the dose of 5 g/L in 5 days. The initial sharp decrease in the organic matter was due to the adsorption of the organic compounds in the micro-pores. Zhou et al. (2011a) used GAC and powdered activated carbon (PAC) to treat the ROC and achieved 88% and 95% DOC removal, respectively, at the carbon dose of 5 g/L. However, both of them could not remove hydrophilic organic compounds of large molecular weight even at higher dose of 5 g/L. Although high organic removal can be achieved by activated carbon, the process does not seem to be cost effective because of high energy consumption in carbon regeneration, high activated carbon usage and longer treatment time.

Adsorption using magnetic ion exchange resin (MIEX) has been considered as another option to treat ROC. MIEX is a strong base anion exchange resin with macroporous polyacrylic matrix in the chloride form. Bagastyo et al. (2011) used MIEX (10, 15 mL/L) to treat two ROC samples of different initial organic concentrations. They showed that colour removal efficiency was ~80% at 20 min contact time for both ROC samples, and a high dosage (15 mL/L) of resin could remove 43% of DOC of one ROC sample (initial DOC 62 ± 5 mg/L) and 10 mL/L of resin could remove 24% of DOC of another ROC sample (initial DOC 42 ± 4 mg/L). Comstock et al. (2011) also used MIEX at different doses of 5, 10, 20 mL/L to treat ROC. In that study at the resin dose of 10 mL/L, UVA_{254} removal was 52% at 5 min contact time and was increased to 82% at 30 min contact time. However, the DOC removal (43%) was comparatively lower than UVA_{254} at the same dose of resin at 30 min contact time. The study on use of MIEX has not been extensively used and more investigations on organic matter, nutrients removal, suitability for high salinity ROC and cost benefits are required.

2.5 Biological processes

The application of biological treatment, mainly biological activated carbon (BAC) has been reported as post-treatment for municipal wastewater concentrate. The details on removal mechanism for organic matter and nutrients by BAC are discussed in following sections 2.6.

Ghyselbrecht et al. (2012) studied the potential of willow fields for reducing nutrients (N & P) and organic content (TOC and COD) of ROC. The study reported that willow test field of 28.33 m^2 and 500 L/hectare of ROC, can reduce 20% of TOC but total nitrogen and total phosphorus reduction rates are limited to 32%.

Most recently, Wang et al. (2016) studied use of microalgae for simultaneous removal of nitrogen, phosphorus, calcium and magnesium ions from the municipal ROC using microalgae cultivation and algal biomass production. The study reported that microalgae (*Chlorella* sp. ZTY4 and *Scenedesmus* sp. LX1) grew well in municipal ROC and these microalgae effectively removed nitrogen and phosphorus by 89.8% and 92.7% respectively. This study provides new opportunities for treating municipal ROC using algal process.

2.6 Biological activated carbon (BAC)

In recent years, biological activated carbon (BAC) technology has emerged as a potentially cost-effective treatment option for municipal wastewater ROC. The BAC system provides simultaneous adsorption of non-biodegradable matter and oxidation of biodegradable matter in a single reactor with microbial activity in a granular activated carbon (GAC) system (Walker and Weatherley, 1999). The process can be economical and cost effective because the single reactor can be used with less needs in the regeneration of carbon, consequently lowering the energy requirement and operating cost (Ying and Weber Jr, 1979).

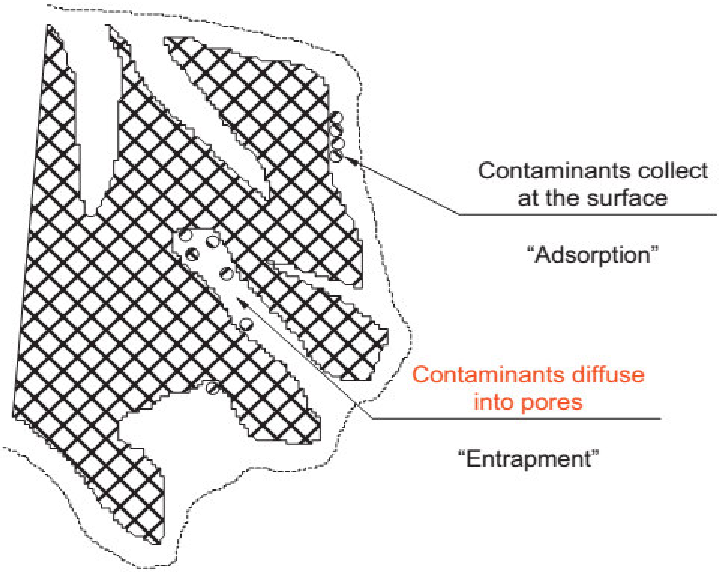


Figure 2.1 Granular activated carbon surface adsorption and pore entrapment (Simpson, 2008).

The BAC process utilises GAC as its water filtration media to remove unwanted microorganism and organic/inorganic matter via adsorption into the pores of GAC. The GAC offers an effective means to remove organic matter due to its irregular creviced, porous particle shape and ability to attract specific contaminants as depicted in following Figure 2.1 (Scholz and Martin, 1997, Simpson, 2008).

GAC has high adsorption capacity with high surface to volume ratio (Pelekani and Snoeyink, 1999). Adsorption of organic matter primarily takes place in mesopores (2-50 nm width) and micropores (1-2 nm width) (Lee et al., 1981, Summers and Roberts, 1988). As the pores of GAC media become slowly filled, the rough porous surfaces of GAC media are amenable to microbial (bacterial) colonization that could grow significant biomass or biofilm forming BAC (Scholz and Martin, 1997). The biofilm can process and biodegrade significant fraction of entrapped waterborne nutrients in the GAC pores, dissolved organic matter (DOM) adsorbed to the GAC surfaces and other contaminants, minerals and microorganisms contained in water source (Dussert and Van Stone, 1994, Zhang and Huck, 1996). The GAC media, which is slightly electro-positively charged, offers maximum adsorption of organic matter, which is electro-negatively charged due to its higher surface area of 600-1000 m^2 or higher (Simpson, 2008).

The establishment of BAC filters can be divided into three steps, Firstly, a significant amount of organic matter is removed by adsorption by the activated carbon in its pores; secondly, growth of bacteria takes place to form the biofilms and thirdly, the biofilm grows and increases becoming biologically active leading to reduction in the adsorption capacity of the carbon. Eventually, the organic matter removal reaches a relatively steady state where biological oxidation is the dominant mechanism for organic matter removal (Simpson, 2008).

The BAC biofilm comprises of microbial cells either immobilised at the surface or the GAC (substratum) or embedded in an extra cellular microbial organic polymer matrix (Ghosh et al., 1999). Bacterial and fungi cells in the biofilm secrete extracellular polymeric substances to form a cohesive, stable matrix. The extracellular matrix is comprised of polysaccharides, proteins, nucleic acids and lipids (Branda et al., 2005, Lazarova and Manem, 1995).

The BAC process offers many advantages to traditional water treatment processes that rely heavily on chemical disinfection (Simpson, 2008). Due to the high organic matter removal efficiency, the BAC process produces water that is more easily disinfected since it has much lower chlorine demand (Takeuchi et al., 1997). The BAC process is less likely to produce undesirable disinfection by products and bacterial re-growth in water distribution systems and more likely to maintain a stable residual chlorine value in the water distribution systems (Scholz and Martin, 1997, Dussert and Van Stone, 1994). The BAC process eliminates the need for coagulant application that is traditionally used for water filtration processes (Hillis, 2000). The biofilm can biodegrade significant proportions of waterborne substances such as DOC, assimilable organic carbon (AOC), organic chemicals (e.g., atrazine, PCBs, simazine), disinfection by products and inorganics (e.g., ammonia) (Dussert and Van Stone, 1994, Takeuchi et al., 1997; Ghosh et al., 1999; Scholz and Martin, 1997; Okabe et al., 2002). The BAC process has shown good potential to remove wide ranges of micropollutants (>90%) and toxicity (bioluminescence inhibition test with *Vibrio fischeri*) even without oxidation pre-treatment (Reungoat et al., 2011). Furthermore, the BAC process is effective to biodegrade algal toxins along with algal/organic matter related taste and odour substances (Simpson, 2008). Moreover, the BAC process is capable of partly breaking down and removing amines/aliphatic aldehydes and phenols/chlorinated phenols (Dussert and Van Stone, 1994). BAC application can remove inorganics such as ammonia significantly by an increasing population of nitrifying bacteria, together with removal of dissolved organic carbon lowering the chlorine demand in finished water (Scholz and Martin, 1997).

In the past, the BAC process was widely used in drinking water treatment (Urfer et al., 1997) and tertiary wastewater treatment (Walker and Weatherley, 1999, Kalkan et al., 2011) for the removal of organic matter. In recent years, investigations on the use of BAC have been increasing to treat municipal RO concentrate because of its effectiveness in removing organic matter. In most of the studies, the BAC process has been used to treat pre-oxidised ROC. The following sections briefly describe the use of BAC in treating municipal ROC.

2.6.1 Integrated treatment option for ROC using BAC

As most of the organic substances present in the municipal wastewater ROC are bio-refractory, pre-treatment options such as oxidative treatments such as AOPs and coagulation may play important roles for improving the overall organic matter removal by the BAC treatment. Table 2.4 provides a summary of the recent studies of the BAC based treatment processes using AOPs on municipal wastewater ROC.

Lee et al. (2009a) studied the BAC system to treat a ROC from water reclamation facilities. The removal efficiencies for TOC, COD and UVA_{254} by BAC alone at EBCT of 60 min were 23.5%, 31.5% and 18.6%, respectively. The coupling of BAC with ozone pre-treatment with an ozone dosage ranging from 3 to 10 mg/L and contact times of 10 and 20 min increased the biodegradability of the RO brine by 1.8-3.5 times. With the average ozone dose of 6 mg/L and contact time 20 mins, the combined process improved the TOC removal from 23.4 ± 6.3% to 69.8 ± 8.1%. This study reported that biological removal is the dominant process in organics removal than ozonation alone. Furthermore, the combined effluent was subjected to CDI treatment and it could remove more than 80% anion and cation.

Ng et al. (2008) obtained 20% TOC removal for a ROC from a water reclamation facility in Singapore using a BAC system with 40 min EBCT. The TOC and TN removal increased to 78% and 91%, respectively, coupled with a downstream capacitive deionization (CDI) process.

Lu et al. (2013) treated a municipal wastewater ROC of TDS ~10,000 mg/L with oxidation using UV/H_2O_2 (UV fluence 12.89 mJ/s/cm^2, 30 min irradiation, 3 mM H_2O_2) followed by BAC (EBCT 60 min). The removal efficiency of the treatment system on DOC (60%), UVA_{254} (78%) colour (64%), COD (48%), TN (24%) and TP (17%) showed the potential for treating the higher salinity ROC. The study further suggested a synergistic effect of the combined treatment for DOC reduction, and no ecotoxicity was detected for the ROC after the combined treatment. The study also confirmed that that BAC process can be used to treat higher salinity municipal ROC.

Recently, Justo et al. (2015) compared UV/H_2O_2-BAC and O_3-BAC treatment systems for treating a ROC with initial DOC of 24 mg/L and TDS 7 mS/cm). The study showed DOC and COD removal efficiencies were 58% and 46%, respectively, for UV/H_2O_2-BAC and 70% and 54% respectively for O_3-BAC treatment. The study also showed the BAC filter could achieve the removal up to 90% for some pharmaceuticals (for e.g., Naproxen, Gemfibrozil, Atenollo).

In a more recent study by Umar et al. (2016b) using sequential coagulation with either alum or ferric chloride, UVC/H_2O_2 followed by BAC treating highly saline municipal ROC (TDS

17,245 mg/L) reported that BAC treatment mainly removed low molecular weight (LMW) neutral molecules indicating that biodegradation is the predominant mechanism of organic matter removal. Furthermore, the study also reported that implementation of coagulation process as a pre-treatment can markedly reduce the electrical energy dose (*E*ED) by 6-8 times for the UVC/H_2O_2 process.

Table 2.4 DOC removal by various integrated treatments for ROC

Treatment process	**EBCT (min)**	**Oxidant dose (mg/L)**	**Oxidant contact time (min)**	**DOC removal (%)**	**TDS (mg/L) or Conductivity (mS/cm)**	**References**
BAC+CDI	40	--	--	78% TOC		Ng et al. (2008)
O_3+ BAC	60	6	20	69.8	1218 mg/L	Lee et al. (2009a)
UVC/H_2O_2+ BAC	60	4 mM	30	60	10,200 mg/L	Lu et al. (2013)
UVC/H_2O_2+ BAC	44.7	0.82mg H_2O_2/mg DOC	98	58	7.3 mS/cm	Justo et al. (2015)
O_3+ BAC	44.7	2.2 mgO_3/mg DOC	19	70	7.3 mS/cm	Justo et al. (2015)
$FeCl_3$+UVC/H_2O_2+BAC	60	3 mM	30	68	17,245 mg/L	Umar et al. (2016b)
Al^{3+} + UVC/H_2O_2+BAC	60	3 mM	30	62	17,245 mg/L	Umar et al. (2016b)

Most of the previous studies have been focused on the removal of organic compounds from the ROC. Relatively less attention has been paid to the effect for nutrient (N & P) removal, particularly for the ROC with very high salt content. High concentration of salt in wastewater can greatly affect the biological treatment system as the salt concentration greater than 1% causes disintegration of cells and cell dehydration due to plasmolysis, the result of outward flow of intracellular water across the cell due to difference in osmotic pressure (Dinçer and

Kargi, 2001). High salinity in the secondary effluent has been found to affect the biological activity greatly, decreasing COD removal, nitrification and denitrification processes (Kargi and Dinçer, 1997). Several pre-treatment options such as ion exchange, reverse osmosis and coagulation have been studied to treat hyper saline wastewater effluent but the processes were high in energy consumption, start-up cost and running cost (Lefebvre and Moletta, 2006). Several aerobic, anaerobic and combined treatment options have also been studied as potentially more cost-effective options for hyper saline industrial wastewater effluent. However, there is generally a lack of knowledge about biological treatment of high salinity ROC so far.

Although, it has been reported that salinity of wastewater negatively affects the microbial activities, it is possible for activated sludge to be acclimated to high salinity environments. In the study by Lu et al., (2013) the BAC process was acclimatised by recirculating a mixture of diluted ROC and activated sludge through GAC columns to treat municipal ROC over 30 d period. The salinity of ROC was gradually increased from TDS 500 to 10,000 mg/L. The gradual increase in salinity may reduce the negative impacts on microbial activities as suggested by Lefebvre and Moletta (2006). Bassin et al. (2011) suggested that when salinity is gradually increased, better ammonia removal could be achieved along with organic removal because of biomass acclimation in the saline condition. The microorganisms could i) survive or tolerate the salinity up to 10 g/L without any acclimation in the conventional water treatment; ii) survive salt in the range of 30-50 g/L by acclimation but the stability of the microorganism would be lost when there is change in salinity; and iii) for higher salinity (>50 g/L), use of halophiles or salt tolerant microorganisms such as *Halobacter* species or *Staphylococcus* species would be beneficial in terms of organic removal (Dinçer and Kargi, 2001, Kubo et al., 2001, Lefebvre et al., 2005, Abou-Elela et al., 2010, Lay et al., 2010).

The salinity greatly affects the phosphorus and nitrogen removal in biological treatment system as mentioned in the literature review by Lay et al. (2010). Phosphorus removal is greatly inhibited by increasing salinity (>5 g/L) (Uygur and Kargı, 2004, Sharrer et al., 2007). Nitrification process may be impaired by elevated salinity, whereas elevated salinity does not affect the denitrifiers as denitrification could take place in the range of 300 g/L NaCl (Glass and Silverstein, 1999, Sharrer et al., 2007). Nitrifiers could survive up to 30 g/L by acclimation; higher salt concentration requires addition of halophiles for satisfactory organic and nutrients removal.

Previous studies mostly reported organic matter and nutrient removals from highly saline wastewaters other than municipal ROCs (except for Lu et al. (2013)) from different biological processes. The following sections mainly report some investigations on nutrient removals from BAC processes treating municipal ROCs.

2.6.2 Nutrient removal during AOP and BAC processes

The nutrient removal from the ROC by AOP and BAC processes are reviewed by studying the existing literature including the basic principles, applications in drinking water and industrial wastewater, with a view to gaining a better understanding about the potential of the two treatment methods on ROC streams, particularly those of high salinity.

2.6.2.1 Nitrogen removal during AOP

In AOP treatment, the nitrogen in organic compounds is usually oxidised to nitrate or to free N_2, sulphur is oxidised to sulphate, and cyanide is oxidized to cyanate, which is then further oxidized to CO_2 and NO_3 or N_2 (Munter, 2001). Dwyer et al. (2008) studied the simultaneous degradation of dissolved organic nitrogen (DON) and associated colour from wastewater containing melanoidins using UV/H_2O_2 based treatment process. They reported with the application of 3300 mg/L of H_2O_2 dose and initial concentration of melanoidin of 2000 mg/L, maximum colour removal was achieved (99%), whereas removals for DOC and DON were only 50% and 25%, respectively. The study also found that most DOC and DON were present in the fraction of colour causing compounds with molecular weight greater than 10 kDa, indicating that the largest molecular weight fractions are more susceptible to oxidation.

Chen et al. (2011b) studied the efficiency of UV/H_2O_2 treatment on selected nitrogenous compounds along with organic degradation and resultant disinfection by products. The study showed that DON removal was only 20-30% from nitrogenous compounds such as atrazine, caffeine, dimethylaminopropyl, methacrylamidem, diltiazem, histamine and triethanolamine. Kurniawan and Lo (2009) used integrated H_2O_2 and GAC to treat stabilized landfill leachate. The integrated treatment process improved the COD and NH_4^+-N removal by 82% and 59% respectively with the H_2O_2 dose of 3-3.5 g/L, which was extremely high dose. The experiment suggested that H_2O_2 alone could not remove NH_4^+-N unlike organic compounds as there are no unsaturated double bonds in NH_3-N causing difficulty for $HO^{\cdot}$ radical to break down the N-H bonds of NH_4^+-N through the electrophilic attack. Thus, increasing peroxide dose could not help to improve ammonia removal. As such, for the complete removal of NH_4^+-N, subsequent biological treatment was recommended using nitrifying bacteria. The study also recommended removing the residual peroxide that could negatively impact the microbiological processes in the biological processes. Bagastyo et al. (2011) investigated removals of dissolved organic nitrogen (DON) from two different ROCs, which were 27% and 32% with 400 mg/L H_2O_2 with UV fluence of 3.1 kWh/m^3 and 120 min contact time. The study suggested that since the organic nitrogen is the most difficult one to eliminate with the oxidation process, a biological treatment can be potentially used for its removal.

The above-mentioned studies investigated on nitrogen removal using AOP but none of the studies were carried out for phosphorus removal. Moreover, studies on nutrient removal from ROC are very few.

2.6.2.2 Nitrogen removal in BAC process

Nitrogen may present in different forms such as ammonia, nitrate, nitrite etc. in secondary effluent of wastewater treatment plant. Nitrogen related compounds have adverse effects on the environment and human health as mentioned elsewhere in this chapter (section 2.3). Nitrogen removal occurs by two steps process, nitrification and denitrification. In nitrification, ammonium is converted into nitrite by ammonium oxidising bacteria (AOB) given by equations 2.14 and 2.15, and then nitrite is converted into nitrate by nitrite oxidizing bacteria (NOB) (Hagopian and Riley, 1998). In denitrification, the nitrate is finally converted into nitrogen gas by denitrifying bacteria. The nitrification and denitrification processes are depicted in Figure 2.2. Nitrification process is carried out by autotrophic bacteria, whereas denitrification process is carried out by heterotrophic bacteria.

$$\underbrace{NH_4^+\text{-N} \longrightarrow NO_2^-\text{-N} \longrightarrow NO_3^-\text{-N}}_{\text{Nitrification}}\ \underbrace{\longrightarrow NO_2^-\text{-N} \longrightarrow N_2}_{\text{Denitrification}}$$

Figure 2.2 Overall biological nitrification-denitrification process

$NH_3 + 1.5O_2 \quad \longleftrightarrow \quad NO_2^- + H_2O + H^+ + 84\ \text{kcal/mol}$ Equation 2.14

$NO_2^- + 0.5O_2 \quad \longleftrightarrow \quad NO_3^- + 17.8\ \text{kcal/mol}$ Equation 2.15

The biological denitrification can take place by the reduction of nitrate to nitrogen gas by facultative anaerobes under anoxic conditions in which nitrite is reduced to elemental nitrogen gas with the production of intermediate compounds nitric oxide (NO) and nitrous oxide (N_2O) as in Figure 2.3 (Payne, 1973, van Rijn et al., 2006).

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

Figure 2.3 Denitrification process

As mentioned previously in section 2.3, the ROC contains elevated nutrients (N & P) increasing potential risks to aquatic organisms, human health and environment. When ammonia nitrogen in the range of 0.18-2 mg/L or even more, it significantly increases the demand for chlorine at the disinfection stage (Radjenovic et al., 2011). In this instance, the BAC filters oxidise ammonia with nitrifying bacteria (nitrifiers) that are embedded in the biofilm or found in bulk water. This removal lowers the overall chlorine demand at the disinfection stage and, eventually, lowers the DBP formation potential.

The study of N & P removals present in wastewater and ROC by the BAC process has been little addressed. Kalkan et al. (2011) used BAC to treat pre-ozonated surface water. The study found that nearly complete removal of NH_4^+-N was achieved by nitrification due to the presence of wide varieties of nitrifiers. Li et al. (2007) investigated treatment of secondary effluent by O_3 and UV/O_3 processes alone were not effective in terms of NH_4^+-N removal (only 6% and 9%), compared to DOC removal. The experiment found that with subsequent BAC treatments, NH_4^+-N removals were not improved too much with only 23% and 25% with O_3 and UV/O_3 processes respectively, which could be because of lower EBCT (only 15 mins) and so less contact time for microbes to use carbon available for growth of nitrifying and denitrifying bacteria.

There are very few researches on municipal ROC treatment using BAC processes to reduce nutrients present in it. Ng et al. (2008) reported that BAC-CDI process could remove DOC and TN by 78% and 91% respectively from ROC. Lu et al. (2013) obtained removals of only 24% total nitrogen (TN) and 17% total phosphorus (TP) with UV/H_2O_2-BAC, and only 12% TN and 7% TP were achieved with BAC alone processes from a high salinity ROC. The less removal of N & P in this study could be due to recalcitrant nature and higher salinity of the ROC used in the study as mentioned earlier.

The previous studies have shown that BAC process has the potential to be used for the degradation/removal of nutrients together with the organic content of ROC. Investigation of the application of BAC process to treat ROC at pilot scale is therefore needed to determine its applicability at industrial scale. Moreover, study on microbial communities residing in the BAC media may also help to optimise and enhance the treatment efficiency of the BAC process.

2.7 Microbiological study of BAC systems

As discussed earlier, biological nutrient removal and organic matter removal are possible due to co-existence of different groups of microorganism in the biological treatment system. Bacteria are able to grow at different salt concentrations. Based on their tolerance to NaCl, they may be classified as non-halophiles which grow optimally at NaCl concentration up to 2%, slight halophiles (2-3%), moderate halophiles (5-10%) and extreme halophiles (>10%). Halotolerant organisms are capable of growing in the absence as well as in the presence of salt (Ventosa et al., 1998). These organisms are of great significance in nutrient cycling under conditions of fluctuating salinity as well as in the degradation or transformation of organic pollutants.

For this purpose, some culture-independent methods such as quantitative polymerase chain reaction (qPCR), fluorescent *in situ* hybridization (FISH) and gene clone library analysis have been used to investigate the microbiota involved in the BAC treatment of drinking water (Yapsakli et al., 2010). Other studies have utilised another culture-independent method, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), to characterise the microorganisms in the BAC process for removing methanol (Babbitt et al., 2009) and dissolved organic matter (Jin et al., 2013).

Yapsakli et al. (2010) used qPCR and slot-blot hybridization techniques to study the diversity of nitrifiers (AOB and nitrite oxidation bacteria (NOB)) in a BAC system fed with raw and pre-ozonated drinking water. They found that most of the AOB belonged to the genera *Nitrosomonas* and *Nitrosospira*, and *Nitrospira* species were the dominant NOB in the BAC columns. Stewart et al. (1990) isolated easily cultivable bacteria such as *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus, Acinetobacter, Aeromonas* and *Chromobacterium* species from GAC treated drinking water. Han et al. (2013) observed that *α-Proteobacteria* and *β-Proteobacteria* were the dominant groups in the BAC effluents operated in upflow and downflow conditions treating drinking water. Similarly, there were other bacterial groups belonging to *Acidimicrobidae*, *Acidobacteria*, *Actinobacteridae*, *Bacilli*, *Clostridia*, *Flavobacteria*, γ-*Proteobacteria* in the BAC media in that study.

There is a lack of information on different microbial communities present in the BAC systems treating municipal ROC in organic matter and nutrient removal, which is crucial for any biological treatment process. Recently, Justo et al. (2015) used FISH technique and observed that β-*Proteobacteria* co-exist with γ-*Proteobacteria* in a BAC filter fed with pre-ozonated RO concentrate. However, this study has limited information on contribution of bacterial communities for organic matter and nutrient removal.

The above studies show that study on diversity of microbial communities treating municipal ROC is in primitive stage and more research is required. As the biofilm in the BAC systems is comprised of different bacteria it is essential to characterise the microbial communities involved to allow a better understanding of the removal mechanisms for organic and nutrient content from the ROC.

2.8 Summary of Literature review

The ROC streams generated from municipal wastewater reclamation plants can contain a high concentration of harmful organic pollutants and considerable amounts of nutrients. The characteristics of the ROC depend on water recovery, source and nature of influent, pre-treatment methods, chemical additives during the RO processes etc. For safe disposal to the receiving environment or reuse, the ROC should be properly treated to reduce the contaminants to accepted levels.

Several treatment options such as AOPs including UV/H_2O_2, ozonation, UV/TiO_2, and electrochemical oxidation have been studied to treat municipal ROC at lab scale. Ozonation is promising process for reducing organic content of municipal ROC and improving biodegradability as it preferentially oxidises molecules with low oxidation state and high degree of unsaturation (low H/C ratio) (These and Reemtsma, 2005). However, high ozone dose is required to achieve higher removal of organic matter in ROC and there is potential of formation of bromate, a potential human carcinogen, from water containing bromide (Benner et al., 2008). Other treatments such as UVA/TiO_2 systems and electrochemical oxidation have shown good potential to treat municipal ROC at lab scale. However, the separation of the TiO_2 from the treated water and its recycling needed to be addressed for the large scale application if UVA/TiO_2 to be used. Similarly, more research is required in terms of optimisation of current density, treatment time, cell design and pH available for electrochemical oxidation. The formation of hazardous by-products is one of the main concerns associated with electrochemical oxidation process. The use of these techniques to treat municipal ROC is in the early stage. More research is required to understand the economic feasibility and effectiveness of these techniques for the sustainability of the RO-based wastewater treatment and reclamation processes.

AOPs such as UV/H_2O_2 have been proven as an effective treatment for removing wide ranges of organic pollutants. The technique produces highly oxidising hydroxyl radicals that have potential to oxidise most of the organics into simpler forms. The UV/H_2O_2 process has many advantages such as non-selectivity, shorter treatment time, low capital investment and easy operation (Legrini et al., 1993). The UV/H_2O_2 technique generates easily biodegradable organic compounds such as organic acids which are easily consumed by microorganisms. The several studies have shown that biodegradability of ROC can be improved by incorporating UV/H_2O_2 followed by biological process such as BAC. This approach would aid in enhancing overall organic matter removal and reducing the energy consumption, thereby improving the overall treatment cost-effectiveness. As the BAC process has been widely used in full scale worldwide in water treatment and water reuse facilities as discussed earlier, it has potential to be used in the wastewater reclamation facilities to treat municipal ROC. To date, the use of UV/H_2O_2 process followed by BAC for combine organic matter and nutrient removal have not been studied for sustainable management and treatment of municipal ROC of varied salinity. Furthermore, there is no information available on impact of varied salinity in treating municipal ROC using BAC.

Coagulation is another promising technique to treat ROC as pre-treatment. Coagulation can significantly improve DOC reduction and phosphorus removal. Although, some studies investigate the reductions of organic matter in ROC using coagulation, limited attention has been paid on nutrient removal with post treatment of ROC using BAC to further improve organic matter removal, together with nutrient (especially phosphorus) removal.

The effectiveness and robustness of a UV/H_2O_2–BAC process for removing organic matter and nutrients from municipal wastewater ROC samples of varied salinity with different compositions in terms of organic matter and nutrient removal for reclamation purposes and safe disposal of municipal ROC has been investigated in this study. The existence of different microbial communities for organic matter and nutrient removal has also been investigated to improve the sustainability of the RO-based wastewater treatment and reclamation processes.

Chapter 3 - Materials and Methods

3.1 Analytical Methods

3.1.1 Dissolved organic carbon

DOC concentration was determined using a Shimadzu TOC-L analyser. The analyser has an accuracy of 0.01 mg/L. Each sample was analysed in duplicate. If the variation was >5%, the samples were re-analysed; results were reported as average values. Chloride concentration over 0.05% may inhibit the oxidation of organics during DOC analysis, which could result in a lower reading than the actual value (APHA, 2005). Therefore, samples were diluted using MilliQ water (Milli-Q Gradient A10 unit Millipore) prior to DOC analysis to lower the chloride concentration to below that limit.

3.1.2 Colour

Colour was determined with the Platinum-Cobalt Standard Method 8025 using a Hach spectrophotometer DR 5000 at the wavelength of 455 nm. For true colour measurement, the samples were pre-filtered (PVDF 0.45 μm membrane, Millipore) prior to taking the readings. The results were reported in average values in mg/L Pt-Co unit. The variation in the readings was usually <2%.

3.1.3 Ultraviolet Absorbance (UVA_{254})

All photometric measurements were carried out using a double beam scanning UV/vis spectrophotometer (UV2 Unicam), with a matched pair of 1 cm path length quartz cuvettes. Absorbance at 253.7 nm was used as an indication of the aromaticity and the presence of conjugated double bonds in the samples. Average values of duplicate readings were reported. The variation in the readings was usually <2%.

3.1.4 Specific UV absorbance (SUVA)

Specific UV absorbance (SUVA, presented as L/mg.m) was calculated by dividing UVA_{254} by the DOC value.

3.1.5 Alkalinity and chloride

Alkalinity was determined using titration method 2320 B (APHA, 2005). Concentrated H_2SO_4 solution (Ajax, AR, 95-98%) was used for preparing the titrant, a solution of 0.1 M H_2SO_4. The titrant was standardised using 0.05 M Na_2CO_3 solution (Ajax, AR, 99.9%). The concentration of the titrant was determined as 0.11 M as the average from 72 triplicate analyses. The volume of 0.1 M H_2SO_4 consumed to achieve the end-point pH of 4.5 was recorded for calculating the total alkalinity of the samples, expressed as mg/L $CaCO_3$. Chloride concentration was determined by Mohr's Method. The sample was titrated against 0.1 M silver nitrate solution using potassium chromate (0.25 M) as an indicator. The formation of red-brown precipitates was regarded as the end point. The average value of triplicate measurements was reported. The variation in the readings was usually <5%.

3.1.6 Liquid Chromatography-Organic Carbon Detection (LC-OCD)

The LC-OCD analyses were conducted at the University of New South Wales (UNSW) using the DOC-Labor Model 8 fitted with a Toyopearl TSK HW-50S column. Samples of 1000 μL were injected into the column and the mobile phase was phosphate buffer at pH 6.4 (2.5 g/L KH_2PO_4 and 1.5 g/L $Na_2HPO_4.H_2O$) at a flow rate of 1.1 mL/min. The LC-OCD separates the organics to give quantitative values for 5 chromatographic fractions with the various retention times which correspond to the organic compounds with different molecular sizes: biopolymers (>20,000 Da), humics (~1000 Da), building blocks (300-500 Da), low molecular weight (LMW) acids and humics, and LMW neutrals (<350 Da).

3.1.7 Determination of residual H_2O_2 and quenching of H_2O_2

Residual H_2O_2 is known to interfere with several analytical measurements including COD and therefore needs to be removed prior to their analyses. The concentration of residual H_2O_2 in the water samples was determined using Merckoquant® peroxide test strips (Merck) as a quick colorimetric indication over a limited range (0, 0.5, 2, 5, 10 and 25 mg/L) as given in Figure 3.1. The possibility of interference by the organic and inorganic content of ROC in the measurement of H_2O_2 was examined by running a control test in which Milli-Q water and ROC were spiked with H_2O_2 at varying concentrations. No interference was observed in the measurement of residual H_2O_2 when using these strips.

Figure 3.1 Colorimetric indication of hydrogen peroxide concentration in ROC using Merckoquant ® test strips

For the removal of residual H_2O_2, catalase (Sigma, bovine liver, activity 4000 units/mg dry weight) was used. The stock solution of catalase was prepared using 25 mg of catalase powder dissolved in 25 mL phosphate buffer (1.17 g Na_2HPO_4 and 0.57 g KH_2PO_4 in 250 mL MilliQ water, pH 7). The prepared solution was divided into several proportions and kept frozen for up to 1 month to maintain its activity. Prior using the catalase, it was thawed and any remaining thawed catalase was discarded. To remove residual peroxide from the sample, 10 µL of the catalase solution was added to every 25 mL of sample followed by 2 h of shaking at 100 rpm at room temperature (20 ± 2ºC); this amount of catalase accounted for the addition of less than 1 mg/L of COD and 0.05 mg/L of DOC. The concentration of residual H_2O_2 was reduced to less than 0.5 mg/L, checked using the test strips, which would have negligible effect on other analyses (Kang et al., 1999).

3.1.8 pH, conductivity and DO

A laboratory pH meter (Hach Sension 156 pH meter) was used to determine the pH of samples. Calibration was done periodically using buffer solutions at pH 4.0, 7.0 and 10.0 (Ajax Chemicals). Dissolved oxygen (DO) was measured with Hach DO meter (LDO 101) and conductivity was measured with Hach conductivity meter (CDC 401).

3.1.9 Total dissolved solids (TDS)

TDS was determined using Standard Method 2540 C (APHA, 2005). A well-mixed sample (100 mL) was filtered using a Whatman® glass-microfiber filter (2 µm) with applied vacuum. The filter paper was then washed with three successive 10 mL volumes of reagent-grade water, allowing complete drainage between washings, and suction was continued for about 3 min after filtration was complete. The total filtrate (with washings) was transferred to a weighed beaker and evaporated to dryness at 105° C for 24 h followed by drying at 180º C for 2 h. The residue was then cooled in a desiccator and immediately weighed. The concentration of TDS was calculated using the equation below.

$$\text{mg TDS / L} = \frac{(\mathrm{A}-\mathrm{B})*100}{\text{sample volume (mL)}}$$

where,

A = weight of dry residue and beaker, mg, and

B= weight of empty beaker, mg

3.1.10 High Purity Water (MilliQ)

The high purity water used in this study was produced by a Milli-Q Gradient A10 unit (Millipore). The Milli-Q water had a TOC concentration of <5 ppb and an electrical conductivity of approximately 0.05 μS/cm.

3.1.11 Chemical oxygen demand (COD)

COD was measured using the low range reagents (0-150 mg/L) supplied by Hach (Method 8000). Replicate samples of 2.0 mL were added to each reagent vial and digested for 2 h at 150° C in a Merck COD digester. Chloride ion is the most common interfering during COD determination as it reacts with silver ion to precipitate silver chloride thus inhibiting the catalytic activity of silver (APHA, 2005). The sample was diluted to bring chloride level below 2,000 mg/L and addition of 0.5 g $HgSO_4$ was made to reduce the interference of chloride ions. Duplicate (triplicate in some cases) measurements were conducted for each sample and average values were reported (variation was ±10%).

3.1.12 Total nitrogen (TN)

TN measurement was performed using a Hach low range reagent kit (0-25 mg/L, item code 2672245). Replicate samples of 2.0 mL were added to each total nitrogen hydroxide digestion reagent vial and digested for 30 min at 105°C following the Hach procedure. Samples were analysed in duplicate (triplicate in some cases) following the Hach procedure and average values were reported (variation was ±10%).

3.1.13 Total phosphorus (TP)

TP measurement was performed using a low range reagent kit (0-3.5 mg/L, item code 2742645, phosVer3 with Acid Persulfate Digestion) supplied by Hach. Replicate samples of 2.0 mL were added in total phosphorus test vial and digested for 30 min at 150° C. Samples were then analysed in duplicate (triplicate in some cases) following the Hach procedure and average values were reported (variation was ±10%).

3.1.14 NH_4^+-N, NO_3^--N and NO_2^--N determinations

3.1.14.1 NH_4^+-N determination

The ammonium nitrogen (NH_4^+-N) was measured colorimetrically at the wavelength of 660 nm via indophenol formation with sodium salicylate method described by Verdouw et al. (1978). A standard curve was prepared using the absorbance at 660 nm and concentration of standard solution. The concentration was then calculated from the standard solution as an example is given in Appendix 1. The samples were diluted to 1 in 20 and then analysed in triplicate and the average values were taken (variation was ± 5%).

The NH_4^+-N concentration of a water sample was then measured as:

NH_4^+-N = (Absorbance at 660 nm / 0.472) *dilution factor (Appendix 1)

3.1.14.2 NO_2^--N determination

The NO_2^--N was measured using Standard Method 4500B (APHA, 2005). A standard curve was prepared using the absorbance at 543 nm and concentration of standard solution. The sample concentration was then calculated from a standard curve as given in Appendix 1 as an example. The samples were diluted in 1 in 25 and/or 1 in 50 and then analysed in triplicate and the average values were taken (variation was ± 5%).

The NO_2^--N concentration of a water sample was then measured as:

NO_2^--N = (Absorbance at 543 nm / 3.0387) *dilution factor (Appendix 1)

3.1.14.3 NO_3^--N determination

The NO_3^--N was measured colorimetrically at the wavelength of 420 nm with sodium salicylate method described by Scheiner (1974). A standard curve was prepared using the absorbance at 420 nm and concentration of standard solution. The concentration was then calculated from the standard solution as an example is given in Appendix 1. The samples were diluted to 1 in 5 and/or 1 in 10 and then analysed in triplicate and the average values were taken (variation was ± 5%).

The NO_3^--N concentration of a water sample was then measured as:

NO_3^--N = (Absorbance at 420 nm / 0.0305) *dilution factor (Appendix 1)

3.1.15 Fluorescent Excitation Emission Matrix spectra

A PerkinElmer LS 55 fluorescence spectrometer was employed to obtain the EEM spectra of the water samples. The EEM spectra allow differentiation of fluorescent humic acid-like (HA-like) and fulvic acid-like (FA-like) materials, proteinaceous materials, and SMPs based on excitation and emission wavelengths. The band width for both excitation and emission was set at 5 nm. The excitation and emission slits were maintained at 7 nm and the scanning speed was set at 1200 nm/min. The data obtained were processed with the FL WinLab package software (Version 4.00.03, PerkinElmer) and Origin software (Origin 3) to generate the 3D EEMs and to export the data for fluorescence regional integration (FRI). The EEM spectra was divided into five regions and EEM volumes were calculated from FRI according to Chen et al. (2003).

3.2 UV reactor

A batch UV reactor was used in this study (Figure 3.2). The annular reactor was fitted with a centrally mounted lamp (Figure 3.2) placed within a quartz glass tube. Samples were placed in the sample chamber as shown in the figure 3.2. The reactor had a working volume of 900 mL and an average irradiated area of 464 cm^2, with a path length of 1.94 cm. The low-pressure UVC lamp (39 W) was purchased from Australian Ultra Violet Services (Victoria, Australia). The lamp emitted monochromatic light at 253.7 nm. Approximately 50% of the total energy input is converted to radiation at 253.7 nm, 2% to visible light while 48% is transformed into heat (Technical data sheet, Australian Ultra Violet Services). The average UV fluence was determined as 8.9 mW/cm^2. The water sample inside the reactor was aerated and mixed by humidified air that was introduced into the reactor via a Teflon air diffuser. Cooling water from a 20 L cooling water reservoir and a chiller (Aqua-Medic, Titan 1500) was circulated through the reactor jacket to keep the temperature constant (20 ± 2^o C). The full design specifications of the reactor can be found from elsewhere (Thomson, 2002).

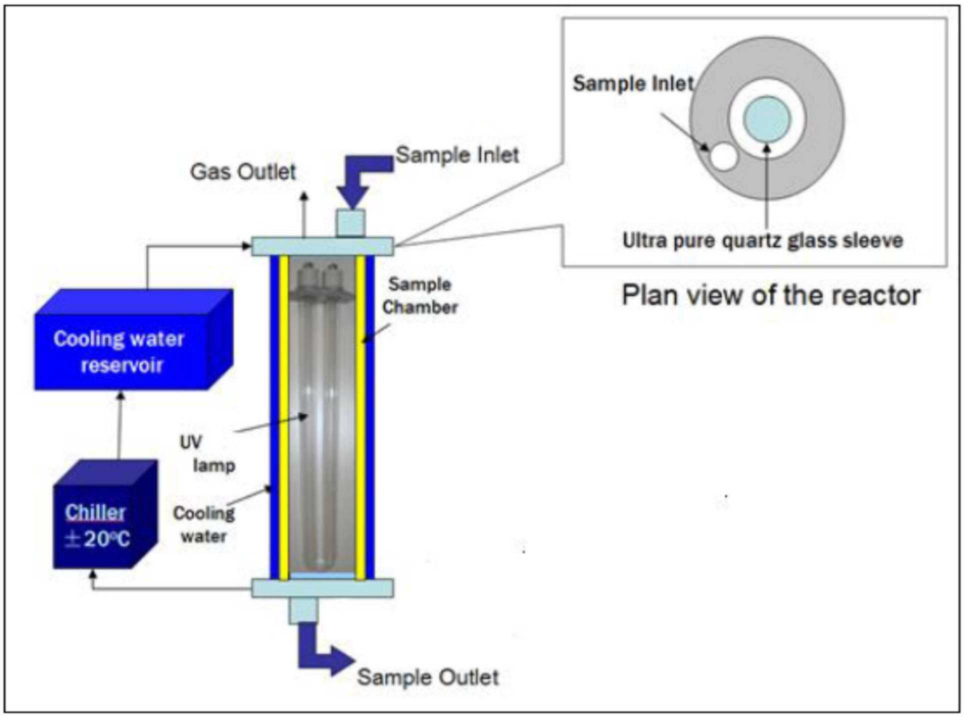


Figure 3.2 Schematic diagram of the UV experimental rig

3.2.1 Coagulation of ROC with Ferric Chloride ($FeCl_3$)

$FeCl_3$ stock solution was prepared using $FeCl_3.6H_2O$ (Chem-supply. Pty Ltd., Australia). A fixed dose of 1 mM $FeCl_3$ at pH 5 was used based on a preliminary coagulation experiment, which was in accordance with the suggested optimum coagulation conditions (Umar et al., 2016a). Coagulation was conducted with a laboratory jar test apparatus (Phipps and Bird, PB-700). The ROC sample (2 L) with the added coagulant was rapidly mixed for 2 min at 250 rpm followed by slow mixing for 25 min at 20 rpm, and then settling for 3 hours. The supernatant of coagulated ROC was then collected and stored at 4°C for further experiment.

3.3 BAC treatment and reactor set -up

Four BAC columns were set up at different experimental study periods. Initially, two BAC columns were set up and operated at 60 min EBCT and then after few months, another two BAC columns were set up and run at 30 min EBCT. The columns had an inner diameter of 1.5 cm and an effective packing height of 12 cm. Coal-based granular activated carbon (GAC) (Activated Carbon Technologies Pty Ltd, Australia, GS 1300) with the effective size of 1.2-1.4 mm, density 0.2-0.3 g/cm^3 and surface area >1200 m^2/g, uniformity coefficient <1.4, micropore 93%, mesopore 5% and macropore 2% was used. The activated carbon (GAC 1300) with surface area >1200 m^2/g, is manufactured for maximum biological activity and is normally used for BAC filters to treat wastewater, including ROC. The microporous (< 2 nm GAC) is suitable for removal of a large proportion of organic pollutants via adsorption and biodegradation as it provides a surface appropriate for biofilm growth. The carbon was sieved to remove the very fine particles, washed repeatedly with deionized water until the remaining fine particles were removed. It was then dried in an oven at 110 °C for 2 days.

The granular activated carbon was then inoculated over 4 days by mixing the carbon particles with activated sludge under aeration condition. The activated sludge was obtained from the wastewater treatment plant which supplied the secondary effluent to the reclamation facility. Nutrients including N, P and C sources were added to the system to promote the growth of microorganisms during the inoculation period (glucose 0.78 g/L, ammonium chloride 0.11 g/L, potassium dihydrogen phosphate 0.033 g/L) (Amann et al., 1996). The carbon was then transferred into glass columns which were fed with the UV/H_2O_2-treated ROC and raw ROC under different experimental conditions. The TDS of the ROC was increased gradually from

0.2 to 16 g/L over four weeks and the DOC and UV absorbance at 254 nm were continuously monitored to ensure the BAC columns were biologically active. The BAC columns were then used to treat the ROC with original salinity. All BAC tests were run at room temperature (22-28°C) in down flow mode and were always in the fully submerged conditions. Peristaltic pumps were used to feed the ROC sample to the columns. The flow rate of the effluent from the BAC columns was monitored daily and regulated using the outlet valve to maintain the fixed exposure time and submerged condition for different EBCTs. All BAC columns were kept in dark by wrapping them with aluminium foil to avoid algal growth. The columns were backwashed for 10 min every two weeks to remove the excessive biomass and reduce the clogging of the media bed.

3.3.1 Microbiological study using PCR-DGGE, sequencing and data analysis

Samples of the BAC column packing were collected on completion of the various experiments for characterisation of the bacteria. As collection of the carbon media from various depths was not feasible due to the narrow column diameter, the carbon packing was removed, thoroughly mixed, and duplicate 5 g samples collected into sterile 50 mL tubes.

The carbon media samples were centrifuged for 5 min and the liquid discarded. The DNA was then extracted from the media using a MoBio PowerSoil® DNA Isolation Kit following the manufacturer's instructions. The purity of extracted DNA was then checked on a Nanodrop-1000 spectrophotometer.

The extracted DNA was subjected to polymerase chain reaction (PCR) in a Bio-RAD T100™ Thermo cycler. The total bacterial community was evaluated using universal primers 341 FGC and 518R on 16S rDNA (Muyzer et al., 1993). The PCR program consisted of initial denaturation at 95°C for 5 min, followed by 20 cycles of 95°C for 30 s, primer annealing at

58°C for 30 s and elongation at 72°C for 60 s. After the last cycle, a final elongation at 72°C for 20 min took place and the amplification ended at 12°C. A negative control with no template DNA was included in the PCR run. Denaturing gel gradient electrophoresis (DGGE) analysis was performed using the Universal Mutation Detection System (BioRad) with a 9% urea-formamide denaturant gradient polyacrylamide (40–60% denaturing gradient). The gel was run at 60 °C and 60 V for 20 h, then silver stained (Girvan et al., 2003).

The culture-dependent microbial isolation technique for sequencing used 10-1,000 fold dilutions of the carbon media samples in 0.85% NaCl. Aliquots from each dilution were plated on dilute Nutrient Agar (1:100) plates and incubated at 25 ± 1 °C for 24-48 h. The most prominent bacteria on these plates were isolated and spread plate cultures obtained after incubation at 25 °C for 24 h. The DNA from the isolates was extracted as described above The extracted DNA (2 μL) was then subjected to PCR using the primers 63F and 1389R (Osborn et al., 2000). The thermocycling conditions used for this primer were set as initial denaturation for 5 min at 94° C, followed by 30 cycles of 94° C for 1 min, 55° C for 1 min, 72° C for 2 min and a final extension of 72 °C for 10 min. A negative control with no template DNA was included in the PCR run. The quality of the PCR product was checked using 1.2% agarose gel electrophoresis. The PCR product was then cleaned up with the DNA clean up kit (Promega) following the manufacturer's instructions, and sent to the Australian Genome Research Facility for sequencing. The sequences from the pure culture PCR were analysed using the method described by Adetutu et al. (2011), and the aligned sequences were submitted to BLASTN from GenBank for generating similarity searches. Phylogenetic trees were generated by performing the neighbour-joining algorithm using MEGA 6 software.

Images of the DGGE gels were analysed with Phoretix 1D software to generate a dendrogram using the unweighted pair group method with mathematical averages (UPGMA). The Shannon diversity index (H') was calculated from DGGE profiles using the formula

H' = - Σpi LN pi (Girvan et al., 2003). Principal component analysis (PCA) was also performed using IBM SPSS software (version 22) on the matrix data obtained from the Phoretix 1D software. Analysis of variance (ANOVA) was performed on the experimental data using the IBM SPSS software. Mean values separation was performed using the Least Significant Difference (LSD) test ($p = 0.05$), where the F-value was significant.

3.4 Characteristics of the ROC with high salinity

The ROC with high salinity used in this study was collected from a wastewater reclamation facility at a local municipal wastewater treatment plant (WWTP), and stored at 4°C. The WWTP treats the raw sewage biologically using the Intermittently Decanted Extended Aeration (IDEA) to produce secondary effluent, which was then treated using an ultrafiltration (UF, 0.04 μm)-RO system to remove salts and other contaminants to produce recycled water. The wastewater treatment plant generates 9 ML recycled water per day by treating 13 ML/day of sewage, i.e, the recovery of the RO system was about 75%. The extended aeration process operates with sludge age of 12 days. Antiscalant and biocide are added prior to the RO process at concentrations of up to 4 mg/L to avoid membrane scaling and microbial growth. Acid is added to maintain the pH at 7. The secondary effluent was high in salinity due to the infiltration of salty ground water to the sewer system, and consequently resulted in high salinity of the ROC.

The characteristics of the ROC collected over the experimental study time period are given in Table 3.1.

Table 3.1 Characteristics of ROC samples with high salinity collected between Jan 2013 to May 2014

Date	Jan, 2013	Mar, 2013	May, 2013	Aug, 2013	Sep, 2013	Nov, 2013	Dec, 2013	Feb, 2014	May, 2014
Parameter	R1	R2	R3	R4	R5	R6	R7	R8	R9
DOC (mg/L)	32	33	34	39	38	35	35.91	33.84	45.13
COD (mg/L)	-	105	118	102	105	127	120	180	150
pH	7.1	7.8	7.66	8	7.7	7.9	7.7	7.8	7.98
Colour (mg Pt.Co/L)	146	164	151	140	151	135	139	134	133
Chloride (g/L)	7.96	7.25	8.84	7.98	8.21	7.28	8.40	7.50	8.36
TDS (g/L)	17.60	15.65	18.20	16.55	17.24	16.03	16.22	15.98	14.78
UVA_{254} (1/cm)	0.62	0.64	0.634	0.62	0.65	0.64	0.616	0.618	0.61
SUVA (L/mg/m)	1.9	1.88	1.86	1.59	1.76	1.83	0.017	0.018	0.014
Alkalinity (as $CaCO_3$, mg/L)	430	350	-	426	418	608	638	620	640
Conductivity (mS/cm)	25.2	22.6	23.4	22.3	23.5	22.74	23.4	22.3	21.81
NH_4^+-N (mg/L)	-	-	-	-	-	3.97	5.27	4.91	4.34
Total nitrogen (mg/L)	-	-	27	-	-	22	18.5	16	23
Total phosphorus (mg/L)	-	-	-	-	-	33.1	26.3	-	-
DO (mg/L)	9.8	10	11	11	9	8	11	10.55	9.93

A total 9 batches of ROC were collected over the period of Jan 2013 to May 2014. The characteristics of ROC samples varied which may be due to seasonal variation, addition of different concentration of antiscalants during membrane cleaning processes and variation in influent characteristics. However, the DO and pH were relatively consistent throughout the collection time period.

3.5 Characteristics of the ROC with low salinity

The ROC with low salinity was collected from a reclamation facility of another wastewater treatment plant in Victoria, Australia. The reclamation facility receives the secondary effluent from a biological sewage treatment process which treats the influent containing domestic wastewater and the trade wastes mainly from a petrochemical processor (30% v/v). The secondary effluent is treated with a process with UF, UV and RO to produce recycled water. The water recovery of the RO system was approximately 75%. The general characteristics of the ROC are given in Table 3.2. The collected samples were stored at 4°C and brought to room temperature before use.

Table 3.2 Characteristics of the ROC samples with low salinity

	December, 2014	**March, 2015**	**June, 2015**
Parameter	R1	R2	R3
DOC (mg/L)	55	53	50
COD (mg/L)	132	150	110
pH	7.8	7.41	7.46
Colour (mg Pt.Co/L)	175	160	135
Chloride (g/L)	2.86	2.60	2.34
TDS (g/L)	4.46	4.56	4.32
UVA_{254} (1/cm)	1.18	1.11	0.83
SUVA (L/mg/m)	2.16	2.10	1.67
Alkalinity (as $CaCO_3$, mg/L)	575	600	540
Conductivity (mS/cm)	7.8	7.2	7.4
Total nitrogen (mg/L)	28	30	19
Total phosphorus (mg/L)	>35	59	45
DO (mg/L)	8.6	10	8.8

Chapter 4 - Removing organic and nitrogen content from a highly saline municipal wastewater reverse osmosis concentrate by UV/H_2O_2-BAC treatment

The aim of this study was to investigate the effectiveness and robustness of a UV/H_2O_2-BAC process for removing organic matter and nitrogen species from a municipal wastewater ROC with very high salinity over an extended period of operation (230 d). The impact of the process variables, including EBCT and residual H_2O_2 in the BAC influent, on the process performance was also studied to gain a better understanding about the bio-treatment. The treatment effectiveness was evaluated in terms of reductions in DOC, UVA_{254}, colour, COD, total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N). Some findings from this study were published in *Chemosphere* entitled 'Removing organic and nitrogen content from a highly saline municipal wastewater reverse osmosis concentrate by UV/H_2O_2-BAC treatment' (Chemosphere, 136 (2015), 198-203)

4.1 Characteristics of ROC used in the study

The characteristics of the ROC samples used in this study are given in Table 4.1. For studying the impact of contact time on organic matter and nutrient removal by the UV/H_2O_2-BAC treatment, the BAC process has been continuously run from January to October, 2013 including the acclimation period. Breakthrough was reached after the acclimation period of 45 days of operation (Jan -Feb, 2013) for organic matter removal. After that, biodegradation was the predominant mechanism for organic matter removal.

Table 4.1 Characteristics of ROC samples used in the study

Parameter	Value
pH	7.7 ± 0.4
DO (mg/L)	10.2 ± 1.3
DOC (mg/L)	36.0 ± 4.0
UVA_{254} (1/cm)	0.62 ± 0.02
Colour (mg Pt-Co/L)	148 ± 10.0
COD (mg/L)	120 ± 19
TN (mg/L)	21.4 ± 4.5
TP (mg/L)	28.5 ± 1.1
TDS (g/L)	16.6 ± 0.8
Chloride (g/L)	7.7 ± 1.8
Conductivity (mS/cm)	23.5 ± 1.3

Note: Values are presented as mean ± 1 standard deviation

4.2 Overall Removal efficiencies for organic matter and nutrients by the UV/H_2O_2-BAC treatment

The UV/H_2O_2-BAC treatment system was operated for 230 days (February to October, 2013) with EBCT of 60 min on multiple batches of ROC sample to evaluate its efficiency for the removal of organics and nutrients (N and P) (Table 4.2). As a reference, the raw ROC was treated by BAC alone using another column under the same operating conditions. After UV/H_2O_2 pre-treatment of the ROC, there were marked reductions in UVA_{254} and colour (60% and 86%, respectively), whereas only 15% reductions were achieved for DOC and COD, respectively. The UVA_{254} reflects the presence of conjugated bonds and aromatic content of organic matter (Chen et al., 2011a). The result was consistent with previous studies which showed great cleavage but limited mineralisation of the organic matter in the municipal wastewater ROC using such treatment (Liu et al., 2012, Umar et al., 2013).

For the BAC alone treatment, the removal efficiency for DOC and COD was markedly higher (38% and 32%, respectively) than for the UV/H_2O_2 treatment, although the reductions in UVA_{254} and colour (64% and 80%, respectively) were comparable for the two treatments. The considerable reduction in DOC by the BAC alone treatment was associated with biodegradation and adsorption. Although the organic molecules in the ROC are generally regarded as low in biodegradability as they were primarily derived from the biologically treated secondary effluent, it was possible that some of the molecules could be removed by the microbes with sufficient contact time.

Using the combined treatment, the removal efficiency for the organic matter was increased significantly, with 57% DOC, 46% COD, 81% UVA_{254} and 95% colour removed from the ROC. The great improvement in organic matter removal was mainly attributed to the partial degradation of the organic molecules by the oxidative treatment, leading to the production of simpler molecules which could be readily removed by the microbes embedded in the BAC column (Lu et al., 2013). A comparison of the organic matter removal efficiency obtained in the present study and the work done by Lu et al. (2013) using the same treatment train showed that the treatment performance was fairly comparable, although the ROC salinity was markedly different. This might indicate that the change in the TDS of ROC in the range of 10-16 g/L did not have any significant impact on the organic matter removal. The results suggested that a synergistic effect existed for the combined treatment in reducing DOC from the ROC. It is worth noting that the UV/H_2O_2–BAC treated ROC had similar DOC and COD levels to the secondary effluent used as the influent of the RO-based reclamation process (i.e., 15 cf. 14.5 mg DOC/L and 64.7 cf. 64 mg COD/L), but significantly lower UVA_{254} and colour (i.e., 0.12 cf. 0.19 /cm and 7.2 cf. 120 mg Pt-Co/L).

In terms of nutrient removal, minimal reduction was obtained by the UV/H_2O_2 treatment. The small reduction in TN during the oxidation process was likely due to the oxidation of some nitrogen species to nitrogen gas (Dwyer et al., 2008). With BAC treatment alone, the removal efficiency was fairly high for TN (71%) but still low for TP (8%). TN reduction for the combined treatment system was lower (60%) compared with the BAC only treatment, although TP reduction was improved slightly. This study showed a higher TN removal (60%) compared with the study by Lu et al. (2013), in which the reduction in TN was only 24% for the combined treatment system with the same EBCT for the BAC treatment. The higher removal of TN in the present study may be related to the use of a lower H_2O_2 concentration (3 mM cf. 4 mM), and thus less residual H_2O_2 after the oxidative process. The impact of residual H_2O_2 on the removal of organic content and nitrogen was therefore investigated further in this work and reported in Section 4.5. Nevertheless, the TP removal was comparable in both studies (15% in this study) for the combined treatment system. It is known that phosphorus removal is greatly inhibited by increasing salinity (>5 g /L) (Uygur and Kargı, 2004). As the salinity of ROC used in both studies was very high (TDS 16.6 g /L and 10 g /L), lower removal of TP would then be anticipated. Since chemical coagulation is generally an effective means for TP removal, it could be employed prior to the oxidative treatment for achieving the required removal efficiency for TP, and additional reduction in organic content. The DO of the effluent was ~6 mg/L for the combined and the BAC alone treatment, which was lower than for the influent (~10 mg/L), suggesting that the activated carbon adsorbed DO and the adsorbed DO was utilized by the microorganisms to biodegrade the contaminants (Jin et al., 2013).

During the 230-d operation of the combined treatment, six batches of ROC sample were used for the study. The TDS of the ROC samples varied from 15.6 to 18.2 g/L and the DOC varied from 32 to 38 mg/L. It was observed that the average DOC removal efficiency of the combined treatment over the ROC sample batches varied only slightly (54-59%), whereas the TN removal efficiency varied a little more (55-65%). This indicates that the UV/H_2O_2-BAC process was a robust treatment system for the ROC for organic matter and nitrogen removal.

Table 4.2 Overall water quality characteristics of the ROC after the different treatments over the 230 days of stable operation

Parameter	**Raw ROC**	**UV/H_2O_2**	**Treatment**	
			BAC	**UV/H_2O_2+BAC**
pH	7.7 ± 0.4	8.0 ± 0.2	8.4 ± 0.2	8.4 ± 0.2
DO (mg/L)	10.2 ± 1.3	12.1 ± 0.9	7.0 ± 0.9	7.2 ± 1.2
DOC (mg/L)	35.0 ± 5.0	30.0 ± 4.0 (15%)	21.0 ± 4.0 (38%)	15.0 ± 2.0 (57%)
COD (mg/L)	120.0 ± 9.0	102.0 ± 8.0 (15%)	82.0 ± 11.0 (32%)	65.0 ± 7.0(46%)
UVA_{254} (1/cm)	0.623 ± 0.025	0.246 ± 0.033 (60%)	0.23 ± 0.06 (64%)	0.12 ± 0.03 (81%)
Colour (mg Pt-Co/L)	138.0 ± 10.0	20.0 ± 8.0 (86%)	28.0 ± 8.0 (80%)	7.0 ± 5.0 (95%)
TN (mg/L)	21.0 ± 3.0	18.0 ± 3.0 (13%)	6 ± 1.0 (71%)	9.0 ± 2.0 (60%)
TP (mg/L)	29.0 ± 2.0	28.0 ± 2.0 (2%)	26.0 ± 1.0 (8%)	24.0 ± 1.0 (15%)

Note: Data based on the 57 samples collected during February to October, 2013. Values are presented as mean ± standard deviation, average removal efficiencies (%) are shown in the brackets.

4.3 Impact of EBCT on BAC for organic matter removal

The impact of EBCT on the BAC treatment was evaluated over a 50 d period by feeding another two established BAC columns with UV/H_2O_2-treated and raw ROC, respectively, at the EBCT of 30 min. The experimental data from the two BAC columns which were operated with the EBCT of 60 min over the same period, as mentioned in Section 4.2, were used for the comparison (Figure 4.1). For the ROC without oxidative treatment, the reduction efficiency was similar, with only 32% (EBCT 30 min) and 36% (EBCT 60 min) of DOC removed, respectively. The lower DOC removal in the BAC system was mainly due to the recalcitrant nature of the organic matter present in the ROC, leading to its limited biodegradation by the microorganisms. For the UV/H_2O_2-treated ROC, DOC removal increased significantly, with averages of 48% to 58% removed for EBCT of 30 and 60 min, respectively. It can be inferred that the organic matter partially oxidized during the oxidation process had been consumed by the microbes for their metabolism, and the longer contact time facilitated the utilisation of the oxidised organic compounds.

The UVA_{254}, colour and COD removals followed a similar trend as the DOC removal. For the raw ROC, increasing the EBCT from 30 to 60 min led to increased average reductions from 25% to 32% for UVA_{254}, 40% to 53% for colour and 19% to 37% for COD. For the UV/H_2O_2-treated ROC, the average reductions increased from 70% to 75% for UVA_{254}, 84% to 90% for colour and 34% to 48% for COD with increasing EBCT from 30 to 60 min with the BAC treatment. The results suggested that sufficient contact time was required for the microbes to biodegrade and consume partially oxidised organic molecules from the oxidation process in the BAC system, and hence maximise the removal efficiency for organic content.

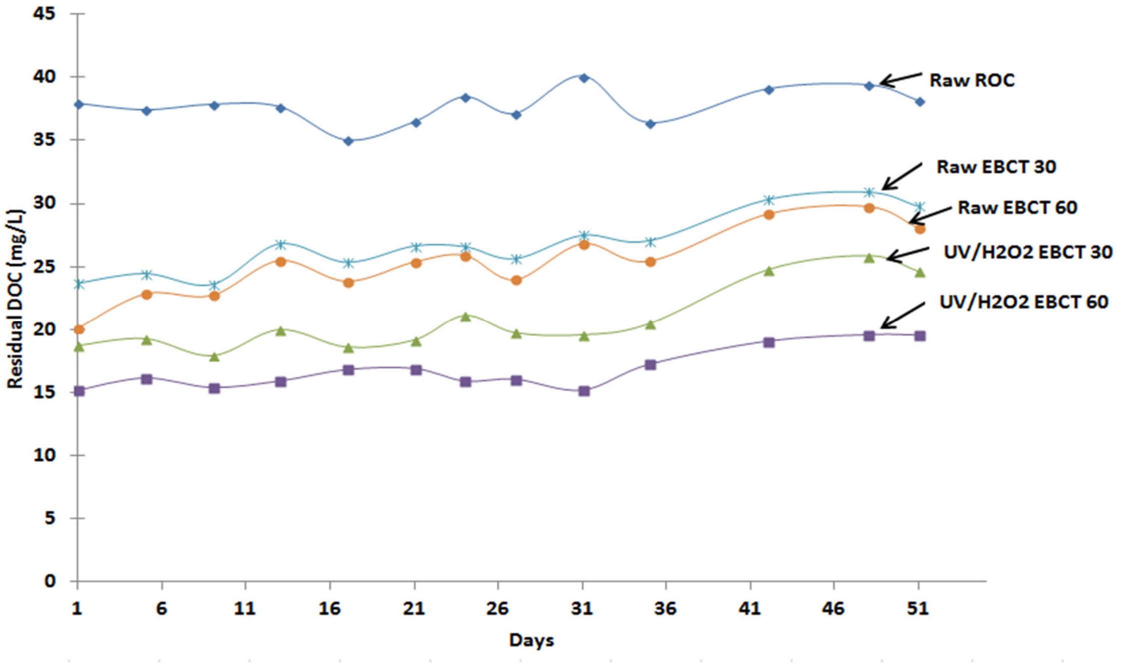


Figure 4.1 Impact of EBCT on DOC removal by various treatments

4.4 Characterisation of nitrogen removal

To obtain a better understanding of the nitrogen removal, the treatments were characterised in terms of TN, NH_4^+-N, NO_2^--N and NO_3^--N over the 50 d period (Figure 4.2 a and b). The

UV/H_2O_2 treatment alone was not very effective for removing total nitrogen, with only 15% of TN removed. NO_2^--N was removed almost completely, whereas NO_3^--N concentration increased by ~1 mg/L after the oxidation treatment. The increase in NO_3^--N concentration was most likely due to the conversion of NO_2^--N to NO_3^--N in the presence of oxidant as suggested by Munter (2001). Since NH_4^+-N has no unsaturated double bonds, it would be difficult for the $HO^\bullet$ radical to break down the N-H bonds through electrophilic attack (Haag et al., 1984), hence the slight reduction in the NH_4^+-N during the treatment was probably due to its volatile nature.

With the combined UV/H_2O_2-BAC treatment, the reduction in TN, NH_4^+-N and NO_3^--N improved markedly. The removal of nitrogen species in the BAC system was possibly due to nitrification-denitrification process (Kalkan et al., 2011). Nitrification is carried out by two different groups of bacteria. The first group, ammonia oxidising bacteria (AOB), oxidises ammonium to nitrite and the second group, nitrite oxidising bacteria (NOB), further oxidises nitrite to nitrate. During the denitrification process, the nitrate is consumed by the denitrifiers, thereby converting nitrate to nitrous oxide (N_2O) or nitrogen gas (N_2) (Lee and Welander, 1996)

In the present study, it was shown that the longer EBCT led to a marked improvement in TN (51% at EBCT 30 min cf. 66% at 60 min), NH_4^+-N (44% at EBCT 30 min cf. 90% at 60 min), NO_2^--N (8% at EBCT 30 min cf. 36% at 60 min) and NO_3^--N (51% at EBCT 30 min cf. 62% at 60 min) removal with the combined treatment, although the total removal in NO_2^--N was significantly lower compared with the oxidative treatment alone. In the present work, higher nitrification was achieved at longer contact time for the combined system. This was consistent with the findings of Krasner et al. (2009) who reported that longer retention time is required for the stable retention of nitrifiers, and for nitrification. Nitrification is carried out by autotrophic bacteria in the presence of sufficient DO (3-4 mg/L) (Tchobanoglous et al., 2003).

In the present work, there was sufficient DO in the BAC influent (6-10 mg/L) for effective nitrification. Kalkan et al. (2011) achieved 65% ammonium removal using a BAC system for treating secondary effluent and suggested that simultaneous nitrification-denitrification could actually take place in the BAC system. The changes in nitrite and nitrate in the effluent from each column (Figure 4.2 a & b) demonstrated that there was effective nitrification taking place. According to Yapsakli et al. (2010), most ammonium removal occurred as soon as the influent entered the BAC column, indicating that most of the nitrification took place in the uppermost part of the filter media. The results inferred that nitrifying bacteria can adapt to the extremely high salinity as there was high removal of ammonium by the combined system at longer contact time, which is in accordance with the fact that the nitrifiers could survive at TDS up to 30 g/L by acclimation (Glass and Silverstein, 1999). The lower removal of nitrite might be due to inhibition of NOB by hydroxylamine produced during conversion of ammonia to nitrite by the AOB and/or due to the H_2O_2 present in the BAC influent as suggested by Stüven et al. (1992) and Rosa et al. (1998). The changes in nitrite and nitrate after BAC treatment (Figure 4.2 a & b) demonstrated that there was effective nitrification taking place.

For the BAC treatment alone, NH_4^+-N removal remained fairly consistent with both EBCTs with 90% and 84% removals at 60 min and 30 min respectively. Longer EBCT also led to improvements in TN and NO_3^--N removals from 62% to 73% and 56% to 65%, respectively. However, in the case of NO_2^--N, with BAC treatment alone, 72% of it was achieved at 60 min EBCT, whereas NO_2^--N concentration increased at 30 min EBCT.

It should be noted that some part of the ammonium removal might have been removed by magnesium ammonium phosphate (MAP) precipitation, with very little (only 8%) phosphate removal because stoichiometrically, ~12.46 mg/L and 16.75 mg/L oxygen is required for complete ammonia oxidation and complete nitrification, respectively (Tchobanoglous et al., 2003), and the measured DO of ROC was 10-11 mg/L for complete ammonia oxidation.

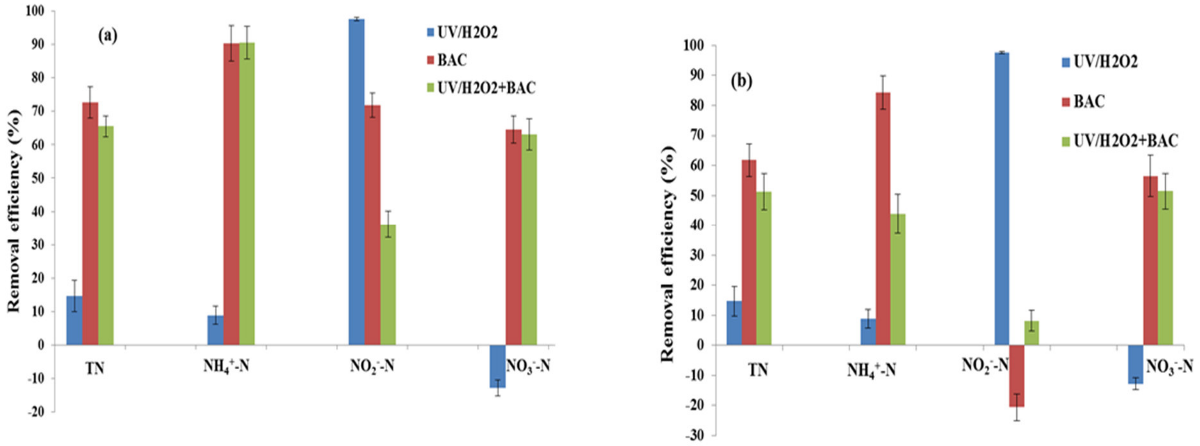


Figure 4.2 Impact of EBCT on nitrogen removal by various treatments at (a) 60 min and (b) 30 min EBCTs respectively (Influent characteristics TN 21 ± 3 mg/L; NH_4^+-N 4.3 ± 0.8 mg/L; NO_2^--N 1.2 ± 0.2 mg/L; NO_3^--N 9.1 ± 3.4 mg/L).

In this study, almost complete nitrification and partial denitrification had taken place. The denitrifiers would be harboured deep inside the biofilm and towards the bottom of the BAC column, where the concentration of DO would be minimal. The COD:N ratio for complete denitrification varies between 7 and 10 (Carrera et al., 2004, Fontenot et al., 2007). In the present study, the ratio was 10, which was sufficient for denitrification. However, complete denitrification was not achieved despite sufficient carbon being available in the influent. This was most likely due to the significant DO concentration which was 6-7 mg/L for the effluent for the BAC system. Complete denitrification would normally take place when DO is <1.5 mg/L. However, Virdis et al. (2010) observed complete denitrification of synthetic wastewater at DO of 4.35 mg/L. The removal of nitrate under aerobic conditions was possibly due to denitrifying activity in anoxic microenvironments that may develop under aerobic conditions. Although, biofilm thickness was not measured, anoxic microenvironments may have developed in the BAC system, so that the denitrification could take place as depicted in Figure 4.3. When the biofilm developed on the BAC was getting thicker, there was insufficient oxygen supply internally, thereby creating anaerobic and/or anoxic conditions deep inside and promoting denitrification. The concentration of oxygen decreases with the depth of biofilm. Nitrate diffuses through the aerobic zone and serves as electron acceptor for oxidation of organic matter in an inner anoxic zone (Hagedorn-Olsen et al., 1994).

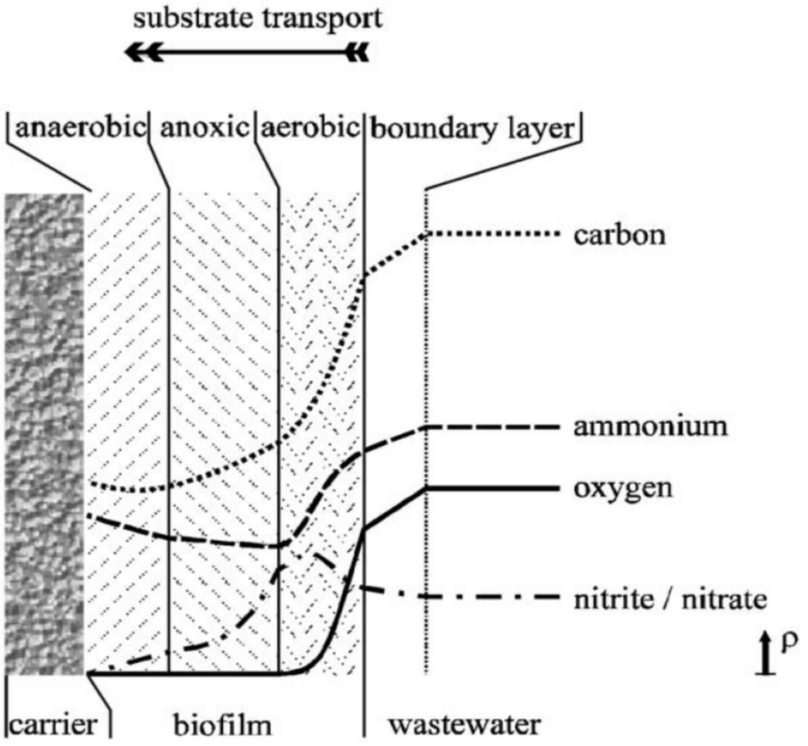


Figure 4.3 Schematic representation of a biofilm structure (Walter et al., 2005).

The results for the BAC alone treatment indicated that the denitrifying bacteria were inhibited more than the nitrifying bacteria by the high salinity of the ROC, as was reported in the study of the nitrification and denitrification of a saline wastewater by Dinçer and Kargi (1999). The slightly lower TN removal by the combined treatment compared with the BAC alone appeared to be due to the lower NO_2^--N removal which could be related to the potential negative impact of the residual peroxide in the UV/H_2O_2-treated ROC on the nitrification and denitrification (Pedersen and Pedersen, 2012). The impact of residual H_2O_2 on BAC treatment is reported section 4.5.

4.5 Impact of residual H_2O_2 on BAC treatment

4.5.1 Impact on DOC, UVA_{254} and colour removal

The residual H_2O_2 after the oxidation process was 50 ± 10 mg/L, which was measured according to Bader et al. (1988) and Merckquant® test strips. Since catalase is a widely used quenching agent for H_2O_2, it was used to remove the residual H_2O_2 from the UV/H_2O_2-treated ROC prior to the BAC treatment in order to study its impact on the BAC process.

For the columns fed with ROC with no residual H_2O_2, the DOC removal decreased from 57% to 50% (Figure 4.4).

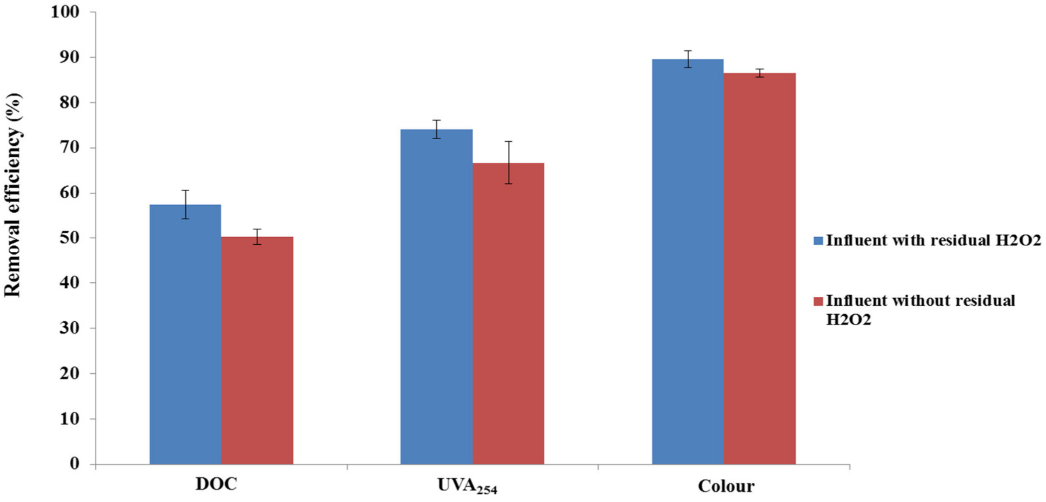


Figure 4.4 Organic matter removal by BAC for the influent with and without residual peroxide (Influent characteristics DOC 38 ± 1.0 mg/L; UVA_{254} 0.609 ± 0.023/cm, and Colour 137 ± 10 mg Pt-Co/L)

The trend for UVA_{254} and colour removal was similar. This suggested that the residual H_2O_2 was beneficial to the removal of organic content as H_2O_2 is generally considered to be an extra supplier of oxygen to the aerobic bacteria and so enhances the bioactivity and thus removal of organic matter. The better removal of organics in the presence of residual H_2O_2 may also be due to the improved biodegradability resulting from the organic intermediates produced from H_2O_2 oxidation (Ksibi, 2006). It is known that microorganisms dealing with external H_2O_2 can release antioxidants and several kinds of enzymes such as catalase, peroxidases and superoxide dismutases for the protection, interception and repair of the microorganisms from radical damage by the H_2O_2 (Angel et al., 1999).

4.5.2 Impact on nitrogen removal

Contrary to the trend for DOC, UVA_{254} and colour removal, the removal efficiency for TN, NO_3^--N and NO_2^--N increased markedly, and the removal efficiency for NH_4^+-N was less when the BAC feed was switched to the ROC with no residual peroxide (Figure 4.5). The lower removal of nitrogen species in the presence of residual H_2O_2 was likely due to the suppression of or other detrimental effect of higher concentration of residual H_2O_2 on the nitrogen-removing bacteria as suggested by Lu et al. (2013) who observed an even lower TN removal. Schwartz. et al. (2000) showed 80% reduction in ammonium removal in a fluidised sand bed filter at a H_2O_2 concentration of 100 mg/L. Møller et al. (2010) found that the nitrification process could be moderately affected with a low dose of H_2O_2 (10-13 mg/L) in a biofilter for a recirculating aquaculture system, thereby reducing ammonia and nitrite removal. They noted that the nitrification process was severely inhibited when the biofilter was exposed to H_2O_2 for a prolonged period even with a lower H_2O_2 concentration. They also showed prolonged nitrite accumulation due to the effect of H_2O_2 on the nitrite oxidising bacteria. In the present study, it appeared that the NOB were more vulnerable to residual H_2O_2 than AOB. A statistical analysis of the experimental data was conducted using analysis of variance (ANOVA) at 95% confidence level (α=0.05) for each N species in the presence and absence of residual H_2O_2. It was shown the impact of the residual H_2O_2 was significant for all N species (i.e., all *p*-values were less than 0.05), with the decreasing significance level in the order of TN (*P*-value 0.00026), nitrite (*p*-value 0.0019), nitrate (*p*-value 0.008) and ammonium N (*p*-value 0.037).

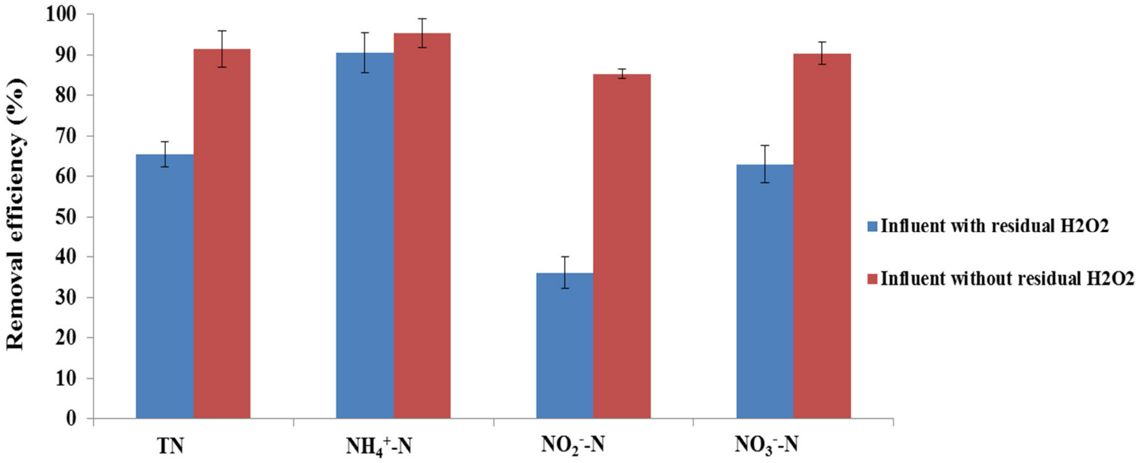


Figure 4.5 Nitrogen removal by BAC for the influent with and without residual peroxide (Influent characteristics TN 21 ± 3 mg/L; NH_4^+-N 4.3 ± 0.8 mg/L; NO_2^--N 1.2 ± 0.2 mg/L; NO_3^--N 9.1 ± 3.4 mg/L).

4.5.3 Verification of the impact of residual peroxide on BAC treatment

To verify the findings from the foregoing study on the impact of residual peroxide, the residual H_2O_2 of the UV/H_2O_2-treated ROC was removed with a rapid BAC filtration method to avoid the potential impact of the catalase on the BAC treatment. Urfer et al. (1997) mentioned that biologically active filters can remove H_2O_2 reliably within a short EBCT. The same concept was used to remove the residual H_2O_2 in the present study. The pre-oxidised ROC was passed through a BAC column (BAC 1) with EBCT of 5 min which was sufficient for 100% removal of the residual H_2O_2 with only very minimal loss of organic and nitrogen contents. The DO removed was also very minimal (~0.05 mg/L). The effluent with no residual peroxide was then fed to another BAC column (BAC2a) with EBCT of 60 min. As a comparison, peroxide was added to some of the effluent with from BAC 1 to resemble the residual peroxide condition before passing it to an identical BAC column (BAC2b) at the same EBCT. The same trend for the impact of residual peroxide on the BAC treatment was shown in the absence of peroxide. The reduction decreased from 55% to 50% for DOC, 42% to 36% for COD, 74% to 73% for UVA_{254} and 90% to 88%, whereas the removal efficiency for nitrogen increased, i.e., from 69% to 85% for TN, 97% to 99% for ammonium, 60% to 83% for nitrate and 42% to 91% for nitrite.

Table 4.3 Characteristics of the ROC after the various treatments for verification of impact of residual H_2O_2 on organic matter and nitrogen

Parameters	**Raw ROC**	**UV/H_2O_2**	**Treatment**		
			BAC1 EBCT5	**BAC2a EBCT60 (without H_2O_2)**	**BAC2b EBCT60 (with added H_2O_2)**
DOC (mg/L)	33.9 ± 0.5	29.8 ± 0.8 (12%)	24.6 ± 0.6 (27%)	16.9 ± 0.3 (50%)	15.1 ± 0.4 (55%)
Colour (mg Pt-Co/L)	145.8 ± 3.8	57.3 ± 13.7 (61%)	46.8 ± 11.7 (68%)	17.5 ± 2.1 (88%)	15.0 ±7.1 (90%)
UVA_{254} (1/cm)	0.62 ± 0.01	0.32 ± 0.01 (48%)	0.24±0.01 (61%)	0.17 ± 0.03 (73%)	0.16 ± 0.02 (74%)
TN (mg/L)	17 ± 0.75	15.7 ± 0.87 (8%)	15.33±1.94 (10%)	2.5±0.00 (85%)	5.25 ± 1.06 (69%)
NH_4^+-N (mg/L)	4.02 ± 0.7	3.06 ± 0.8 (23%)	1.63 ± 0.5 (59%)	0.05 ± 0.00 (99%)	0.095 ± 0.063 (97%)
NO_3^--N (mg/L)	9.18 ± 0.75	9.8 ± 0.36	9.5 ± 0.54	1.52 ± 0.5 (83%)	3.70 ± 0.90 (60%)
NO_2^--N (mg/L)	1.52 ± 0.14	0.14± 0.3 (81%)	1.40 ± 0.35 (6%)	0.135 ± 0.02 (91%)	0.90 ± 0.07 (42%)

Note: Values are presented as mean ± standard deviation; average removal efficiency (%) is shown in the bracket

4.6 Conclusions

The study demonstrated that combined UV/H_2O_2-BAC treatment of an extremely high salinity municipal ROC led to effective reduction in organic content and nitrogen species. The treated ROC had similar levels of DOC, COD and TN to the secondary effluent which was used as the influent for the reclamation process, but better water quality in terms of UVA_{254} and colour. However, the process was ineffective for TP removal due to the high salinity, and coagulation prior to the combined treatment may be required to achieve target TP removal. Coagulation of the ROC would also provide additional removal of organic content for the combined treatment.

It was demonstrated that the biological process could be acclimated to the very high salinity wastewater, and sufficient contact time (i.e., EBCT 60 min) was essential for the BAC system to achieve satisfactory performance for the removal of organic content and nitrogen species. It was shown that greater nitrification-denitrification took place in the combined treatment system, without addition of a carbon source or aeration of the BAC system.

This study revealed that the presence of residual H_2O_2 in the oxidised ROC was beneficial to DOC removal, whereas it had an inhibiting effect on nitrogen removing bacteria. More detailed microbiological characterisation of the BAC system operated under various conditions including salinity, initial concentrations of organic matter and nutrients of the ROC would help to obtain better insight into the removal mechanisms, and hence to further optimise the process for cost-effective treatment of the highly saline ROC.

The next chapter investigates the impact of various salinity levels of municipal ROC on organic matter and nutrients removal with the combined treatment of UV/H_2O_2-BAC with the change in microbial communities at various salinity levels.

Chapter 5 - Impact of salinity on organic matter and nitrogen removal from a municipal wastewater RO concentrate using biologically activated carbon coupled with UV/H_2O_2

The main objective of this study was to investigate the impact of salinity (TDS at 7, 10 and 16 g/L) of a RO concentrate (ROC) on the treatment efficiency of a biological activated carbon (BAC) system after pre-oxidation with UV/H_2O_2 in terms of removal of organic matter and nitrogen species, and the bacterial communities. The TDS of the ROC collected from the wastewater reclamation facility could vary from 5 to 19 g/L, which was based on a 4-year sampling program and the typical TDS level was 10-16 g/L; however, under some extreme conditions such as heavy rainfall the TDS of the ROC could be as low as 5-8 g/L. According to the existing literature, the salinity of the ROC used in this study was much higher compared with that for most previous studies (i.e., TDS 2-10 g/L). Microbiological characterisation using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and culture based techniques were used to investigate the diversified bacterial communities present in the BAC system.

The findings from this study were published in *Water Research* entitled 'Impact of salinity on organic matter and nitrogen removal from a municipal wastewater RO concentrate using biologically activated carbon coupled with UV/H_2O_2' (Water Research, 94 (2016), 103-110).

5.1 Collection, preparation of various salinity of ROC and characterization of ROC used in the study

Raw ROC and secondary effluent used for the preparation of the ROC samples for the tests was collected from the same treatment plant as in Chapter 4. The general characteristics of the ROC samples and secondary effluent used for this study are given in Table 5.1.

To study the impact of ROC salinity on the organic and nutrient removal efficiency of the BAC treatment, ROC samples with three different levels of salinity were prepared: low (TDS ~7 g/L), medium (TDS ~10 g/L) and high salinity (TDS ~16 g/L) ROC. Sample preparation involved diluting the raw ROC with secondary effluent in the ratio of 1:2 to make the low salinity ROC (Table 5.1). To obtain the medium and high salinity ROC with the same content of organics and nutrients as the low salinity ROC, NaCl and $MgSO_4$ were added to the diluted ROC in the same ratio (5:1) as for the original ROC. The electrical conductivity values of the low, medium and high salinity ROC samples were approximately 11, 16.8 and 24 mS/cm, respectively.

Table 5.1 Water quality of raw ROC, secondary effluent and diluted ROC

Parameters	**Raw ROC**	**Secondary effluent**	**Diluted ROC (Low salinity)**
DOC (mg/L)	43	9	20.3
Colour (mg Pt-Co/L)	134	36	68.2
UVA_{254} (/cm)	0.60	0.15	0.30
TN (mg/L)	23	3	13.4
NH_4^+-N (mg/L)	4	2	3.75
NO_2^--N (mg/L)	2	0	0.55
NO_3^--N (mg/L)	12	1	9.80
COD (mg/L)	150	35	75
Chloride (g/L)	8.37	2.18	3.53
DO (mg/L)	10	9	9.8
Conductivity (mS/cm)	22	7	11
TDS (g/L)	14.0	4.12	7.12

The ionic compositions of raw ROC and secondary effluent are given in Table 5.2.

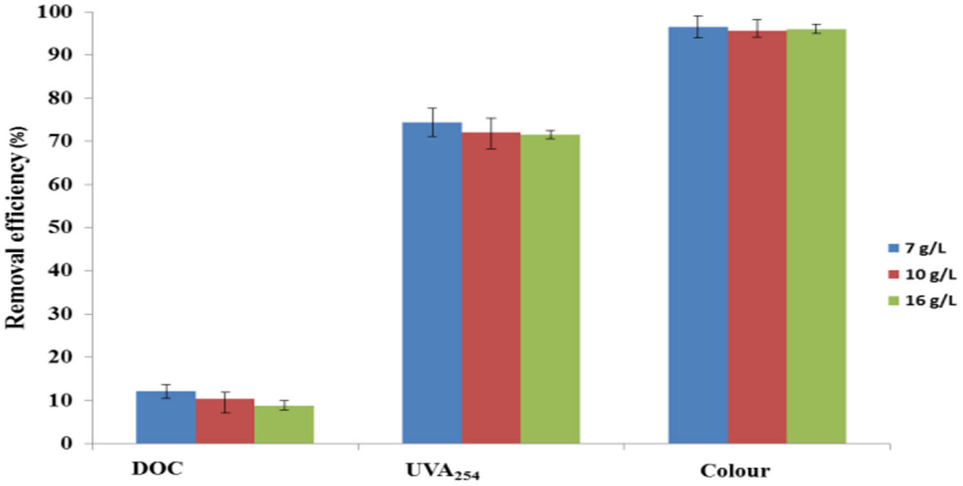
Table 5.2 Ionic composition of raw ROC and secondary effluent used in the study

Ion (mg/L)	Raw ROC	Secondary effluent
Na^+	433.0	32.9
Mg^{2+}	87.5	6.28
K^+	12.3	0.975
Ca^{2+}	29.5	2.04
Mn^{2+}	0.013	--
Fe^{3+}	<1.52	<1.52
Ni^{2+}	0.003	0.003
Cu^{2+}	0.001	--
Zn^{2+}	3.22	1.00
Mo^+	1.37	0.32
Pb^{2+}	<0.015	--

5.2 Impact of salinity on organic matter removal by UV/H_2O_2-BAC treatment

The three BAC columns were equilibrated with the original ROC which had been pre-treated with UV/H_2O_2 and their comparable performance confirmed. Then the ROC at the three salinity levels (TDS of 7, 10 and 16 g/L) was subjected to UV/H_2O_2 followed by BAC treatment.

Comparable organic matter removal was obtained for each of the three ROC preparations after the UV/H_2O_2 treatment. There was a marked reduction in UVA_{254} (72-74%) and colour (96%) on average, and 9-12% DOC removal (Figure 5.1a). This was consistent with previous studies which showed extensive cleavage and thus reduction in colour, but limited mineralisation, for the organic matter in a municipal wastewater ROC using the same treatment (Liu et al., 2012, Umar et al., 2013). Liu et al. (2012) observed that organic matter removal was not greatly affected by salinity over the range 4.4-11.2 mS/cm, and Umar et al. (2013) observed little impact of the salinity (8.3-23 mS/cm) on DOC and COD removals. In this study the reductions in DOC at low salinity were marginally higher than for medium and high salinity as shown by one way ANOVA analysis (*p*-value was 0.016 ($\alpha<0.05$) at 95% confidence level), whereas differences in the reduction of UVA_{254} and colour at different salinity were not significant.



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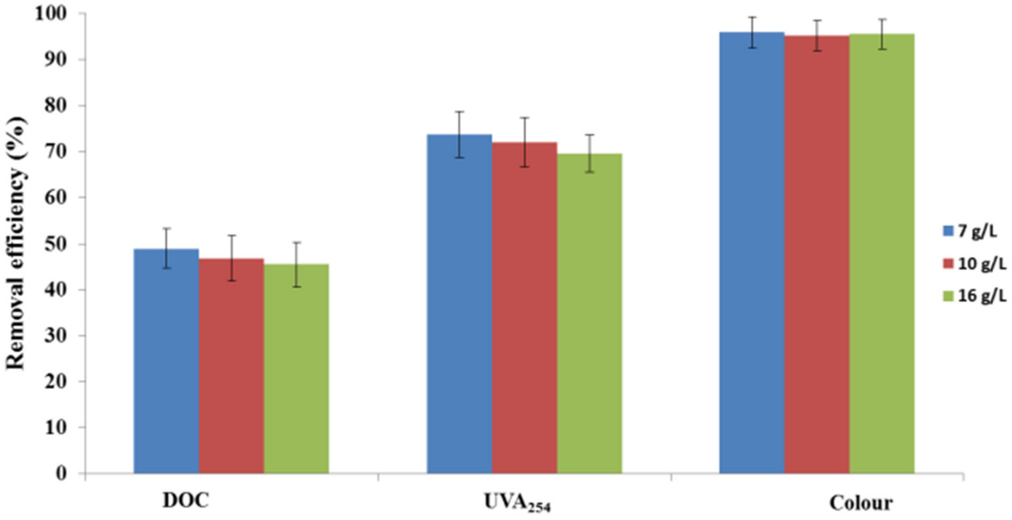


(b)

Figure 5.1 Organic matter removal by (a) UV/H_2O_2 pre-treatment and (b) sequential UV/H_2O_2 and BAC treatment for the ROC of various salinity (number of analyses, n=12 for each salinity level).

After the treatment with BAC, the DOC removal increased significantly (to 45-49%) (Figure 5.1b). The increase in DOC removal in BAC treatment was mainly due to consumption of previously oxidised and mineralised organic matter by the microorganisms. Although there appeared to be a trend toward reduced removal with increased salinity, one way ANOVA analysis showed that there was not a significant difference (*p*-value of 0.216, $\alpha > 0.05$ at 95% confidence level).

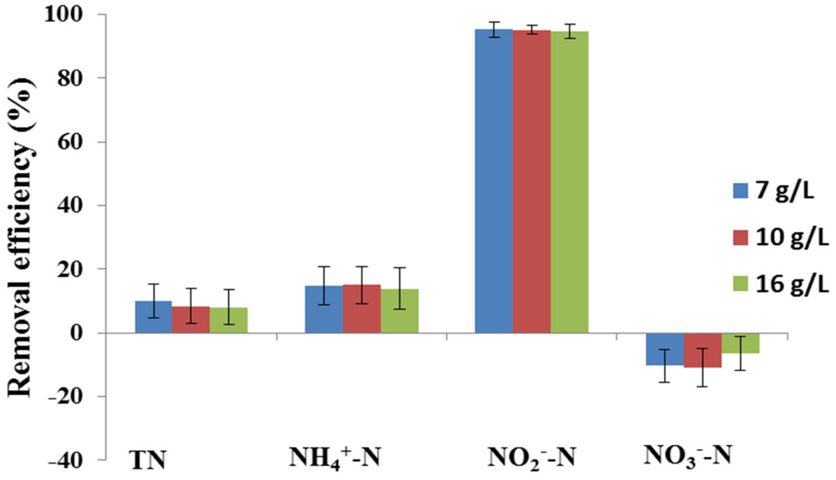
The UVA_{254} reduction was similar for low and medium salinity with subsequent BAC treatment. For high salinity, there was a slight decrease in UVA_{254} reduction which may be attributed to the release of soluble microbial products (SMPs) due to cell lysis and decaying biomass at the high salinity (Azami et al., 2012). The colour removal followed the same trend as for reduction in UVA_{254}.

5.3 Impact of salinity on nitrogen removal by UV/H_2O_2-BAC treatment

Similar to organic matter removal, there was very little difference in the removal efficiency of nitrogen species after the UV/H_2O_2 treatment of the ROCs with different salinity (Figure 5.2a) and this was confirmed by one-way ANOVA analysis (*p*-values were >0.05). The reduction in TN (~9%) and NH_4^+-N (~15%) was fairly low, whereas the reduction in NO_2^--N was very high (approximately 95%) which was due to the oxidation of NO_2^--N to NO_3^--N (Munter, 2001). The small reduction in NH_4^+-N during the UV/H_2O_2 treatment could be due to its volatile nature. The small reduction in TN was most likely the result of the oxidation of some nitrogen species to gaseous N_2 during the treatment (Dwyer et al., 2008).

After the sequential treatment, TN removal increased significantly, with the highest removal of 38% for the high salinity compared with the low salinity (31%) ROC (Figure 5.2b). Over 90% NH_4^+-N and around 80% NO_2^--N removal was obtained for the three different ROC salinity levels. It can be inferred that complete nitrification and partial denitrification had taken place in the BAC media. Nitrate removal increased considerably with increased ROC salinity, with 39% removal for the high salinity sample. Although one way ANOVA analysis showed significant increase in the removals of TN and nitrate between low/medium and high salinity ROC (*p*-values 0.001 and 0.007 ($\alpha < 0.05$) at 95% confidence level), this was not so for ammonia and nitrite (*p*-values 0.360 and 0.855 respectively ($\alpha > 0.05$) at 95% confidence level).

Overall, it appeared that the salinity of the ROC did not affect the ammonium removal, which is consistent with the AOB being able to adapt to and grow over the salinity range of 0-35% (Glass and Silverstein, 1999). In this study, the extent of denitrification was lower than nitrification, which could be because of high DO present in the influent (~ 10 mg/L) as DO of the effluent from the BAC process at different salinities was 6-7 mg/L, and complete denitrification would normally take place when DO < 1.5 mg/L. Most of the nitrate removal would have taken place by denitrifying bacteria residing deep inside the media and biofilm where anoxic microenvironments had developed. The greater denitrification achieved at high salinity may indicate that the dominating denitrifiers were a range of halotolerant denitrifying bacterial species which had been previously acclimated to high salinity (Glass and Silverstein, 1999). The result was aligned with that obtained by Yoshie et al. (2006), where greater denitrification was achieved at 10% than at 2% salinity due to the presence of halotolerant denitrifying bacteria.



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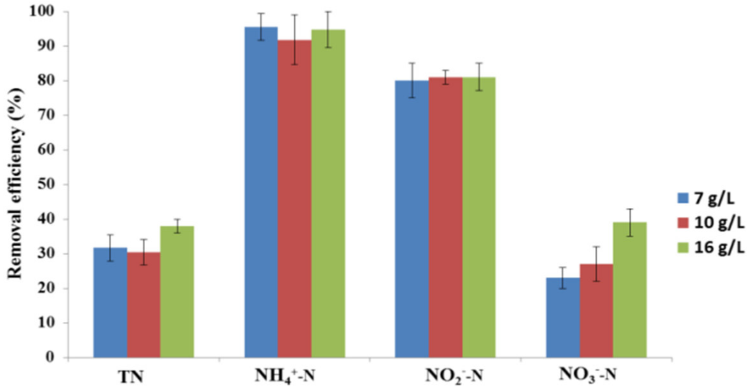


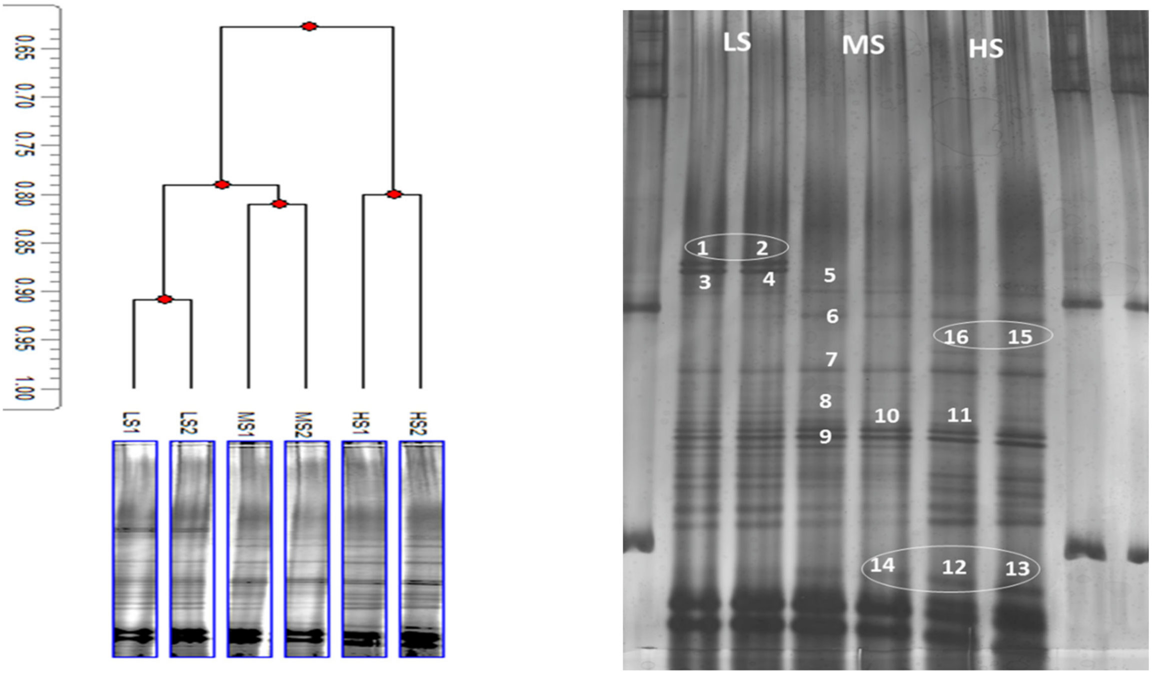
(b)

Figure 5.2 Nitrogen species removal by (a) UV/H_2O_2 pre-treatment and (b) sequential UV/H_2O_2 and BAC treatment for the ROC of various salinity (number of analyses, n=12 for each salinity level).

5.4 Microbiological characterisation of BAC media

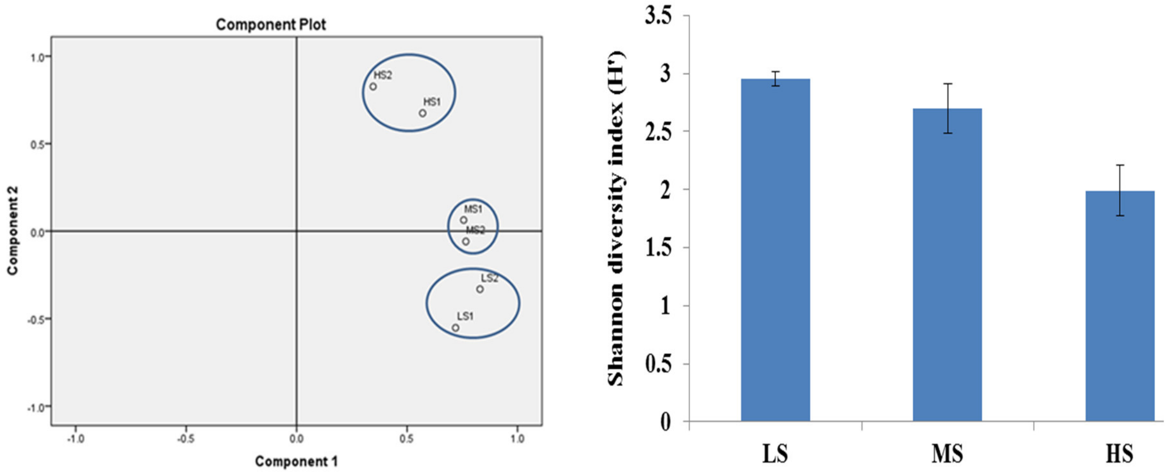
In order to gain better insight into the impact of salinity on the BAC treatment, bacterial communities residing in the BAC media and exposed to the ROC of different salinity were examined using PCR-DGGE. UPGMA dendrograms (cluster analysis), unique bands from DGGE profiles, principal component analysis (PCA) and Shannon diversity index (H') from microbial community fingerprinting were obtained and used for the characterisation.

The bacterial DGGE profiles showed that while a large number of bands were common for the different ROC salinity levels, some unique bands were observed (Figure 5.3a &b). For example, bands 1 and 2, and 15 and 16, were present only at low and high salinity, respectively; band 12 was present only for medium and high salinity. The appearance and disappearance of unique bacterial DNA bands was attributed to the adaptation of major bacterial communities to the change in salinity. Cluster analysis showed that the bacterial banding pattern for the ROCs of low and medium salinity was different from that for the ROC of high salinity. PCA analysis of the bacterial communities at different salinity showed the presence of diverse groups of bacteria within the BAC system (Figure 5.4a). These distinct bacterial communities at the different salinity conditions reflected the selection process for organic matter and nitrogen removal. PCA analysis showed that the bacterial communities exposed to the ROC of low and medium salinity were more closely related than those for the high salinity ROC. This was in accordance with the Shannon diversity index (H') values, where H' values obtained for ROC of low and medium salinity were much closer (2.958 and 2.695) than for the high salinity ROC (H' = 1.99) (Figure 5.4b). The higher H' value demonstrates greater bacterial diversity at lower salinity. The greater difference in the diversity indices for low and medium salinity compared with high salinity indicated that there were different bacterial species in each BAC column. This was supported by one way ANOVA analysis of the H' results which showed significant differences in the diversity of the bacterial communities present in the three BAC columns (*p*-value 0.027 <0.05).



(a) (b)

Figure 5.3 (a) Cluster analysis of bacterial communities using the UGPMA method and (b) location of unique bands from DGGE profiles of BAC media on the various ROC salinity. LS, MS and HS denote low, medium and high salinity ROC samples, respectively



(a) (b)

Figure 5.4 (a) PCA and (b) H' calculated from DGGE profiles of total bacteria in the BAC media for the various ROC salinities. LS, MS and HS denote low, medium and high salinity ROC samples, respectively.

Sequence analysis and phylogenetic trees of the isolates from the culture-based isolations showed the various bacterial species present in the BAC systems (Table 5.3 and Figure 5.5). It was shown that *Bacillus* sp. belonging to the phylum *Firmicutes* was common in all three BAC columns. *Rhodococcus* sp. belonging to the phylum *Actinobacteria* was present in low and high salinity BAC media, whereas *Brevundimonas* sp. (phylum *α-Proteobacteria*) was found only in medium salinity BAC media. This showed that the bacterial communities adapt, and/or new bacterial communities develop to adapt, to the changed salinity environment. *Bacillus* sp. are known to be very resistant to various adverse conditions such as high salinity and to be responsible for nitrification-denitrification and organic matter removal (Choi et al., 2002). This was in accordance with the results showing carbon and nitrogen removal by the BAC systems at various salinity in the present study. *Bacillus cereus,* which was observed in this study (Figure 5.5) is a halotolerant bacterium that could grow in hyper saline conditions (15% NaCl) according to Kubo et al. (2001). Similarly, *Pseudomonas* sp. can perform both nitrification and denitrification in a single reactor (Kim et al., 2008). They also reported that the aerobic denitrifier *P. putida* could remove ammonia and organic carbon, as well as perform aerobic denitrification under DO conditions of 5-6 mg/L, thereby reducing nitrate to nitrogen in a synthetic wastewater. The high effluent DO levels (6-7 mg/L) and the presence of *Pseudomonas* sp. in the BAC system suggests that aerobic denitrification was a major mechanism for carbon and nitrate removal in the present study. *P. xanthomarina* was identified as being present (Figure 5.5). It has been described as a new species with only one representative strain, which is located in the same 16S rDNA phylogenetic branch as *P. stutzeri* with sequence similarities above 98%, and reported to grow in 0-8% NaCl and be responsible for aerobic denitrification by Romanenko et al. (2005). The higher denitrification together with carbon removal obtained for the ROC of high salinity was likely due to the prevalence of *Bacillus* sp. and *Pseudomonas* sp., as indicated by the phylogenetic tree.

Rhodococcus sp., which were found in the BAC systems in this study, could perform heterotrophic nitrification and aerobic denitrification in wastewater treatment as reported by Chen et al. (2012). Similarly, *Brevundimonas* sp., which were detected in the BAC media treating the medium salinity ROC, can grow optimally in 5-20 g/L NaCl Abraham et al. (1999).

According to microbiological characterisation, there were diverse groups of different bacterial communities present in the BAC filters in this study, suggesting that the selection of halotolerant bacteria appeared to start at low salt concentrations and some can also exist at high concentrations as indicated by the presence of *Bacillus* sp. under all salinity conditions studied.

Table 5.3 Summary of bacterial isolates detected at different salinities in BAC media with culture-based isolation.

Nearest taxon	Accession No	Similarity (%)	Phylum	Salinity
Pseudomonas sp	KF769958	100	γ-*Proteobacteria*	HS
Pseudomonas stutzeri	JX177716	99	γ- *Proteobacteria*	
Bacillus thuringiensis	JF512478	99	*Firmicutes*	
Rhodococcus sp	KC291615	99	*Actinobacteria*	
Paenibacillus azoreducens	JX290553	99	*Firmicutes*	MS
Brevundimonas sp	HM584265	99	α-*Proteobacteria*	
Bacillus sp	LN680100	99	*Firmicutes*	
Pseudomonas sp	EF198405	94	γ-*Proteobacteria*	
Bacillus sp	KJ943984	99	*Firmicutes*	LS
Rhodococcus erythropolis	KM670434	99	*Actinobacteria*	
Rhodococcus sp	KC291615	99	*Actinobacteria*	

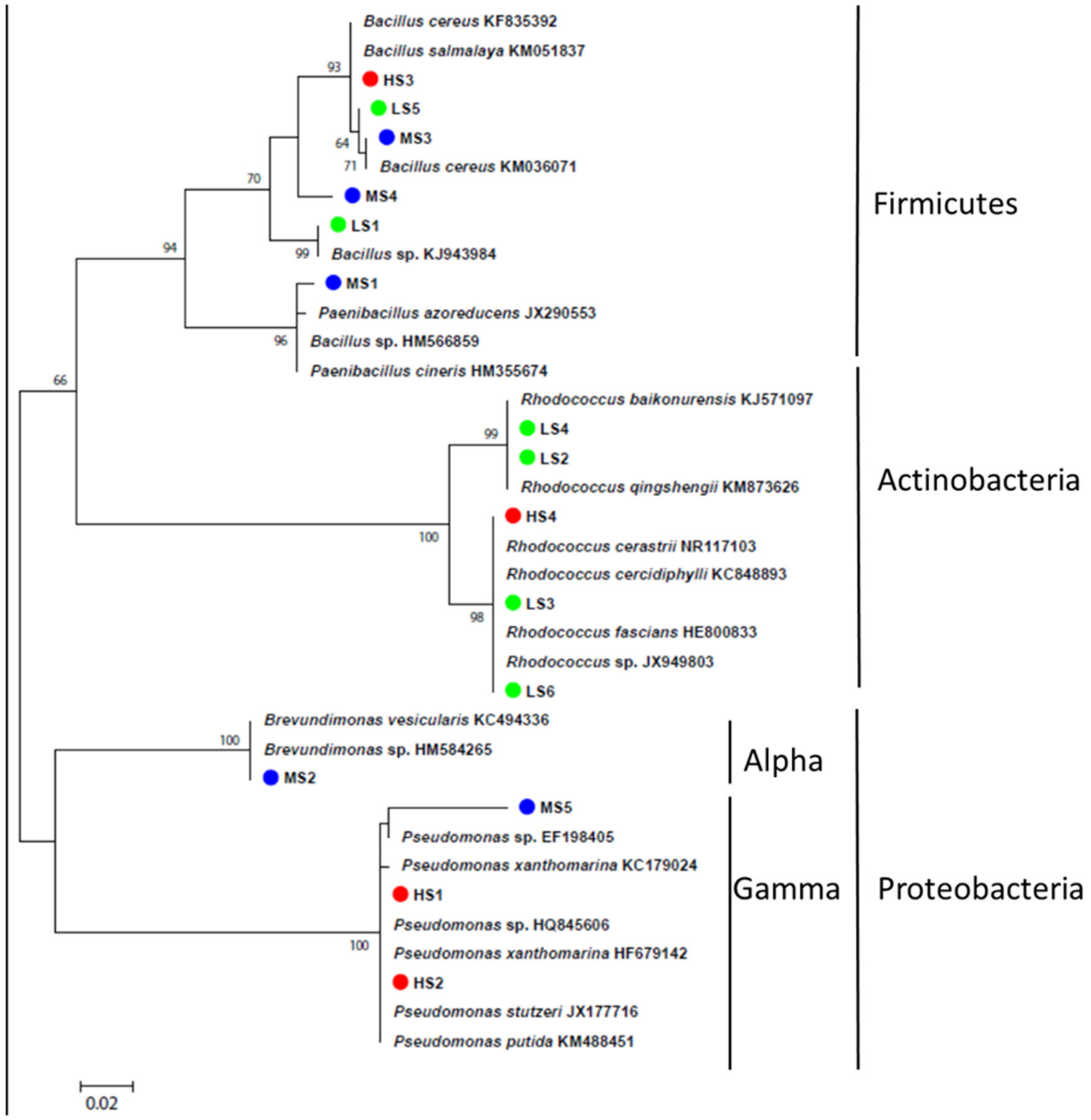


Figure 5.5 Phylogenetic trees of bacterial communities derived from pure culture isolates at different salinities. Distances were calculated with the maximum likelihood model in PhyML. Only partial sequences that could be aligned were used for the phylogenetic tree and bootstrap values ≥ 0.50 are shown

The total bacterial populations present in the BAC media exposed to the ROC of different salinity were determined as $\log_{10}$ CFU/g of dry media (Table 5.4). There were 6.36, 5.45 and 6.21 bacteria for ROC of low, medium and high salinity respectively. This may indicate that the different ROC salinity over the tested range did not greatly affect the number of bacteria, but led to the significant changes in the type of bacterial consortia.

Table 5.4 Total viable count of bacterial cells in BAC media on Nutrient Agar

	Salinity		
	Low	**Medium**	**High**
Bacteria count ($\mathbf{Log_{10}}$CFU/g media)	6.36 ±0.30	5.45 ± 0.15	6.21 ± 0.66

5.5 Conclusions

This study showed that ROC salinity can have various impacts on the removal of organic matter and nitrogen species using the sequential UV/H_2O_2 and BAC process. Comparable DOC removal (45-49%) was achieved by the UV/H_2O_2-BAC treatment for the ROC with high to low salinity. Considerably higher removal in TN and nitrate was achieved by the treatment for the high salinity ROC compared with low and medium salinity. This was likely due to the presence of a wider range of denitrifying bacteria. Denitrification was more prevalent at high salinity, which might suggest the denitrifying bacteria contributing to the better TN removal were more halotolerant. It was shown that salinity did not affect nitrification as indicated by removals of more than 90% and 80% of ammonia and nitrite, respectively, by the treatment process. Microbiological characterisation of the BAC system revealed that a diverse range of bacterial communities belonging to phyla *Firmicutes*, *Actinobacteria* and α- and γ-*Proteobacteria* were present in the BAC systems and so were likely contributors to the organic matter removal and nitrification and denitrification processes.

Chapter 6 - Treatment of a reverse osmosis concentrate containing a significant proportion of petrochemical process wastewater using BAC-UV/H_2O_2

BAC coupled with UV/H_2O_2 was evaluated as a treatment for removing organic and nutrient content from a ROC derived from a wastewater containing domestic wastewater and a significant proportion of trade waste from a petrochemical processor (accounting for 1/3 of influent flow). The physical and chemical characteristics of the ROC used in this study were largely different from the ROC investigated in the previous work, reported in Chapters 4 and 5 such as TDS~4.5 g/L cf. ~16 g/L , chloride~2.7 g/L cf. 7.7 g/L and conductivity~7.8 mS/cm cf. 23 mS/cm. Similarly, the DOC concentration (~52 mg/L) of the ROC used in this study was also significantly different from the ROC investigated in the previous work (DOC~36 mg/L) as reported in Chapters 4 and 5. During the 100-d lab-scale testing, the combined treatment was performed under similar experimental conditions as used previously (e.g., EBCT 60 min) along with microbiological characterisation using PCR-DGGE and sequencing for a better understanding of the microbial process in the BAC treatment. In evaluating the treatment efficiency, ecotoxicity and the potential petrochemical contaminants such as total petroleum hydrocarbon (TPH), polyaromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylenes (BTEX) and phenol were also examined. Additional information about the treatment was obtained thought an advanced molecular weight/size distribution characterisation of the changes in organic matter using Liquid Chromatography-Organic Carbon Detection liquid (LC-OCD) analysis.

6.1 Introduction

Some municipal wastewater ROC may contain significant amounts of contaminants from industrial sources such as petrochemical production processes. The wastewater generated from petrochemical processors is commonly known as ‘produced water’. Produced water usually contains dissolved and dispersed compounds including BTEX, naphthalene, phenanthrene, and dibenzothiophene (NPD), polyaromatic hydrocarbons (PAHs) and phenols, and other production related chemicals (Veil et al., 2004). Total dissolved solids (TDS) concentrations of produced water can range from 0.1 g/L to as high as 300 g/L (Tellez et al., 2002). Many countries have implemented stringent regulatory standards for discharge of produced water to onshore, offshore and to coastal areas (Tellez et al., 2002). Total petroleum hydrocarbons (TPH), phenol, chlorophenols and BTEX pose a great threat to the environment, as they are toxic to aquatic organisms, carcinogenic, mutagenic even at small concentration (Shukla et al., 2010). It has been reported that the metals and hydrocarbons present in produced water are toxic to the ecosystem, negatively affecting organs and fertility of aquatic organisms (Igunnu and Chen, 2012).

A wide range of processes have been adopted for treating produced water. These include physical treatment through adsorption using activated carbon, zeolites and resins, membrane processes, chemical treatment such as coagulation, oxidation and electrochemical processes, and biological processes such as activated sludge, trickling filters, sequencing batch reactors, biological aerated filters and oxidation ponds/lagoons(Fakhru'l-Razi et al., 2009).

BAC processes have been reported as being effective in treating the wastewater from industrial sources such as printing and dyeing, food production, pharmaceuticals and petrochemical processes (Walker and Weatherley, 1999, Jin et al., 2013). Walker and Weatherley (1999) studied the treatment of an industial effluent from a carpet printing plant comprising of a ternary solution of acid dyes using BAC, and reported the BAC treatment outperformed the GAC treatment process for dye removal due to the growth of bacterial communities such as *Pseudomonas putida*. Lin et al. (2001) studied the degradation of bio-refractory compounds and the growth of biofilm in a BAC treatment system integrated with pre-ozonation for treating synthetic wastewater containing phenol, benzoic acid, aminobenzoic acid and real petrochemical industry wastewater. They reported that the pre-ozonation increased the biodegradability of the wastewater as indicated by BOD_5:COD ratio and converted biorefractory organic components into biodegradable ones. They also reported that the BAC treatment system can remove 70-90% of COD (influent COD 100-350 mg/L) from the secondary treated effluents from petrochemical plants, and suggested the enhanced COD removal was due to not only acclimated bacteria but also species succession of bacteria in biofilm. Augulyte et al. (2009) studied the efficiency of a BAC system for treating wastewater polluted with petroleum products such as PAHs and TPH. They reported that the BAC system removed 96-99.7% of the sum of 36 PAHs (ranging from 19 to 46 μg/L in influent) and 18-89% of TPHs (with hydrocarbons ranging from C_{10}-C_{40}, 1.03-4.57 mg/L in influent). The authors attributed the removal of PAHs to sorption, whereas removal of TPHs to biological activities. These studies demonstrated the potential of BAC in treating the wastewater generated from petrochemical industries. However, there is generally a lack of information about the treatment of municipal wastewater ROC streams in which significant amounts of petrochemical contaminants are present.

The aim of the present study is to examine the performance of a UV/H_2O_2-BAC process in treating a ROC derived from a municipal wastewater with a significant proportion of trade waste from a petroleum processor, and to establish a good understanding of the biological treatment on such type of ROC through the microbiological characterisation with the PCR-DGGE and sequencing techniques.

6.2 Source and characteristics of the ROC

Raw ROC was collected from a reclamation facility of a wastewater treatment plant in regional Victoria. The reclamation facility receives secondary effluent from a biological sewage treatment process which treats the influent containing domestic wastewater and the trade waste primarily from a petrochemical processor (30% v/v). The secondary effluent is treated with a process with ultrafiltration (UF), UV and RO to produce recycled water. The general characteristics of the ROC samples used for this study are given in Table 6.1. The ROC used in this study was significantly lower in salinity (TDS~ 4.5 g/L) compared with the ROC used in the previous studies (i.e., TDS ~16 g/L) reported in Chapters 4 and 5. However, the ROC used in this study was relatively higher in DOC (~52 mg/L) and UVA_{254} (~1.12 /cm) compared with the ROC used in the previous work (DOC ~36 mg/L and UVA_{254} ~0.62 /cm). The variances of salinity and organic matter between two ROC samples were primarily due to different sources and nature of sewage. The water quality characteristics of the ROC are shown in the following Table 6.1, which were based on the analytical results of the 22 number of analyses for the ROC collected during January to April, 2015.

Table 6.1 Water quality of raw ROC

Parameters	Raw ROC
DOC (mg/L)	52 ± 2
Colour (mg Pt-Co/L)	167 ± 4
UVA_{254} (1/cm)	1.12 ± 0.02
SUVA (L/mg. m)	2.18 ± 0.07
COD (mg/L)	141 ± 18
TN (mg/L)	28 ± 9
NH_4^+-N (mg/L)	2.2 ± 1.0
NO_2^--N (mg/L)	0.11 ± 0.03
NO_3^--N (mg/L)	22 ± 2
TP (mg/L)	52 ± 7
Chloride (g/L)	2.7± 0.13
DO (mg/L)	8.6 ± 0.2
pH	7.5 ± 0.3
Conductivity (mS/cm)	7.8 ± 0.2
TDS (g/L)	4.5 ± 0.07

6.3 Effects of UV/H_2O_2 pre-treatment, BAC and combined treatment on organic matter removal

The UV/H_2O_2-BAC treatment system has been operated for 106 days (January to April, 2015) with this ROC, including acclimation period of 45 d for the BAC system at the EBCT of 60 min on three batches of ROC. In the inoculation of the microorganisms to the GAC media, the activated sludge obtained from the regional Victorian wastewater treatment plant was utilised as the source of the seeding microbes. As a reference, the raw ROC was treated by an identical BAC system under the same operating conditions. The mean values of the organic matter removals with the various treatments are presented in Figure 6.1.

With UV/H_2O_2 pre-treatment, there was a markedly greater reduction in UVA_{254} (63%) and colour (85%) than in DOC (20%) and COD (27%). The BAC treatment gave greater removals for DOC (37%) and COD (47%), but lower removals for UVA_{254} (54%) and colour (74%) compared with stand-alone UV/H_2O_2 treatment. The organic matter removal efficiency was improved significantly by the combined UV/H_2O_2-BAC treatment, with 57%, 82%, 94% and 59% reduction in DOC, UVA_{254}, colour and COD, respectively. The greater organic matter removal for the combined treatment was mainly attributed to the partial degradation of complex organic matter by the oxidative treatment, leading to the production of simpler molecules that were readily consumed by microorganisms residing in the BAC media.

In the UV/H_2O_2 treatment, the higher reduction in UVA_{254} and colour but lower reduction in DOC and COD was mainly attributed to the effective cleavage but limited mineralisation of the organic compounds in the ROC (Liu et al., 2012, Umar et al., 2013). By comparing the organic matter removal efficiency in this study with some previous studies using the same treatment process on ROC with significantly different characteristics, it appeared that the UV/H_2O_2 treatment gave slightly high reduction in organic matter for the ROC with lower TDS. The DOC (20%) removal in this study was only slightly higher than that obtained in

Chapter 4 (DOC removal 15%) using a ROC of TDS ~16 g/L and DOC ~36 mg/L with the similar condition of UV/H_2O_2 treatment (i.e., UV fluence 8.9 mW/cm^2, 3 mM H_2O_2). Lu et al. (2013) also obtained 15% DOC removal for the ROC of TDS 10 g/L and DOC 44 mg/L with UV fluence of 12.89 mW/cm^2, 4 mM H_2O_2 and 30 min irradiation time. The COD removal was higher for the ROC used in this study compared with the ROC used in the work reported in Chapter 4 (i.e., 27% cf. 15%). The considerable difference in organic matter removals between this study and previous studies with UV/H_2O_2 pre-treatment was primarily attributed to the different nature of the organic contaminants from the different sources. The ROC used in this study contained hydrocarbons containing two or more aromatic rings and have a volatile nature, such as PAHs (Augulyte et al., 2009). Some of these hydrocarbons could have volatilised during secondary treatment, leaving more easily degradable organic matter in the secondary effluent. Thus, the ROC generated may have comprised of easily degradable organic matter and hence, a higher organic matter removal was achieved.

With the BAC treatment alone, DOC removal (37%) in this study was comparable with that of the previous studies, but COD removal was significantly higher in this study (47% cf. 29-32% in study by Lu et al. (2013) and the work reported in Chapter 4). With the combined treatment, although the reductions in DOC (57%) and UVA_{254} (82%), were comparable with the previous studies (Lu et al., 2013) and Chapter 4, COD (59%) and colour (94%) removals were significantly higher, which imply that the characteristics of the ROC such as TDS and the organic matter composition could significantly affect the removal efficiency with the BAC treatment system. The BAC treatment system appeared to be more resilient compared with the chemical oxidation in treating the ROC containing a significant proportion of the industrial trade waste, which was significantly different in characteristics compared with the ROC studied previously.

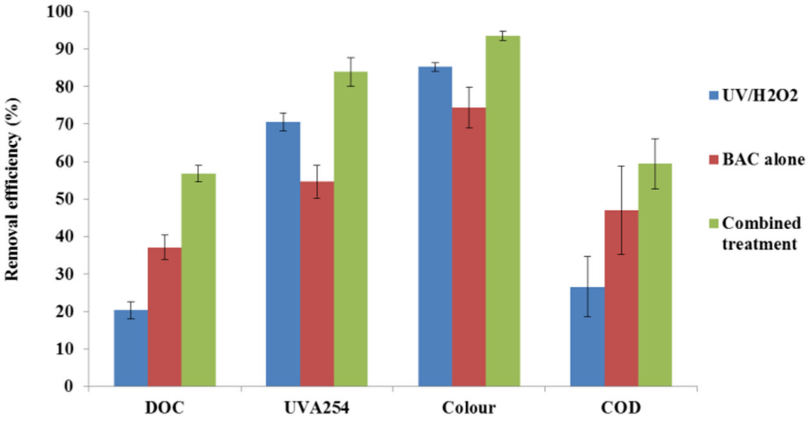


Figure 6.1 Organic matter removal by the various treatments (n= 13)

6.4 Effects of UV/H_2O_2 pre-treatment, BAC and combined treatment on nutrient removal

The removal efficiency of nutrients by the various treatments was characterised in terms of TN, NH_4^+-N, NO_3^--N, and TP (Figure 6.2). As observed for previous ROC, the UV/H_2O_2 pre-treatment alone was not very effective in reducing TN (7%), NH_4^+-N (12%) and TP (9%). NO_3^--N increased slightly (1 mg/L) after the oxidation treatment. As noted previously, the increase in NO_3^--N concentration was most probably due to the conversion of oxidation of some nitrogen species to NO_3^--N in the presence of oxidant (Munter, 2001) and the smaller reduction in TN and NH_4^+-N during the UV/H_2O_2 treatment was likely due to the oxidation of some nitrogen species into gaseous nitrogen (Dwyer et al., 2008) and volatile nature, respectively.

For the combined treatment, TN removal was slightly improved with 15% reduction, whereas NH_4^+-N removal was improved markedly to 89%. NO_3^--N removal was minimal with only 5% removed. For the BAC alone treatment, TN removal was 21%, NH_4^+-N removal was 93% and, NO_3^--N removal was only 7%. In this study, TN and NH_4^+-N removals were lower compared to these nitrogen species removals achieved Chapters 4 and 5, despite the lower salinity of ROC. The lower nitrogen species removal could be related to the different sources of ROC which contains different organic matter, existence of different consortia of microbes in BAC media with different affinity for organic matter consumptions, C:N ratio etc.

The combined treatment and the BAC alone treatment have better TP removals with 60% and 64%, respectively. Better TP removal was obtained compared with previous studies on the ROC with a significantly higher TDS level used in Chapter 4. This was attributed to the lower salinity of the ROC in this study as phosphorus removal by the microbes can be greatly inhibited when salinity >5 g/L (Uygur and Kargı, 2004).

Nitrogen removal in the BAC media can take place by nitrification-denitrification as described in the previous chapters. Higher NH_4^+-N removal was obtained as a result of the nitrification process through ammonia oxidizing bacteria (AOB) residing in the BAC media as well as the high DO (>2 mg/L) which can enhance the nitrification. At the DO of ~ 9 mg/L, denitrification could not be achieved as demonstrated by the low nitrate removal efficiency. However, in Chapter 4, higher removals for nitrogen species were obtained with 60% TN, 90% NH_4^+-N and 62% NO_3^--N removal at the similar DO level with the same UV/H_2O_2-BAC treatment of a highly saline municipal ROC with no significant input of industrial wastewater. In some other studies, denitrification was achieved at a DO of 4.5 mg/L in treating a synthetic wastewater (Virdis et al., 2010). Denitrification at higher DO is possible due to micro-environment deep inside BAC pores where the oxygen penetration is limited (Jin et al., 2013). In this study higher TP removal and lower TN removal was achieved with both treatment conditions which could be due to competition between poly-phosphate accumulating organisms (PAOs) and denitrifying bacteria on same carbon material (Vlekke et al., 1988). It was possible that PAOs outcompeted denitrifying bacteria present in the BAC media, and negatively affected nitrogen removal. In addition, lower denitrification could be due to lower C:N ratio which was only 4.37 for the ROC, which was much less than the required ratio of 7-10 for denitrification purpose (Carrera et al., 2004, Fontenot et al., 2007). The microbiological characterisation (as described in section 6.5) showed that bacterial communities present in the BAC media were mainly responsible for phosphorus and hydrocarbon removal, and this could indicate that nitrogen removal had been limited by the growth of phosphorus and hydrocarbon removing bacteria.

Also, the typical chemical compounds such as aliphatic and aromatic hydrocarbons present in the ROC were likely to negatively affect nitrogen removal, as these chemical compounds are generally not readily biodegradable, and toxic to microorganisms (Oller et al., 2011).

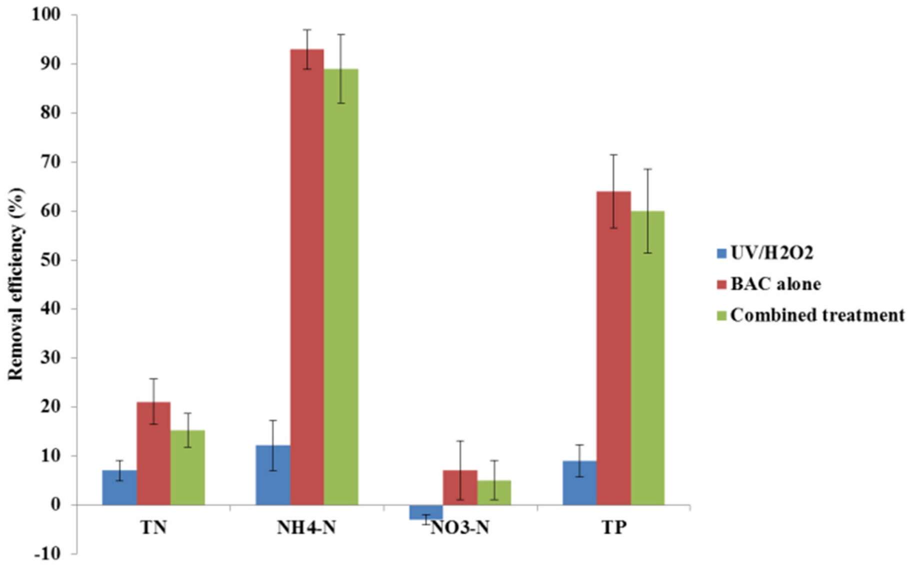
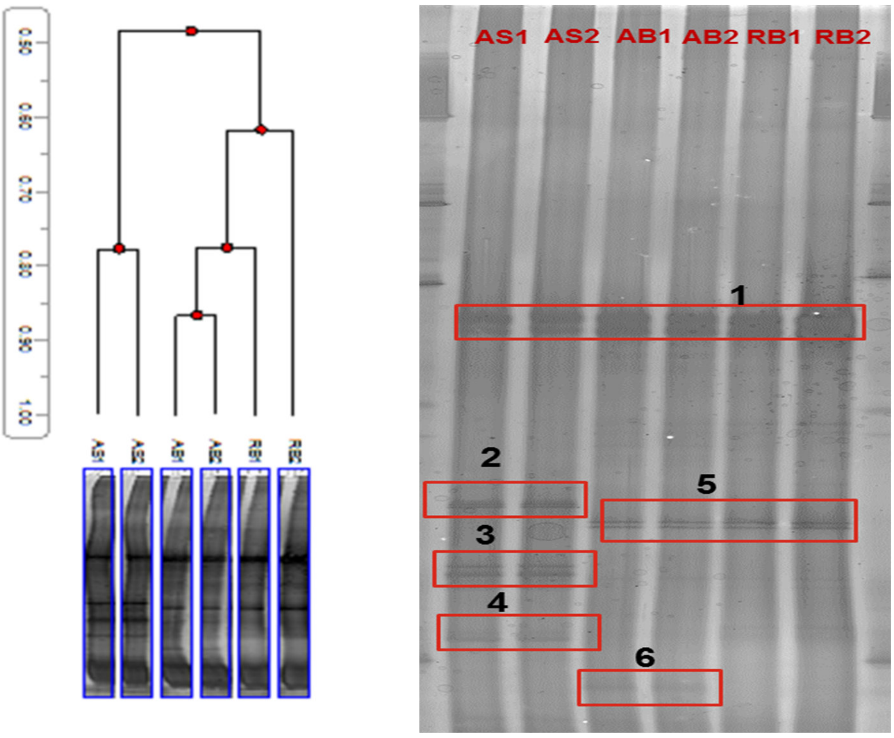


Figure 6.2 Nutrients removal by the various treatments (n= 13)

6.5 Microbiological characterisation of BAC media using PCR-DGGE

The microbiota in the BAC media exposed to raw ROC and the UV/H_2O_2 treated ROC as well as the activated sludge used for BAC inoculation were examined using PCR-DGGE. Similar to the work reported in Chapter 5, UPGMA dendrograms (cluster analysis), unique bands from DGGE profiles, principal component analysis (PCA) and Shannon diversity index (H') from microbial community fingerprinting were obtained and used for the characterisation. The bacterial DGGE profiles showed that most bands were in common for

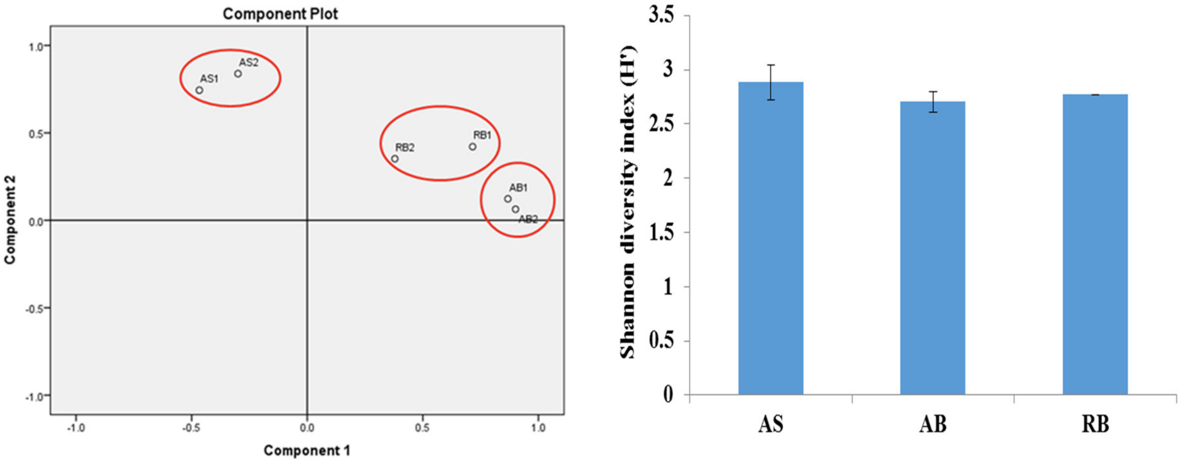
BAC media exposed to the raw and UV/H_2O_2 treated ROC, while some unique bands were observed (Figure 6.3a). The DGGE profiles of the activated sludge showed some unique bacterial bands that were not present in the BAC media. For example, in Figure 6.3b, Band 1 was common in all samples, Bands 2, 3 & 4 were present only in activated sludge samples, Band 5 was present in BAC media exposed to both the UV/H_2O_2 treated and raw ROC and Band 6 was present only in BAC media exposed to UV/H_2O_2 treated ROC. The activated sludge had been used to inoculate the BAC media at the beginning of the experiment. The appearance and disappearance of unique bacterial bands for different samples were attributed to the adaptation of major bacterial communities under various experimental conditions.



(a) (b)

Figure 6.3 Cluster analysis of bacterial communities using the UGPMA method and (b) location of unique bands from DGGE profiles of activated sludge and BAC media on the various experimental conditions. AS, AB, RB denote activated sludge, UV/H_2O_2-BAC and BAC alone media samples, respectively.

Cluster analysis (Figure 6.3b) showed that the bacterial banding pattern for activated sludge was different from the BAC media treating raw ROC and UV/H_2O_2 treated ROC. The PCA analysis of the bacterial communities present in the BAC media and activated sludge showed that diverse bacterial communities were present in these samples (Figure 6.4a). The bacterial communities in the BAC media exposed to the raw and UV/H_2O_2 treated ROC were more closely related than those present in activated sludge as suggested by the PCA analysis. This appeared to be consistent with the comparable nutrient removals under those experimental conditions. Shannon diversity indices (H') of bacterial communities were determined as 2.88, 2.70 and 2.77 for activated sludge, UV/H_2O_2-BAC and BAC only treatment systems, respectively (Figure 6.4b). The H' values of the bacterial communities were not significantly different in the activated sludge, and the BAC systems fed with the UV/H_2O_2 pre-treated ROC and raw ROC as the p-value (0.363) >0.05.



(a) (b)

Figure 6.4 (a) PCA and (b) H' calculated from DGGE profiles of total bacteria in the activated sludge and BAC media on the various experimental conditions. AS, AB, RB denote activated sludge, UV/H_2O_2-BAC and BAC alone media samples, respectively.

Sequence analysis of the isolates from the culture-based isolation showed various bacterial species present in the activated sludge and the BAC system under different experimental conditions (Table 6.2). The activated sludge sample comprised of *Chryseobacterium indologenes*, *Chryseobacterium* belonging to *Flavobacteriia* with 98-99% similarity. *Chryseobacterium* is a gram-negative, rod-shaped bacterium, and was reported to give enhanced phosphorus removal from wastewater in a sequencing batch reactor (Kämpfer et al., 2003). Since good phosphorus removals were obtained by the BAC treatment under the different experimental conditions, this bacterium was most likely retained in the BAC media during the inoculation/acclimation with the activated sludge. The common bands of bacteria (labelled as 1) in DGGE profile (Figure 6.3b) and higher removal of phosphorus with the BAC treatments indicated that the bands were likely to belong to *Chryseobacterium*, although the bacterium was not detected in the BAC media with the sequence analysis. This might be due to the biased nature of culture dependent method (DNA isolation) to detect all bacterial communities on the commonly applied media (Liu et al., 1997).

The BAC media exposed to the UV/H_2O_2 treated ROC hosted different bacterial consortia such as *Micrococcus* belonging to *Actinobacteria*, *Ralstonia* belonging to β-*Proteobacteria* and *Agrobacterium* belonging to α-*Proteobacteri*a. Beer et al. (2006) suggested that phylum Actinobacteria were the major PAO in aerobic reactors containing Polyphosphate as detected using FISH analysis. *Micrococcus,* and *Pseudomonas* can accumulate a large amount of phosphorus under aerobic condition and have a good phosphorus eliminating capacity from municipal wastewater (Nakamura et al., 1991, Li et al., 2003). Apart from the phosphorus removing capacity, *Micrococcus* is the potential degrader of polynuclear aromatic hydrocarbons (PAHs) which are commonly generated from petroleum refinery processes and are recalcitrant hydrophobic compounds (Stringfellow and Alvarez-Cohen, 1999). Wei et al. (2015) also reported that *Micrococcus*, *Psuedomonas*, α- and β-*Proteobacteria* can degrade PAHs for the treatment of petrochemical nanofiltration concentrate (NFC) wastewater of salinity 6.6-7.3% and of low biodegradability. Similarly, *Ralstonia* sp. and *Agrobacterium* sp.

can uptake phosphorus as polyphosphate, thereby remove phosphorus from the wastewater (Lee and Choi, 1999, Seviour et al., 2003).

Table 6.2 Summary of bacterial isolates detected in BAC media exposed to raw ROC and UV/H_2O_2 treated ROC and activated sludge samples with culture-based isolation.

Samples	Nearest taxon	Accession no	Similarity (%)	Phylum
Activated sludge	*Chryseobacterium indologenes*	KC189901	99	*Flavobacteriia*
	Chryseobacterium sp.	KC252829	98	
UV/H_2O_2 – BAC media	*Micrococcus* sp.	KP345959	99	*Actinobacteria*
	Ralstonia sp.	KM056760	99	β-*Proteobacteria*
	Agrobacterium sp.	KF836041	97	α-*Proteobacteria*
Raw-BAC media	*Pseudomonas* sp.	KP462872	99	γ-*Proteobacteria*;
	Sphingopyxis sp.	KM253169	99	α-*Proteobacteria*

For the BAC media exposed to the raw ROC, *Pseudomonas* belonging to γ-*Proteobacteria* and *Sphingopyxis* belonging to α-*Proteobacteria* were detected. *Pseudomonas* is ubiquitous bacteria in soil and wastewater treatment plants and can play vital roles in nutrient recycling, and biodegradation of broad range of synthetic and natural organic compounds (Sarró et al., 2005). *Pseudomonas* and *Flavobacterium* have the capacity of phosphorus uptake in activated sludge (Fuhs and Chen, 1975). Besides, *Pseudomonas* can adsorb and degrade a wide range of organic chemicals such as phenol, petroleum hydrocarbons, aromatic hydrocarbons and polycyclic aromatic hydrocarbons, and polychlorinated bisphenyls in soil and water environments (Zhang et al., 2011) .

The microbiological characterisation demonstrated that the diverse bacterial communities present in the BAC media were responsible for phosphorus removal, and this was in accordance with the higher phosphorus removals, which were most likely achieved through bio-absorption and bio-mineralisation.

6.6 Ecotoxicity and TPH, PAH, BTEX &Phenol analysis

Examination of the ecotoxicity, TPH, PAH, BTEX and phenol was conducted by a commercial laboratory (ALS Environmental, Melbourne, Australia). Ecotoxicity assessment was performed using the Microtox® assay which employs the luminescent marine bacterium *Vibrio fischeri*, according to the protocol provided with the Microtox® 500 Analyser. *Vibrio fischeri* has the property of emitting part of the energy released in the metabolic reaction (ascorbic acid cycle) as light. Any disruption in this metabolic reaction as a result of the presence of toxic substances will result in a change in the amount of light emitted. The analyses of TPH, PAH, BTEX and phenol were performed using GC/MS.

The Microtox® assessment showed that raw ROC, the ROC treated by BAC and the ROC treated by UV/H_2O_2-BAC process were non-ecotoxic. However, the ROC after stand-alone UV/H_2O_2 treatment showed toxicity (EC_{50} value of 46% which is considered as highly toxic). The partial oxidation of organic matter present in the raw ROC during oxidation process could have led to the formation of toxic compounds (Umar et al., 2016b). However, the BAC treatment seemed to be effective in removing toxic by-products (Umar et al., 2016b)

The concentrations of PAH, BTEX and phenol in the raw, AOP treated ROC and BAC treated ROC were found to be very low (<0.001 mg/L). The total TPH (hydrocarbon>C_{10}-C_{40}) concentration was 0.7 mg/L in raw ROC and it was reduced to < 0.1 mg/L with UV/H_2O_2 treatment and the combined treatment of UV/H_2O_2-BAC. The UV/H_2O_2 process is reported as effective in reducing hydrocarbons present in wastewater due to the generation of hydroxyl radical (Munter, 2001). The TPH concentration in the raw ROC was much less than the maximum allowable concentrations to be discharged in the sewerage system according to trade waste management policy for Central Highlands Water, Victoria (30 mg/L) and according to National Pollutant Discharge Elimination System (NPDES) (5 mg/L), USA. Nevertheless, the BAC alone treatment reduced the TPH concentration by 71%, demonstrating the potential of BAC process in biodegrading TPH present in wastewater. The depletion of TPH by the BAC filter was likely due to the presence of *Pseudomonas* as revealed by the PCR-DGGE and sequencing analyses.

6.7 Impact on molecular size of the organic content

The changes in the molecular size of the organic components in the ROC during the various treatments were investigated using LC-OCD. The LC-OCD separates DOC into five different chromatographic fractions: biopolymers (≥ 20,000 Da), high MW humic substances (1,000-20,000 Da), building blocks (300-500 Da), low MW (LMW) acids and humic substances (<350 Da) and LMW neutrals (<350 MW) (Huber and Frimmel, 1996).

As shown by the LC-OCD chromatograms (Figure 6.5), biopolymers (0.6%) were almost negligible, whereas humics (51%) were the major constituents followed by LMW neutrals (22%) and building blocks (8%) in the raw ROC. A small portion (17%) of the humic substances of raw ROC was removed by UV/H_2O_2 treatment. During the oxidative process, building blocks and LMW acids and humic substances increased which was due to the molecular breakdown of the larger organic matter present in the ROC. A further removal of the humics took place in the subsequent BAC treatment. The combined UV/H_2O_2-BAC treatment reduced humics (57%), building blocks (30%) and LMW neutrals (60%) markedly. Nearly complete removal of LMW and HS was achieved by the combined treatment. The BAC alone treatment removed only 11% of humic-like compounds and 14% of LMW neutrals, and was ineffective in removing the building blocks and LMW acids and HS. The enhanced reductions in humics, building blocks and LMW neutrals by the combined treatment were attributed to the breakdown of the recalcitrant organic matter (e.g., humics and humic-like substances) with the oxidative treatment, which were then biodegraded in the BAC treatment by microorganisms (Lu et al., 2013) .

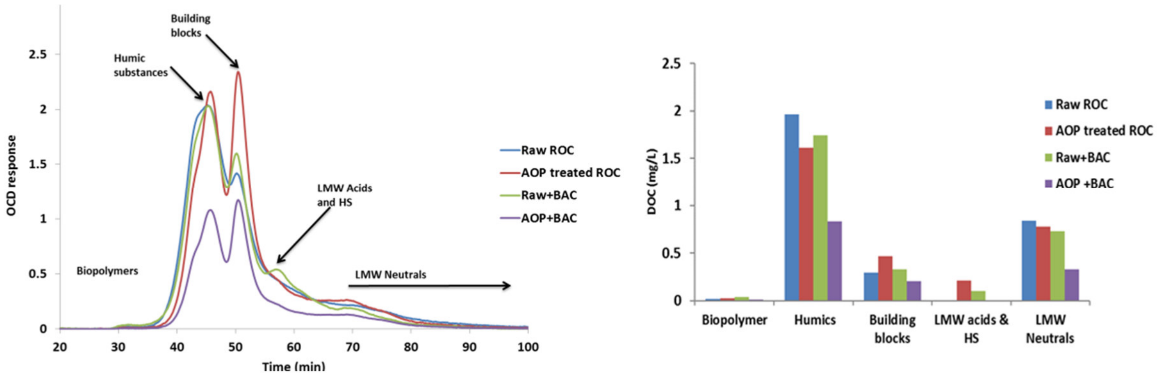


Figure 6.5 LC-OCD chromatograms of ROC with various treatments ; and DOC concentration of the fractions with and without UV/H_2O_2 pretreatment of ROC

6.8 Conclusions

This study showed the combined UV/H_2O_2-BAC treatment resulted in significantly greater reductions in organic and phosphorus content of the ROC comprising domestic wastewater and a significant amount of petrochemical processing wastewater compared with the ROC studied previously, which was significantly higher in salinity/TDS and contained no significant amounts of industrial trade waste. Although, similar high ammonium removal (89-93%) was achieved by the combined treatment on this type of ROC, nitrate removal was minimal (5-7%) and this led to the significantly lower total nitrogen removal. The different treatment efficiency was related to the significantly different natures of the ROC, which were likely to have significant impact on both the oxidative and the biological treatments.

The microbiological study revealed the existence of various phosphorus removing bacteria, also known as PAOs, including *Micrococcus*, *Ralstonia*, *Pseudomonas*, *Agrobacterium,* resulting in the enhanced the phosphorus removal. The study also implied that PAOs could outcompete the denitrifying bacteria, limiting their growth and hence activity. The PAOs could have utilised the organic carbon for cell synthesis. Furthermore, the better TP removal achieved was related to the markedly lower salinity of the ROC, which would be more beneficial to the PAOs. The presence of some bacterial genera such as *Micrococcus* and *Pseudomonas* was thought to help the organic matter removal as they have been reported to be effective in degrading the hydrocarbons present in the ROC.

The BAC based process was demonstrated as an effective treatment for removing TPH from the ROC. The BAC process was also shown to reduce the ecotoxicity of the ROC after the oxidation process, implying the possible utilisation of the BAC process as a barrier for toxic substances in the ROC.

In order to gain more insights into the impact of the characteristics of ROC and the microbial processes on the treatment efficiency, a further comparison of the results of the two different ROC types, including an analysis of the degradation kinetics in the BAC treatment, is made and discussed in Chapter 7.

Chapter 7 - A comparison of organic matter and nutrient removals from two types of municipal wastewater ROC using UV/H_2O_2-BAC treatment

Chapters 4 and 5 report on the efficiencies of the UV/H_2O_2-BAC treatment of a highly saline (TDS~16 g/L) municipal wastewater ROC and the impact of varied salinity of ROC on the BAC based treatment, respectively. In Chapter 6, the treatment of a lower salinity ROC (TDS ~4.5 g/L) derived from a wastewater containing domestic wastewater and a significant proportion of petrochemical process wastewater (accounting for 1/3 of influent flow) with the same process was reported. This chapter presents a comparison of the treatment on the two types of ROC, with a view to obtaining further insights into the organic matter and nutrient removal by the BAC based treatments. The two types of ROC represented the wastewater streams of significant different characteristics in terms of organic and inorganic composition, and concentration. For the convenience of the comparison, the two types of ROC were simply denoted as ROC A (used in the study of chapters 4 and 5) and ROC B (used in the study of chapter 6). The degradation kinetics of the organic matter in the BAC-based treatment on the two types of ROC was also studied and reported in this chapter.

7.1 Introduction

The organic and inorganic constituents and their concentrations in the municipal wastewater ROC may vary significantly, depending on their sources and catchment conditions. The presence of the organic and inorganic matter in the ROC, as indicated by the water characteristics, may greatly affect chemical and biological treatment efficiencies. Some recent studies on the removal of organic matter and nutrients from highly saline municipal ROC streams (TDS 10-17 g/L) have been conducted using UV/H_2O_2 pre-treatment followed by BAC (Lu et al., 2013, Umar et al., 2013, Umar et al., 2016a). These studies showed marked reductions in organic matter, but limited nutrient removal at the high ROC salinity. In some other studies, good reductions in organic matter from relatively lower salinity ROC have been reported (Dialynas et al., 2008, Lee et al., 2009a). However, there is generally a lack of comparative studies on the AOP-BAC treatment of ROC streams with significantly different physical-chemical characteristics, and hence relatively limited knowledge on their impact to the treatment efficiency.

The understanding of degradation kinetics for substrate in the biological treatment systems would be advantageous for the integration of the unit processes to treat industrial and municipal wastewater. In general, kinetic models allow (i) exploration and prediction of the combined process performance under distinct circumstances, (ii) determination of optimal operating regions for given discharge levels and (iii) determination of appropriate practical limits for discharge (Scott and Ollis, 1996). The degradation kinetics can contribute to the establishment of overall engineering models for predicting the effect of system variables including flow rates, reactor volumes, and organic loading rates for the effective treatment of wastewater streams (Esplugas et al., 2004).

Municipal and industrial wastewaters contain numerous substrates and their compositions can vary greatly. Biodegradable COD (bsCOD) has been used as a substrate for evaluating kinetics in biological treatment system (Tchobanoglous et al., 2003). Scott and Ollis (1997) stated that BOD or TOC of industrial and domestic wastewater could be included in kinetic models for multiple-step treatment systems such as the AOP-biological treatment.

Researchers have previously focussed mainly on modelling AOP treatment of wastewater, paying relatively less attention to the downstream biological treatment processes. There were fewer studies on the modelling of BAC-based wastewater treatment processes (Ying and Weber Jr, 1979, Walker and Weatherley, 1997). Ying and Weber Jr (1979) first developed four predictive models under the Michigan Adsorption Design and Applications Model (MADAM) program for mathematic description of the dynamics of bioactive adsorbers. The models assumed that the flow in the BAC systems is plug flow with axial dispersion for the liquid phase, biological growth limited by the availability of substrate only, and wash–off of biomass from the carbon surface is negligible during initial stages of growth. Walker and Weatherley (1997) developed a kinetic model for BAC beds treating textile industry wastewater using Monod equation. In this work, degradation of colour causing compounds such as azo and di-azo dyes was studied with the BAC on three different bacteria: *Bacillus benzeovorans*, *Bacillus gordonae* and *Pseudomonas* sp. They reported that these bacterial communities had different specific growth rates, and the higher specific growth rate resulted in higher capacity to decolourise azo and di-azo dyes.

A few studies have been conducted on the kinetic modelling for combined treatment systems (Beltrán et al., 1997, Ledakowicz et al., 2001). However, there is a lack of study on kinetic modelling for the treatment of ROC using UV/H_2O_2 followed by BAC.

The aim of the present study is to compare (i) the organic matter and nutrients removal from the ROC of two types; (ii) the bacterial communities in the BAC systems; and (iii) the degradation kinetics for the combined treatment system for the two types of ROC.

7.2 Comparison of the characteristics of the two ROC

As indicated in the previous chapters, the ROC samples used in this comparative study were collected from the two different wastewater reclamation plants in Australia. ROC A was collected from a wastewater reclamation facility treating mostly domestic wastewater; and ROC B was collected from a reclamation facility receiving secondary effluent from a biological sewage treatment process which treats the influent containing domestic wastewater and the trade waste primarily from a petrochemical processor (30% v/v). The general characteristics of the two types of ROC are given in Table 7.1. The concentrations of inorganics (as indicated by the TDS, chloride and electrical conductivity) of ROC A were markedly higher than ROC B, whereas the concentrations of organics (as indicated by DOC, COD, colour and UVA_{254}) of ROC B were significantly higher than ROC A. The concentrations of nutrients (except for NH_4^+-N) of ROC B were higher than those of ROC A. The ionic concentrations in two types of ROC are given in Table 7.2.

Table 7.1 Water quality of ROC A and ROC B

Parameters	**ROC A**[a]	**ROC B**[b]
DOC (mg/L)	36.0 ± 4.0	52 ± 2
Colour (mg Pt-Co/L)	148 ± 10.0	167 ± 4
UVA_{254} (/cm)	0.62 ± 0.02	1.12 ± 0.02
COD (mg/L)	120 ± 19	141 ± 18
TN (mg/L)	21.4 ± 4.5	28 ± 9
NH_4^+-N (mg/L)	4.3 ± 0.8	2.2 ± 1.0
NO_3^--N (mg/L)	9.1 ± 3.4	22 ± 2
TP (mg/L)	28.5 ± 1.1	52 ± 7
Chloride (g/L)	7.7 ± 1.8	2.68 ± 0.13
DO (mg/L)	10.2 ± 1.3	8.6 ± 0.2
pH	7.7 ± 0.4	7.5 ± 0.3
Conductivity (mS/cm)	23.5 ± 1.3	7.8 ± 0.2
TDS (g/L)	16.6 ± 0.8	4.5 ± 0.70

a- Average values from the analyses of 6 batches of ROC sample

b- Average values from the analyses of 3 batches of ROC sample

Table 7.2 Ionic concentrations of the two ROC types

Ion (mg/L)	ROC A	ROC B
Na^+	433	50.7
Mg^{2+}	87.5	5.58
K^+	12.3	2.50
Ca^{2+}	29.5	6.4
Mn^{2+}	0.013	0.0058
Fe^{3+}	<1.52	-
Ni^{2+}	0.003	-
Cu^{2+}	0.001	-
Zn^{2+}	3.22	-
Mo^+	1.37	-
Pb^{2+}	<0.015	-

7.3 Comparison of organic matter removal with UV/H_2O_2, BAC and their combination

The removal efficiency of organic matter from ROC A and ROC B in terms of DOC, COD, UVA_{254} and colour is summarised in Table 7.3. The data presented here are derived from the studies reported in Chapters 4 and 6.

Table 7.3 Removal efficiency (%) of organic matter by UV/H_2O_2, BAC and their combination

	Reduction (%)					
	UV/H_2O_2		BAC		UV/H_2O_2-BAC	
Parameter	**ROC A**	**ROC B**	**ROC A**	**ROC B**	**ROC A**	**ROC B**
DOC	14 ± 7	20 ± 2	38 ± 5	37 ± 3	57 ± 8	57 ± 2
COD	15 ±13	27 ± 9	32 ± 7	47 ± 12	48 ±5	59 ± 7
UVA_{254}	61 ± 5	71 ± 2	64 ± 5	55 ± 4	81 ± 5	82 ± 2
Colour	86 ± 5	85 ± 1	80 ± 6	74 ± 5	95 ± 4	95 ± 1

The UV/H_2O_2 treatment led to considerably higher reductions in DOC, COD and UVA_{254} for ROC B compared with ROC A. However, the colour removals were comparable for the two ROC types. With the BAC alone treatment, comparable DOC removal was achieved for both ROC types, whereas COD removal (47%) was higher for ROC B compared with ROC A (32%). However, colour (80%) and UVA_{254} (64%) removals were greater for ROC A than ROC B (74% and 55% for colour and UVA_{254}, respectively). The trend in DOC, UVA_{254} and colour reduction on ROC A and ROC B was similar for the UV/H_2O_2-BAC treatment, whereas the reduction in COD was higher for ROC B (59%) compared to ROC A (48%). The greater reduction in COD for ROC B could be due to combined effects of lower salinity of the ROC and/or the presence of readily biodegradable organics in it. Dinçer and Kargi (2001) also reported a decrease in COD removal (from 96% to 43%) when salinity of wastewater increased from 2 g/L to 13 g/L.

The UV/H_2O_2 oxidation alone was able to degrade some organic matter and a great amount of colour-causing compounds including aromatic fractions in both ROC, but the extent of removal in organic matter was slightly greater for ROC B compared to ROC A, with approximately 6% DOC, 12% COD and 10% UVA_{254} differences between them. This could imply that ROC salinity did not affect greatly on organic matter mineralisation. This appeared to be consistent with some previous studies where salinity showed much less impact on organic matter mineralisation in the similar oxidation processes (Liu et al., 2012). In the study reported in Chapter 5 of this thesis, it was also shown that the reduction in DOC at a low ROC salinity (TDS 7 g/L) was only slightly higher than that at a high salinity (TDS 16 g/L). Liu et al. (2012) reported that organic matter reductions were not greatly affected by salinity over the electric conductivity (EC) range 4.4-11.2 mS/cm, and Umar et al. (2013) observed little impact of the salinity in the EC range of 8.3-23 mS/cm on DOC and COD removals in the UV/H_2O_2 treatment of a wastewater ROC. This slightly lower DOC removal for the ROC A could be due to the presence of high concentration of ionic compounds (Table 7.2) that could reduce the oxidation rate of organics to the final products due to the resultant lower UVT (Bagastyo et al., 2011, Umar et al., 2016). As the ROC B contained some petrochemical compounds with aromatic rings and some compounds were volatile in nature, it was possible that these compounds could be more easily degraded or stripped from the ROC during the AOP treatment, resulting in the higher organic matter removal compared with the ROC A which did not contain any significant amounts of petrochemical compounds.

7.4 Comparison of nutrient removal with UV/H_2O_2, BAC and their combination

The removal efficiency of nutrients with UV/H_2O_2, BAC and their combination for the two types of ROC was characterised in terms of TN, NH_4^+-N, NO_3^--N, and TP, and is summarised in Table 7.4.

Table 7.4 Removal efficiency (%) of nutrients by UV/H_2O_2, BAC and their combination

	Reduction (%)					
	UV/H_2O_2		**BAC**		**UV/H_2O_2-BAC**	
Parameter	**ROC A**	**ROC B**	**ROC A**	**ROC B**	**ROC A**	**ROC B**
TN	13 ± 8	7 ± 2	71 ± 9	21 ± 5	60 ± 6	15 ± 4
NH_4^+-N	13.5 ± 8	12 ± 5	90 ± 7	93 ± 4	91 ± 6.5	89 ± 7
NO_3^--N	-17.3 ± 4.3	-3 ± 1	63 ± 13	7 ± 6	61± 16	5 ± 4
TP	2.2±1.5	10 ± 3	8 ± 4	64 ± 5	15 ± 2	60 ± 8

The UV/H_2O_2 alone treatment led to much less removal of nutrients for both ROC A and ROC B. It was observed there was around 1-2 mg/L increase in nitrate after the oxidation treatment on both ROC. As previously noted, this was attributed to the conversion of some nitrogen species into NO_3^--N in the presence of oxidant (Munter, 2001).

With the BAC alone treatment, comparable high ammonium removal was obtained for both ROC, with 90-93% reduction. However, higher TN removal was achieved for ROC A compared with ROC B (71% cf. 21%). Nitrate removal was markedly lower for ROC B with only 7% reduction and higher for ROC A with 61% reduction. TP removal was significantly higher for the ROC B compared with ROC A (i.e., 64% cf. 8%).

With the combined treatment, markedly lower reductions in TN and NO_3^--N were achieved for ROC B compared with ROC A (15% cf. 60% for TN, and 5% cf. 61% for NO_3^--N). It was shown the denitrification was much less for ROC B than for ROC A as indicated by less nitrate removal, affecting the overall nitrogen removal. However, there were comparable high ammonium removals for both ROC with 89-91% reduction. For both ROC, denitrification was less than nitrification, which was likely due to the higher DO of the ROC (typically 6-7 mg/L) than what required for denitrification (i.e., DO should be less than 1.5 mg/L). The denitrification was thought to be a result of the micro-environment deep inside BAC pores where the oxygen penetration is limited (Jin et al., 2013).

In contrast to the nitrogen species, a significantly higher TP removal was achieved with the combined treatment for ROC B (60%) compared with ROC A (15%). The opposite trends for nutrient removals for the two different types of ROC could be related to the factors such as salinity level, organic constituents, different bacterial communities present in the BAC systems and the C:N ratio. Higher phosphorus removal was possible for ROC B as phosphorus removal is greatly inhibited by increasing salinity to >5 g/L (Uygur and Kargı, 2004). At higher salinity, plasmolysis of poly-phosphate accumulating organisms (PAOs) can take place, causing lower phosphorus removal. The much lower nitrate removal for ROC B could be due to competition between denitrifiers and PAOs on the same organic substrates (Vlekke et al., 1988). It was possible that PAOs outcompeted denitrifying bacteria present in the BAC media, and negatively affected nitrogen removal. In addition, lower denitrification for ROC B could also be due to lower C:N ratio of 5 in the influent, which was much less than the required value of 7-10 for denitrification purpose (Carrera et al., 2004, Fontenot et al., 2007).

7.5 Comparison of bacterial communities present in the BAC media exposed to the two ROC types

The PCR-DGGE–sequencing analysis of BAC media exposed to ROC A and ROC B showed diverse microbial communities from different bacterial groups with different functions (Table 7.5). Different BAC columns were set up for the two types of ROC as mentioned in previous chapters.

Table 7.5 Bacterial communities in the BAC media exposed to ROC A and ROC B

Nearest taxon	Accession No	Similarity (%)	Phylum	ROC
Pseudomonas sp	KF769958	100	γ-*Proteobacteria*	A
Pseudomonas stutzeri	JX177716	99	γ-*Proteobacteria*	
Bacillus thuringiensis	JF512478	99	*Firmicutes*	
Rhodococcus sp	KC291615	99	*Actinobacteria*	
Micrococcus sp.	KP345959	99	*Actinobacteria*	B
Ralstonia sp.	KM056760	99	β-*Proteobacteria*	
Agrobacterium sp.	KF836041	97	α-*Proteobacteria*	
Pseudomonas sp.	KP462872	99	γ-*Proteobacteria*	
Sphingopyxis sp.	KM253169	99	α-*Proteobacteria*	

It was shown *Pseudomonas* sp. belonging to γ-Proteobacteria was common in the BAC systems treating for ROC A and ROC B. *Pseudomonas* are ubiquitous bacteria in soil and wastewater treatment plants that can play vital roles in nutrient recycling, biodegradation of a broad range of synthetic and natural organic compounds, degrade a wide range of organic chemicals such as phenol, petroleum hydrocarbons, aromatic hydrocarbons and polycyclic aromatic hydrocarbons, and polychlorinated bisphenyls in soil and water environments (Sarró et al., 2005). However, their functions varied in nutrient removals for the two types of ROC. *Rhodococcus* sp., and *Micrococcus* sp. belonging to Actinobacteria were present in the BAC media exposed to ROC A and ROC B, respectively. *Rhodococcus* sp. can perform heterotrophic nitrification and aerobic denitrification in wastewater treatment plant (Chen et al., 2012). *Micrococcus,* and *Pseudomonas* can accumulate a large amount of phosphorus under aerobic condition and has a good phosphorus eliminating capacity from municipal wastewater (Nakamura et al., 1991, Li et al., 2003). Apart from the phosphorus removing capacity of *Micrococcus*, the bacterium is the potential degrader of polynuclear aromatic hydrocarbons (PAHs), which are generated from most petroleum refinery and are recalcitrant hydrophobic compounds (Stringfellow and Alvarez-Cohen, 1999). Since the ROC B was derived from wastewater treating the trade waste mostly from a petrochemical process along with domestic wastewater, the presence of *Micrococcus, Pseudomonas* can be anticipated (Wei et al., 2015). The other bacterial communities present in the BAC media treating ROC B contained *Ralstonia* belonging to β-*Proteobacteria*, *Agrobacterium* and *Sphingopyxis* belonging to α-*Proteobacteri*a. These bacterial communities were more similar to PAOs that can uptake phosphorus as poly P, thereby reducing phosphorus (Lee and Choi, 1999, Seviour et al., 2003). It was observed that even though the bacterial communities belong to same groups, their functions varied in terms of nutrients removal.

Furthermore, bacterial communities present in the activated sludge that had been used to inoculate the respective BAC media were also investigated. *Bacillus* sp. belonging to *Firmicutes* was detected in the activated sludge that was used to inoculate the BAC media treating ROC A and *Chryseobacterium* belonging to *Flavobacteriia* was detected in the activated sludge which was inoculate the BAC media treating ROC B. *Chryseobacterium* sp. can enhance phosphorus removal from wastewater (Kämpfer et al., 2003) and *Bacillus* sp. are responsible for nitrification-denitrification and organic matter removal under adverse conditions such as higher salinity (Choi et al., 2002). The presence of different bacterial communities in the activated sludge and BAC media showed that microbes can adapt themselves with varied treatment conditions. These different bacterial species have different affinity towards organic matter and nutrients consumption at different rates, thereby affecting organic matter and nutrients removal at different salinities. The kinetic rates during the BAC treatment processes for ROC A and ROC B have been calculated and described in Section 7.7.

7.6 Comparison of the molecular size changes on the two types of ROC

The changes in the molecular size of the organic components in the ROC during the various treatments were investigated using LC-OCD for ROC A and ROC B. A comparison of LC-OCD chromatograms shows the significantly different nature of the organic content in the two types of ROC (Figure 7.1). The ROC A contained a measurable amount of biopolymers, whereas the amount of biopolymers was almost negligible in ROC B (Figure 7.1 a-d). It was shown that the humics were the major constituents in ROC A, accounting for 30% of the total DOC followed by LMW neutrals (21%), building blocks (7%) and biopolymers (2%). Similarly, humics (51%) were the major constituents in ROC B followed by LMW neutrals (22%), building blocks (8%) and biopolymers (0.6%). A small proportion of the humic substances (~17%) of both ROC samples were removed by UV/H_2O_2, leading to effective mineralization of chromophoric organic content of the two ROC types with simultaneous high colour removals (85-86%). Slightly higher organic matter (DOC and COD) degradation of ROC B could be due to greater concentrations of humic like substances that degraded at faster rate compared with ROC A with UV/H_2O_2 treatment. The reduction of the humics were accompanied by a corresponding increase in building blocks and LMW acids and humic substances by UV/H_2O_2 treatment of both ROC and decreased with subsequent BAC treatments of both ROC streams. The removal of LMW neutrals was apparently higher for ROC A (31%) than for ROC B (9%). Removal of biopolymers removal was 17% for ROC A and there was no removal for ROC B. It can be inferred that organic matter degradation for ROC A could be mostly due to mineralisation of LMW neutrals of organic content during UV/H_2O_2 treatment.

The combined UV/H_2O_2 and BAC treatment reduced humics (52%), building blocks (54%) and LMW neutrals (77%) for ROC A, and for ROC B the reductions in humics, building blocks and LMW neutrals were 58%, 30% and 60%, respectively. The combined process completely removed LMW acids and humic substances for both ROC. The BAC process enhanced the removals of different fractions of organic matter of both ROC (mostly LMW neutrals) and these removals were higher for ROC A (except for humics). The higher removal in LMW neutrals for ROC A could be due to their adsorption on the BAC pores or biofilm. The organic matter removal for ROC A was attributed to greater reductions in building blocks and LMW neutrals. The removal of biopolymers was higher for ROC B (45% cf. 29% for ROC A).

The BAC alone treatment was ineffective in removing the biopolymers in both ROC samples. The reductions in humics (11%) and LMW neutrals (14%) were lower for ROC B compared with ROC A (20% humics and 70% LMW neutrals), which was consistent with the higher colour and UVA_{254} removal for ROC A. The BAC alone treatment could not remove building blocks for ROC B but removed 50% of it for ROC A. The biopolymers increased enormously by 4-fold (295%) for ROC A compared with only 90% for ROC B. The increase in biopolymers for both ROC could be due to breakdown of biopolymers by the microorganisms and then possible accumulation of those molecules by the biofilm attached to the carbon particles. Some of these accumulated biopolymers (such as carbohydrates) might have washed off from the BAC column (Umar et al., 2016b).

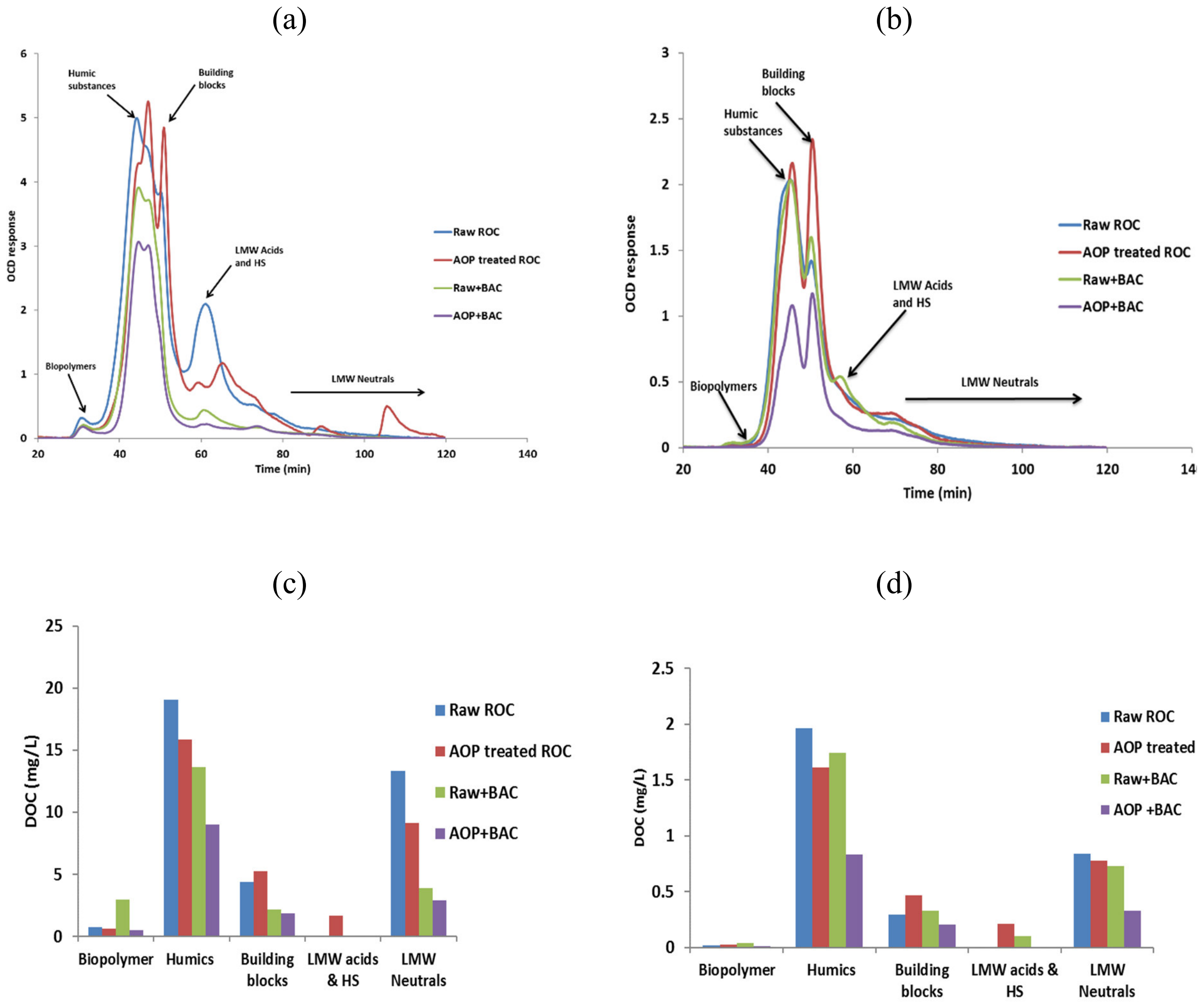

(a) (b) (c) (d)

Figure 7.1 LC-OCD chromatograms of (a) ROC A (b) ROC B; and DOC concentration of the fractions (c) ROC A (d) ROC B with AOP alone , BAC alone and combined treatments

7.7 Comparison of degradation kinetics of the two ROC under the BAC based treatment

The degradation kinetics for both ROC were determined and then compared. According to Scott and Ollis, (1995), specific industrial wastewater treatment requires kinetic models to be developed to determine the optimal operating conditions for low-cost high efficiencies when changing the parameters. BOD or TOC were widely used to develop the kinetic models for industrial and domestic wastewater (Scott and Ollis, 1996). Ledakowicz et al. (2001)

suggested that Monod's biodegradation kinetic equation was suitable for the combined treatment of chemical oxidation followed by biological treatment of wastewater, and reported that the AOPs pre-treatment decreased the Monod constant and increased maximum growth rate in activated sludge. For the BAC treatment system, Monod's kinetic models has been used in previous studies (Ying and Weber Jr, 1979). Ying and Weber Jr (1979) assumed that the flow in BAC is plug flow with axial dispersion for the liquid phase, biological growth limited by availability of substrate only, and wash–off of biomass from the carbon surface is negligible during initial stages of growth. Due to the complexity of simultaneous biodegradation and carbon adsorption, following assumptions were made in the determination of the degradation kinetics in this work (Ying and Weber Jr, 1979, Walker and Weatherley, 1997):

a) Plug flow with axial dispersion for the liquid phase
b) Two resistance external mass transport and intra-particle solid phase diffusion control the rate of adsorption
c) Biological growth is limited only by substrate availability
d) No diffusional resistance within the biofilm
e) A steady state biofilm thickness is attained.

The kinetic constants were then calculated using Monod's equation for the combined treatment on ROC A and ROC B. It should be noted there were certain limitations in the calculation of kinetics for this work, as the biomass in terms of volatile suspended solids (VSS) in the BAC treatment were not measured, so that maximum growth of biomass (μ_{max}) could not be measured experimentally. As such, most parameters were adopted from (Tchobanoglous et al., 2003) to predict the Monod's kinetics. Monod's biodegradation model can be expressed by equation 7.1. The model describes the effect of nutrient concentration 'S' on the rate of microbial growth. This model fits experimental data for biological processes in wastewater treatment. As the molecular composition of industrial wastewater is usually poorly characterized and the organic loading rate varies widely, COD has been widely used to describe the organic loading of the wastewater (Ledakowicz et al., 2001). In this study, COD concentration has been used as substrate for calculation as most kinetic parameters were based on COD in Tchobanoglous et al. (2003) to predict the Monod constant.

$$\mu = \mu_{max} . \frac{S}{K_s + S} \qquad \text{Equation 7.1}$$

where,

μ = specific growth rate (1/d)

μ_{max} = maximum specific growth rate (1/d)

S = concentration of the limiting substrate or nutrient (g/m^3)

K_s = saturation coefficient or Monod constant

When the substrate is being used at its maximum rate, the bacteria are also growing at their maximum rate. The maximum specific growth rate of the bacteria is thus related to the maximum specific substrate utilization rate as follows (Tchobanoglous et al., 2003):

$$\mu_{max} = k.Y, \qquad \text{Equation 7.2}$$

where,

Y = yield coefficient (g/g) and k = maximum substrate utilization rate (g/g.d)

In this work, Monod's kinetic model was used to predict the specific growth rate of biomass in the BAC systems to treat the UV/H_2O_2 treated and the untreated ROC for the two types of ROC. Some parameters such as k, K_s and Y were adopted from Tchobanoglous et al. (2003) and are given in Table 7.6.

Table 7.6 Monod's kinetic parameters adopted from Tchobanoglous et al. (2003)

Coefficients	**unit**	**Values**	
		Range	**Typical**
k	gCOD/gVSS.d	2-10	5
K_s	mgCOD/L	10-60	40
Y	mgVSS/mgCOD	0.3-0.6	0.5

Using the values for k, K_s and Y,

μ_{max}= 5*0.5 = 2.5 /d

This μ_{max} was used to calculate specific growth rates for biomass in the BAC media treating UV/H_2O_2 treated ROC and raw ROC alone for ROC A and ROC B, respectively, as given in Table 7.7.

Table 7.7 Calculated specific growth rate of biomass in BAC based treatment of the ROC, when K_s =40 mg/L

μ	**ROC A**	**ROC B**
UV/H_2O_2+BAC	1.80 /d	1.84 /d
Raw+BAC	1.87 /d	1.94 /d

The specific growth rates of biomass in the BAC were lower with the UV/H_2O_2 treated ROC compared with the ROC without pre-treatment for both types of ROC. The higher specific growth rates for ROC B may be attributed to higher microbial degradation activities and so higher substrate removal in the BAC mediated processes was achieved. The specific growth rate can also be affected by BAC biofilm thickness as higher specific growth rate can be achieved with lower biofilm thickness (Walker and Weatherley, 1997). Although thickness of

BAC biofilms were not measured directly in this study, it can be postulated that thickness of BAC biofilm treating ROC B could be lower than thickness of BAC biofilm treating ROC A because BAC treatment of ROC A was operated for longer period of time (230 days) compared with 100 days' operation for ROC B. Consequently, the specific growth rate for ROC A may have been decreased after a long period of time due to lack of nutrients, possible inhibition due to intermediate compounds and lack of DO deep inside the BAC biofilms and within activated carbon particles (Walker and Weatherley, 1997). Furthermore, upon gradual decrease in the saturation coefficient or Monod constant values from 40 to 10 mg/L for both ROC, increase in specific growth rates for the UV/H_2O_2 treated ROC and Raw ROC for both ROC were observed as depicted in Figure 7.2. Nevertheless, the specific growth rate for UV/H_2O_2-BAC combination was lower than that for Raw-BAC combination of both ROC samples. The gradual decrease of specific growth rates with higher saturation constant suggested that various organic pollutants were biodegraded at different rates. The lower specific growth rates when pre-oxidised ROCs were fed to the BAC system could be due to the generation of some toxic by-products with the UV/H_2O_2 treatment of both ROC as suggested by Umar et al. (2016a). The pre-oxidised ROC could be highly toxic (EC_{50} value was 13%, interpreted as extremely toxic) (Umar et al., 2016a) and the ROC used in the work reported in Chapter 6 was also toxic after UV/H_2O_2 treatment (EC_{50} value was 46%, interpreted as highly toxic). The toxic by-products that may have been generated during partial oxidation process could limit the specific growth rates of biomass. However, Ledakowicz et al. (2001) observed a decrease in Monod constant and an increase in maximal specific growth rate with AOP pre-treatment followed by activated sludge. This could be due to application to wastewater with different nature and organic contaminants with varied degradation rates, different concentration of oxidant doses, different biological treatment systems.

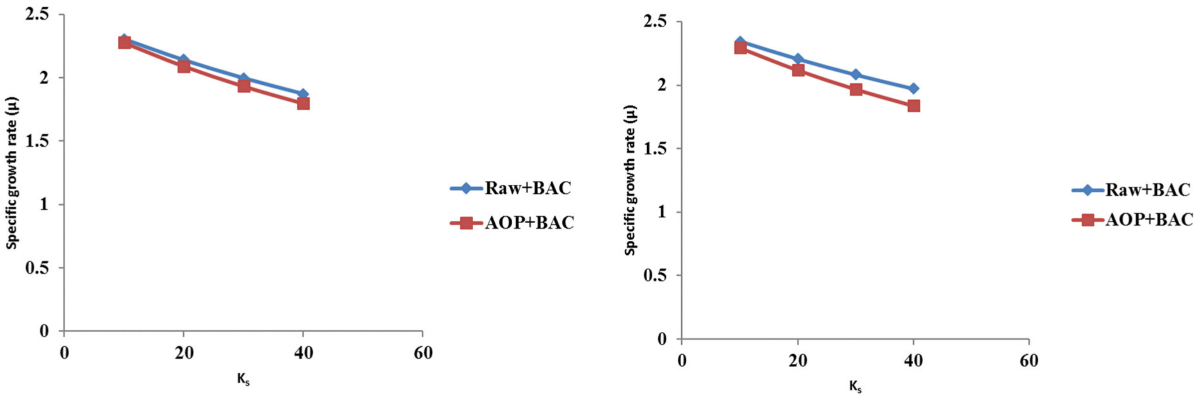

(a) (b)

Figure 7.2 Specific growth rates (µ) at different saturation coefficients for (a) ROC A and (b) ROC B

In the present study, it was also attempted to obtain the reaction constant (K) for UV/H_2O_2 alone, UV/H_2O_2-BAC and Raw-BAC treatments for both ROC samples using plug flow model without considering biodegradation. The reaction rates for reductions in DOC and COD for both ROC samples were calculated (Table 7.8). In the plug flow model, biodegradable substances removed with first-order reaction according to equation 7.3:

$C_e = C_o\, e^{-K.t}$ Equation 7.3,

Where,

K = reaction constant (1/min);

C_o and C_e are influent and effluent DOC concentrations (mg/L) and

t= hydraulic detention time (min).

Equation 7.3, suggested that $C_e = f\ (C_o, K)$ for the given contact time i.e., C_e is directly proportional to C_o.

The reaction rates for DOC and COD reductions were higher for ROC B compared with ROC A with UV/H_2O_2 treatment only, which could be due to effect of salinity as higher salinity reduces the oxidation rate of organics to give the final products (Bagastyo et al., 2011). These higher rate constants for ROC B may be attributed to slightly higher organic matter removal as given in Table 7.3 with UV/H_2O_2 treatment. With the combined treatment, the reaction rates for DOC reductions were fairly similar with similar DOC removal (57-58%) for both ROC samples. The reaction rate for COD reduction was 2.5 times higher for ROC B compared with ROC A. This could imply that UV/H_2O_2 treatment generated the organics of higher biodegradability by removing considerable amount of high molecular weight compounds such as humic like and those organics were degraded at faster rate for ROC B. Scott and Ollis (1996) also reported that higher kinetic value was due to removal of most of the recalcitrant compounds, leaving less recalcitrant compounds for subsequent biological treatment. With the BAC alone treatment also, the rates for DOC reductions were fairly similar for both ROC, but the reaction rate for COD reduction was slightly higher for ROC B, implying that the organics present in ROC B were less recalcitrant and can be degraded at relatively faster rate.

Table 7.8 Reaction rate constants for the reductions in DOC, COD with BAC treatment with and without UV/H_2O_2 for both ROC

Treatment	Parameter	ROC A	ROC B
		K (1/min)	**K (1/min)**
UV/H_2O_2 only	DOC	0.004	0.007
	COD	0.005	0.009
UV/H_2O_2+BAC	DOC	0.012	0.010
	COD	0.004	0.010
Raw+BAC	DOC	0.008	0.007
	COD	0.009	0.0102

7.8 Conclusions

The BAC based treatments of the two different types of ROC were compared for a further understanding of the impact of the characteristics of the ROC on the treatment efficacy. The two ROC samples characteristics were greatly different in terms of salinity, organic matter and nutrients. The UV/H_2O_2 treatment was efficient for ROC B with better organic matter removal, possibly due to lower salinity and less recalcitrant organics of the studied ROC and higher reaction rate constants for DOC and COD reductions. The combined treatment of both types of ROC gave comparable DOC, colour and UVA_{254} removals regardless of salinity levels but COD removal was higher for ROC B which had lower salinity than ROC A. The combined process was highly effective for reducing colour and UV absorbance, and their reductions were attributed to the breakdown of recalcitrant organic matter present in these ROCs.

The nutrients removal was mostly affected by the existence of different bacterial consortia at different salinity levels and different types of ROC sources. The PAOs could exist at lower salinity aligning with previous studies. This study has also shown that the higher specific growth rate of biomass using Monod's equation can be achieved at lower salinity, which could possibly lead to the higher COD removal.

Overall, this comparative study showed that the BAC based treatment can be efficiently used to treat municipal ROC of various salinity levels containing different organic contaminants for removing organic matter and nutrients, and hence potentially reducing the environmental risks associated with ROC.

Further work is required in terms of kinetic modelling of the combined AOP-BAC systems for the treatment of ROC of different composition and salinity levels under different experimental conditions to represent substrate degradation as well as to achieve better process effectiveness and economics.

Chapter 8 - Impact of pre-treatment using coagulation and sequential coagulation-UV/H_2O_2 process on BAC treatment of ROC

Coagulation is a widely used treatment process in the water industry for removing bio-refractory organic matter compounds and nutrients such as phosphate from water and wastewater (Clark et al., 1997). In some applications, coagulation can be used as a post-treatment for controlling disinfection by products (DBPs) precursors (Volk et al., 2000). It is commonly considered as a less energy intensive process compared with oxidative water treatment such as advanced oxidation with UV/H_2O_2 (Bagastyo et al., 2011). As such, coagulation and the sequential coagulation-UV/H_2O_2 were assessed as the pre-treatments for the subsequent BAC process for removing organic matter and nutrients from the ROC. This chapter reports on the experimental investigation on the treatment of the two types of ROC, i.e., ROC A and ROC B as studied early in this work. The results were compared with those obtained from the studies utilising UV/H_2O_2 as the pre-treatment for the BAC process.

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8.1 Introduction

The most commonly used coagulants in the water industry include aluminium based (such as alum) and iron based (such as ferric chloride, ferric sulphate, ferric sulphate, ferrous sulphate) compounds due to their effectiveness in removing organic matter and particulates from water and wastewater, easy availability and low cost. Coagulation process has been used to treat the most bio-refractory wastes not amenable to biological treatment processes (Tatsi et al., 2003). Coagulation process involves colloid destabilisation, precipitation, co-precipitation and/or adsorption on to the flocs (Jacangelo et al., 1995). In recent years, coagulation processes have been studied for treating the municipal wastewater ROC from water recycling facilities (Bagastyo et al., 2011, Comstock et al., 2011). Coagulation process can remove mainly high molecular weight organic compounds, and hence improve the UV transmittance (UVT) of the wastewater for further oxidation treatment (Umar et al., 2016a). Umar et al. (2016a) reported that ferric chloride (1 mM Fe^{3+}) can improve total DOC reduction and UVT of the water, and reduce energy requirement as indicated by the reduced electrical energy dose (*E*ED) for the treatment of a high salinity municipal ROC. Coagulation process can also improve nutrient (N & P) removal (Clark et al., 1997). Coagulation can achieve high phosphorus removal due to the precipitation of phosphate with the metal ion in the form of orthophosphate and/or interaction and adsorption with the flocculated particles (D'Elia and Isolati, 1992). The basic reaction taking part in the phosphorus removal is as given in equation 8.1, associated with a number of secondary reactions (Aguilar et al., 2002).

$$Me^{3+} + H_nPO_4{}^{n-3} \leftrightarrow MePO_4 + nH^+ \qquad \text{Equation 8.1}$$

where Me refers to the metal of the salt used as coagulants.

The coagulants may act in different manners for low and high salinity wastewater. For example, high ion content in high salinity ROC can affect chemical hydrolysis and metal stability during coagulation, thereby affecting colloid destabilisation and removal (Duan et al., 2003, Duan and Gregory, 2003). Similarly, different coagulants may act differently during coagulation process. For example, alum is more soluble in high salinity water than ferric–based ones due to the formation of weak flocs (Edzwald and Haarhoff, 2011).

The coagulation process coupled with UV/H_2O_2 treatment can improve removal of organics as coagulation can improve UVT for the subsequent UV/H_2O_2 treatment process. Therefore, the aim of the present study was to evaluate the impact of coagulation and the sequential coagulation-UV/H_2O_2 process as the pre-treatments for the BAC in removing the organic matter and nutrients from the two types of ROC; ROC A and ROC B as studied in chapter 7.

A fixed dose (1 mM) of ferric chloride at pH 5 removed the most organic matter from the high salinity municipal ROC (Umar et al., 2016a). The same dose (1 mM Fe^{3+}) was used in this study for both ROC A and ROC B for comparison on organic matter and nutrients removals.

8.2 Organic matter removal by coagulation and coagulation-UV/H_2O_2 pre-treatments

The removal efficiency of organic matter in terms of DOC, COD, UVA_{254} and colour from ROC A and ROC B by the treatments is summarized in Table 8.1.

Table 8.1 Removal efficiency (%) of organic matter by the pre-treatments

	Reduction (%)			
	Coagulation		**Coagulation-UV/H_2O_2**	
Parameter	**ROC A**	**ROC B**	**ROC A**	**ROC B**
DOC	26 ± 1	19 ± 4	29 ± 1	40 ± 6
COD	15 ± 7	11 ± 4	16 ± 3	22 ± 8
UVA_{254}	54 ± 2	30 ± 5	79 ± 1	76 ± 5
Colour	90 ± 1	52 ± 2	97 ± 1	90 ± 5

The coagulation had considerably higher removal efficiencies for organic matter for ROC A compared with ROC B. Coagulation led to significantly higher colour removal compared to removals of DOC, COD and UVA_{254} of both ROC samples. Umar et al. (2016a) achieved better DOC removal (42%) for the ROC of similar salinity of the ROC A used in this study using ferric chloride (1 mM) at pH 5. However, higher colour removal (90%) was achieved in this study compared with their study (78%), but UVA_{254} removals were similar between both studies (54% and 53%). These variations in the organic matter removal between these studies were due to the variations in organic fractions present in the ROC.

The sequential coagulation-UV/H_2O_2 treatment resulted in higher removals in DOC (40%) and COD (22%) for ROC B compared with ROC A (29% DOC and 16% COD), whereas, UVA_{254} and colour removals were slightly higher for ROC A. Coagulation followed by UV/H_2O_2 treatment led to 3% and 21% more reduction of DOC for ROC A and ROC B respectively. Colour and UVA_{254} also followed similar trends of improved reductions with sequential coagulation-UV/H_2O_2 treatment. Umar et al. (2016b) also achieved better organic removal (51% DOC, 87% colour and 65% UVA_{254}) with coagulation followed by UV/H_2O_2 treatment of the ROC of higher salinity. The coagulation removed a significant portion of the organic matter of both ROC and the remaining organic matter was further mineralized by UV/H_2O_2 treatment. The significantly high colour removal for ROC A was likely due to the formation of stronger flocs and so the better settlement of the colour compounds at higher salinity than at lower salinity (Duan et al., 2003). Duan et al. (2003) suggested that coagulation with $FeCl_3$ preferentially removed higher molecular weight organic compounds and hydrophobic substances such as humics and leaving lower molecular weight compounds intact. According to them, the coagulant effectively removes organic matter by adsorption of organics on amorphous metal hydroxides, along with charge neutralization. Due to step-wise treatment of coagulation followed by UV/H_2O_2, improved reductions in DOC, UVA_{254} and colour were achieved for both ROC samples, whereas improved reductions in COD was achieved only for ROC B. The enhanced reductions in colour and UVA_{254} by the combined pre-treatment were attributed to the greater breakdown of the chromophores as a result of the improved UVT after coagulation (Umar et al., 2016b).

8.3 Organic matter removal by BAC with and without pre-treatment

The organic matter removals by BAC with and without the pre-treatments are presented in Table 8.2.

Table 8.2 Overall removal efficiency (%) of organic matter for the BAC with and without pre-treatments

Parameter	Reduction (%)					
	Coagulation-BAC		Coagulation-UV/H_2O_2-BAC		BAC	
	ROC A	ROC B	ROC A	ROC B	ROC A	ROC B
DOC	61 ± 3	37 ± 5	67 ± 2	62 ± 5	36 ± 5	37 ± 3
COD	44 ± 10	31 ± 10	46 ± 10	53 ± 14	37 ± 13	47 ± 12
UVA_{254}	77 ± 2	51 ± 5	88 ± 2	82 ± 3	32 ± 5	55 ± 4
Colour	99 ± 1	74 ± 5	99 ± 1	95 ± 3	53 ± 4	74 ± 5

The coagulation-BAC treatment achieved markedly higher reductions in DOC for ROC A (61%) than that for ROC B (37%). The DOC reductions improved from 61% to 67% for ROC A and from 37% to 62% for ROC B for the BAC with the sequential coagulation-UV/H_2O_2 treatment. Similar to DOC reduction, COD reduction improved from 31% to 53% for ROC B for the coagulation-UV/H_2O_2-BAC treatment, however COD reduction did not improve for ROC A. The less reductions in UVA_{254} (51%) and colour (74%) were achieved for ROC B compared with ROC A (77% UVA_{254} and 99% colour) for the BAC with coagulation as the pre-treatment. The UVA_{254} reduction improved with the coagulation-UV/H_2O_2–BAC treatment of both ROC samples with comparable removal (82% for ROC B and 88% for ROC A). The colour removals were consistently high for both ROC samples. The treatment with BAC alone for ROC A and ROC B were already discussed in chapter 7.

The organic compounds present in ROC A and ROC B vary greatly as indicated in the LC-OCD chromatograms as reported in chapter 7. The major constituents in ROC A comprised of humics (30%) of the total DOC followed by LMW neutrals (21%), building blocks (7%) and biopolymers (2%). The major constituents in ROC B comprised of humics (51%), LMW neutrals (22%), building blocks (8%) and biopolymers (0.6%). The coagulation pre-treatment removed a greater proportion of humic and humic-like substances which have high molecular weight (1000-20,000 Da) and of lower biodegradability. Umar et al. (2016b) also reported that the coagulation pre-treatment with 1 mM Fe^{3+} removed a significant portion of humics (63% removal) from a municipal ROC which had the salinity similar to that of the ROC A. The BAC treatment of both ROC mainly removed the LMW compounds present in the coagulated ROC and these results were consistent with the high reduction in DOC of both ROC due to biodegradation. Nevertheless, the organic matter reduction for ROC A was higher than for ROC B with the coagulation-BAC treatment (Table 8.2). This was most likely due to the higher concentrations of remaining high molecular weight organics in the coagulated ROC B, affecting the overall organic matter removal efficiencies. Since, the ROC B contained higher concentration of humics (51%) than ROC A (30%), it was possible that coagulated ROC B contained a greater amount of humics which were not easily biodegraded in the BAC process. It was also observed that the flocs formed after coagulation of ROC B were fragile and poorly settled compared to that of ROC A during experiment. Even though longer settling time was provided for ROC B, the supernatant contained tiny flocs /residue that could overlay the BAC biofilm and could inhibit the adsorption and/or biodegradation process in the BAC system. The sequential coagulation-UV/H_2O_2 treatment of both ROC enhanced the organic matter removal due to the breakdown of remaining high molecular weight compounds to LMW biodegradable compounds during UV/H_2O_2 treatment of the coagulated ROC. The BAC treatment then removed these LMW compounds resulting in the improved organic matter reductions for both ROC.

8.4 Fluorescence excitation –emission spectra

The impact of the various pre-treatments on the BAC treatment was investigated using fluorescence excitation-emission (EEM) spectra and fluorescence regional integration (FRI) technique for both ROC (Figures 8.1, 8.2 and Appendix A2). The DOCs of both ROC samples were adjusted to 7 mg/L to avoid the inner filter effect associated with high DOC levels (>10 mg/L). The EEM-FRI was conducted for ROC A and ROC B after coagulation, coagulation-UV/H_2O_2 pre-treatments as well as for the UV/H_2O_2 pre-treatment as a comparison. The EEM spectra of both ROC was divided into five regions with region I and II associated with aromatic proteins (AP I and AP II), region III associated to fulvic acid-like substances (FA-like), regions IV and V containing soluble microbial products (SMPs) and humic acid-like substances (HA-like), respectively (Chen et al., 2003). There was significantly higher fluorescence intensity for the regions for fulvic acid-like substances (FA-like, III) and humic acid-like substances (HA-like, V) compared with aromatic proteins (API and APII) and soluble microbial products (SMPs, IV) for both raw ROC (Figure 8.1a and 8.2a). The untreated ROC B exhibited extra fluorescence peaks in region III at Ex/Em: 375-400 nm/ 220-260 nm and region V at Ex/Em: 375-400 nm/ 260-285 nm and at Ex/Em: 375-400 nm/ 350-380 nm (Figure 8.2a). These extra peaks could be related to some petrochemical related compounds such as hydrocarbons as this ROC was generated from wastewater which contained significant proportion of petrochemical waste (30%).

The EEM spectra of both ROC after various pre-treatments and BAC treatments are presented in Appendix A2 and the EEM volumes were determined using the Fluorescence Regional Integration (FRI) method (Chen et al., 2003). For untreated ROC B, the EEM volumes for HA-like substances were higher compared with ROC A (Figures 8.1b and 8.2b), which could be due to the presence of extra peaks in HA-like and FA-like region. The EEM volumes of both ROC samples reduced markedly after each pre-treatment (except for coagulation) and both ROC exhibited similar spectral patterns although the rates of reductions were different after the treatment (Figures 8.1b and 8.2b).

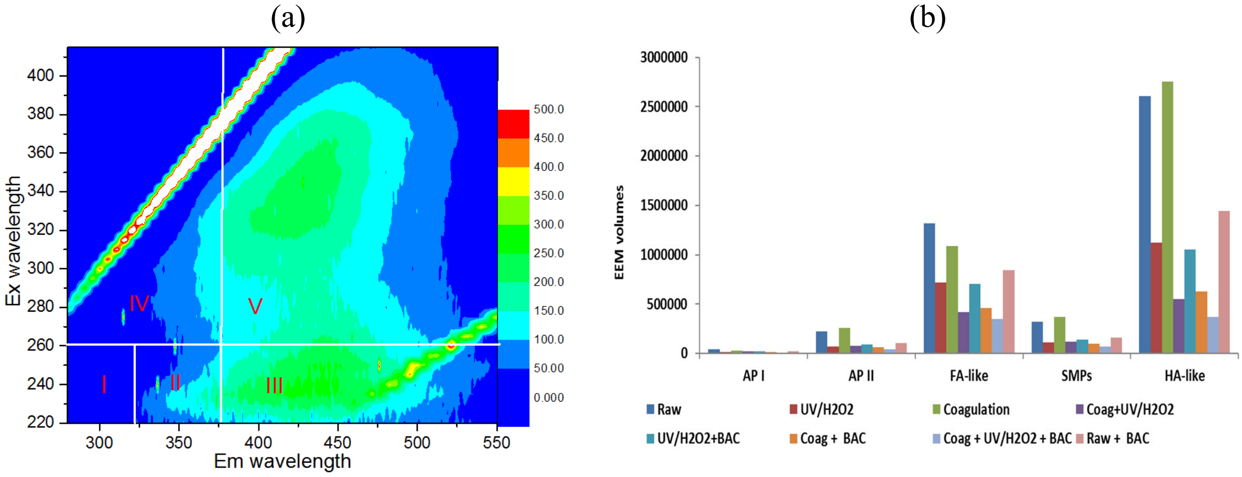

Figure 8.1 (a) EEM spectrum of untreated ROC A and (b) EEM volumes of ROC A with and without different treatments

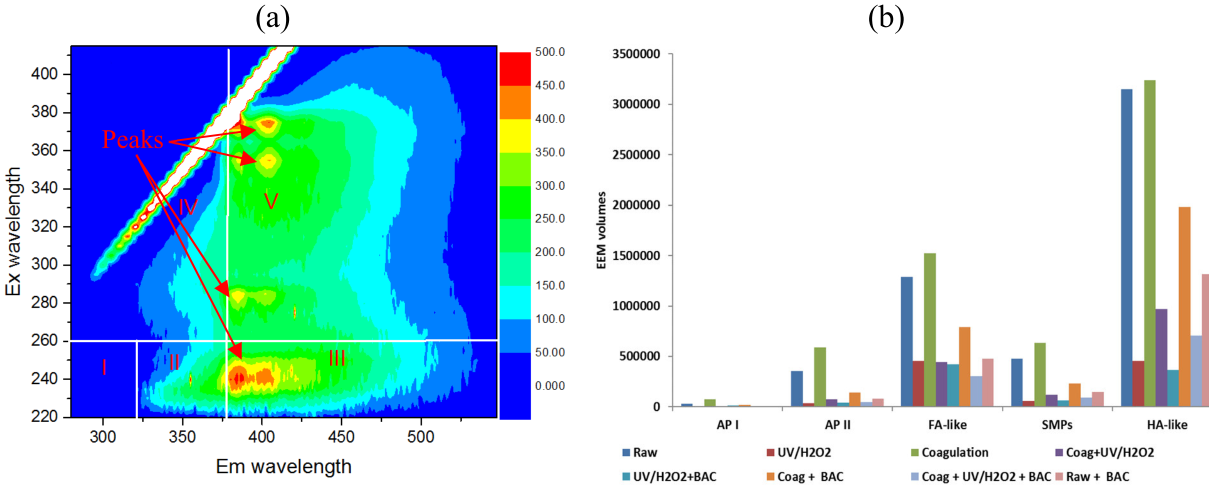

Figure 8.2 (a) EEM spectrum of untreated ROC B and (b) EEM volumes of ROC B with and without different treatments

The UV/H_2O_2 pre-treatment of both ROC reduced the fluorescence in all regions, and thus the EEM volumes also reduced in all regions. The UV/H_2O_2 pre-treatment led to marked reduction in the fluorescence of the humic-like substances (III and V), indicating the breakdown of high MW humic compounds into simpler molecules for both ROC. This was consistent with the marked reductions in colour (85%) and UVA_{254} (61% for ROC A and 71% for ROC B) as reported in chapter 7. There were also marked reductions in fluorescence intensity of extra peaks in HA-like and FA-like substances for ROC B, indicating the breakdown of some of the petrochemical related compounds into simpler molecules that were more biodegradable and amenable to microorganisms (A2). With stand-alone UV/H_2O_2 pre-treatment of ROC B, the reductions in HA-like, FA-like, SMPs, API and APII species were 85%, 65%, 88%, 82% and 91%, respectively. With the same pre-treatment for ROC A, the reductions in HA-like, FA-like, SMPs, API and APII species were only 57%, 45%, 65%, 67% and 57%, respectively. The greater reduction in EEM volumes for different organic species for ROC B indicated that the organics present in ROC B were of higher biodegradability. In addition to this, some of the petrochemical related organics present in ROC B might have volatilised and stripped off during oxidation process as noted in Chapter 7, which led for greater EEM volumes reductions for different species.

The coagulation pre-treatment led to an increase in the fluorescence intensity and thus the EEM volumes in most regions increased for both ROC, with higher increment for ROC B in API, APII, FA-like and SMPS than for ROC A (Figures 8.1b & 8.2b). However, the increase in EEM volumes for HA-like substances was marginally greater for ROC A (5%) than for ROC B (3%). Although, there were considerable reductions in colour and UVA_{254} for both ROC with coagulation, EEM volumes increased significantly for most regions. This was attributed to the complexes formed between anionic humic and cationic coagulant species (Cabaniss, 1992). Umar et al. (2016a) also observed increase in EEM volumes with coagulation treatment (1 mM Fe^{3+}) of municipal ROC of salinity ~16.6 g/L. The fluorescence intensity of the extra peaks in FA-like and HA-like species for ROC B increased with coagulation and the fluorescence intensity also increased towards AP I region, which was most probably due to the formation of complexes with some petrochemical related compounds and the coagulant species.

The coagulation-UV/H_2O_2 pre-treatment led to marked reductions in fluorescence in all regions for both ROC, which was in accordance with enhanced reductions of organic matter. The marked reduction in fluorescence was in accordance to the reduction in EEM volumes in all regions for both ROC. Coagulation mainly removed large molecular weight compounds such as humic like substances which were also preferentially removed by the $HO^{\bullet}$ generated during the UV/H_2O_2 treatment (Umar, 2014). The fluorescence intensity of the extra peaks in regions III (FA-like) and V (HA-like) for coagulated ROC B also markedly reduced with the application of subsequent UV/H_2O_2 treatment suggesting that the oxidation process led to effective breakdown of the complexes which were formed with some petrochemical compounds and the coagulant species during coagulation as described earlier. This was consistent with marked reduction in UVA_{254} and colour for ROC B. The EEM volumes for UV/H_2O_2 treatment of coagulated ROC A revealed a large reduction in all fraction with greater proportional reductions for HA-like (80%), FA-like (61%) and SMPs (68%). For coagulated ROC B, the reductions for HA-like, FA-like and SMPs were 70%, 71% and 81%, respectively. The reductions in APs were higher for ROC B (89% AP I and 86% AP II) than for ROC A (33% AP I and 68% AP II with UV/H_2O_2 treatment of coagulated ROC samples. The lower EEM volume reduction of HA-like substances for ROC B might be attribute to the effect of intense extra peaks in HA-like region of ROC B. It was possible that coagulation removed significant portion of humic-like substance, mostly hydrophobic matter, leading to increased UVT for ROC A as reported by Umar et al. (2016a). The marked reductions in colour (90-97%) and UVA_{254} (76-79%) during UV/H_2O_2 treatment of coagulated ROC samples indicated the effective removal of a major portion of the colour causing compounds of high molecular weight and was in accordance to the LC-OCD analysis of both ROC noted in chapter 7. The two ROC samples revealed different trends in terms of EEM volume reduction and fluorescence intensity, indicating the impact of the different organic composition of the ROC on the efficiency of the pre-treatments.

The EEM spectra of the BAC alone treatment of both ROC led to a reduction in the fluorescent organic matter in all regions (Appendix A2). This occurred due to the adsorption and breakdown of fluorescent molecules. The remaining fluorescent species were mainly humic and fulvic substances due to the low biodegradability of these molecules for both ROC. The reductions in EEM volumes in different regions were higher for ROC B than ROC A, i.e., 58% cf.45% (HA-like), 63% cf. 36% (FA-like), 69% cf. 50% (SMPs), 88% cf. 45% (API) and 78% cf. 54% (APII) reductions. For both ROC streams, the humic substances reductions were lower compared with other organic species, which were expected due to their low biodegradability (Velten et al., 2007, Marhaba, 2000). The fluorescence intensity of extra peaks in regions III and V were not reduced with BAC treatment of ROC B suggesting that some of the petrochemical related compounds present in the ROC were of low biodegradability and recalcitrant nature.

The UV/H_2O_2-BAC treatment of both ROC led to extra reductions in fluorescence intensity in HA-like, FA-like, SMPs, API and APII regions. The BAC further removed fluorescence in all regions of the oxidised ROC (Appendix A2). It should be noted that the fluorescence intensity of the extra peaks for the ROC B also greatly reduced with this combination suggesting that the UV/H_2O_2 treatment facilitated the breakdown of some petrochemical related compounds present in the ROC B into simpler molecules and these molecules were effectively removed by BAC process. The EEM volumes also reduced in all regions with the combined treatment of both ROC, however, reduction in EEM volumes was higher for ROC B than ROC A, implying that the organic content in the oxidised ROC B was more biodegradable compared with ROC A (Figures 8.1b and 8.2b). Nevertheless, some of the organics present in both pre-oxidised ROC might also have been adsorbed in the carbon media of the BAC process leading to enhanced reduction in EEM volumes.

The coagulation-BAC treatment greatly reduced fluorescence intensity in all regions for both ROC, however, reduction in fluorescence intensity was higher for ROC A compared with ROC B (Appendix A2). The BAC treatment of both coagulated ROC facilitated in reducing the fluorescence intensity predominantly due to biodegradation mechanism. The lower reduction in fluorescence intensity for coagulated ROC B was due to the recalcitrant nature of complexes which were formed during coagulation. The BAC treatment of coagulated ROC B led to a minimum reduction in the fluorescence intensity of the extra peaks in HA-like and FA-like and this reduction was possible due to the biodegradation. The coagulation-BAC treatment greatly reduced EEM volumes in all regions for ROC A with 76%, 65% and 70% reductions in HA-like, FA-like and SMPs reductions, respectively, whereas only 37%, 39% 52% reductions, respectively, were achieved in coagulated ROC B (Figures 8.1b and 8.2b). The lower reduction in EEM volumes for ROC B was possibly due to presence of greater concentration of high molecular weight compounds (humic-like) of low organic biodegradability in coagulated ROC B. It should be noted that the trends for EEM volume reductions for the two ROC streams were different after the UV/H_2O_2-BAC and coagulation-BAC treatments, the latter being more effective for ROC A, which was mainly due to enhanced removal of HA-like, mostly hydrophobic matter by coagulation as observed by Umar et al. (2016a).

The sequential coagulation-UV/H_2O_2-BAC treatment reduced EEM volumes in all regions for both ROC (Figures 8.1b and 8.2b). The enhanced reductions of EEM volumes in all regions for both ROC streams were due to enhanced breakdown of the remaining high MW organic matter after coagulation to LMW biodegradable products during UV/H_2O_2 treatment, which were then removed by the subsequent BAC process. This combination also led to enhance reductions of extra peaks present in the HA-like and FA-like substances for ROC B. The two different types of ROC revealed different trends in the reduction of fluorescence and EEM volumes as well as colour and UVA_{254} demonstrating the breakdown of chromophores at different rates indicating differences in the composition and concentration of the organic content. Taking account of the reductions in fluorescence intensity in all regions (including the fluorescence of extra peak) and the EEM volumes, the UV/H_2O_2 treatment greatly improved the biodegradability for coagulated as ROC B.

8.5 Nutrient removal by Coagulation and Coagulation- UV/H_2O_2 pre-treatments

The nutrient removals with the various pre-treatments are presented in Table 8.3. The coagulation and coagulation-UV/H_2O_2 pre-treatments removed more than 90% of phosphorus from both ROC. The UV/H_2O_2 pre-treatment used in the studies reported in Chapters 4 & 6 was ineffective in reducing TP, with only 2-10% removal for both ROC. This was in accordance with the previous studies which demonstrated that coagulation with metal salts could lead to higher phosphorus removal through effective precipitation (Zhou et al., 2008).

Table 8.3 Removal efficiency (%) of nutrient by the pre-treatments

	Reduction (%)			
	Coagulation		**Coagulation-UV/H_2O_2**	
Parameter	**ROC A**	**ROC B**	**ROC A**	**ROC B**
TN	5 ± 3	5 ± 2	5 ± 3	10 ± 3
NH_4^+-N	19 ± 7	22 ± 4	47± 7	45 ± 4
NO_3^--N	5.4 ± 3.7	7 ± 3	-11±7	13 ± 4
TP	92.5 ± 1.3	94 ± 2	93.0 ± 1.3	97 ± 2

In case of total nitrogen (TN) removal, none of the pre-treatments was effective for both ROC. Coagulation removed only 5% of TN from both ROC and the coagulation-UV/H_2O_2 pre-treatment removed only 10% and 5% TN for ROC A and ROC B, respectively. NH_4^+-N removal from the two ROC samples was comparable for both pre-treatments. The NH_4^+-N removal was higher with the sequential coagulation-UV/H_2O_2 pre-treatment which removed 45-47% of NH_4^+-N from ROC samples. Coagulation removed only 5-7% of NO_3^--N for both ROC samples, whereas the sequential coagulation-UV/H_2O_2 pre-treatment increased nitrate concentration for ROC A and reduced it for ROC B.

Nitrogen removal by coagulation is mainly related with colloidal matter removal and on account of this, removed nitrogen would be mainly in the form of proteins/albuminoid type (Aguilar et al., 2002). It was likely that most of nitrogen in the form of colloidal matter might have been removed as flocs during coagulation, leaving some weak and tiny flocs in the supernatant. The coagulation process removes NH_4^+ ion due to the formation of dissolved salt in water or electrostatic attraction onto the surface of the negatively charged colloidal particles at pH 5-7 (Aguilar et al., 2002). The UV/H_2O_2 treatment can decrease nitrogen species due to the oxidation of some nitrogen species into gaseous N_2 (Dwyer et al., 2008). The slight removal of NH_4^+-N with UV/H_2O_2 pre-treatment could be due its volatile nature.

8.6 Impact on nutrient removal by BAC with and without pre-treatments

The overall nutrient removals with the BAC based treatments are presented in Table 8.4. Excellent phosphorus removal (97-99%) was achieved with the treatments involving coagulation on both ROC.

Table 8.4 Overall removal efficiency (%) of nutrients for the BAC with and without pre-treatments

	Reduction (%)					
Parameter	**Coagulation-BAC**		**Coag-UV/H_2O_2 -BAC**		**BAC**	
	ROC A	**ROC B**	**ROC A**	**ROC B**	**ROC A**	**ROC B**
TN	22.4 ± 6	15 ± 6	47.5± 8	34 ± 6	72 ± 9	21 ± 5
NH_4^+-N	96.6 ± 1	90 ± 9	98.5±0.7	90 ± 9	90 ± 7	93 ± 4
NO_3^--N	**-11 ± 8**	18 ± 12	41 ± 12	16 ± 10	63 ± 13	7 ± 6
TP	97 ± 2	99 ± 1	97 ± 0.6	99 ± 1	8 ± 4	58 ± 5

In terms of nitrogen species, ammonium removal was consistently high (≥90%) under the various treatment scenarios on both ROC A and ROC B. The highest NH_4^+-N removal was obtained with the sequential coagulation-UV/H_2O_2-BAC treatment (98%) for ROC A. High ammonium removal (90-93%) was also achieved with BAC alone treatment on both ROC. The higher ammonium removal with the BAC treatments was attributed to effective nitrification in the BAC systems.

The sequential coagulation-UV/H_2O_2-BAC treatment resulted in the overall TN removal of 47%, whereas coagulation-BAC treatment gave only 22% TN removal for ROC A. The sequential coagulation-UV/H_2O_2-BAC and coagulation-BAC removed only 34% and 15% of TN, respectively, for ROC B.

The coagulation-BAC combination did not remove nitrate from ROC A, and removed only 18% nitrate from ROC B. Nitrate removal was high (41%) for ROC A and very low (16%) for ROC B with sequential coagulation-UV/H_2O_2-BAC treatment. The extent of nitrate removal was lower compared with TN removal from both ROC samples with sequential coagulation-UV/H_2O_2-BAC treatment implying that partial denitrification took place in the BAC system.

The increase in NO_3^--N after coagulation-BAC for ROC A was attributed to the combined effect of higher DO, pH and a short operation time (12 days) for the denitrifiers to become adapted to the coagulated ROC. Apart from the influence of the high DO, the low denitrification in the BAC treatment treating ROC A with this combination could also be attributed to release of soluble microbial products (SMPs) during the biodegradation process involving the nitrifiers (Kindaichi et al., 2004, Krasner et al., 2009). SMPs are the pool of organic compounds that result from substrate metabolism and biomass decay during the complete mineralization of simple substrates, they act as the substrates for another group of microorganisms and are normally not readily biodegradable (Schiener et al., 1998, Azami et al., 2012). The SMPs can be utilised by heterotrophs (denitrifiers) as an extra substrate supply. Since the SMPs are not normally readily biodegradable, they could have impacted the denitrification process. However, much less nitrate removal (5-18%) was achieved for ROC B with different pre-treatments followed by BAC, even though experiment run time was longer (30 days). As nitrate removal was extremely low for ROC B, lower TN removal was achieved for ROC B. The BAC treatment systems treating ROC B with different pre-treatments can remove more phosphorus than nitrogen due to the competition between denitrifiers and phosphorus removing bacteria for same organic carbon in which phosphorus removing bacteria might have outcompeted the denitrifiers.

8.7 Conclusions

Pre-treatments with coagulation or sequential coagulation-UV/H_2O_2 significantly improved the organic matter removal for the BAC based process of the ROC generated from the different wastewater sources. This was attributed to the effective decrease in the non-biodegradable organic matter in the ROC. The DOC removals were comparable for ROC A and ROC B under the sequential coagulation-UV/H_2O_2–BAC (62-67%) treatment. Coagulation facilitated the removal of humic like substances, and the subsequent UV/H_2O_2

treatment facilitated the breakdown of some remaining large MW compounds into LMW compounds that were readily removed by the downstream BAC process for both ROC. However, coagulation-BAC treatment was more effective in removing DOC from ROC A than that of ROC B (61% cf. 37%). This was mainly due to the presence of higher concentration of low biodegradable organics in the coagulated ROC B, which were not amenable to microorganisms present in the BAC process. In terms of nutrient removal, coagulation pre-treatment led to excellent TP removal for the BAC based treatment process (> 90%) as a result of effective precipitation of phosphates during the coagulation. ROC A exhibited better TN removal than ROC B under the various treatments, suggesting that salinity as well different organic constituents played important roles in nutrient removal. This study showed that better nitrogen removal at higher salinity but higher phosphorus removal was achieved only at lower salinity (< 5g/L), which was in line with previous studies as phosphorus removing organisms are vulnerable to high salinity. The study also showed that the BAC treatment was resilient with regard to ammonium removal, with over 90% reduction achieved by all treatment options on the two ROCs.

Chapter 9 - Conclusions and Recommendations

9.1 Conclusions

The effectiveness of the BAC based treatment processes for removing organic matter and nutrients from two municipal wastewater ROC with significantly different characteristics were investigated in this study. The main findings from the study are as follows.

As a pre-treatment of ROC of different characteristics for the BAC process, stand-alone UV/H_2O_2 treatment effectively broke down the organic matter as indicated by the greater reductions in colour and UVA_{254}. The resulting reduction of DOC and COD was however low compared with colour and UVA_{254} reductions with UV/H_2O_2 treatment for both ROC implying that partial degradation of organic matter had taken place with the oxidation process. The loss of colour and UVA_{254} correlated well with the decrease in high molecular weight compounds such as humic-like and biopolymers, and concurrent increase in the concentration of low molecular weight compounds as demonstrated by LC-OCD analysis and are amenable to microorganisms in the subsequent BAC process. Compared with organic matter, nutrient removal was markedly lower with stand-alone UV/H_2O_2 treatment over the salinity range studied.

UV/H_2O_2 followed by BAC treatment led to the enhanced organic matter removal for a highly saline ROC (ROC A), with 57% DOC, 81% UVA_{254} and 95% colour removal achieved. This indicates the partially oxidised organic matter was readily biodegraded by the microorganism in the BAC system. The BAC alone treatment resulted in comparable UVA_{254} and colour removal, but DOC removal was lower (38%) compared with the UV/H_2O_2-BAC treatment. For the combined treatment system with UV/H_2O_2-BAC, high molecular weight compounds and low molecular weight compounds were further reduced due to their formation in the oxidation process and subsequent utilisation by the microorganisms and some adsorption on the GAC. In terms of nutrient removal, higher total nitrogen (60%) and moderate total phosphorus removal (15%) were achieved on the highly saline ROC. Ammonium nitrogen removal was consistently high (90%) for BAC alone and the combined treatments due to the high degree of nitrification. The BAC treatment was consistently effective for organic matter removal under the high salinity environment (i.e., TDS ~16 g/L).

In the study of the impact of ROC salinity at low (TDS 7 g/L), medium (10 g/L) and higher (16 g/L) levels on the UV/H_2O_2-BAC treatment, it was observed organic matter removal was comparable over the tested salinity ranges showing the robustness of the BAC process, but higher total nitrogen removal was achieved for the ROC at higher salinity compared with low and medium salinity. It was revealed by the microbiological analysis that diverse bacterial communities such as *Bacillus* sp. (*Firmicutes*), *Pseudomonas* sp. (γ-*Proteobacteria*) and *Rhodococcus* sp. (*Actinobacteria*) were present in the BAC system over the salinity range studied, confirming these bacterial communities were responsible for carbon and nitrogen removal. Denitrification appeared to be more prevalent at higher salinity of ROC, suggesting that the denitrifying bacteria contributing to the greater total nitrogen removal were more halotolerant. Nitrification was not affected by salinity as indicated by consistently high ammonium nitrogen removal (> 90%) over the salinity range of studied.

UV/H_2O_2-BAC treatment was also conducted on another ROC (denoted as ROC B) which was derived from a municipal wastewater containing a significant proportion of petrochemical processing wastewater. The ROC B had significantly different water characteristics from the ROC A in terms of organic concentrations (such as DOC and COD) and inorganic contents (such as TDS, chloride and conductivity). The UV/H_2O_2-BAC treatment of ROC B also resulted in a significantly greater organic matter reduction compared with the stand-alone UV/H_2O_2 treatment, with 58% DOC, 82% UVA_{254} and 94% colour removal achieved. The treatment led to higher phosphorus removal (60%) but lower total nitrogen removal (15%), indicating that higher ROC salinity (TDS >5 g/L) could greatly inhibit the phosphorus removal. The microbiological analysis revealed the BAC system contained the bacterial communities closely related to PAOs such as *Micrococcus* sp. (*Actinobacteria*), *Ralstonia* sp. (β-*Proteobacteria*), *Agrobacterium* sp., *Sphingopyxis* sp. (α-*Proteobacteria*) and *Pseudomonas* sp. (γ-*Proteobacteria*). The UV/H_2O_2-BAC treatment effectively reduced the TPH present in the ROC, implying the suitability of such treatment for the ROC containing the petrochemical compounds.

The UV/H_2O_2-BAC treatment resulted in comparable DOC, colour and UVA_{254} removals on both types of ROC (ROC A and ROC B), although COD removal was higher for ROC B. The BAC treatment effectively removed low MW compounds due to biodegradation as shown by LC-OCD analysis of both ROC. In terms of nutrient removal, higher total phosphorus and lower nitrogen removal was achieved for ROC B, whereas the opposite was observed for ROC A. The specific growth rate of biomass was consistently higher for ROC B compared with ROC A, implying that the degradation of substrate (COD) took place at a higher rate as calculated with Monod's equation. The reaction rates for UV/H_2O_2, BAC and their combination were also higher for ROC B than ROC A, implying that the organics present in ROC B were more easily biodegradable than those present in ROC A. The decrease in colour and UVA_{254} correlated well with the decrease in high molecular weight humic-like compounds for both ROC as demonstrated by the LC-OCD analysis, and with the resultant increase in biodegradability of the ROC.

In the study of the impact of the various pre-treatments on the overall treatment efficiency of the BAC based processes, coagulation removed considerable organic matter from both ROC. Coagulation pre-treatment resulted in some increase in the fluorescence response due to the complexation between anionic HA-like substances and coagulant species for both ROC (ROC

A and ROC B). The increase in the fluorescence response was higher for ROC B than for ROC A. The BAC treatment of both coagulated ROC resulted in higher reduction in the fluorescence intensity in all regions, with was accompanies with organic matter removal. However, the removal in organic matter was greater for ROC A than for ROC B, implying that the complexes formed for ROC B were of low biodegradability. The coagulation+UV/H_2O_2 pre-treatment enhanced the reduction of fluorescence intensity in all regions for both ROC indicating that the interaction was weak. This combination also led to enhanced reduction in the extra peaks present in the ROC B, showing the potential of UV/H_2O_2 treatment in removing petrochemical related compounds. The sequential coagulation-UV/H_2O_2-BAC treatment further enhanced the organic matter removal due to the improved UVT with coagulation for both ROC and successive removals of organic matter by each treatment with 62-67% DOC reductions achieved. Coagulation improved total phosphorus removal to over 90%. The coagulation-BAC treatment achieved better organic removal for ROC A than for ROC B due to greater removal of high molecular weight compounds by coagulation leaving lower molecular weight compounds intact, which were further removed by BAC. Unlike the total phosphorus, total nitrogen removal was improved with the BAC based treatments on both types of the ROC. Ammonium nitrogen removal was also consistently high (>90%) for both ROC with these treatments, implying the pre-treatments did not negatively impact the effectiveness of nitrification of the BAC system.

The differences in the observed trends for the treatments on the two types of ROC were attributed to the differences in the nature of organic matter and other physico-chemical properties such as salinity levels of the studied ROC. Although there was a moderate increase in ecotoxicity for the ROC after the UV/H_2O_2 treatment, the downstream BAC treatment led to the total removal of the toxicity, suggesting the biological treatment could be utilised as an effective barrier to the toxic compounds present in the ROC. The nutrient removal from the different types of ROC was dependent on bacterial communities in the BAC system. Although coagulation enhanced phosphorus removal from both types of ROC, the organic matter removal was improved for ROC B, which suggests that the treatment process should be optimised depending upon the nature of the ROC.

9.2 Recommendations for future work

Although the study demonstrated that the BAC based treatment processes had the potential for treating municipal wastewater ROC streams to reduce their environmental and health risks on disposal or reuse, more work should be done in order to justify the technological viability of the BAC based processes and gain better understanding about the processes with a view to maximising the treatment efficiency.

The following are recommended for future studies.

1. Although the overall removal of organic matter by the BAC based treatments was assessed, it would be useful to investigate their removal efficiencies for some harmful micropollutants and petrochemical compounds of interest.
2. More advanced microbiological tools such as quantitative PCR (q-PCR) and cutting-edge metagenomics characterisation with MiSeq should be used for better insights into the bacterial communities governing the organic and nutrient removal, with a view to improving the treatment performance.
3. In this study, a fixed dose of ferric chloride (coagulant) was used for both ROC based on previous study for a higher salinity ROC (Umar et al., 2016a). Further work on optimisation of the coagulant dose at different pH using different coagulants for lower salinity ROC should be assessed for enhanced organic matter removal. More work may be needed to examine and mitigate the impact of residual coagulant on the biological treatment.

4. The economic feasibility of using UV/H_2O_2, coagulation and their combination as the pre-treatments for the BAC should be conducted.
5. Other treatment options such as using ozonation, UV/TiO_2 may be assessed for organic matter removal from different types of ROC, as well as economic feasibility of these treatment options.
6. The process should be further developed and trialled at larger scales such as pilot plants to gain more operational data for the possible full-scale applications.

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List of Appendices

Appendix A-1 Standard curve for NH_4^+-N, NO_3^--N and NO_2^--N

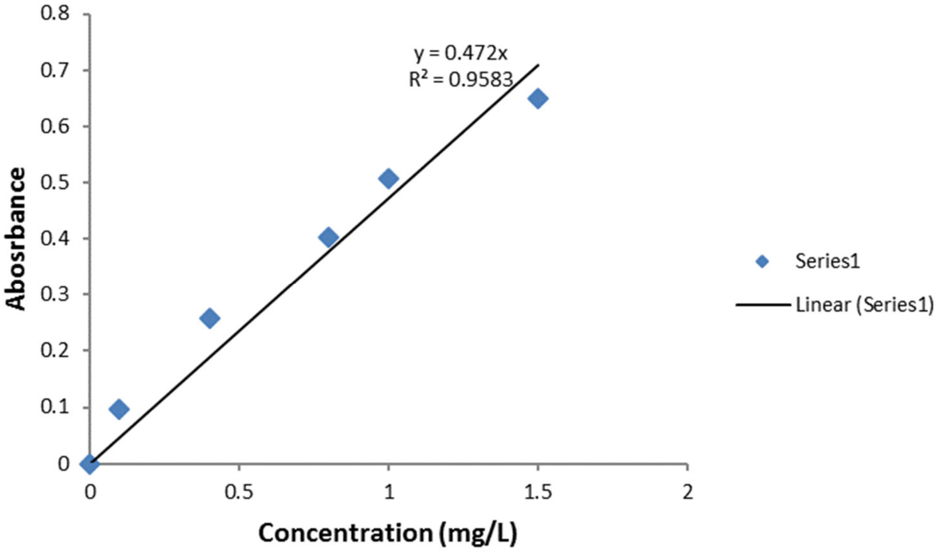


Figure A1-1 Standard curve for ammonium nitrogen NH_4^+-N measurement

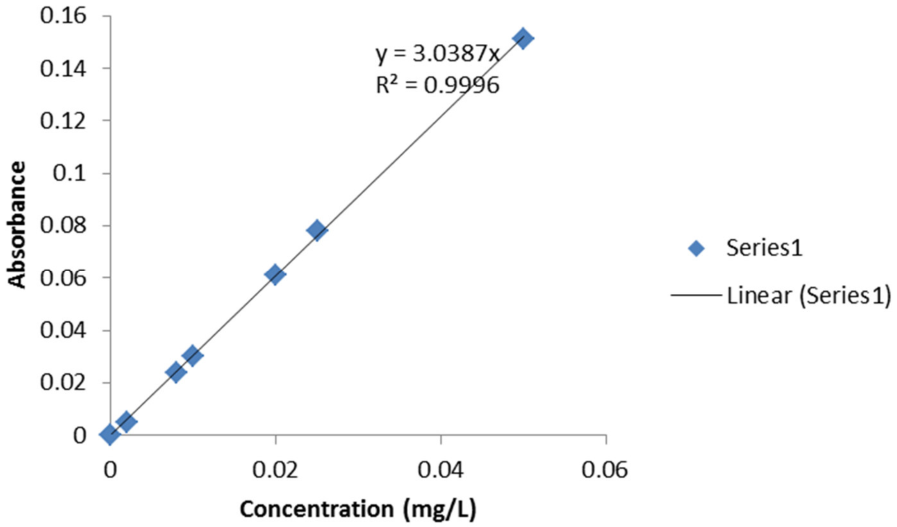


Figure A1-2 Standard curve for nitrite nitrogen NO_2^--N measurement

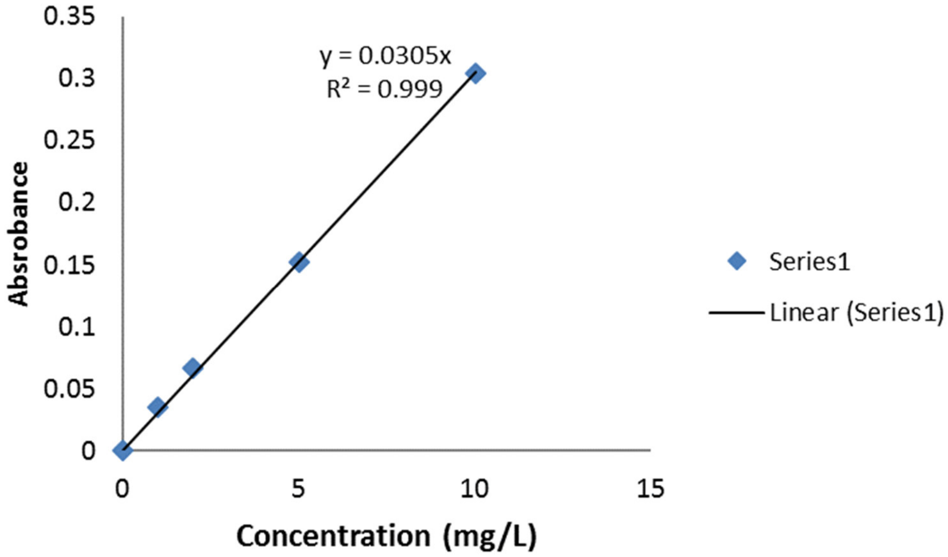
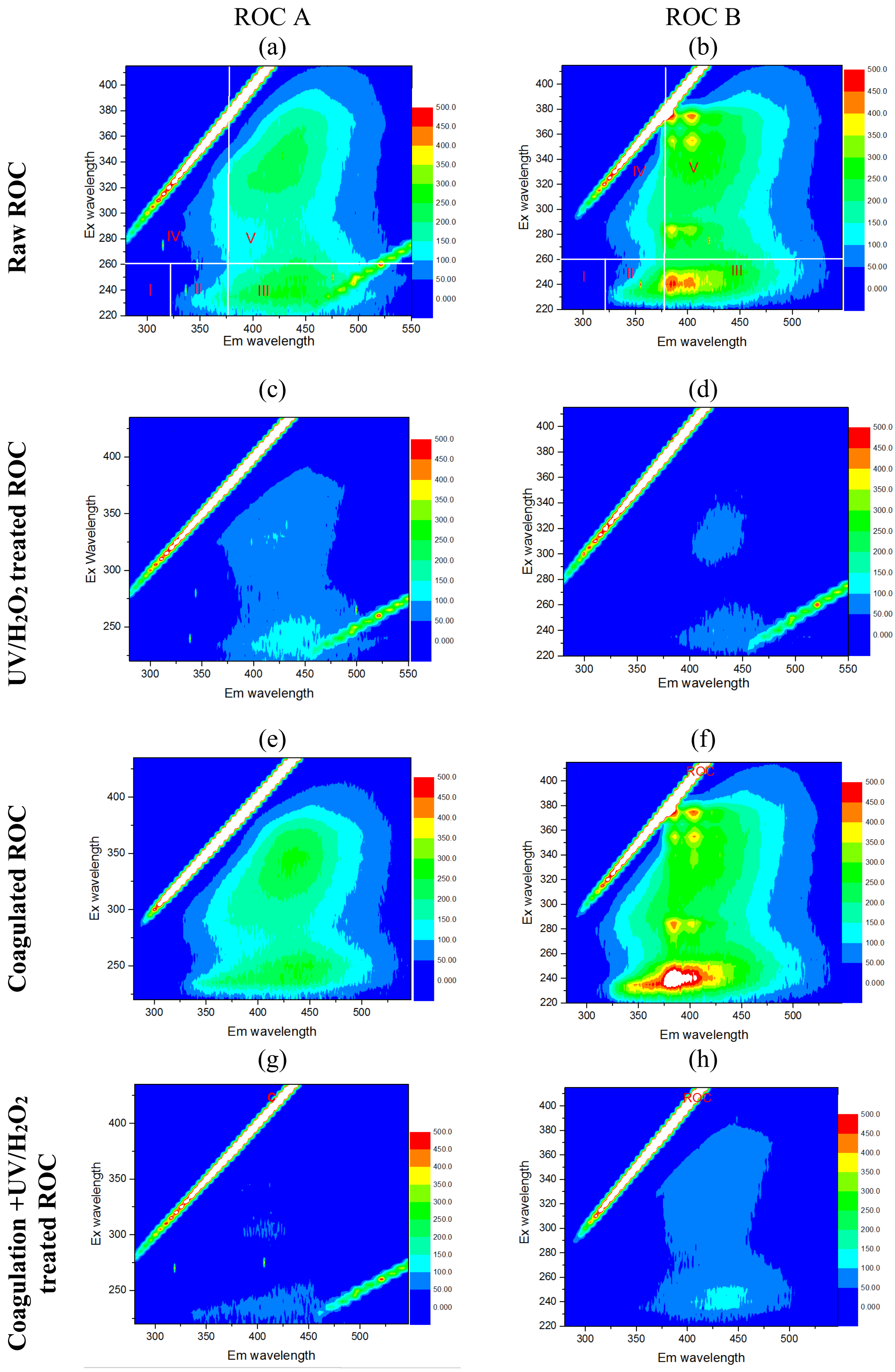
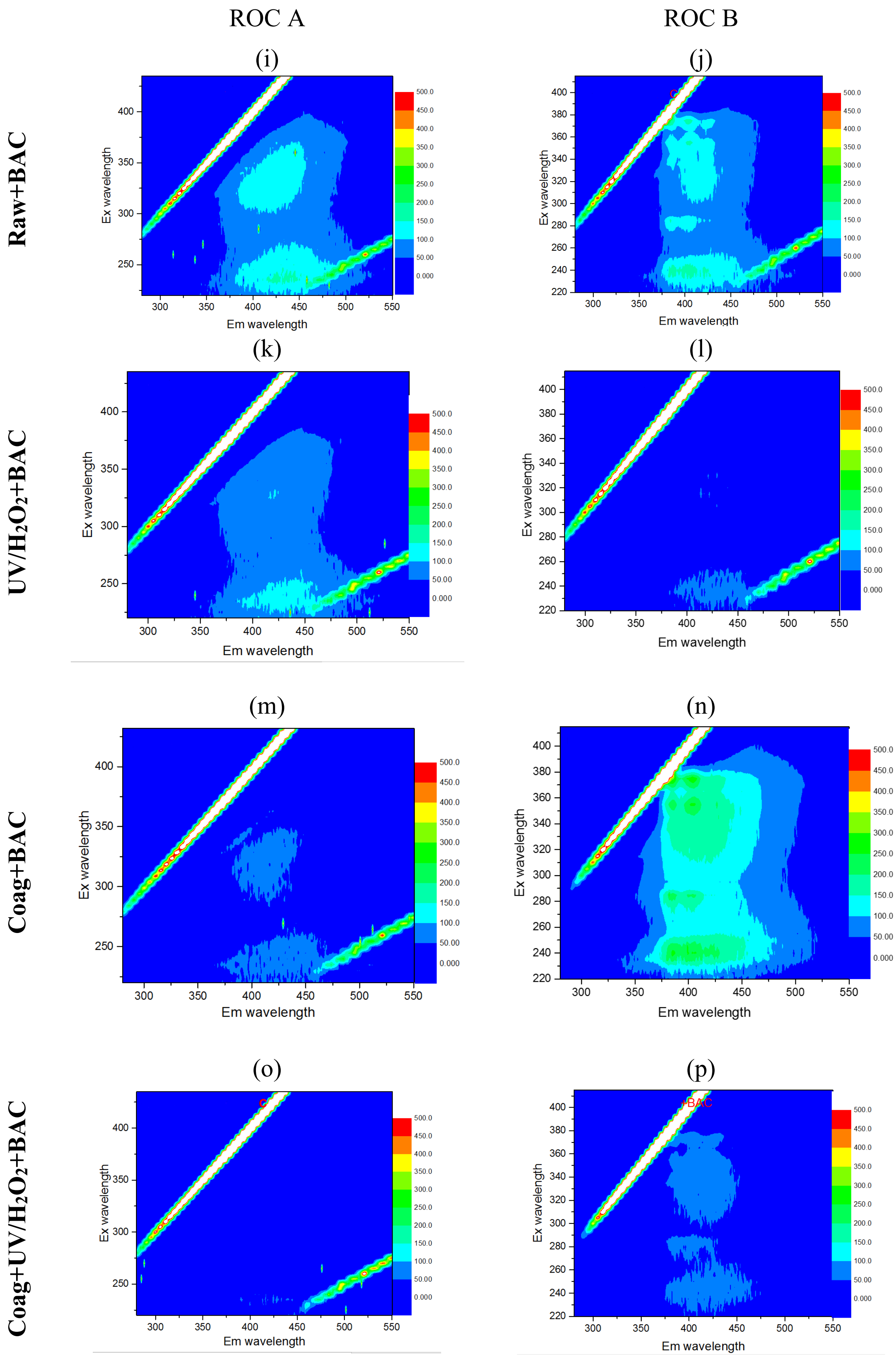


Figure A1-3 Standard curve for nitrite nitrogen NO_3^--N measurement

Appendix A-2 EEM spectra with different pre-treatments and BAC combinations of ROC A and ROC B




ROC A
ROC B
Raw+BAC
UV/H_2O_2+BAC
Coag+BAC
Coag+UV/H_2O_2+BAC
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(p)
Ex wavelength
Em wavelength
300
350
400
450
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550
220
240
250
260
280
300
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150.0
100.0
50.00
0.000
C
+BAC