CRANFIELD UNIVERSITY

Eleanor Claire Raper

Biological treatment of coke making wastewater

School of Water, Environment and Energy
Research Degree

PhD
Academic Year: 2013 - 2017

Supervisor: Dr Ana Soares and Prof Tom Stephenson
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This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

ABSTRACT

Production of coke for steel manufacturing produces a wastewater containing total nitrogen (TN) (up to 600 mg/L) alongside toxic compounds phenol (60 - 400 mg/L), thiocyanate (SCN^-) (100 - 400 mg/L), polycyclic aromatic hydrocarbons (PAHs) (Σ6PAHs:179±35 µg/L) and trace metals. Emission limits introduced by the Industrial Emissions Directive (IED) in 2016 require treated coke effluent to contain <50 mg/L TN, <4 mg/L SCN^-, <0.5 mg/L phenol and <50 µg/L Σ6PAHs which cannot be consistently met by the conventional activated sludge process (ASP). Treatment process modifications were investigated to ensure compliance.

Activated carbon addition to the ASP (400 mg/L) increased Σ6PAHs removal by 20% enabling emission compliance whilst increasing nickel, chromium and cadmium removal. The addition of 0.5 g/L of a commercial bioaugmentation product increased dissolved Σ6PAHs removal by 51%. Biostimulation (addition of micronutrients/alkalinity) enabled SCN^- and phenol emission compliance. Survival of supplemented exogenous bacteria in a simulated river water discharge was investigated for the first time showing limited survivability.

Thiocyanate degradation mechanisms were poorly understood but were important to ascertain, especially as SCN^- degradation produces ammonia increasing TN loading. Control of influent ammonia and phenol concentration was important enabling SCN^- degradation under anoxic and aerobic conditions. Deoxyribonucleic acid sequencing of the mixed culture identified a new species of *Thiobacillus* which had metabolic similarities to *T. thioparus* and *T. denitrificans*.

Nitrification was limited (41%) confirming the importance of intrinsic alkalinity availability in the wastewater, however, sodium carbonate addition (300 mg/L as

$CaCO_3$) increased efficiencies to 96%. An anoxic-aerobic ASP was investigated for TN removal enabling an effluent TN <50 mg/L when the soluble chemical oxygen demand (sCOD):TN ratio was maintained above 5.7. Acetic acid was identified as a suitable source of carbon addition to maintain this ratio.

An anoxic-aerobic ASP combined with AC and bioaugmentation can ensure compliance with the IED.

Keywords:

Coke wastewater; total nitrogen; thiocyanate; polycyclic aromatic hydrocarbons; phenol; trace metals; activated sludge process; activated carbon; bioaugmentation; biostimulation.

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Lastly, and most importantly, I would like to express my gratitude to Dr Ana Soares, lead academic supervisor, for always providing me with encouraging words and invaluable support throughout my research.

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AC	Activated carbon
AOB	Ammonia oxidising bacteria
ASP	Activated sludge process
BAT	Best available technique / Best available technology
BOD	Biological oxygen demand
BREF	Best available techniques reference document
CAPEX	Capital expenditure
COD	Chemical oxygen demand
sCOD	Soluble chemical oxygen demand
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
GAC	Granular activated carbon
GEM	Genetically engineered microorganisms
HRT	Hydraulic retention time
ICP-MS	Inductively coupled plasma-mass spectrometry
IC_{50}	50% inhibition concentration
IED	Industrial emissions directive
MGE	Mobile genetic elements
MLSS	Mixed liquor suspended solids
NOB	Nitrite oxidising bacteria
NO_x	Oxidised nitrogen
OPEX	Operational expenditure
OTU	Operational taxonomic unit
PAC	Powdered activated carbon
PAHs	Polycyclic aromatic hydrocarbon
PCR	Polymerase chain reaction
PHS	Priority hazardous substance
PNP	p-nitrophenol
PS	Priority substance
RAS	Return activated sludge
RBC	Rotating biological contactor
RNA	Ribonucleic acid

rRNA	Ribosomal ribonucleic acid
SAC	Special area of conservation
SCN^-	Thiocyanate
sCOD	Soluble chemical oxygen demand
SP	Specific pollutants
SPA	Special area of protection
SRT	Sludge retention time
SS	Suspended solids
TN	Total nitrogen
VSS	Volatile suspended solids
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

Chapter 1 Introduction

1.1 Background

Water is an increasingly valuable resource in high demand due to the increasing world population, declining water quality and mismanagement of water resources (United Nations Environment Programme, 2010). Only 2.5% of water on Earth is freshwater. Of this 2.5%, 70% is frozen in Antarctica and Greenland's icecaps making it inaccessible whilst most of the remaining freshwater is inaccessible groundwater. Therefore only 1% is available for withdrawal and human use (United Nations Environment Programme, 2010). As such, careful management of water resources is required. In Europe, industry accounts for 40% of total water abstractions (Eurostat, 2016). Due to the large volumes of water used by industry, industrial activity holds the potential to cause significant pollution of freshwater resources (United Nations Environment Programme, 2010). Consumption levels vary drastically between countries. For instance, the steel industry in India consumes 8-10 times more water per ton of steel than that consumed in developed countries (Rande and Bhandari, 2014). In many developing countries, more than 70% of industrial wastewaters are discharged untreated into freshwater bodies leading to decreased ecosystem and human health, contaminated food and water and increased production costs (United Nations Environment Programme, 2010). In developed countries, however, industrial wastewaters are heavily regulated. For example, in Europe, industrial wastewaters are regulated under the Industrial Emissions Directive (IED) whilst in the United States they are regulated under the Clean Water Act (European Commission, 2015; US.EPA, 2015).

In 2015, the UK alone produced 10,907 thousand tonnes of steel whilst the EU as a whole produced 166,120 thousand tonnes (World Steel Association, 2016).

North America and China contribute significantly more at 110,945 and 803,825 thousand tonnes respectively (World Steel Association, 2016). Overall, world steel production in 2015 stood at 1,597,473 thousand tonnes. Current production levels suggest similar trends for 2016 and 2017 (World Steel Association, 2016). As a result of high production levels and the associated high volumes of wastewater, careful regulation of their discharge is required. The production of coke for use in the steel industry results in significant quantities of wastewater. Each ton of coke produced results in 0.1 - 1 m^3 of wastewater (European Commission, 2013; Pal and Kumar, 2014).

1.2 Coke wastewater characterisation

Coke wastewaters are composed of nitrogenous compounds including ammonia (NH_4^+), thiocyanate (SCN^-) and cyanide (CN^-) as well as organic pollutants such as phenol, polycyclic aromatic hydrocarbons (PAHs) and trace metals (Vázquez *et al.*, 2006; Staib and Lant, 2007; Marañón *et al.*, 2008; Kim *et al.*, 2011). Coke wastewaters are typically characterised by ammonia concentrations of 50 - 500 mg/L, thiocyanate concentrations of 100 - 400 mg/L and phenol concentrations of 60 – 400 mg/L (Vázquez *et al.*, 2006; Staib and Lant, 2007; Marañón *et al.*, 2008; Bai *et al.*, 2010). Burmistrz and Burmistrz (2013) reported the sum of 16 PAHs of 255 - 312 µg/L whilst Zhang et al. (2012) reported concentrations of 5470 ± 907 µg/L for the sum of 18 PAHs. Additionally, the sum of 6 PAHs has been reported at 179 ± 35 µg/L (Raper *et al.*, 2017). Trace metals have been reported at 4216 µg/L ranging from 0.13 µg/L for cadmium to 3612 µg/L for iron (Raper *et al.*, 2017).

The high concentration of nitrogen containing compounds is problematic due to the potential for the eutrophication of receiving environments (Camargo and Alonso, 2006). Thiocyanate can additionally contribute a significant proportion of nitrogen as its degradation products include ammonia (Kim et al., 2009).

Although SCN^- is less toxic than CN-, it still represents a concerning level of toxicity and has been attributed to enzyme inhibition and nervous system damage (Paruchuri, Shivaraman and Kumaran, 1990; Boening and Chew, 1999). Phenolic compounds are toxic and are capable of interfering with biochemical functions within humans (Nuhoglu and Yalcin, 2005). Aquatic organisms have been shown to suffer from extreme toxic effects from phenol at parts per million whilst effects, albeit less severe, are still reported at parts per billion (Guerra, 2001). Polycyclic aromatic hydrocarbons are widely known for their toxicity. The higher their molecular weight, the greater the toxicity and recalcitrance. Approximately 98% of the PAHs found in coke wastewater were reported to be high molecular weight compounds (Zhang et al., 2012; Raper et al., 2017). Additionally, whilst trace metals are essential for metabolic processes, they also have an affinity for sulphur and nitrogen which make up proteins providing them with plentiful potential binding sites within cells. This in turn makes trace metals toxic in large concentrations as they can bind to proteins preventing their usual metabolic roles (Rainbow, 2002).

1.3 Regulation

New emission limits are now being enforced for coke wastewater, under the IED which requires total nitrogen (TN) (the sum of ammonia-nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite-nitrogen (NO_2^--N)) to be reduced to <50 mg/L (European Commission, 2013). Thiocyanate is required to be reduced to <4 mg/L whilst easily released CN^- should be reduced to <0.1 mg/L. Phenols are required to be reduced to <0.5 mg/L, chemical oxygen demand (COD) <220 mg/L and biological oxygen demand (BOD) <20 mg/L. Limits for PAHs consider the sum of 6 PAHs (Fluoranthene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene and Benzo[g,h,i]perylene) which should be <50 µg/L. Trace metals are required to be reduced to <1000 µg/L (sum of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and mercury (Hg)). Additionally, both PAHs and trace

metals occur on the list of priority pollutants under the Water Framework Directive (WFD) (European Commission, 2014).

Consequently, there is a need for research into treatment systems which will enable compliance with the new coke wastewater emission limits. Activated sludge processes, specifically the pre-denitrification (anoxic-aerobic) activated sludge process (ASP), are highlighted as the best available technique (BAT) (European Commission, 2013) and therefore the optimisation of an anoxic-aerobic ASP for nitrogen is of notable importance. Other additional technologies may also be required to ensure compliance for other priority pollutant emission limits including bioaugmentation, biostimulation and adsorption by activated carbon (AC).

1.4 Aims and objectives

Aim: To optimise the activated sludge treatment process for coke wastewater treatment for nitrogen removal to comply with the 50 mg/L total nitrogen (TN) emission limit of the IED whilst maintaining and, or improving the removal of priority pollutants including PAHs, phenol, thiocyanate and trace metals.

Consequently, the following objectives were identified:

1. To critically review the application of bioaugmentation to activated sludge processes treating industrial wastewaters to understand current knowledge and enable method development for its application to coke wastewater.
2. To evaluate the role of AC in the removal of PAHs and trace metals from coke wastewater in an ASP treatment system.
3. To elucidate the degradation pathway of SCN^- in order to understand the requirements of SCN^- degrading bacteria.

4. To evaluate the ability of bioaugmentation and biostimulation to enhance the removal of SCN^-, PAHs and trace metals. Furthermore, to investigate the ability of exogenous bacteria to survive in a simulated river water discharge system.
5. To identify suitable carbon sources for stable nitrification and denitrification.
6. To characterise the optimal operational conditions for TN removal in an anoxic-aerobic ASP.

1.5 Thesis plan

The thesis is presented as a series of chapters formatted as journal papers (published, submitted or in preparation). The connection between the chapters and objectives is schematised in Figure 1-1. Each chapter was written by the first author Eleanor Claire Raper with comments from supervisors Dr Ana Soares and Prof Tom Stephenson. Industrial supervision, from Tata Steel, was provided by Dr David Anderson and Ray Fisher. Pilot-scale and laboratory-scale reactors were operated by Eleanor Claire Raper. Laboratory work was undertaken by Eleanor Claire Raper with partial laboratory support in Chapter 3, 4, 5, 6 and 7. Deoxyribonucleic acid sequencing in Chapter 4 and 5 was completed through polymerase chain reaction (PCR) analysis and was completed by an external lab under the direction of Eleanor Claire Raper. Polycyclic aromatic hydrocarbon and trace metal analysis in Chapter 3 and 5 and suspended solids analysis in Chapter 6 and 7 were completed through an external laboratory or named author under the direction of Eleanor Claire Raper.

Chapter 2 reviews the application of bioaugmentation to wastewaters regulated under the IED. The review considers the root causes behind bioaugmentation failures and techniques to overcome such problems. The review highlights key

areas for development and assisted methodology development for Chapter 5. Chapter 2 was written as a review article and is ready to submit to Process Safety and Environmental Protection.

Chapter 3 considered the application of AC to the ASP in order to enhance the removal of the PAHs and trace metals (priority pollutants). This chapter was submitted to the Journal of Chemical Technology and Biotechnology - *E. Raper, A. Soares, J. Chen, A. Sutcliffe, E. Aries, D. R. Anderson, T. Stephenson. (2017) Enhancing the removal of hazardous pollutants from coke making wastewater by dosing activated carbon to activated sludge process. Journal of Chemical Technology and Biotechnology*. This study targeted a gap in literature on the application of AC to ASP processes for the removal of PAHs and trace metals in continuous processes.

Chapter 4 aimed to identify the mechanism of SCN^- removal in the ASP. Deoxyribonucleic acid (DNA) microbial sequencing, through polymerase chain reaction, was used to identify SCN^- degrading species in the activated sludge mixed culture enabling a better understanding of SCN^- degradation pathways, to be better understand the requirements to obtain significant removal efficiencies. Additionally, the chapter highlighted necessary operational requirements for SCN^- degradation and investigated the roles of potentially inhibitory compounds. This chapter was written up in preparation for submission to Applied Microbiology and Biotechnology.

Chapter 5 was a scoping study to investigate the application of bioaugmentation and biostimulation to coke wastewater. The ability of bioaugmentation to improve the removal of PAHs, trace metals, phenol and SCN^- was investigated. Additionally, microbial sequencing was used to identify the operational taxonomic unit (OTU) abundance whilst flow cytometry was used to investigate the ability of inoculated bacteria cells to survive in the treated effluent and after

discharge to the receiving environment. This chapter was written up in preparation for submission to the Journal of Chemical Technology and Biotechnology.

Chapter 6 investigated the requirements of both nitrification and denitrification for coke making wastewater. Different compounds were considered for the supply of inorganic carbon to nitrifying bacteria. The optimal compound was then selected and the required dose rate optimised. The provision of additional organic carbon was investigated for denitrification. Two alternative carbon compounds, glycerol and acetic acid, were compared for their ability to support the denitrification of coke wastewaters. Knowledge gained through this chapter was applied to Chapter 7. This chapter was written up for submission to Environmental Technology.

Chapter 7 investigated the optimisation of a pilot-scale pre-denitrification (anoxic-aerobic) ASP. The optimal conditions required for TN removal were identified to enable compliance with the IED emission limit. Chapter 7 was in part presented at “Ecotechnologies for Wastewater Treatment (ecoSTP)” the 3rd IWA Specialized International Conference (2016). This chapter brings together findings from Chapter 4 and 6 and was written up for submission to the Journal of Hazardous Materials.

Chapter 8 brings together results from each chapter and discusses the requirements for the removal of TN, SCN^- and phenol. Treatment options are then discussed for PAHs and trace metal removal. The chapter then synthesises the requirements and highlights the operational conditions required for an anoxic-aerobic ASP to enable compliance with the IED emission limits. Contributions to knowledge are reported and areas for future work highlighted.

Chapter 9 brings together the overall conclusions of the work in relation to the initial objectives.

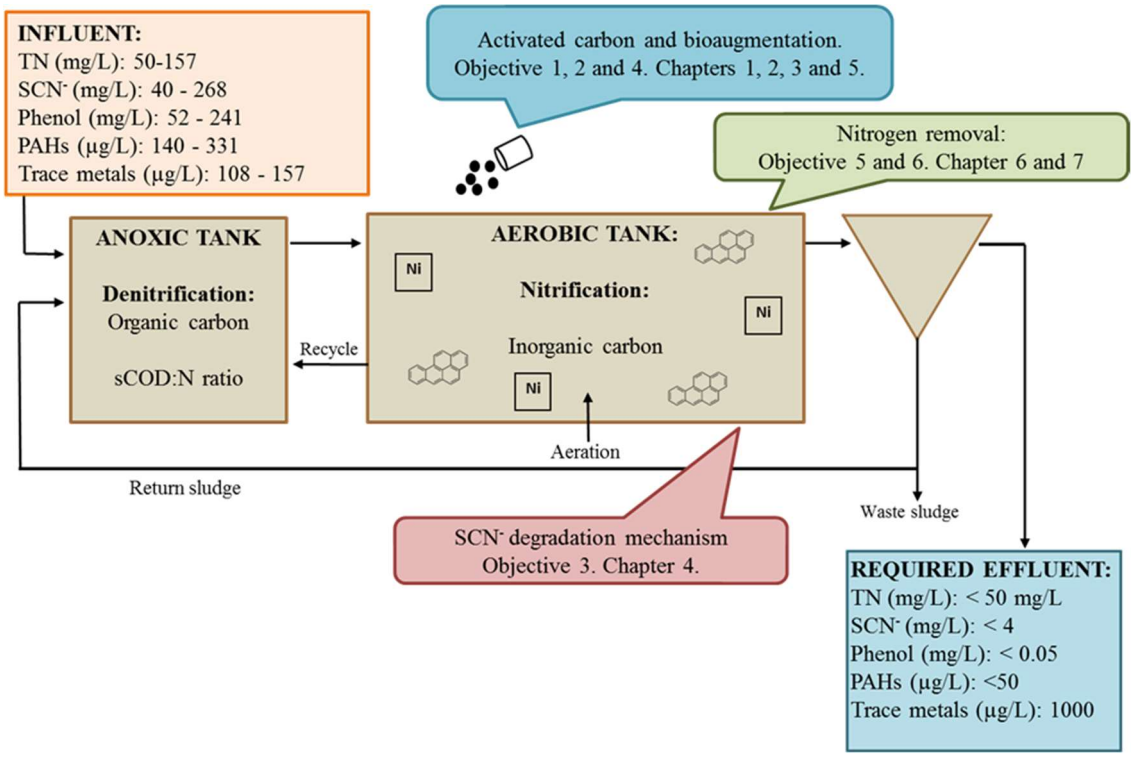

Figure 1-1: Schematic representation of thesis structure demonstrating the incorporation of activated carbon/bioaugmentation into the ASP, the impact of carbon availability on nitrogen removal and the consideration of SCN^- degradation pathways.

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Chapter 2 Industrial wastewater treatment through bioaugmentation

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Abstract

Bioaugmentation of activated sludge processes through the addition of microorganisms is used with the aim of enhancing treatment, in particular removal of priority pollutants. With industrial wastewaters, studies have covered target pollutants including ammonia and polycyclic aromatic hydrocarbons (PAHs): compounds that are regulated around the globe. However, bioaugmentation is a technique that has been associated with doubt over its ability to benefit treatment processes. Failure of bioaugmentation has been reported to be associated with numerous factors that include growth rate being lower than rate of washout, insufficient inoculum size and substrate availability. Limitations of bioaugmentation can be overcome through techniques that include increased inocula dosing, pre-acclimatisation of inocula in sidestream reactors, addition of nutrients and surfactants and applying sufficient acclimatisation periods. Surveys of the literature show that a key area for further research is better understanding of the degradation pathways where bioaugmentation is being applied. There also remains a need to undertake bioaugmentation efficacy studies at full-scale with test and control streams and more reports on the economic viability of the technique.

Keywords: Bioaugmentation; industrial wastewater; nitrogen; polycyclic aromatic hydrocarbons

2.1 Introduction

Industrial wastewaters account for a large proportion of pollution to freshwater systems and therefore are regulated across the globe. For example, in Europe, industrial wastewaters are regulated under the Industrial Emissions Directive (IED) whilst in the United States they are regulated under the Clean Water Act (European Commission, 2015b; US.EPA, 2015). Under the IED, the compounds regulated vary for each industrial process and are reported along with their associated emission limit in the best available techniques reference document (BREF) (European Commission, 2011). An activated sludge process (ASP) is identified as the best available technique (BAT) for the treatment of a number of such wastewaters. This includes wastewaters from the milk and food industry, waste gas treatment, refinery of mineral oil and gas, iron and steel coke processing and glass manufacturing (European Commission, 2003, 2006, 2012, 2013a, 2013b, 2014, 2015a). Emission limits for such wastewaters are summarised in Table 2-1.

The suspended microbial mass in an ASP is responsible for the biodegradation of organic compounds via the metabolic reactions of the bacteria (Zhang et al., 2014a). Many industrial wastewaters contain a mixture of toxic compounds and also compounds which are recalcitrant and have the potential to persist in effluents after an ASP. It is therefore necessary to establish treatment methods which can cope with the complex mixture of compounds typically associated with industrial wastewaters. Bioaugmentation, the addition of supplementary microorganisms with their associated biodegradative capacities, may allow improved performance of ASPs (Semrany *et al.*, 2012).

Table 2-1: Industrial Emission Directive emission limits for wastewaters for which an activated sludge process is recognised as the best available technique.

Wastewater origin	BAT emission limit (mg/L)	Reference
Produced Water (Oil and gas wastewater)	Hydrocarbon oil index: 0.1 – 2.5 COD: 30 – 125 TN: 1 -25	(European Commission, 2014)
Food and Milk: e.g. Raw dairy, Cheese, Mixed dairy, palm oil mill effluent.	Oil and grease: < 10 COD: <125 BOD_5: <25 TN: < 10 TP: 0.4-5	(European Commission, 2006)
Glass manufacturing	COD: < 5-130 Total hydrocarbons: <15 Ammonia (as NH_4): < 10 Phenol: < 1	(European Commission, 2012)
Coke making wastewater:	COD: < 220 BOD_5: <20 SCN: < 4 PAHs*: 0.05 Phenols: 0.5 TN: <15-50	(European Commission, 2013a)

*Sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene

Industrial wastewaters represent some of the most challenging to treat and therefore offer insight into some of the more complex situations in which bioaugmentation may be implemented. Despite this, the huge diversity of microorganisms and their associated degradative pathways, means that bioaugmentation can be adapted to an infinite array of wastewaters containing many different pollutant compounds. Benefits may include more stable operational conditions, better flocculation characteristics, decreased start-up times, resistance to shock loads and better cold weather performance (Stephenson and Stephenson, 1992; Van Limbergen, Top and Verstraete, 1998; Guo *et al.*, 2009; Bartrolí, Carrera and Pérez, 2011; Qu *et al.*, 2011). Bioaugmentation historically gained mixed reviews and has been reported to be unpredictable (Boon *et al.*, 2000). Despite this, many advances have been

made within the field and it is important to reflect upon this progress and apply the knowledge gained. Previously a number of concepts have been highlighted for their role in the likelihood of successful bioaugmentation; including strain selection, addition and maintenance techniques, knowledge of the molecular biology and the capabilities of commercial products (Stephenson and Stephenson, 1992; Van Limbergen, Top and Verstraete, 1998; Thompson *et al.*, 2005; Herrero and Stuckey, 2014).

2.1.1 Strain selection

The selection of a suitable strain is essential to the success of bioaugmentation. The selected strain(s) must be able to withstand the environmental conditions imposed on them within a treatment process including; temperature, pH, dissolved oxygen, nutrient availability, toxicity and microbial pressures (Bitton, 2011). It is well recognised that without an understanding of conditions within the treatment process bioaugmentation is likely to fail due to the poor survival of the inoculum and or competition from indigenous microbial populations (Thompson *et al.*, 2005; Herrero and Stuckey, 2014). The selection and isolation of a strain from the indigenous location has progressively become the favoured approach as this increases the likelihood of success as the strain is already adapted to survive in the selected environment (Ueno *et al.*, 2007). This approach can be taken when a species is present in a treatment process but in insufficient numbers for adequate treatment. Selection of a strain from an alternative site may be the only option when a compound cannot be degraded by species present in the indigenous location, however, success may be limited if the environmental conditions are not conducive to the survival of the inoculated strain (Thompson *et al.*, 2005).

Applications may include the use of a single strain or a combination of strains used to produce a suitable mixed consortium. An individual strain may be

selected for its ability to degrade a specific compound or due its role in a more complex degradation pathway. A number of strains may be used to replicate a natural community, enhance or replicate a catabolic pathway with numerous stages and, or, degrade a number of target pollutants within the same wastewater (Van Limbergen, Top and Verstraete, 1998; Thompson *et al.*, 2005). Increasingly, mixed consortia are selected for bioaugmentation applications as degradation processes are frequently built upon the combined action of numerous species, especially for the degradation of complex xenobiotic compounds (Stroo, Leeson and Herb Ward, 2013).

The success of a mixed consortium was demonstrated by Khehra et al. (2005) for the treatment of recalcitrant dyes released from the textile processing industry. In laboratory investigations, single strains and the consortium were supplemented with 20 mg/L of dye. Whilst individual strains were able to decolorize 3 of the 6 dyes, to a range of degrees, the consortia resulted in the decolourisation of all of the dyes. Further to this the time required for the decolourisation was reduced from 24 hours to 8 hours. Due to the structural diversity of the dyes the synergistic actions of the consortium proved to have a beneficial role. Similarly the synergistic actions of a consortium (previously developed by Chhatre et al. (1996) was recognised as important by Domde, Kapley and Purohit (2007) in the treatment of petroleum wastewater. In this application, a combination of isolates worked together to solubilise and then degrade various components of crude oil. One isolate was responsible for producing a biosurfactant and the emulsification of the crude oil which then made long chain aliphatics and aromatics available to the other two isolates for degradation. This combination of isolates resulted in an overall degradation rate of 65-70% and an increase in COD removal from 15% without bioaugmentation to 52.2% with the consortium (Chhatre *et al.*, 1996; Domde, Kapley and Purohit, 2007).

Genetic manipulation has provided further opportunities for the degradation of compounds for which a pollutant-degrading strain does not exist (Stroo, Leeson and Herb Ward, 2013). Microorganisms can be genetically engineered to over-express degradation genes or have increased survivability techniques (McClure, Fry and Weightman, 1991; Nüβlein *et al.*, 1992; Stroo, Leeson and Herb Ward, 2013). Such a technique opens up the possibility of designing microorganisms to assist with the treatment of pollutants which require numerous degradation steps or for xenobiotic compounds. Knowledge of the degradation pathways involved for such compounds is limited and a naturally occurring species capable of its degradation may not exist (Stroo, Leeson and Herb Ward, 2013). Microorganisms which have been genetically modified have been investigated in groundwater aquifer microcosms (Jain *et al.*, 1987), lake waters (Awong, Bitton and Chaudhryt, 1990) and ASP (McClure, Weightman and Fry, 1989; McClure, Fry and Weightman, 1991). McClure, Weightman and Fry (1989) demonstrated that bacteria which had been genetically engineered were able to survive within a laboratory-scale ASP and did not encourage protozoa reproduction despite large numbers of bacteria being inoculated. Additionally Nüβlein et al. (1992) were able to confirm that microorganisms that were genetically engineered were not only capable of surviving in an ASP but were also able to maintain their genetic information and degrade the required pollutants. Such genetic adaptation may allow the design of microorganisms which are able to assist in the degradation of pollutants which require numerous degradation steps. Further to the genetic modification of microorganisms, gene bioaugmentation which involves the use of catabolic mobile genetic elements (MGEs) has been highlighted for its applicability to bioaugmentation (Stroo, Leeson and Herb Ward, 2013). Mobile genetic elements consist of pieces of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) which can be transferred from one organism to another (Stroo, Leeson and Herb Ward, 2013).

Despite the numerous possible ways in which genetic engineering may improve the future of bioaugmentation applications, current research is heavily

laboratory based and currently field successes cannot be fully assessed due to legislative restrictions resulting from concerns surrounding the risks to both the environment and human health of the uncontrolled spread of microorganisms which have been genetically engineered (Van Limbergen, Top and Verstraete, 1998). Strategies such as the use of a 'suicide element' and immobilisation techniques have been considered in order to reduce such risks (Liu, Huang and Wang, 2008; Stroo, Leeson and Herb Ward, 2013). Suicide techniques for example, may be repressed by an environmental signal such as the pollutant to be degraded. When the signal no longer exists the suicide element is activated. This technique has been shown to be successful in preventing the spread of engineered cells (Torres, Garcia and Diaz, 2003). As a result of concerns surrounding the use of microorganisms which have been genetically engineered there has been little advancement in the field due to legislative constraints (Davison, 2005). Legislation often ignores the ways in which molecular genetics can be used for risk mitigation, and consequently, future research will have to both inform and follow regulations (Davison, 2005; Stroo, Leeson and Herb Ward, 2013).

Commercial inocula are now also widely available. Such products vary greatly in their make-up, cell density, advised dosing rates and the incorporation of other additives e.g. stabilisers and nutrients. These factors need to be considered when selecting a suitable product (Stroo, Leeson and Herb Ward, 2013). The use of commercial inocula may be able to offer a short-term solution to an immediate treatment issue, however, success rates may vary because they are typically produced and tested under stable conditions, which do not reflect the real-life scenario for many industrial wastewaters, in turn reducing the survivability of the inocula (Stephenson and Stephenson, 1992).

2.1.2 Operational considerations

The application of bioaugmentation is more likely to provide a positive result to a treatment system that is well characterised in terms of the wastewater characteristics and operational parameters, as this knowledge helps to identify potential obstacles in the survival of the inoculated bacteria including toxicity and nutrient availability. Without a detailed knowledge of the system the likelihood of a successful integration of the inoculum reduces. Activated sludge processes can differ greatly in their configuration, although, principally they consist of one or more treatment cells containing biomass which may be aerobic, anoxic or anaerobic in nature. Wastewater may also be treated under continuous flow conditions, in well mixed systems, or operated under a batch or plug-flow system. Introduction and maintenance methods should therefore be informed by the design of the treatment system whilst treatment efficiencies and pollutant concentrations will inform decisions on dosing rates (Stephenson and Stephenson, 1992; Park *et al.*, 2008).

2.1.2.1 Dosing technique

Direct dosing involves the addition of the selected microorganisms straight into a treatment vessel. Such a technique represents the most simplistic method of bioaugmentation and can be advantageous as it can be applied as and when required. This can be economically beneficial as it does not require plant modifications and the associated continuous operational costs. Problematic to this approach, however, is the reduced survival rates of inoculated microorganisms due to a lack of acclimatisation to the environmental conditions of the host environment for example toxicity, pH, carbon availability, predation and competition between the indigenous and inoculated bacteria (Chong, Pai and Chen, 1997; Bouchez *et al.*, 2000; Songzhe *et al.*, 2009). The use of a side-stream reactor can help overcome some of the aforementioned problems as it enables the acclimatisation of the inoculated microorganisms to the environmental conditions thus increasing their survival rate (Parker and

Wanner, 2007). The footprint of a side-stream is typically approximately 10% of the main reactor. As the side-stream can enable process intensification this can represent a much smaller investment cost than the cost associated with expanding a treatment works to cope with a higher capacity. Despite this in some instances the additional land requirements may still be disadvantageous in some situations (Salem *et al.*, 2003). The use of encapsulation techniques can assist the incorporation of inoculated bacteria into the existing flocs (Stormo and Crawford, 1992). Bouchez et al. (2009) mixed the inoculum with an alginate solution, forming bead structures which were inoculated into the reactor. The beads allowed the inoculated bacteria to remain in the system and protected them from the intense grazing that was experienced without such encapsulation. The beads were observed to break into fragments by day 8 but such fragments were incorporated into flocs of the indigenous sludge allowing their successful incorporation into the system.

2.1.2.2 Dosing location

The success of bioaugmentation has been shown to be influenced significantly by the location to which the selected microorganisms are dosed. Dosing location should be selected based on a careful consideration of the environmental conditions that the selected micro-organisms require or will face. Determination of the most suitable location may be more critical in industrial wastewaters which frequently contain single or multiple pollutants known for their toxic effects. The impact of the correct location identification was demonstrated by Jianlong et al. (2002) during the treatment of coke making wastewater. The wastewater, characterised by the presence of multiple toxic compounds, was treated through an ASP with an anaerobic, anoxic and aerobic reactor. *Burkholdiera pickettii*, a quinoline degrading species, was shown to have a beneficial role at any location, nevertheless, this positive impact was higher when *Burkholdiera pickettii* was added to the aerobic reactor. The provision of a suitable food source and the lower toxicity, as a result of the degradation of co-occurring compounds in previous treatment cells to smaller

compounds, resulted in higher degradation efficiencies. Similar conclusions were drawn for the removal of 2-4-dichlorophenol in a laboratory-scale ASP. A mixed culture was developed through the enrichment of sludge taken from two wastewater treatment plants. The mixed culture was then added to a separate reactor with a carrier-system of plastic lace strings (Quan, Shi, Liu, Wang, *et al.*, 2004). Removal was higher at 90.3% when the bioreactor was added after the aeration cell than when the bioreactor was situated before the aeration cell (86.2% removal). It had been assumed that locating the bioreactor before the aeration cell would allow the removal of 2-4-dichlorophenol which in turn would improve the removal efficiency of other pollutants as a result of the lowered toxicity of the wastewater. Despite this, the 2-4-dichlorophenol removal decreased when the bioreactor was placed before the aeration cell as a lack of easily degradable compounds resulted in a decrease in the removal of the targeted 2-4-dichlorophenol. When the bioreactor was placed after the aeration cell the bioaugmented culture was able to specialise in the removal of 2-4-dichlorophenol increasing the treatment efficiency.

2.1.2.3 Dosing size and regime

Dosing sizes and regimes vary considerably between different applications. The first dose that requires consideration is the initial inoculum size which should be sufficiently large enough to overcome initial predation pressures whilst not large enough to result in a disturbance to the ecosystem equilibrium. Ramadan, El-Tayeb and Alexander (1990) reported that p-nitrophenol containing wastewaters required a high initial dose (4.3×10^4 cells per ml) in order to overcome predation pressures. In contrast, Bouchez et al. (2000) reported a disturbance of the ecosystem balance as a result of increased pressures from a large inoculation. Secondly, the use of maintenance doses may be necessary in order to maintain a population of the inoculated bacteria which may decrease over time as a result of routine sludge wastage or poor survival rates. The need for a maintenance dose varies from application to application. Boon et al. (2003) noted that bioaugmentation was not a permanent process when investigating

the removal of 3-chloroaniline whilst Martín-Hernández, Suárez-Ojeda and Carrera (2012) reported that maintenance doses were not necessary when the initial dose was sufficiently high to overcome initial predation pressures.

2.1.3 Bioaugmentation failures and associated improvement techniques

Successful reports of bioaugmentation have equally been associated with reports of unsuccessful bioaugmentation attempts. Fundamental to the success of any application is the ability of the inoculated bacteria to survive and prosper. Numerous factors have been given for the failure of bioaugmentation (Table 2-2) including the growth rate of the microorganism being lower than the rate of washout (Boon *et al.*, 2000), insufficient inoculum size (Ramadan, El-Tayeb and Alexander, 1990), insufficient substrate (Goldstein, Mallory and Alexander, 1985; Bouchez *et al.*, 2000; Tyagi, da Fonseca and de Carvalho, 2011; Martín-Hernández, Suárez-Ojeda and Carrera, 2012), predation by protozoa (Goldstein, Mallory and Alexander, 1985; Boon *et al.*, 2000; Bouchez *et al.*, 2000), competition between the inoculated and indigenous bacteria (Stephenson and Stephenson, 1992; Bouchez *et al.*, 2000; More *et al.*, 2010), the presence of other inhibiting substances (Goldstein, Mallory and Alexander, 1985; Bouchez *et al.*, 2000; Tyagi, da Fonseca and de Carvalho, 2011), the availability of alternative substrates (Goldstein, Mallory and Alexander, 1985; Chitra *et al.*, 1995; Quan, Shi, Liu, Wang, *et al.*, 2004; Mahin *et al.*, 2011), the need for an acclimatisation period (Stephenson and Stephenson, 1992) and extremes in environmental factors such as temperature and pH (Tyagi, da Fonseca and de Carvalho, 2011). An understanding of the root cause of bioaugmentation failures is important to allow the advancement of bioaugmentation applications.

Grazing was held responsible for the failure of *M. aerodenitrificans* to become established in an aerobic nitrifying sequencing batch reactor by Bouchez et al. (2000). The added bacteria were associated with the liquid phase of the reactor rather than any incorporation into bacterial flocs. As a result of their suspended nature they were targeted by grazing protozoa which have grazing rates which were proportionate to the fast rates of decline seen in the system. Ramadan, El-Tayeb and Alexander (1990) also saw a decline in the inoculated bacterial abundance which coincided with the multiplication of protozoa in the treatment of p-nitrophenol (PNP) containing wastewaters. Similarly, an overgrowth of protozoa as a result of bioaugmentation was reported by Songzhe et al. (2009) during the removal of ammonia from marine aquaculture wastewaters. Furthermore, a rapid decline of the denitrifier *Pseudomonas stutzeri* TR2 was once again associated with probable predation during the treatment of piggery wastewater (Ikeda-Ohtsubo, Miyahara, Kim, *et al.*, 2013). Songzhe et al. (2009) concluded that a form of protection e.g. encapsulation from grazing was necessary. An alternative investigated was the ability of heat treatment to protect the inoculated bacteria from predation (Ikeda-Ohtsubo, Miyahara, Kim, *et al.*, 2013). Results showed that adapting reactor conditions allowed predation problems to be overcome. When the temperature of the treatment reactor was reduced to 35^{o}C predators were able to proliferate. During this period, there was a rapid ten-fold increase in their associated genes. When the temperature was increased to 40-44^{o}C there was no increase in the number of genes representing predators and therefore *Pseudomonas stutzeri* TR2 was protected from predation.

Contrary to reports of the negative effects of grazing to bioaugmentation Yu, Peng and Ren (2011) demonstrated that grazing did not have a significant impact on nitrogen removal. Nitrification efficiencies were monitored in a bioaugmentation system where protozoa were inhibited and one where protozoa were not inhibited. Although initially protozoa numbers increased rapidly in the non-inhibited reactor, their numbers then declined gradually over

the duration of the study and complete nitrification was possible in both reactors. The increased time requirement, from 71 days with protozoa inhibition to 76 days without protozoa inhibition, was not considered to be significant.

Nutrient limitation is a particularly important factor in the treatment of industrial wastewaters which frequently have a lack of essential nutrients required for microbial development (Burgess, Quarmby and Stephenson, 1999). Nutrient limitations have been held responsible for failed bioaugmentation attempts due to competition resulting between the indigenous and inoculated bacteria. Ramadan, El-Tayeb and Alexander (1990) demonstrated that the supplementation of nutrients could increase the likelihood of successful bioaugmentation as the addition of nitrogen and phosphate allowed low densities of inoculum to remove p-nitrophenol (PNP) potentially increasing their growth rates and resistance to higher protozoa numbers. Such nutrient addition is referred to as biostimulation. Biostimulation, however, can also include the addition of other stimulants such as surfactants. Nikolopoulou, Pasadakis and Kalogerakis (2013) demonstrated that the presence of a biosurfactant could increase degradation rates in oil contaminated sites through enhancing the solubility of the hydrocarbons. Under such treatment systems it is important, however, to have adequate controls in order to be able to assess what degree of improvement is as a result of the biostimulation and what improvement is as a result of the bioaugmentation. Due to the complementary action biostimulation has therefore become a technique that is frequently reported for its use alongside bioaugmentation (Wenderoth *et al.*, 2003; Olaniran, Pillay and Pilay, 2006; Tyagi, da Fonseca and de Carvalho, 2011; Nikolopoulou, Pasadakis and Kalogerakis, 2013; Shoji *et al.*, 2014; Sun *et al.*, 2014).

Industrial wastewaters are frequently characterised by changing load rates which results in fluctuating concentrations of the target compounds. Some bioaugmentation failures have been linked to long periods of starvation from the target pollutant. One means by which to tackle this problem is to select an initial

dose which is high enough to allow a proportion of bacteria to persist in the treatment system until the load rate increases again. This approach was investigated by Martín-Hernández, Suárez-Ojeda and Carrera (2012) during the treatment of p-nitrophenol in a laboratory scale sequencing batch reactor. Using a dose rate of 2% and a dose rate of 5% respectively, it was found that the higher initial dose rate allowed the inoculated bacteria to survive the 20 days of starvation and maintain treatment. Importantly the dose rate of 5% was still practical in terms of its application to full-scale treatment works. In contrast Duque et al. (2011) found that periods of substrate inhibition did not cause failure during the treatment of 2-fluorophenol in a rotating biological contactor.

For some bioaugmentation applications failure lies in the inadequate adaptation of the inoculum to the host environment. Chong, Pai and Chen (1997) reported that a mixed culture, designed to treat petroleum wastewater, was unable to proliferate in the system showing no benefit to treatment under pH shock conditions and complete failure under continuous high pH conditions. The failure was linked to biomass washout as a result of growth retardation or death of the inoculated population. Biomass washout, as a result of poor reactor conditions, including an inadequate carrier system and violent air bubbling, was also reported by Park et al. (2008) in the treatment of cyanide wastewater. Additionally, Songzhe et al. (2009) reported that inoculated bacteria were unable to form an adequate biofilm due to the interaction of other indigenous bacteria resulting in biomass washout and the failure of bioaugmentation. Inadequate adaptation is of increased likelihood in industrial wastewaters and highlights the requirement of understanding treatment conditions and method adaptation techniques.

Table 2-2: Reasons for bioaugmentation failures and possible improvement techniques.

Problem	Technique to overcome problem
Predation (Overgrowth of protozoa) (Goldstein, Mallory and Alexander, 1985; Ramadan, El-Tayeb and Alexander, 1990; Songzhe *et al.*, 2009; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	High initial doses (Ramadan, El-Tayeb and Alexander, 1990) Protection from grazing (Songzhe *et al.*, 2009) Heat treatment (Ikeda-Ohtsubo, Miyahara, Yamada, *et al.*, 2013)
Competition for nutrients between indigenous and inoculated bacteria (Ramadan, El-Tayeb and Alexander, 1990; Yu *et al.*, 2005; Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Supplementation of nutrients (biostimulation) (Ramadan, El-Tayeb and Alexander, 1990)
Insufficient inoculations (Ramadan, El-Tayeb and Alexander, 1990)	Repeated inoculations (Boon *et al.*, 2003) Continual inoculations (Abeysinghe *et al.*, 2002)
Poor biofilm formation (Park *et al.*, 2008; Songzhe *et al.*, 2009)	Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, *et al.*, 2004)
Wash-out (Chong, Pai and Chen, 1997; Park *et al.*, 2008)	High initial doses (Ramadan, El-Tayeb and Alexander, 1990) Immobilisation/encapsulation (Stormo and Crawford, 1992; Quan, Shi, Liu, Lv, *et al.*, 2004)
Decline of inoculated bacteria due to toxins (Goldstein, Mallory and Alexander, 1985)	Protection from adverse environmental conditions (Songzhe *et al.*, 2009) Allow acclimatisation period (Stephenson and Stephenson, 1992) Use autochthonous bioaugmentation (Ueno *et al.*, 2007)
Alternative substrates available (Goldstein, Mallory and Alexander, 1985; Chitra *et al.*, 1995; Quan, Shi, Liu, Lv, *et al.*, 2004; Mahin *et al.*, 2011)	Detailed understanding of ecological background (Songzhe *et al.*, 2009)
Large inoculations disturbing balance of ecosystem (Bouchez *et al.*, 2000)	Careful consideration of dose rate
Periods of starvation (Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Higher dose rate to allow survival in the system for longer time periods (Martín-Hernández, Suárez-Ojeda and Carrera, 2012)

2.1.4 Applications of bioaugmentation to pollutants regulated by the Industrial Emissions Directive

A wide variety of wastewaters are regulated under the IED, all of which could potentially benefit from the application of bioaugmentation. An improved understanding of the capabilities of bioaugmentation could therefore offer widespread opportunities to industrial wastewater treatment. Industrial wastewaters can encompass a wide variety of pollutant compounds although typically some commonalities exist between the different wastewaters. Nitrogen compounds are common to many types of wastewater particularly those from milk and food industries and coke processing. Levels of ammonia in coke making wastewater can vary from 123 mg/L up to 4,500 mg/L (Ganczarczyk, 1983; Gould, 1986). Ammonia concentrations vary between sites due to variations in operational conditions but also temporally due to variations in production levels (Marañón *et al.*, 2008). High concentrations of ammonia are also characteristic of dairy wastewaters. As with coke making wastewaters they are subject to both spatial and temporal variations due to difference in the products produced and the treatment methods in place. Furthermore, these wastewaters are often produced intermittently (Vidal *et al.*, 2000).

Nitrogen is a key target pollutant as it can be responsible for the eutrophication of receiving waters. Nitrifying bacteria are slower growing than the general heterotrophic community and are less resistant to toxicity and consequently may be outcompeted. Supplementation through bioaugmentation may therefore be beneficial to treatment systems characterised by high nitrogen loading. As the removal of nitrogen occurs in a two-step process involving the oxidation of ammonia to nitrite and then the subsequent oxidation of nitrite to nitrate, nitrifying treatment processes require process stability in order to allow the two steps to remain in synchronisation and prevent accumulation of the more toxic nitrogen species nitrite. Abeysinghe et al. (2002) investigated the ability of bioaugmentation to support the nitrification process when operating under

stress conditions. At a solids retention time of two days, the treatment system was operating near washout conditions, but the addition of 45 and 67.5 mg/L of ammonia oxidisers, as COD, allowed effluent ammonia concentrations to be reduced from 4.5 mg/L to <1 mg/L. The application of bioaugmentation can therefore be an effective and convenient tool to support industrial treatment systems which are frequently operating under stress conditions.

Obbard and Shan (2001) also reported the use of bioaugmentation to support the treatment of prawn aquaculture ponds which are characterised by high nitrogen loading rates but experience high levels of nitrifier washout as a result of the regular pond water exchange which is used to prevent the build-up of toxins in such ponds. Inert media has been reported to enhance treatment by increasing bacterial populations through biofilm formation (Stephenson *et al.*, 2013). This technology was selected in order to tackle the problem of washout. Indigenous nitrifiers were immobilised onto porous clay pellets allowing total ammoniacal nitrogen to be reduced from 3 mg/L to 0.5 mg/L and allowing the pond concentration to fall below the required concentration necessary for optimum prawn growth (1.33 – 1.53 mg/L) (Table 2-3). Treatment of high nitrogen loads through bioaugmentation was reported by Onyia et al. (2001) for the treatment of palm oil wastewater (Table 2-3). Palm oil wastewaters are characterised by organic nitrogen loads of 180 – 1820 mg/L and treatment of the wastewaters was time intensive with more than 11 days being required in order to achieve 50% nitrification, however, the addition of 15 mg/L of a mixed nitrifying culture increased efficiency to 100% within seven days.

Employment of a carrier material has also been applied to support bioaugmentation. In the treatment of petrochemical wastewater Ma et al. (2009) used a carrier system, of polyurethane foam, to encourage the inoculated bacteria to form a biofilm (Table 2-3). The resulting biofilm prevented the washout of the inoculated bacteria and prevented the gradual decrease in their numbers as a result of predation. Additionally, the inoculated bacteria were

provided with organic substrates and inorganic trace elements to support their growth. Consequently, the bioaugmented reactor showed better performance with increased start up times (20 days compared to 30 days without bioaugmentation), a higher resistance to shock-loads of COD, higher treatment efficiencies of refractory organic compounds (reduced to 21 compared to 46 without bioaugmentation) and a reduction of effluent ammonia concentrations (4.1 mg/L compared to 12.4 mg/L).

Table 2-3: Examples of bioaugmentation applied to compounds present in industrial wastewaters.

Compound	Scale	Application	Conclusions
Nitrogen			
(Onyia *et al.*, 2001)	Laboratory	Palm oil effluent	15 mg/L of mixed cultures led to 100% increase in nitrification. Reduced HRTs led to 20% reduction in land requirement.
(Obbard and Shan, 2001)	Laboratory	Prawn aquaculture wastewaters	Immobilised bacteria allowed total ammoniacal nitrogen reduced from 3 mg/L to 0.5 mg/L allowing ponds to remain at optimal conditions.
(Ma *et al.*, 2009)	Full-scale	Petrochemical wastewaters	Immobilisation prevented washout of nitrifiers. National discharge limits met in 20 days compared to 30 days. Effluent ammonia concentrations fell from 12.4 mg/L to 4.1 mg/L.
Aromatic compounds			
(Qu *et al.*, 2011)	Laboratory	Synthetic alkaline wastewaters	Addition of Pseudomonas sp. JY-2 allowed improved start-up times (90% removal compared to 65% after 1.5 days) and increase long-term treatment efficiency (90% compared to 80%).
(Fang *et al.*, 2013)	Laboratory	Coal gasification wastewater	Bioaugmentation increased removal efficiencies from 66% to 80% despite high variation in levels of phenol (500-3000 mg/L).
(Duque *et al.*, 2011)	Laboratory	2-fluorophenol wastewaters	2-fluorophenol degrading species FP1 allowed treatment of waters subjected to shock loads of up to 50 mg/L of 2-fluorphenol.

(Martín-Hernández, Suárez-Ojeda and Carrera, 2012)	Laboratory	p-nitrophenol (PNP) wastewaters	Bioaugmentation allowed immediate removal of shock loads of PNP. Without bioaugmentation PNP removal took 4 days to reach 100% and then failed after 8 days.
(Straube *et al.*, 2003)	Laboratory and pilot-scale	PAH contaminated soil	Bio-surfactant producer *Pseudomonas aeruginosa* strain 64 increased PAH degradation from 23% to 34%. Bioaugmentation and biostimulation increased degradation to 87%. Biostimulation alone increased degradation to 86%.
(Sun *et al.*, 2014)	Pilot	Former coke works contaminated soil	Total PAH levels fell by 24% in the control, 35.9% with bioaugmentation, and 59% with biostimulation. Bioaugmentation was responsible for the increased removal of heavy molecular weight molecules.

Bioaugmentation has also been used for the treatment of aromatic compounds including phenols and polycyclic aromatic hydrocarbons (PAHs) which are present in a wide variety of industrial wastewaters including those from agrochemical, pharmaceutical, petrochemical, coal gasification, coke processing, insecticide, hydrocarbon and produced wastewaters amongst others (Table 2-3). Aromatic compounds are regulated under the IED and are also listed as Priority Substances within the European Union (European Union, 2013).

Coal gasification wastewater is subject to high variability in phenol concentrations from 500 – 3000 mg/L as a result of fluctuations in the pre-treatment performance. Such variability can be problematic to biological treatment due to the changing substrate levels and therefore the decline in bacterial numbers during periods of limited food supply. However, system stability is of increasing importance as emission limits continue to be lowered further. Addition of phenol degrading bacteria by Fang et al. (2013) (Table 2-3) allowed phenol treatment efficiencies to increase from 66 to 80% and allowed increased resistance to fluctuating loads. Ammonia removal also improved (5% to 25%) although fluctuating ammonia load rates required a higher recovery

time. Resistance to shock-loading of phenolic compounds was also seen to improve due to bioaugmentation by Duque et al. (2011) in the removal of 2-fluorphenol. Interestingly Duque et al. (2011) promoted biofilm formation in a rotating biological contactor (RBC) through the batch application of the inoculum. This technique provided a means by which the system was able to stabilise and long-term bioaugmentation was not required. This allowed improved resistance to shock-loads and furthermore resistance to periods of starvation (Table 2-3). Although improved resistance to shock-loads of p-nitrophenol was also seen by Martín-Hernández, Suárez-Ojeda and Carrera (2012) resistance to starvation periods was determined by the size of the initial inoculum dose (Table 2-2).

The stable removal of both pyridine and quinoline from coke making wastewater was observed after the inoculation of a laboratory scale sequencing batch reactor filled with modified zeolite (Zhang *et al.*, 2014b). Removal of both compounds remained at 100% whereas removal efficiencies could vary from 0 to 93% without bioaugmentation. This was attributed to an improved bacterial diversity which increased resistance to shock loadings. The interaction of species in a mixed culture of four species (*Paracoccus* sp. BW001, *Shinella zoogloeoides* BC026 and *Pseudomonas* sp. BC001) was believed to be responsible for the success of bioaugmentation for the removal of pyridine and quinoline in coke making wastewaters (Bai *et al.*, 2010).

Polycyclic aromatic hydrocarbons (PAHs) can be found in both oil and gas wastewaters and coke making wastewaters and are typically difficult to treat as they accumulate in the suspended solids of ASP, reducing their bioavailability to microbial degradation (Douben, 2003). Examples of bioaugmentation to enhance removal of PAHs typically focus on the treatment of contaminated soils and groundwater (Vogel, 1996; Straube *et al.*, 2003; Yu *et al.*, 2005; Jacques *et al.*, 2008; Silva *et al.*, 2009; Teng *et al.*, 2010). Useful knowledge may be

gained from these applications, however, as PAHs are mainly associated with the suspended solids in ASPs.

Straube et al. (2003) and Sun et al. (2014) both considered the role of bioaugmentation and biostimulation for the removal of PAHs from soil (Table 2-2). Biostimulation was applied in order to overcome environmental limitations. Straube et al. (2003) demonstrated the ability of the bio-surfactant-producer *Pseudomonas aeraginosa* strain 64 to stimulate the autochthonous PAH degraders in soil samples. After 11 months bioaugmentation led to an increase in PAH degradation from 23% to 34%. Biostimulation and bioaugmentation led to an increase in the PAH degradation to 87%. At pilot-scale, after 16 months PAH removal increased from 12% in the control to 87% with bioaugmentation and biostimulation. Despite this 86% removal could be achieved with biostimulation alone. Sun et al. (2014) found comparable results to Straube et al. (2003) when researching the impact of bioaugmentation and biostimulation on former coke works. Over 3 months total PAH levels fell by 24% in the control, 35.9% with bioaugmentation and 59% by biostimulation. The combination of bioaugmentation and biostimulation only brought about small improvements in comparison to biostimulation alone. The removal of heavy molecular weight PAHs, however, was noticeably higher with bioaugmentation than with biostimulation alone. This is of significance due to the increased resistance of heavy molecular weight PAHs to degradation.

2.2 Discussion

The consistent and stable removal of priority pollutants from industrial wastewater is essential. Whilst close system monitoring and process control may have historically been the most important factors in achieving stable operation and meeting emission limits this alone may no longer suffice. Process control needs to be optimised to minimise shock loadings where possible,

however, such process control has to be within an economically viable range. Even with optimal process control the inherent variability of industrial wastewaters can still result in emission variability. Compliance with increasingly stringent emission limits therefore requires the application of new technologies to meet reduced consents and respond to transient treatment issues. Whilst achieving effluents of increasingly high quality is important in the long-term it is equally important that techniques are developed to re-gain treatment promptly after transient issues. Without the development of technologies which can bring about quick process recoveries, industries will be faced with increasingly high costs as a result of producing effluents out of compliance. Bioaugmentation should be considered as one such technique.

Compliance with nitrogen effluent standards impacts a wide variety of industries including palm oil effluent, aquaculture wastewaters, coke making wastewaters and petrochemical wastewaters. Nitrification is well known for its process instability due to the requirement for the close linking of bacterial species responsible for different parts of the removal process (Philips, Laanbroek and Verstraete, 2002). Low growth rates of nitrifying bacteria and uncoupling of the nitrification chain can be problematic in any treatment but those of an industrial nature are much more susceptible to upsets as a result of their characteristic variations in loading and often the presence of toxic compounds. Bioaugmentation has been shown to offer a potential technique in which to stabilise nitrification and particularly deal with transient treatment problems. Abeysinghe et al. (2002) demonstrated the ability of bioaugmentation to improve ammonia removal during stress conditions. Similarly Ma et al. (2009) demonstrated the improved capability of a bioaugmented ASP treating petrochemical wastewater to deal with shock-loadings of COD. Recovery from shock-loading was also 50% faster. Compliance can also be problematic for priority pollutants which are persistent and toxic as the biomass not only requires acclimation but can still be negatively impacted by a sudden shock-load of the toxic compound. As with nitrogen, bioaugmentation has been

demonstrated to have some success in the treatment of such compounds. Qu et al. (2011) observed improved long-term stability of treatment systems treating aromatic compounds. The addition of Pseudomonas sp. JY-2 led to 90% removal efficiencies compared to 80% without with the additional benefit of increased start up times. Both Duque et al. (2011) and Fang et al. (2013) also found improved resistance of treatment systems to fluctuating phenol levels with the application of bioaugmentation.

Despite the benefits which have already been reported caution must be applied to findings from numerous reported investigations. For instance under the stress conditions reported by Abeysinghe et al. (2002), daily dosing was required to maintain sufficient levels of microorganisms. Bioaugmentation was therefore capable of dealing with transient issues but would be uneconomic for the long-term treatment of an unstable treatment system. Similarly, although Ma et al. (2009) showed improved nitrogen removal efficiencies bioaugmentation was conducted in a system with immobilisation and was compared against a conventional reactor. The reduced washout, which was the main benefit of the system, could therefore potentially have been achieved through the use of carrier media alone to simply support biofilm formation. It is important therefore that the purpose of bioaugmentation is clearly defined before success is determined e.g. short-term solution technique or for long-term benefits.

A significant benefit of bioaugmentation is the ability to treat on demand. Direct dosing can provide an immediate solution to a wide array of failing treatment systems. Where space is an issue and treatment systems are already operating at their maximum capabilities, bioaugmentation may be the only way in which to maintain effluent compliance without resorting to halting upstream operations. Direct dosing may make use of commercial products, however, commercial products have been associated with a tendency to fail to produce the reported benefits of the product and or require higher dosing rates than suggested by the manufacturer (Stephenson and Stephenson, 1992). These products may be

able to offer a short-term solution to an immediate problem but because of the problems associated with inadequate adaption of the microorganisms to the environment and the high dosing levels required they may not be able to meet the requirements of long-term use. As the economic costs associated with treatment processes become more pertinent, the use of commercial products may therefore become less viable. The use of side-stream technologies is becoming increasingly common due to the advantages it has for bacterial adaptation and for long-term bioaugmentation applications (Krhutková *et al.*, 2006; Smith *et al.*, 2008; Yu, Peng and Pan, 2012).

Despite some positive reports of the impact of bioaugmentation on process performance there are still substantial areas that require more research which are required in order to be able to make well founded conclusions. Firstly, one of the most important aspects requiring research is development in the understanding of degradation pathways. Without understanding the degradation pathway of a compound the possibility of finding a suitable species to inoculate are reduced. The area of strain development has previously been highlighted for its importance (Thompson *et al.*, 2005). It is not only important to consider which strain(s) may be required but also other requirements that the strain may have for it to operate successfully. Under some circumstances the use of biostimulation may be necessary in order to provide nutrients, or other critical components such as biosurfactants, in order to allow an inoculum to be successful. The synergistic action of a mixed consortium was highlighted by Khehra et al. (2005) whilst the importance of the combined action of a biosurfactant and a pre-adapted consortium was reported by Nikolopoulou et al. (2013). More research in this field may be applicable to supporting the degradation of wastewaters containing polycyclic aromatic hydrocarbons where complex compounds of a number of different molecular weights can occur simultaneously. Developments in the field of genetic engineering may also assist in the development of strains suitable to target xenobiotic compounds for which removal is currently limited. Van Der Gast et al. (2003) also reported that

treatment performance was more reproducible for a constructed consortium than an undefined community, a factor that is of increasing importance. Despite this, concerns around the release of genetically modified bacteria have significantly impacted progress in this area (Davison, 2005).

The success of bioaugmentation is ever increasingly being linked to the effective incorporation of the inoculated strain into the host environment, the success of which is influenced from strain selection and introduction strategy through to the ability to survive within the introduced environment (Herrero and Stuckey, 2014; Thompson et al., 2005). The importance of having a detailed knowledge of the treatment system has been emphasised through numerous applications (Goldstein, Mallory and Alexander, 1985; Bouchez *et al.*, 2000; Songzhe *et al.*, 2009; Martín-Hernández, Suárez-Ojeda and Carrera, 2012). An understanding of the conditions in a treatment process offers a way in which to prevent an inoculum being negatively influenced by environmental factors such as pH and temperature as well as exposure to toxic compounds, allowing the selection of a dosing strategy or location to minimise exposure to negative conditions. Such detailed knowledge can also help inform on possible solutions to any problems that arise. Industries such as dairy processing, where each site encompasses different process operations, would particularly benefit from this. As bioaugmentation methodologies can vary greatly the technique allows the individuality of different treatment processes to be recognised and catered for.

Appropriate dosing rates also lack sufficient research. Although many references have been made to over-dosing and/or under-dosing huge variations can be seen in dose rates that have been successful between applications which essentially appear to be very similar. In the treatment of pyridine and quinoline in laboratory-scale SBRs, both treating wastewater from the same site and achieving a 99% removal rate, Bai et al. (2010) reported a dose of 0.007 – 0.0200 g/L in comparison to a dose of 0.223 g/L (Zhang *et al.*, 2014b). Of three species used in each study, two of the species applied were the same in both

applications. Whether the relatively large difference in dose can be accounted for by the third species remains unknown. Research is also contradictory in the need for repeated inoculations as maintenance doses. Both Boon et al. (2003) and Abeysinghe et al. (2002) reported the need for repeated inoculations as maintenance doses whilst Martín-Hernández, Suárez-Ojeda and Carrera (2012) reported that this was unnecessary if the initial dose rate was sufficiently sized to overcome initial survival pressures. High dosing rates have equally been criticised as they have been linked to disturbances in the balance of an ecosystem (Bouchez *et al.*, 2000). For this reason, it is important that investigations take place which consider a variety of different dosing regimen for identical wastewater treatment facilities.

The bioaugmentation strategies also require further investigation. Many different potential configurations are reported with the most critical difference being whether the inoculum is developed in situ or ex-situ (Parker and Wanner, 2007). In situ schemes have been associated with greater success because such biomass has developed under conditions which are similar to the mainstream conditions. Whilst the general schemata of the bioaugmentation process needs consideration, the introduction location and strategy also require further investigation. Technologies that support the incorporation of the inoculum into the host environment have proven to be effective in maintaining the population in the treatment system overcoming issues such as predation pressures, washout and loss due to toxic effects (Stormo and Crawford, 1992; Quan, Shi, Liu, Wang, *et al.*, 2004; Bouchez *et al.*, 2009). Although some techniques such as encapsulation may result in higher capital costs they may also bring about long term economic gains. The use of side-stream reactors has become common practice in the Czech Republic. Wastewater treatment quality in the Czech Republic gains international attention due to the geographical conditions, whereby all of the rivers originate in the country and therefore any pollution entering the surrounding countries can be directly linked back to the Czech Republic, leading to strict emission limits (Wanner *et al.*, 1996). Due to land

shortages process intensification has been required and the use of side-streams has become common (Wanner, Kos and Novák, 2009). Such technology has allowed winter treatment to be stabilised and has also proven to be cost effective with a 25% reduction in required tank sizes resulting in a 15% decrease in investment costs and a further 5 - 50% saving on operational costs (Krhutková *et al.*, 2006).

Despite the ever-increasing importance of the costs associated with technologies the economics of bioaugmentation is an area that requires considerable attention and has been significantly over-looked. So far, economic assessments of bioaugmentation have been limited in extent (Gerrard and Stephenson, 1990; Stephenson and Gerrard, 1990). The economic benefits associated with direct dosing and the use of a side-stream were considered by Stephenson and Gerrard (1990) who conducted economic modelling of the two systems. The careful optimisation of a side-stream reactor was highlighted as an important factor in determining the economic viability of side-stream reactors. Although some economic benefits have been seen due to reduced land requirements and due to reduced operational costs after the investment in a side stream reactor significant investigations into CAPEX and OPEX costs remain unreported, especially for costs associated with strain selection, culture development and immobilisation techniques (Onyia *et al.*, 2001; Krhutková *et al.*, 2006).

The complexity of industrial wastewaters increases the challenge of identifying the most effective techniques as many interacting processes can be taking place simultaneously. Despite this, industries should take on the opportunity of learning from previous bioaugmentation successes and failures to gain from the benefits that may be obtained from bioaugmentation. Research has already increased the understanding of the complex interactions between introduced microorganisms and the host environment leading to improved application success. Many of the problems that have arisen in the field of bioaugmentation

have proven to be overcome through process development (Table 2-2). For the field of bioaugmentation to move forward it is now essential for key gaps in the research field to be addressed. Overall, when considering whether bioaugmentation is successful it must be firstly considered what the aim of the bioaugmentation is i.e. short-term solution to a treatment issue or the long-term improvement of a system. Current research has been limited by focusing on laboratory-scale investigations, synthetic wastewaters and failing to have adequate controls in place. Understanding in the field would be enhanced significantly by operating parallel studies. Full-scale investigations have been limited in extent and such investigations have also lacked controls (Parker and Wanner, 2007).

2.3 Conclusion

The field of bioaugmentation continues to be surrounded by controversy over its ability to improve wastewater treatment processes. The technique has seen many advances in the areas of strain selection, inoculum introduction strategies and treatment configurations. Furthermore, it has been shown to be applicable to a wide variety of different industries. Despite this bioaugmentation has failed to make the key transition from laboratory applications to full-scale applications with limited full-scale reports being available. In order for the benefits of bioaugmentation to become more widely understood and recognised, developments need to take place in some key areas. Firstly, applications need to recognise the importance of understanding the degradation pathways of target compounds. Without an understanding of the degradation pathway and the associated bacterial requirements selection of a suitable inoculum is problematic. Previous case studies have demonstrated success when the inoculum has been selected for its specific abilities. Secondly, studies with adequate controls are essential in order to get a true representation of the benefits acquired through bioaugmentation. Finally, the economics of the technique requires consideration. The success and widespread use of

bioaugmentation in the Czech Republic demonstrates the benefits of the technique, however, under which conditions it is successful requires substantial reporting.

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Chapter 3 Enhancing the removal of hazardous pollutants from coke making wastewater by dosing activated carbon to a pilot-scale activated sludge process

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Abstract

Powdered activated carbon (PAC) was investigated for its ability to remove 6 polycyclic aromatic hydrocarbons (Σ6PAHs) (fluoranthene, benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene), trace metals and colour from coke making wastewater when dosed to a pilot-plant activated sludge process (ASP). The ASP had a volume of 0.68 m^3 and was operated to simulate the full-scale ASP treating coke wastewater from a steel works. Operational conditions included a flow rate of 0.78 m^3/day, a hydraulic retention time of 21 hours, a sludge retention time of 38 days and a temperature of 27^oC. The ASP was operated for a control period before PAC was dosed directly into the aeration cell at a dose of 400 mg/L. Powdered activated carbon addition resulted in a 20% increase in removal efficiency of the Σ6PAHs. Removal efficiency of trace metals was variable, but increased for nickel, chromium and cadmium by 22.6%, 20.5% and 12.4%, respectively. Improvement in colour removal efficiency was marginal at 5%. PAC addition allowed the improvement of treatment efficiencies in the ASP process at relatively low capital and operational costs, which may assist in reaching tighter effluent emission limits set for the industry.

Keywords: Activated carbon, Polycyclic aromatic hydrocarbons, Trace metals, Activated sludge process, Coke wastewater

3.1 Introduction

Coke making wastewaters originate from the quenching of hot coke masses and washing of ammonia stills as well as the cooling and washing of coke oven gases (Kim *et al.*, 2008). Coke making wastewater contains numerous compounds capable of causing toxic effects in the environment including ammonia (NH_4^+), thiocyanate (SCN^-), cyanide (CN^-), phenols, polycyclic aromatic hydrocarbons (PAHs) and trace metals (Vázquez *et al.*, 2006; Marañón *et al.*, 2008; Wei *et al.*, 2012; Zhu, Tian and Chen, 2012; Zhang *et al.*, 2013; Pal and Kumar, 2014; Chen *et al.*, 2015). Characteristics of coke making wastewater vary between production plants in response to both the composition of the coals used and differences in plant operation (Marañón *et al.*, 2008). Ammonia concentrations have been reported from as low as 123 mg/L (Ganczarczyk, 1983) up to 4500 mg/L (Gould, 1986). Thiocyanate was reported by Gould (1986) in the range of 130 to 860 mg/L with values of ca. 200 mg/L being reported at other treatment plants (Ganczarczyk, 1983; Staib and Lant, 2007). Phenol concentrations also vary between plants from as low as 60 mg/L (Staib and Lant, 2007) to 1900 mg/L (Gould, 1986) with average values of ca. 250 mg/L (Gould, 1986; Bai *et al.*, 2010). Typically chemical oxygen demand (COD) averages at 2300 mg/L (Gould, 1986; Staib and Lant, 2007; Bai *et al.*, 2010) whilst biological oxygen demand (BOD) shows large degrees of variation from 683 mg/L (Bai *et al.*, 2010) to 2800 mg/L (Gould, 1986).

As a result of increasing concern over pollutant emissions to the aquatic environment emission limits for industrial wastewater have become increasingly stringent. New emission limits were introduced in 2016 for coke making wastewaters under the Industrial Emissions Directive (IED) (European Commission, 2013). Six PAHs (Σ6PAHs: fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene) and some metals have been identified in the best available technology (BAT) reference document for the production of iron and

steel (European Commission, 2013) but there is little information available on their concentrations in coke making wastewater. Zhang et al. (2012) reported Σ18PAHs of 5470 ± 907 µg/L and 4.7 ± 0.4 µg/L in untreated and treated wastewater, respectively. Recently Σ6PAHs were reported for treated effluent from a UK steel making wastewater with average concentrations of 36.3 µg/L and a range of 8.5 - 99.4 µg/L (Chen *et al.*, 2015). Burmistrz and Burmistrz (2013) reported EPA Σ16PAHs in untreated coke wastewater varying between 255 - 312 µg/L and 0.94 - 4.46 µg/L in treated effluent. Chen et al. (2015) also reported trace metal emission factors for cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), iron (Fe), copper (Cu), zinc (Zn) and arsenic (As) of 0.04, 4.7, 6.0, 11.7, 1003, 1.1, 410 and 4.6 mg/tonne of coke, respectively.

A number of trace metals and PAHs frequently occurring in coke making effluents have been listed as priority substances (European Commission, 2014) (Table 3-1) under the Water Framework Directive (WFD) (European Parliament, 2000). The new achievable emission limits introduced for coke making wastewaters based on emissions associated with the BAT (European Commission, 2013) consist of <220 mg/L COD, <20 mg/L BOD_5, <4 mg/L SCN^-, <0.1 mg/L easily released cyanide ion (CN^-), <50 µg/L Σ6PAHs and <15-50 mg/L for the sum of ammonia-nitrogen, nitrate-nitrogen and nitrite-nitrogen. In particular, the high variability of PAH concentrations in coke making effluent reported by Chen et al. (2015) means consistently achieving the emission limit may be problematic and consequently there is a need to improve pollutant removal efficiencies.

The treatment of coke making wastewater typically takes place through biological treatment using activated sludge processes (ASP), although, many treatment plants also use initial pre-treatment methods e.g. steam stripping and chemical coagulation (Jianlong *et al.*, 2002; Pal and Kumar, 2014). Different treatment plants can also use different combinations of anoxic, anaerobic and aerobic treatment cells in order to reduce target pollutants.

Table 3-1: Priority Hazardous Substances (PHS), Priority Substances (PS) and Specific Pollutants (SP) associated with coke wastewaters.

Priority Hazardous Substances	Anthracene
	Cadmium
	Benzo[a]pyrene
	Benzo[b]fluoranthene +Benzo[k]fluoranthene
Priority Substances	Fluoranthene
	Lead
	Naphthalene
	Nickel
Specific Pollutants	Iron
	Arsenic
	Chromium
	Zinc

The use of activated carbon (AC) has received considerable attention for a number of decades for the removal of pollutants found in both industrial (Kunz and Giannelli, 1976; De Walle, Chian and Small, 1977; Chao, Yeh and Shieh, 1986; Meidl, 1997; Goel *et al.*, 2005; Amuda, Giwa and Bello, 2007; Crisafully *et al.*, 2008; Augulyte *et al.*, 2009) and domestic wastewaters (Joyce, Allen and Sukenik, 1966; Nicolet and Rott, 1999; Bansode *et al.*, 2004; Hussain *et al.*, 2011; Boehler *et al.*, 2012) and may allow improved treatment efficiencies of coke making wastewater. Activated carbon can be applied as a pre-treatment method, dosed directly into the treatment process or applied as a post-treatment method (Bornhardt, Drewes and Jekel, 1997; Çeçen and Aktaş, 2011). It can also be in either a granular (GAC) or powdered (PAC) form. Due to its larger pore size GAC is more suitable for heavy molecular weight pollutants (Valderrama *et al.*, 2008). When added into the ASP, it acts as an absorbent whilst also providing a surface for microorganisms to grow in a biofilm (Abu-salah *et al.*, 1996; Augulyte *et al.*, 2009). This allows pollutants to be absorbed and then diffused out gradually, allowing increased contact times between the

microorganisms and pollutant, which enhances the removal of less easily degradable compounds which may require a higher residence time (Abu-salah *et al.*, 1996).

Activated carbon has been successfully applied to PAHs and trace metal removal in a variety of industrial as well as domestic wastewaters using a range of AC types and operational configurations (Leyva Ramos *et al.*, 2002; Amuda, Giwa and Bello, 2007; Crisafully *et al.*, 2008; Augulyte *et al.*, 2009; Budinova *et al.*, 2009; Karnib *et al.*, 2014). Removals of 96.9 - 99.7% of Σ36PAHs have been demonstrated in a batch mode pilot-scale biologically activated carbon system treating wastewaters containing petroleum products (Kliaugaite, Jankunaite and Racys, 2008). Furthermore, the benefit of addition of AC to coke making wastewaters has been demonstrated in a laboratory-scale continuous flow reactor. A dose rate of 300 mg/L and a hydraulic retention time (HRT) of 20 h resulted in a 26% increase in COD removal and more stable cyanide removal (Chao, Yeh and Shieh, 1986). More recently a batch study using activated coke (carbonaceous material activated by steam and similar to AC but with a lower surface area) demonstrated improved removals of COD (91.6%) and colour (90%) at a dose rate of 200 g/L (Zhang *et al.*, 2010).

The majority of research focussing on PAH and trace metal removal with AC has been completed in batch systems. Goel et al. (2005) identified the limitations of applying those results to real treatment applications, explaining that in batch tests adsorption continues until an equilibrium is established but in a continuous treatment system this equilibrium is never achieved, resulting is significantly different efficiencies. Therefore, the objective of this study was to address the current gap in knowledge on the ability of AC to improve removal efficiencies of PAHs and trace metals from coke making wastewater in a continuous process at a significant scale (0.68 m^3) pilot-plant. Results from this study can also offer insight on the treatability of other types of wastewaters that contain PAHs and or trace metals through an ASP with AC addition.

3.2 Materials and Methods

3.2.1 Wastewater and activated sludge seed

Coke making wastewater and return activated sludge (for seeding the pilot-plant) were sourced from a UK, full-scale, industrial wastewater treatment plant (WWTP) that processed coke making wastewater. The full-scale WWTP included an inlet reservoir and an activated sludge process with 4 aeration tanks, followed by 2 clarifiers. Each aeration tank had a volume of 570 m^3 and received wastewater at a flow rate of 680 m^3/day allowing for a HRT of 21 hours and a sludge retention time (SRT) of 38 days. Return activated sludge was recirculated from the clarifier to the aeration cell.

3.2.2 Pilot-plant

In order to investigate the ability of PAC to treat coke making wastewater, an ASP pilot-plant was established following the configuration shown in Figure 3-1. The pilot-plant followed the same configuration as the full-scale treatment plant running at an equivalent HRT and SRT of 21 hours and 38 days, respectively. The wastewater entered the aerobic cell at 0.78 m^3/day with an overflow into the clarifier. The settled sludge was recirculated at a flow rate of 1.6 m^3/day and waste sludge removed manually at a rate of 6 L/day. The temperature and dissolved oxygen was maintained at ca. 27^{o}C and 2 - 4 mg/L respectively, the same conditions as the full-scale plant. The pH within the system averaged at 6.8.

The pilot-plant was operated for a control period, equivalent to 14 times the HRT, with samples collected 3 times per week for PAH, trace metal, suspended solids and colour analysis. After the control period the pilot-plant was dosed with 400 mg/L of PAC directly into the aeration cell in batch mode 3 times per week. The PAC used was, a commercial product, produced from lignite and

characterised by a grain size (D_{50}) of 24 µm, a bulk density of 0.44 g/cm^3, a specific surface area of 300 ± 30 m^2/g and a 0.5% moisture content. The PAC dose rate was based on a previous application by Chao, Yeh and Shieh (1986) of 300 mg/L, being increased to reflect the slightly higher average total phenol and thiocyanate levels in the studied coke making wastewater (Table 3-2) Samples were taken for PAH, trace metal, suspended solids and colour analysis 3 times per week. Removal efficiencies of total phenol and SCN^- were monitored throughout to ensure no negative impacts occurred due to PAC addition as the degradation of these compounds is an essential requirement for the successful treatment of coke making wastewater.

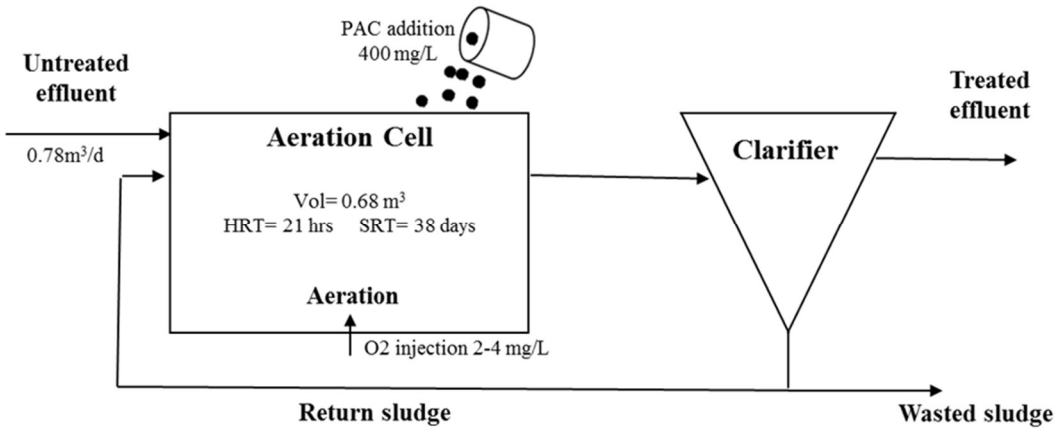

Figure 3-1: Schematic representation of the activated sludge pilot-plant with PAC addition.

3.2.3 Analysis

3.2.3.1 Total phenol

Total phenol analyses were completed by adding Folin Ciocalteu reagent and sodium carbonate (20% w/v) to filtered samples (1.25µm filter) and quantified by measurement in a spectrophotometer at wavelength of 760 nm (20 mm cell) (Cecil CE 1011) (The Institution of Gas Engineers, 1967).

3.2.3.2 Thiocyanate

Samples were filtered (1.25 µm filter) and the SCN^- concentration measured using a Galley photometric system (Thermo Scientific, United States) that was calibrated using potassium thiocyanate (0-500 mg/l) (based on The Institute of Gas Engineers analytical method for thiocyanate) (The Institution of Gas Engineers, 1971).

3.2.3.3 Colour

Samples were filtered through a 0.45µm filter and the residual colour was then analysed using a Jenway 6300 spectrophotometer (Staffordshire, UK) at a wavelength of 470 nm (Clesceri *et al.*, 1998).

3.2.3.4 Suspended solids

Suspended solids were analysed according to standard methods (Eaton, 2005). A 100 ml sample was filtered (1.25 µm filter) and dried at 105 ± 5^oC for 1 hour. Filter papers were then weighed and dried until a constant weight was achieved.

3.2.3.5 PAHs

Samples were collected and stored at 2 - 8^oC. Analysis was based on ISO 11338-2 (ISO, 2003). Samples were filtered (Whatman GF/C 70 mm diameter) to separate the particulate matter from the liquid phase. The particulate matter was dried for 12 h in a fume cupboard and subsequently extracted by accelerated solvent extraction with dichloromethane at 2000 psi and 150^oC using a Dionex ASE 200 (Runcorn, UK). Filters were placed into the cells where they were filled with dichloromethane. The solvent containing the organics was then flushed out under a nitrogen stream. The dichloromethane extract was then concentrated, solvent exchanged with methanol, added to the liquid phase and then extracted using an AutoTrace solid phase extractor (Caliper Life

Sciences). The PAH SPE extraction cartridges (750 mg/3 ml) were conditioned with methanol and water. The samples were loaded and PAHs were retained on the cartridge which was rinsed with methanol and water (20/80 v/v) and dried under a stream of nitrogen. The PAH fraction was then eluted using tetrahydrofuran and hexane. The extract was then solvent exchanged with hexane and analysed using GC-MS (Agilent 6890 Gas Chromatograph coupled to an Agilent 5973 MSD Mass Spectrometer). A capillary column (Agilent DB-5MS) was used with a constant flow rate operating under temperatures between 50^{o}C and 310^{o}C at specified rates and intervals over a 55 minute timescale. The Σ6PAHs quantified were fluoranthene, benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene.

3.2.3.6 Trace metals

Samples were analysed for total metals, aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) based on ISO method 17294-1 (ISO, 2006). For the analysis of total metal concentrations, 3 ml of nitric acid were added to 27 ml of sample and placed on a hotplate for 2 h at 90^{o}C. Each sample was then digested using a CEM Microwave Assisted Reaction System (MARS) using XP-1500 Plus High-Pressure Digestion vessels. Samples were then analysed using an Agilent 7500cx ICP/MS (Stockport, UK) with an Octopole Reaction System in order to overcome interferences (Agilent Technologies, no date).

3.2.3.7 Statistical analysis

Due to the high degree of natural variation in PAH concentrations measured in the wastewater feeding the pilot-plant both PAH and trace metal data was analysed statistically using an f-test, to ensure that the data fitted within a normal range of variance. PAH data was analysed using a paired t-test at a 90% confidence level. Trace metal data was analysed using a paired t-test with 95% confidence level.

3.3 Results and Discussion

3.3.1 Wastewater characterisation

On average the full-scale coke making wastewater contained 219 mg/L of total phenol and 195 mg/L of thiocyanate (SCN^-) (Table 3-2). Trace metal concentrations ranged from 0.13 µg/L Cd to 3612 µg/L Fe. Therefore, Fe constituted the majority of the total trace metals measured at 4216 µg/L (Table 3-2). The Σ6PAHs presented typical total concentrations of 179 µg/L. Individual PAHs varied from 13.6 µg/L (benzo[g,h,i]perylene) to 64.4 µg/L (fluoranthene). The new emission limit for the Σ6PAHs to achieve following treatment is 50 µg/L, hence effective treatment of PAHs in the treatment facility is crucial to achieve the required limit (European Commission, 2013).

3.3.2 Comparison of treatment efficiencies of the pilot-plant and full-scale treatment works during the control period (without PAC dosing)

During the control period the ASP achieved total phenol and SCN^- removal efficiencies comparable to the full-scale treatment works. Effluent SCN^- averaged at 0.7 mg/L leading to an average removal efficiency for SCN^- was 99%. Removal efficiencies remained stable despite fluctuations in inlet feed concentrations allowing compliance with the 4 mg/L emission limit (Figure 3-2).

Total phenol removal reached 97%, which was in the same order of magnitude as the full-scale treatment removal efficiency of 99% (Figure 3-2). Such results confirmed that the pilot-plant was operating effectively. Removal efficiencies of total phenol and SCN^- were maintained during the addition of PAC demonstrating that PAC did not impact the removal of the biodegradable pollutants SCN^- and total phenol (Figure 3-2). Similarly, Chao, Yeh and Shieh (1986) reported removal of total phenol to <1 mg/L and therefore observed no improvement in total phenol removal at dose rates of 200 -1000 mg/L. Although both SCN^- and total phenol were efficiently degraded by the well adapted biomass under control conditions it was equally important that no negative impacts were observed as emissions of both SCN^- and total phenol are regulated under the IED.

Table 3-2: Typical characteristics of coke making wastewater before and after treatment in the ASP pilot plant during control operational conditions (without PAC treatment) and their associated treatment efficiencies.

	Inlet	SD	Outlet	SD	Treatment efficiency (%)
Phenols (total) (mg/L)	219	15	6.3	0.4	97
Thiocyanate (mg/L)	195	24	0.6	0.2	100
Total trace metals (µg/L) *	4216	568	2878	220	35
Total of 6 PAHs (µg/L) **	179	35	53.6	21	58
Suspended Solids (mg/L)	23	8	20	3	-
Colour (Absorbance 470 n)	0.32	0.03	0.31	0.01	2.04

* Sum of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb.

** Sum of PAHs identified in BREF as associated with coke making- fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene.

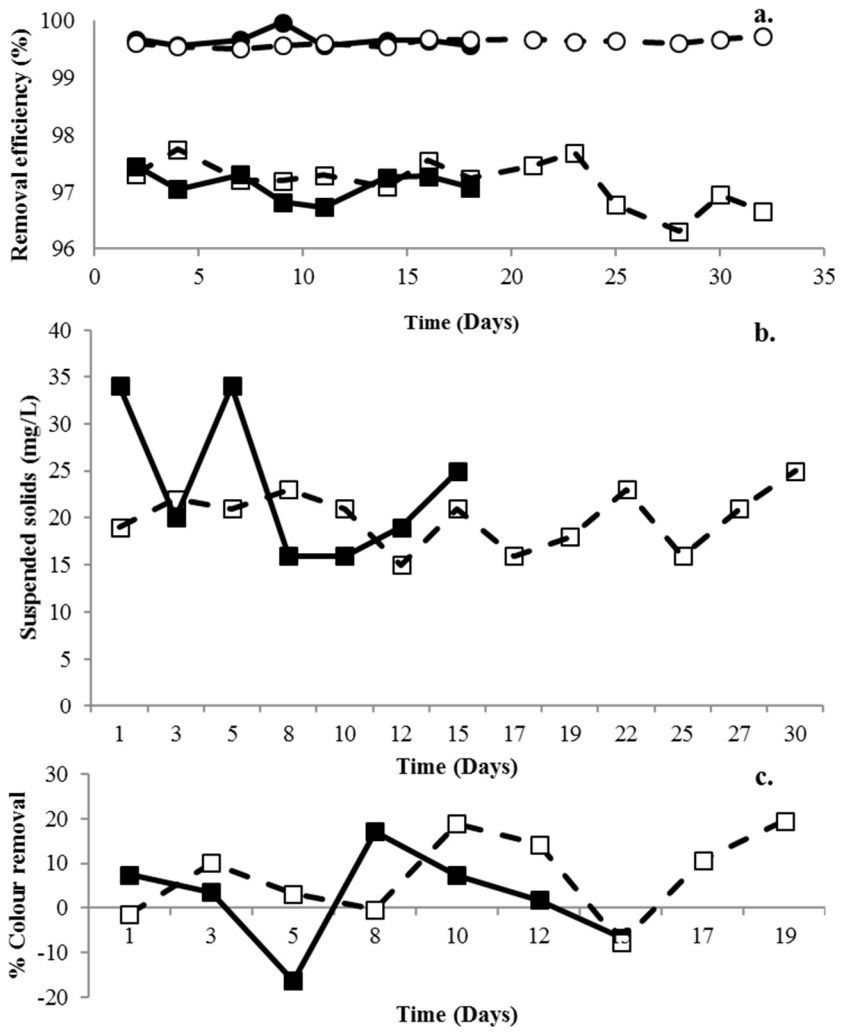

Figure 3-2: a- Removal efficiency of total phenol and SCN- during the control ASP and ASP dosed with 400 mg/L PAC. ● SCN^- without PAC, ○ SCN^- with 400 mg/L PAC, ■Total phenol without PAC, □ total phenol with 400 mg/L PAC. b- Suspended solid concentrations in outlet during the control ASP and ASP dosed with 400 mg/L PAC. ■ Suspended solids without PAC, □ suspended solids with 400 mg/L PAC. c- Colour removal efficiency during the control ASP and ASP dosed with 400 mg/L PAC. ■Colour removal efficiency without PAC, □ colour removal efficiency with 400 mg/L PAC.

3.3.3 Impact of PAC addition on suspended solids (SS) and colour removal

The SS removal showed a small increase from 20 mg/L to 24 mg/L, before and after PAC dosing, respectively. Despite this, the standard deviation of the SS measurement after PAC dosing (SD) decreased from 11 to 7 mg/L, suggesting a small improvement on sludge settling characteristics. The use of AC in ASP processes has been reported to promote rapid settling and enhance sludge dewatering, which are added benefits of the AC dosing (Çeçen and Aktaş, 2011). Addition of PAC resulted in a 5% improvement in colour removal, which was lower than other values previously reported. The use of PAC in the treatment of steel mill coke effluent at a dose of 300 mg/L resulted in a 50% removal of colour (Chao, Yeh and Shieh, 1986). Zhang et al. (2010) reported a 90% colour removal when treating coking wastewater with activated coke, however, this was at a much larger dosing rate of 200 g/L and under batch rather than continuous operational conditions.

3.3.4 Impact of PAC addition on trace metals removal

Addition of PAC to the ASP led to variable removals of trace metals (Table 3-3). Cadmium, a Priority Hazardous Substance (PHS), showed improved removal with average efficiency, increasing from 45.4% to 57.8%, after PAC dosing. Cadmium removal was also more stable with the removal efficiency standard deviation decreasing from 16% to 11%. Trace metal removal has been strongly associated with the pH of a solution due its role in influencing the ion charge and associated characteristics (Mohan and Singh, 2002). The pH in this study was not at the optimum for Cd removal averaging at 6.8. During batch tests with GAC Leyva-Ramos et al. (1997) reported a twelve fold increase in adsorption and associated removal of Cd(II) with a pH change of 3 to 8.

Table 3-3: Average treatment efficiencies for the removal of trace metals, in the continuous activated sludge pilot plant with and without PAC, showing a significant increase in the removal of Ni using t-test statistical analysis.

	No activated lignite		400 mg/L activated lignite		t-test	
	Av. removal (%)	SD	Av. removal (%)	SD	P-value	Significant (95%)?
Al	41.6	10.2	24.3	17.0	0.07	N
Cr	16.7	40.7	37.2	14.0	0.33	N
Mn	37.1	5.4	35.9	9.0	0.80	N
Fe	29.8	6.0	24.7	5.9	0.16	N
Co	18.4	1.6	12.5	8.8	0.10	N
Ni	46.9	12.3	69.5	7.7	0.00	Y
Cu	65.5	6.2	61.5	9.2	0.42	N
Zn	65.7	5.2	69.1	8.1	0.43	N
As	12.6	6.3	0.5	17.9	0.10	N
Cd	45.4	16.0	57.8	11.0	0.18	N
Pb	57.4	13.6	56.0	22.7	0.90	N

The largest improvement in trace metals removal was observed for Ni (Figure 1-1). Addition of PAC to the ASP process led to a significant improvement in Ni with an increase in average removal efficiency of 22.6%. As with Cd, the average removal efficiency became more stable with the standard deviation being observed to fall from 12.3% to 7.7% (Table 3-3). Through batch tests with AC, Karnib et al. (2014) found that Ni showed the greatest removal efficiency in comparison to Pb, Zn and Cd for each of the tested metal concentrations of 30 - 200 µg/L. PAC was also identified as the best absorbent for Ni in batch tests due to its higher surface area (710 m^2/g) compared to other tested AC products which were characterised by lower surface areas (420-485 m^2/g) (Rao, Parwate and Bhole, 2002). The PAC selected in this investigation had a lower surface area than the PAC products tested by Rao, Parwate and Bhole (2002),

however, it managed to achieve higher removal rates at lower dose rates. Rao, Parwate and Bhole (2002) also reported an optimum pH of 8 for maximum Ni removal (56% with 1000 mg/L PAC). As stated earlier the studied ASP process, with a pH of 6.8, was operating below the reported optimum of 8 but despite this a dose rate of 400 mg/L was capable of a removal efficiency of 69.5%. Powdered activated carbon addition resulted in a 20.5% improvement in Cr removal. As in the case of Ni and Cd, the observed removal efficiency standard deviation decreased from 40.7% to 14.0%, after PAC dosing.

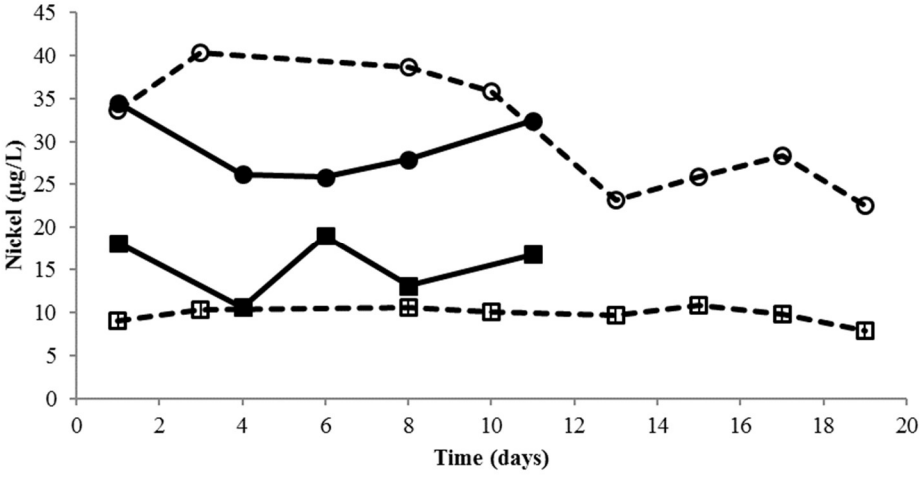

Figure 3-3: Removal of nickel ● Inlet no AC, ○ inlet 400 mg/L AC. ■ Outlet no AC, □ outlet 400 mg/L AC, showing increased stability of outlet Ni concentrations.

The average removal efficiency of Zn increased by just 2% leading to no significant difference. Zn removal has been shown to be dependent on individual treatment process conditions (pH and temperature) and the type of AC (Leyva Ramos *et al.*, 2002). For some AC products temperature has little impact on adsorption whilst for others temperature increases also increased removal (Leyva Ramos *et al.*, 2002). At 27ºC, the temperature in the studied ASP process, should have encouraged Zn removal, however, only small improvements were seen suggesting that the PAC selected in the current application may lack the acidic adsorption sites necessary for Zn adsorption.

Although improvements were seen in the removal of some trace metals after PAC dosing, this was not observed for all metals investigated. Trace metal removals for Al, Mn, Fe, Co, Cu, As and Pb were shown to decline (Table 3-3). The presence of a mixture of trace metals in the wastewater may be responsible for some removal efficiencies being lower than those reported in literature. Many investigations consider the ability of AC to improve the removal of a single trace metal. When numerous trace metals are present, their ability to compete for available active sites impacts the observed removal efficiencies. Petrov, Budinova and Khavesov (1992) reported that the presence of Cu^{2+}, Pb^{2+}, Zn^{2+} and Cd^{2+} in equal quantities resulted in decreased adsorptions of each. This was due to the preferential adsorption of Cd^{2+} over the other metal ions ($Cd^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+}$). Netzer and Hughes (1984) reported the impact of different contact time requirements of different metals, with those requiring less contact time demonstrating higher removals. Lead removal efficiencies, for example, decreased in the presence of other metals such as Cu and Co. Low removal efficiencies of As in control conditions (12.6%) and the further decline in efficiency when PAC was dosed (0.5%) may be the result of such factors.

3.3.5 Impact of PAC addition on PAHs removal

Σ6PAHs entered the pilot-plant at 213 ± 71µg/L (Figure 3-4). Improvements in removal efficiencies were significant for fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene resulting in a significant improvement in the removal of the average Σ6PAHs (58.2% to 77.8%) (Table 3-4). Stability in the treatment of the Σ6PAHs was also improved with a reduction of ca. 50% in the standard deviation (Table 3-4). Overall the concentration of Σ6PAHs fell from an average of 54 ± 21µg/L under control conditions to 34 ± 11µg/L after combined ASP and PAC addition (Table 3-4). Outlet concentrations were therefore in compliance with the new emission limit of <50 µg/L Σ6PAHs (European Commission, 2013), demonstrating the benefit of adding PAC to ASP.

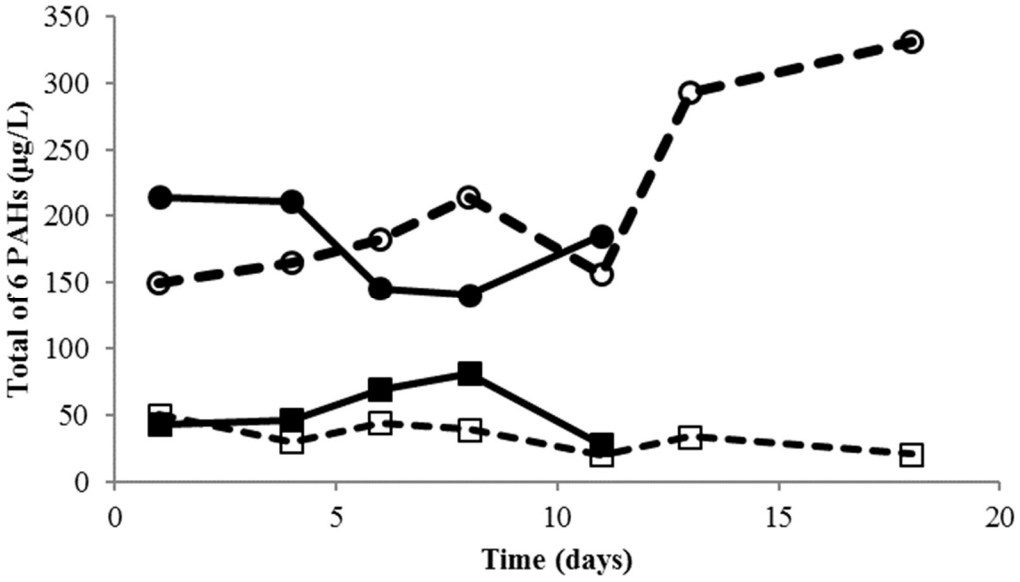

Figure 3-4: Removal of Σ6PAHs. ● Inlet no AC, ○ inlet 400 mg/L AC. ■ Outlet no AC, □ outlet 400 mg/L AC. Standard deviation of method typically equal to 0.15.

Table 3-4: Average removal efficiency for PAHs with and without AC dosing in the continuous activated sludge pilot plant. Improvements in removal efficiency were significant and standard deviations were reduced by 50%.

		Removal efficiency (%)			
	MW (g/mol)	Without AC		400 mg/L AC	
		Mean	SD	Mean	SD
Fluoranthene	202	93.9	3.10	96.6	1.68
Benzo [b + j] fluoranthene	252	53.4	28.1	74.2	12.9
Benzo [k] fluoranthene	252	62.9	22.7	79.0	10.7
Benzo [a] pyrene	252	59.1	26.9	79.3	11.8
Indeno [1,2,3-cd] pyrene	276	42.0	34.7	70.0	18.9
Benzo [g,h,i] perylene	276	38.1	32.1	67.7	18.9
ΣPAHs		58.2	24.4	77.8	12.2

High molecular weight PAHs are known for increased persistence in the environment being characterised by high n-Octanol/water partition coefficients (log K_{ow}) (Abu-salah *et al.*, 1996; Jeon and Madsen, 2013). Of the PAHs investigated log K_{ow} values range from 5.12 - 7.66 demonstrating their lipophilic nature and the likelihood of the PAHs to adsorb (de Maagd *et al.*, 1998; Popp, Bauer and Wennrich, 2001). As AC contains macro as well as micro-pores it is particularly suitable for the sorption of PAHs including those of high molecular weights (Valderrama *et al.*, 2008). If the PAH mixture contains varying sizes there will therefore be an increased removal efficiency for smaller PAH molecules, especially in PAC, due to a higher number of micropores which are suitable for their sorption. The AC product used in this investigation was in powdered form. Consequently, micropores would outnumber macropores which could result in lower removal efficiencies of high molecular weight PAHs (Yuan

et al., 2010). Both indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene have a high molecular weight of 276 g/mol and therefore improved removal of these more resistant PAHs is of notable importance. Despite PAC being characterised by fewer macropores improved removal efficiencies were seen for both indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene (28% and 30% respectively). Research into the effect of multiple PAH compounds occurring simultaneously suggests that the adsorption of PAHs will be influenced by the molecular weight of the compound (Yuan *et al.*, 2010). This is a limiting factor in the current investigation.

Removal of PAHs was associated with adsorption due to their lipophilic nature and associated poor biodegradability (Burmistrz and Burmistrz, 2013). Furthermore, biofilm formation would be limited as a result of the high levels of aeration applied in aeration cell and resulting shear (Mason *et al.*, 2000). Additionally, any further benefit of PAC in relation to biofilm formation would be limited as a result of the high SRT of 38 days present under normal operational conditions and a long-term adaptation period required for biofilm formation which was not accounted for in this investigation (Augulyte *et al.*, 2009). Longer-term investigations are required to investigate whether biofilm formation would result in biological degradation rather than adsorption alone.

3.3.6 Economic feasibility of PAC addition to the ASP

In order to assess the economic feasibility of PAC addition to the ASP, for the removal of PAHs and trace metals from coke making wastewater, the capital expenditure (CAPEX) and operational expenditure (OPEX) were calculated (Table 3-5). Costs were calculated for a full-scale ASP, following the configuration of the tested pilot-plant, based on an assumed plant with a capacity of 2000 m^3/day. A dose rate of 400 mg/L was selected due to the aforementioned comparison with the previous application by Chao, Yeh and Shieh (1986), however, the selected dose rate has an important impact on

observed removal efficiencies. Lü et al. (2011) reported that adsorption of phenol on lignite increased up to 500 mg/L and then remained stable. Similarly, Amuda, Giwa and Bello (2007) showed that Zn adsorption increased up until the point at which the adsorption peak is reached which was impacted by both the adsorbent type (as a result of the specific surface area) and the adsorbent dose. The economic costs are therefore specific to a dose rate of 400 mg/L and would therefore change in response to required dose rate optimisation.

The CAPEX costs consisted of the initial manual PAC dose required to reach a concentration of 400 mg/L in the aeration cell. Hence, an initial 800 kg of PAC would be required representing a cost of £760 (£0.38 per m^3 wastewater). The OPEX costs consisted of the cost of PAC addition required to maintain a dose rate of 400 mg/L. The amount of PAC lost via sludge wasting was therefore calculated. Suspended solids in the wasted sludge were 15,000 mg/L with PAC accounting for 4% of the suspended solids. Assuming sludge was wasted at 5 L/m^3 of treated effluent, this would lead to a total suspended solids loss of 54,750 kg/yr resulting in an annual loss of PAC of 2190 kg/yr, costing £2081/yr. Therefore, loss of PAC through suspended solids represents a cost of £2.85 per 1000 m^3 of wastewater or less than £0.01 per m^3 of wastewater. The excess sludge was returned to the full-scale coke making process, hence no costs of sludge disposal were predicted in this study.

Table 3-5: CAPEX and OPEX cost analysis of PAC dosing to full-scale ASP.

ASP plant configuration:	
Plant capacity (m^3/day)	2000
Suspended solids in wasted sludge (mg/L)	15,000
Sludge wasted (L/day)	10,000
PAC dose rate (mg/L)	400
Costs and dosing associated with PAC addition:	
Cost of PAC per kg (£) (RWE AG Pers.Comm, 2014)	0.95
Initial PAC dose required (kg)	800
Cost of PAC per m^3 of wastewater (£)	0.38
CAPEX (initial dose) (£)	760
PAC lost via sludge wasting (kg/yr)	2190
Cost of PAC loss per 1000m^3 wastewater (£)	2.85
OPEX cost (PAC required for maintenance) (£/yr)	2081

The costs associated with the addition of PAC were then compared to the CAPEX and OPEX costs of alternative techniques available to improve removals of PAHs and trace metals in wastewater. Tiravanti, Petruzzelli and Passino (1997) demonstrated the economic feasibility of ion exchange processes for Cd removal from tannery wastewater. Costs were estimated at ca. £1.85 per m^3 of wastewater. This represented an improvement on the conventional physiochemical treatment method which cost ca. £2.43 per m^3. Heavy metal removal can be achieved through the application of coagulation followed by sand filtration. Høibye et al. (2008) considered the economic viability of sand filtration taking a holistic approach to the cost calculation. This considered both the potential and prevented environmental costs of the application, leading to an expense of £0.04 per m^3. Costs were also compared

to the treatment of textile wastewater, which is considered a complex effluent (Lin and Peng, 1996) as is the case with coke making wastewater. Typical treatment often uses a combination of biological, physical and chemical treatment methods, with costs being reported as £0.28 per m^3. When combined with electrochemical treatment, operating costs were reduced to £0.21 per m^3 (Lin and Peng, 1996). Additionally, the cost of PAC addition was compared to the use of membranes and ozone (Table 3-6). Reports into the economics of these techniques focus on the treatment of domestic wastewater. Pollutants requiring treatment in domestic wastewater are typically 10 - 100 times lower than concentrations observed in coke making wastewater whilst the consent limits are typically higher than those required by the IED for coke making wastewater and therefore this requires consideration when comparing treatment costs. Furthermore, reported costs can vary significantly for the use of membranes due to the varying pre-treatment costs which can be extensive (Black and Veatch, 2008).

Table 3-6: Economic feasibility of PAC addition to the ASP in comparison to other treatment methods.

Technology	Type of wastewater	CAPEX (per m^3) (£)	OPEX (per m^3) (£)	Reference
PAC	Coke making	0.38	<0.01	This study
Ozone	Mixed*	583 - 972	0.17	(Black and Veatch, 2008)
Membrane	Domestic**	810 - 1620	0.22 - 1.33	(Black and Veatch, 2008)

* removal of benzene, toluene, ethylbenzene and xylenes (BTEX) and methyl tertiary butyl ether (MTBE)
** removal of pathogens and priority substances with turbidity removal and taste and odour control

PAC addition therefore offers an economically favourable option to achieve improvements in pollutant removal. Capital expenditure is minimal when PAC is dosed directly into the ASP process as it removes the requirement for plant modifications such as the installation of adsorption columns and pumps. The economic advantage of direct addition to an ASP was also reported by Flynn and Stadnik (1979) who reported savings of ca. £4.5 million in CAPEX costs and a £3.2 million saving in OPEX costs compared to GAC columns. Operational costs are also cheaper than other removal strategies such as sand filtration and ion exchange processes. Treatment processes used for domestic wastewater such as ozonation and membrane filtration have high CAPEX and OPEX costs. As industrial wastewaters, such as coke making wastewater, are characterised by higher pollutant concentrations the use of ozonation and membranes is associated with further economic costs and therefore PAC offers an economically viable treatment method for coke making wastewaters.

3.4 Conclusion

The ASP pilot-plant performed similarly to the full-scale treatment works regarding total phenol and SCN^- with removal efficiencies at 99%, and 97%, respectively, demonstrating effective treatment. Powdered activated carbon dosing to ASP increased the removal efficiency of a range of trace metals and PAHs associated with coke making wastewater. In the case of Ni the removal increased by 22.6% whilst the Σ6PAH removal efficiency increased by ca. 20%. Overall the Σ6PAHs concentration was reduced to 34 µg/L, allowing compliance with the new emission limit of <50 µg/L Σ6PAHs at relatively low capital and operational costs, which may assist in reaching tighter effluent emission limits set for the steel industry. Other wastewaters containing PAH and trace metal, from industries such as agrochemical, pharmaceutical, petrochemical, coal gasification, coke processing, insecticide, hydrocarbon and produced wastewaters amongst others, may benefit from PAC addition in a future of increasingly stringent emission limits. Overall, PAC addition was associated

with a CAPEX cost of £0.38 per m^3 and an OPEX cost of <£0.01 per m^3, offering an economically viable method to remove pollutants from coke making wastewater.

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Chapter 4 Characterisation of thiocyanate degradation in a mixed culture activated sludge process treating coke wastewater

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Abstract

Coke wastewater contains a significant quantity of thiocyanate (SCN^-) which is degraded through microbial metabolism. High treatment efficiencies are required in order to comply with the Industrial Emissions Directive (IED) limit of <4 mg SCN^-/L, however, microbial degradation has been reported to suffer from instability highlighting the need for improved understanding of underlying degradation mechanisms. Deoxyribonucleic acid sequencing analysis revealed that the most abundant species in a mixed culture taken from an activated sludge process (ASP) treating coke wastewater was an uncultured species of *Thiobacillus* (26%). The mixed culture degraded SCN^- under aerobic and anoxic conditions displaying similar metabolic capabilities to *Thiobacillus denitrificans* and *Thiobacillus thioparus*. Overall 100 mg SCN^-/L was degraded in 120 hours. It was hypothesised that the SCN^- degraded to OCN^- and sulphide which were ultimately hydrolysed/oxidised to NH_4^+, HCO_3^- and SO_4^{2-}. Nevertheless, at 360-2100 mg SCN^-/L a breakdown in the degradation pathway was observed, possibly due to a decrease in cyanase activity. Respirometry investigations demonstrated that NH_4^+ was inhibitory to SCN^- degradation (IC_{50}: 316 mg/L). Likewise, phenol (180 mg/L) and hydroxylamine (0.25 - 16 mg/L) reduced SCN^- degradation by 41% and ca. 7% respectively. In contrast, nitrate and nitrite (up to 16 mg/L) and sulphate (up to 2000 mg/L) had no impact on SCN^- removal. Overall, this study demonstrated the capability of a newly characterised *Thiobacillus* sp. to degrade SCN^- under both anoxic and aerobic conditions.

Nevertheless, SCN^-, NH_4^+ and phenol concentrations should be carefully controlled in the ASP to ensure an effluent SCN^- <4 mg/L.

Keywords: Thiocyanate; Coke wastewater, Thiobacillus denitrificans; Thiobacillus thioparus.

4.1 Introduction

Production of coke for steel making, generates wastewater which contains significant quantities of thiocyanate (SCN^-) ranging from 50 to 400 mg/L (Vázquez *et al.*, 2006; Staib and Lant, 2007; Raper, Fisher, *et al.*, 2017). Thiocyanate is generated as cyanide and sulphur react under the high temperatures associated with the coke making process (Kim and Katayama, 2000). Emissions of SCN^- are regulated in coke making wastewater by the Industrial Emissions Directive (IED) and must be reduced to less than 4 mg/L (European Commission, 2013). Removal of SCN^- from the coke wastewater can be achieved through treatment in an activated sludge process (ASP), however, this process is known to be sensitive and instable, which can lead to treatment losses (Vázquez *et al.*, 2006; Staib and Lant, 2007). Staib and Lant (2007) reported SCN^- degradation to be the most sensitive process after nitrification.

Due to the production of SCN^- in many industrial processes there has been an appreciable interest in SCN^- degradation (Hung and Pavlostathis, 1999; Grigor *et al.*, 2006; Grigor, Smirnova and Dulov, 2009; Combarros *et al.*, 2015; Watts and Moreau, 2016). Thiocyanate degrading bacteria have been isolated and identified from a variety of sources including many bacteria from the genus *Arthrobacter*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Thiobacillus*, *Acinetobacter*, *Burkholderia*, *Chryseobacterium*, *Klebsiella*, *Ralstonia* and *Methylobacterium* (Boucabeille, Bories and Ollivier, 1994; Hung and Pavlostathis, 1997; Kelly and

Wood, 2000b; Kim and Katayama, 2000; Sorokin *et al.*, 2001; Lee *et al.*, 2003, 2008; Chaudhari and Kodam, 2010; Huang *et al.*, 2013a). Numerous degradation pathways have been identified for the degradation of thiocyanate (Table 4-1) with reports of degradation via the action of both autotrophic and heterotrophic bacteria. Autotrophic bacteria utilise inorganic carbon from SCN^- as a carbon source whilst heterotrophic SCN^- degraders utilise SCN^- as a source of nitrogen and use organic carbon as an energy source (Watts and Moreau, 2016). Autotrophic pathways are the most commonly reported. Heterotrophic pathways are less commonly reported and have mainly been linked with the use of synthetic wastewaters (Table 4-1) (Watts and Moreau, 2016). Several end products have been reported including ammonia (NH_4^+), sulphate (SO_4^{2-}), carbonyl sulphide and trithionate. Additionally, a number of intermediate compounds have been reported including thiosulphate, tetrathionate and cyanate (OCN^-). As there are several possible degradation pathways, a greater understanding of the degradation pathway and bacterial requirements will provide an understanding of the requirements which need to be met to maintain stable operation in wastewater treatment plants.

Treatment of SCN^- in coke wastewater is further complicated by the presence of multiple compounds, which are associated with toxicity. Coke wastewater contains NH_4^+, phenol, polycyclic aromatic hydrocarbons (PAHs) and trace metals (Vázquez *et al.*, 2006; Marañón *et al.*, 2008; Bai *et al.*, 2010; Raper, Soares, *et al.*, 2017). Concentrations of such pollutants can also vary significantly between and within different treatment works in response to the changing coke composition and operational conditions (Marañón *et al.*, 2008). Ammonia concentrations have been reported to vary between 50 and 500 mg/L with similar fluctuations observed for phenol concentrations (60 – 400 mg/L) (Vázquez *et al.*, 2006; Marañón *et al.*, 2008; Bai *et al.*, 2010).

Table 4-1: Thiocyanate degradation pathways.

	Species	Oxygen availability	SCN^- degradation pathway	Type of wastewater and reference
Autotrophic	Mixed culture	Aerobic	$SCN^- + H_2O \rightarrow HCNO + HS^-$ $HCNO + H_2O \rightarrow NH_4^+ + HCO_3^-$ $HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$ Overall: $SCN^- + 2O_2 + 3H_2O \rightarrow NH_4^+ + HCO_3^- + SO_4^{2-} + H^+$	Photo-processing (Hung and Pavlostathis, 1997) Synthetic photo-processing (Hung and Pavlostathis, 1999) * Metallurgical (synthetic and reused water) (Grigor, Smirnova and Dulov, 2009) Synthetic *Burkholderia* sp., *Chryseobacterium* sp., *Ralstonia* sp.(Huang *et al.*, 2013b)
	Mixed culture: dominated by *Pseudomonas* and *Bacillus*	Aerobic	$SCN^- + 2H_2O + 2O_2 \rightarrow CO_2 + SO_4^{2-} + NH_4^+$ $SCN^- + 2H_2O \rightarrow CO_2 + S^{2-} + NH_4^+$ $SCN^- + 3H_2O + 0.5O_2 \rightarrow CO_2 + S^o + NH_4^+ + 2OH^-$	Coke wastewater (Paruchuri, Shivaraman and Kumaran, 1990)
	Mixed Culture	Aerobic	$SCN^- + 2H_2O + 2O_2 \rightarrow CO_2 + SO_4^{2-} + NH_4^+$	Coke wastewater (Staib and Lant, 2007)
	Thiobacillus thioparus	Aerobic	$SCN^- + 2H_2O \rightarrow COS + NH_3 + OH^-$	Synthetic (Katayama *et al.*, 1992)
	Acinetobacter johnsonii and *Pseudomonas diminuta*	Aerobic	$SCN^- \rightarrow S_2O_3^{2-} \rightarrow SO_4^{2-}$	Synthetic (Boucabeille, Bories and Ollivier, 1994)
	Thialkalivibrio thiocyano denitrificans	Aerobic/ Anaerobic	$5SCN^- + NO_3^- + H_2O + 8H^+ + 5HCO_3^- \rightarrow 5SO_4^{2-} + 5NH_3 + 10CO_2 + 4N_2$	Soda lake sediment (Sorokin *et al.*, 2004)
	Thiobacillus denitrificans	Anoxic	$5SCN^- + NO_3^- + H_2O + 8H^+ + 5HCO_3^- \rightarrow 5SO_4^{2-} + 5NH_3 + 10CO_2 + 4N_2$	(Robertson and Gijs Kuenen, 2006)*
Heterotrophic	*Pseudomonas putida* (strain 21) and Pseudomonas stutzeri (strain 18)	Aerobic	$SCN^- + H_2O \rightarrow NH_3 + CO_2 + S_2O_3^{2-}$ Further converted by P. putilda strain 21 to: $S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{2-}$	Synthetic (Grigor *et al.*, 2006)
	Soil isolate 26B	Aerobic	$SCN^- + H_2O \rightarrow NH_3 + CO_2 + S_2O_3^{2-}$	Synthetic (Stratford, Dias and Knowles, 1994)*
	Klebsiella pneumoniae and Ralstonia sp.	Aerobic	$SCN^- + 2H_2O \rightarrow COS + NH_3 + OH^-$	Synthetic (Chaudhari and Kodam, 2010)

* Inferred from text

Paruchuri, Shivaraman and Kumaran (1990) reported that a mixed culture containing *Pseudomonas* and *Bacillus* species was capable of degrading up to 1,400 mg/L of SCN^- in batch culture over 6 days. Furthermore, they investigated the impact of phenol and NH_4^+ demonstrating that NH_4^+ was shown to have no inhibitory effect up to 2000 mg/L, after which prolonged oxidation was required to maintain SCN^- degradation. Thiocyanate was more sensitive to phenol with 50 mg/L prolonging the oxidation requirement and 500 mg/L resulting in complete inhibition. In contrast, Staib and Lant (2007) suggested that under continuous treatment conditions phenol would exert no inhibitory influence as its degradation rate would exceed the degradation rate of SCN^-. Jeong and Chung (2006) investigated the degradation of SCN^- in a lab-scale continuous process. Coke oven gas was diluted to create a wastewater characterised by SCN^- levels of 3000-7000 mg/L. The wastewater was then passed through a fluidized biofilm reactor with a 40% filling ratio. When the volumetric loading rate of thiocyanate exceeded ca. 4 $kg/m^3.d$ the biodegradation rate slowly declined demonstrating a substrate inhibition effect. Outlet SCN^- concentrations >50 mg/L were correlated with declining degradation rates.

Observations into SCN^- degradation to date have been controversial and further investigation is required to fully understand the process. The objective therefore was to characterise the mixed culture responsible for SCN^- degradation in the ASP treating coke wastewater. Knowledge on the nature of SCN^- degrading community would enable a greater understanding of the conditions required to maintain stable treatment efficiencies and enable compliance with the IED emission limit of <4 mg/L. Inhibition tests would provide insight to the SCN^- degradation pathway as well as required operational conditions.

4.2 Materials and Methods

4.2.1 Wastewater and activated sludge biomass

Coke wastewater and activated sludge biomass was collected from the a full-scale, UK, steelworks ASP operating under aerobic treatment conditions. Activated sludge biomass was taken from the return activated sludge (RAS) feed which was characterised by a sludge retention time (SRT) of ca. 38 days.

4.2.2 Temperature control and SCN^- degradation rate batch tests

Batch tests were conducted to investigate the impact of temperature and concomitant nitrification and SCN^- degradation. Batch tests with a 0.95 L working volume were completed using coke wastewater and activated sludge biomass to produce a mixed liquor suspended solids (MLSS) of 4500 mg/L. Aquatic pumps enabled dissolved oxygen to be maintained at ca. 3 mg/L. Samples were taken systematically to demonstrate the influence of temperature and concomitant nitrification. Temperature was maintained through a water bath at 25^oC to reflect the target conditions on the full-scale ASP, however, to investigate the impact of temperature some batch tests were left without heat provision. These batch tests were therefore subject to diurnal temperature variations (8 - 21^oC).

4.2.3 Respirometry

Activated sludge biomass was collected in advance of each run of the respirometer and was stored at 2 - 5^oC for a maximum of 48 hours. The respirometer (Environmental Services, UK) consisted of ten respirometric cells (450 ml working volume) positioned in a water bath maintained at a temperature of 25 ±1 ^oC to reflect the target operational conditions of the full-scale ASP. Temperature was controlled by a Grant thermostatic circulator (GD120), UK. Oxygen supply to the respirometric cells was enabled via the provision of a

copper sulphate pentahydrate solution (25% w/v). Carbon dioxide was removed by a 2M sodium hydroxide solution. Oxygen consumption data was recorded by a data logger at 20 minute intervals. Oxygen concentration within each respirometric cell was maintained via agitation using a magnetic stirrer. Activated sludge biomass and coke making wastewaters were combined to replicate the mixed liquor concentrations in the full-scale treatment process of 8300 mg/L.

In order to investigate the inhibition of SCN^- the mixed liquor from the coke making process was spiked with solutions of hydroxylamine hydrochloride (NH_2OH), potassium nitrite and potassium nitrate at concentrations varying from 0.25 – 16 mg/L. The impact of SCN^- was investigated through spiking potassium thiocyanate at concentrations from 250 - 2000 mg/L. The impact of ammonia and sulphate were investigated by spiking ammonium chloride and potassium sulphate at concentrations of 250 - 1500 mg/L NH_4^+ and 1000 - 2000 mg/L SO_4^{2-} respectively. Inhibition tests were completed over 4-5 days and repeated in triplicates.

4.2.4 Chemical analysis

Samples were filtered (0.45 µm syringe filters -VWR) and pH recorded (Jenway 3540, UK). Mixed liquor suspended solids were analysed according to standard methods (Eaton, 2005). Merck cell test kits were used to determine the concentration of NO_2^-, NO_3^-, NH_4^+, SO_4^{2-} and soluble chemical oxygen demand (sCOD) following the manufacturer's instructions. Thiocyanate was complexed with iron (III) to produce an orange-red colour based on The Institute of Gas Engineers analytical method for thiocyanate (The Institution of Gas Engineers, 1971). A complex reaction between phenol (mono) and 4-aminoantipyrene was used to determine phenol concentrations (based on ISO 6439:1990) (ISO, 1990). Concentrations of SCN^- and phenol were then determined

colourimetrically on a Jenway 6300 spectrophotometer (Staffordshire, UK) at a wavelength of 465 and 510 nm, respectively.

4.2.5 Molecular microbial ecology

Activated sludge biomass was analysed through polymerase chain reaction (PCR) in order to quantify the microbial diversity in the ASP mixed liquor. The biomass was placed into a lysing matrix tube and the deoxyribonucleic acid (DNA) extracted (MPBIO FastDNA Spin Kit for soil, Santa Ana, USA). The V4 and V5 regions of the 16S ribosomal RNA (rRNA) gene were targeted with the universal primers 515F and 926R (Quince *et al.*, 2011). Error correcting golay barcodes enabled sample multiplexing (Hamady *et al.*, 2012). Polymerase chain reaction products were purified using HighPrep magnetic beads (Magbio, Gaithersburg USA) and QuantiFluor ONE (Promega, Madison USA). An equimolar pool of amplicons was sequenced using Illumina MiSeq with 2x300 v2 chemistry (Illumina, San Diego USA). Quantitative Insights Into Microbial Ecology (QIIME) 1.9 (Caporaso *et al.*, 2010) and the SILVA 16S rRNA gene database v123.1 (Quast *et al.*, 2013) were used for sequence analysis. The 16S rRNA gene sequences were grouped at 97% similarity to create operational taxonomic units (OTUs). Representative sequences from each OTU were then taxonomically assigned using the SILVA database. If the 16S sequences were not found in the database, these were described as “uncultured”, “ambiguous” or “other”. An uncultured sequence was one in which the sequence matched a database sequence but taxonomy was unavailable. An ambiguous species referred to a sequence which had more than a 97% similarity to more than one sequence of the genus. A sequence was referred to as “other” when the sequence could be identified no further than the genus level.

4.3 Results and Discussion

4.3.1 Coke wastewater characterisation

Characteristics of the coke wastewater are shown in Table 4-2. The sCOD of the coke wastewater averaged at 644 mg/L. Thiocyanate and phenol concentrations were 95 mg/L and 20 mg/L respectively. Ammonia contributed the highest concentration of nitrogen to the wastewater at an average of 91 mg/L as NH_4^+-N with small contributions of NO_3^--N and NO_2^--N each at 3 mg/L. The wastewater was characterised by a typical pH of 7.8. Concentrations of pollutants therefore fell within the typical range of coke wastewater previously reported for this site as well as in the wider literature (Vázquez *et al.*, 2006; Staib and Lant, 2007; Marañón *et al.*, 2008; Raper, Fisher, *et al.*, 2017).

Table 4-2: Characterisation of coke wastewater

	Concentration (mg/L) and standard deviation
sCOD	644 ± 130
Phenol	20 ± 2
SCN^-	95 ± 18
NH_4^+-N	91 ± 24
NO_3^--N	3 ± 2.5
NO_2^--N	3 ± 2.9
pH	7.8 ± 0.3

4.3.2 Thiocyanate degradation in the mixed culture

Respirometry tests showed that the mixed culture was capable of SCN^- removal at a range of initial SCN^- concentrations (Figure 4-1). After 120 hours, removal of 110 mg/L SCN^- was complete. For initial SCN^- concentrations of 360 to 610

mg/L, the average SCN^- removal was 19 and 13%, respectively. Hence, as the initial SCN^- concentrations increased, removal efficiencies declined. Whether the observed decline in removal efficiencies was as a result of toxicity or the requirement for longer degradation times deserves further investigation. Despite this, at an almost 20 times increase in the initial SCN^- concentration to 2109 mg/L, 58% of the initial SCN^- was degraded after 5 days demonstrating the ability of the mixed culture to cope with high SCN^- concentrations. The mixed culture therefore had a high SCN^- removal capacity similar to the mixed consortium investigated by Paruchuri, Shivaraman and Kumaran (1990). A co-culture of SCN^- degrading bacteria *Klebsiella pneumoniae* and *Ralstonia* showed decreased removal efficiencies at increased initial concentrations in batch tests conducted by Chaudhari and Kodam (2010). Degradation efficiencies declined from 100% at 500 mg/L SCN^- to 76%, 57%, 42%, and 34% at 1000, 1,500, 2,000, and 2,500 mg/L SCN^- respectively.

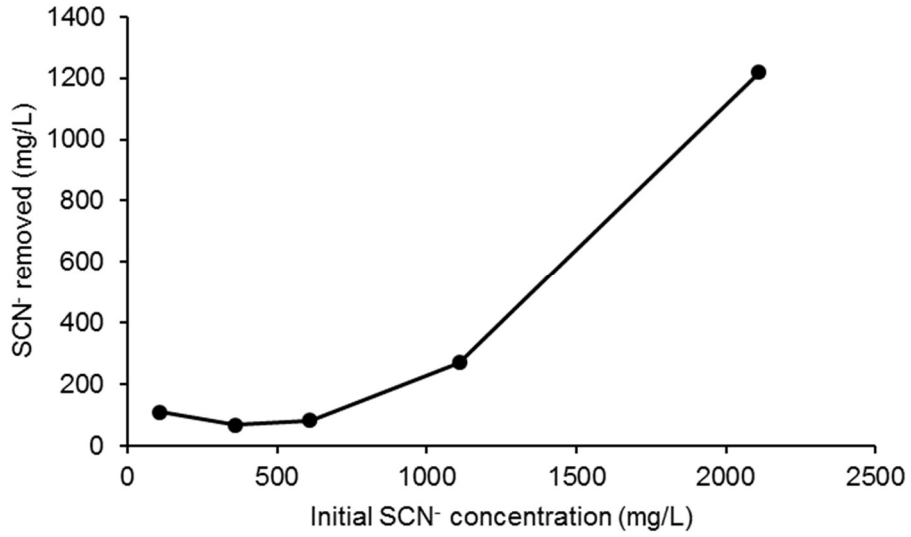

Figure 4-1: Concentration of SCN^- removed by the mixed culture after 5 days in the respirometer at different initial SCN^- concentrations.

On the other side, different SCN^- initial concentrations resulted in different end products (Table 4-3). When the initial SCN^- concentration was 110 mg/L (control conditions), the ammonia was observed to increase from 70 mg/L to

110 mg/L. Ammonia is produced during the degradation of SCN^- through all reported degradation pathways (Table 4-1). For each mole of SCN^- degraded Kim et al. (2008) reported the production of 0.24 moles NH_4^+-N. The mixed culture in the present investigation produced 0.26 moles of NH_4^+-N from each mole of SCN^- degraded. Under control conditions the production of NH_4^+-N was in line with the theoretical NH_4^+-N production expected (28 mg/L). As the initial SCN^- concentration increased, however, there was a decline in NH_4^+-N production suggesting a breakdown in the degradation process. Hung and Pavlostathis (1997) reported that SCN^- degradation proceeds in a series of steps (Table 4-3). Firstly SCN^- is hydrolysed forming OCN^- which is subsequently hydrolysed to form NH_4^+ and bicarbonate (HCO_3^-) whilst sulphide is oxidised to produce SO_4^{2-}. Lower than expected concentrations of NH_4^+-N suggests that SCN^- hydrolysis occurred but OCN^- hydrolysis did not.

Cyanate is hydrolysed by the enzyme cyanase producing CO_2 and NH_4^+ (Kozliak *et al.*, 1995; Douglas Gould *et al.*, 2012). The *E.coli* enzyme is the only cyanase which has been studied in detail (Walsh *et al.*, 2000). Bicarbonate is believed to be involved in a nucleophilic attack on OCN^- which produces CO_2 and carbamate (Walsh *et al.*, 2000). Decarboxylation then takes place producing CO_2 and NH_4^+ (Walsh *et al.*, 2000). Although cyanase is induced by OCN^-, high cyanate concentrations can equally have a toxic effect (Hung and Pavlostathis, 1997). Both HCO_3^- and OCN^- are capable of binding at the other substrate binding site resulting in a dead-end complex (Anderson and Little, 1986). It is therefore suspected that at higher concentrations of SCN^- the hydrolysis of SCN^- proceeded more rapidly producing high concentrations of OCN^- which in turn led to inhibition of cyanase. As a result of the lower cyanase activity OCN^- would accumulate further and no NH_4^+ would be produced (Hung and Pavlostathis, 1997).

Furthermore, degradation of SCN^- during the treatment of coke wastewater has typically been reported to occur in aerobic conditions. Kim et al. (2008, 2011)

reported that SCN^- degradation took place in the aerobic tank of the laboratory-scale anoxic-aerobic ASP. In contrast, however, previous work on the current activated sludge biomass revealed that the biomass was capable of completely removing SCN^- in both aerobic conditions and anoxic conditions (Raper, Fisher, *et al.*, 2017; Raper, Soares, *et al.*, 2017). This SCN^- degradation in the anoxic cell of a pilot-scale anoxic-aerobic ASP was possible with biomass taken from an aerobic process without any acclimatisation period.

Table 4-3: Impact of initial SCN^- concentration on SCN^- removal and end product formation in the mixed culture after 5 days in the respirometer.

Start (mg/L)				End (mg/L)					
SCN^-	NH_4^+-N	NO_3^--N	NO_2^--N	SCN^-	NH_4^+-N	NO_3^--N	NO_2^--N	Theoretical NH_4^+-N concentration *	Difference between theoretical and empirical NH_4^+-N**
109	72	5	2	1	106	3.4	37	100	6
359	″	″	″	291	101	7.2	14	165	-64
609	″	″	″	527	98	10.6	17	230	-132
1109	″	″	″	839	15.4	18.2	21	360	-345
2109	″	″	″	890	4	25.4	17	620	-616

* Empirical data of SCN^- degradation by the studied mixed culture demonstrates that one mole of SCN^- forms 0.26 moles of NH_4^+-N. Theoretical NH_4^+-N concentration calculation: Start NH_4^+-N (mg/L) + (0.26 x Start SCN^- (mg/L).

** Difference between NH_4^+-N formation based on molar ratio of SCN^- to NH_4^+ and empirical NH_4^+-N concentration = End NH_4^+-N (mg/L) - (Start NH_4^+-N (mg/L) + (0.26 x Start SCN^- (mg/L)))

4.3.3 Impact of nitrogen compounds on SCN^- degradation

Nitrification is an essential process in the removal of nitrogen from coke wastewater. Hence compounds associated with the nitrogen cycle were assessed for their impact on SCN^- degradation (Table 4-4). Nitrite (0.25 - 16 mg/L) had no impact on SCN^- degradation efficiency. Likewise, nitrate (0.25 - 16 mg/L) had no impact on SCN^- degradation. Hydroxylamine (NH_2OH) was investigated as it is an intermediate compound produced during nitrification (Gerardi, 2002). A small inhibitory response to hydroxylamine was observed (Table 4-4) with an average 7% reduction in SCN^- degradation at concentrations from 0.25 mg/L to 16 mg/L. Hydroxylamine is typically found at low concentrations (Gerardi, 2002) whilst SCN^- degradation proceeds faster than nitrification therefore any inhibitory impact of nitrification would be minimal. Figure 4-2 demonstrates that SCN^- removal was complete at 72 hours whilst nitrification was <40%, taking 5 days to reach 90%.

Table 4-4: Impact of nitrogen compounds (associated with nitrification) on SCN^- treatment in the mixed culture after 4 days in the respirometer.

	Change in treatment efficiency in percentage (%)					
	Concentration added (mg/L)					
	0.25	**0.5**	**1**	**2**	**4**	**16**
NH_2OH	-7.9*	-6.9*	-6.9*	-5.0*	-4.0*	-8.9*
NO_2^-	-0.4	0	0	-0.3*	0	-0.4*
NO_3^-	0.2	0.1	0.2	0.2	0.4	0

*Negative percentages indicate a decrease in SCN^- removal in relation to control conditions (no nitrogen compound spiked).

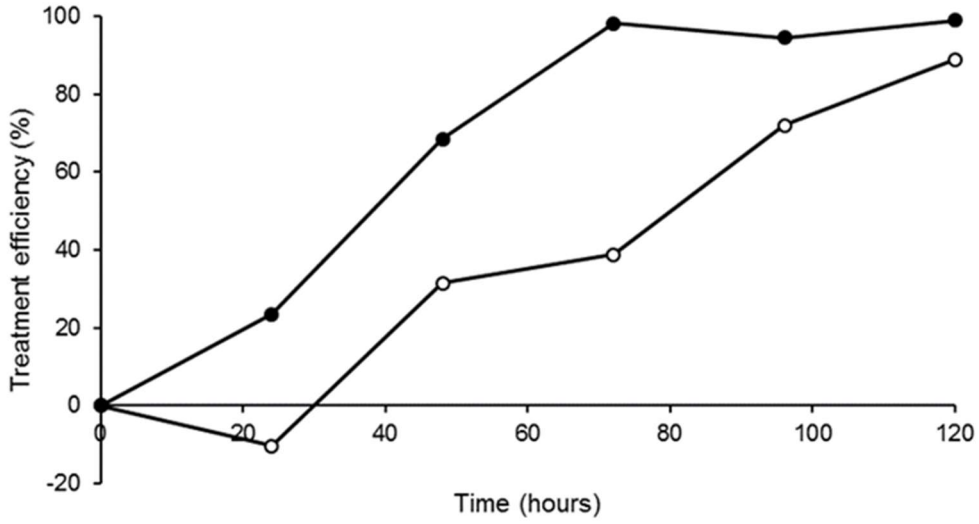

Figure 4-2: Thiocyanate and nitrification treatment efficiency by the mixed culture during batch tests ●- SCN^- removal efficiency ○- Nitrification efficiency.

4.3.4 Impact of ammonia, sulphate and phenol on SCN^- degradation

The average NH_4^+-N concentration at 0 hours in the respirometer was 82 mg/L. Additional NH_4^+ was subsequently spiked (250 - 1500 mg/L NH_4^+) to added to the coke wastewater to assess the impact of NH_4^+ on SCN^- degradation. Ammonia was observed to have an inhibitory influence on SCN^- degradation (Table 4-5). Thiocyanate removal efficiencies declined by 19-24% upon the addition of 250 - 1500 mg/L NH_4^+ and oxygen consumption in the respirometer tests was also observed to decline (Figure 4-3). Decreased oxygen consumption was likely to be associated with NH_4^+ toxicity. The calculated half maximal inhibitory concentration (IC_{50}) for NH_4^+ was 316 mg/L NH_4^+-N. The mixed culture was therefore more sensitive to NH_4^+ concentrations than the mixed culture described by Paruchuri, Shivaraman and Kumaran (1990). Ammonia loading to the ASP is therefore a critical parameter that should be controlled in the operation of ASPs treating coke wastewater.

Table 4-5: Impact of ammonia, sulphate and phenol on SCN^- treatment efficiency in the mixed culture after 4 days in the respirometer.

	Change in % treatment efficiency							
	Concentration added (mg/L)							
	80	**130**	**180**	**250**	**500**	**1000**	**1500**	**2000**
NH_4^+	nd	nd	nd	-24	-19	-22	-43	nd
SO_4^{2-}	nd	nd	nd	nd	nd	2.8	nd	5.8
C_6H_5OH	-29	-38	-41	nd	nd	nd	nd	nd

Sulphate at concentrations between 80 - 2000 mg/L, had little impact on SCN^- degradation suggesting that no inhibition occurred as a result of its formation during the degradation process (Table 4-5). The impact of varying phenol concentration on the SCN^- removal was also investigated due to concerns around its toxicity. The results obtained indicated that phenol had an inhibitory role on SCN^- degradation with the addition of 80 mg/L resulting in a 29% decrease in SCN^- removal (Table 4-5). Thiocyanate removals continued to decline by 38% and 41% with the addition of 130 and 180 mg/L of phenol, respectively. Increased phenol results in higher organic carbon availability which can increase the growth of heterotrophic bacteria which results in increased competition for dissolved oxygen with the slower-growing autotrophic bacteria (Kim et al. 2013a).

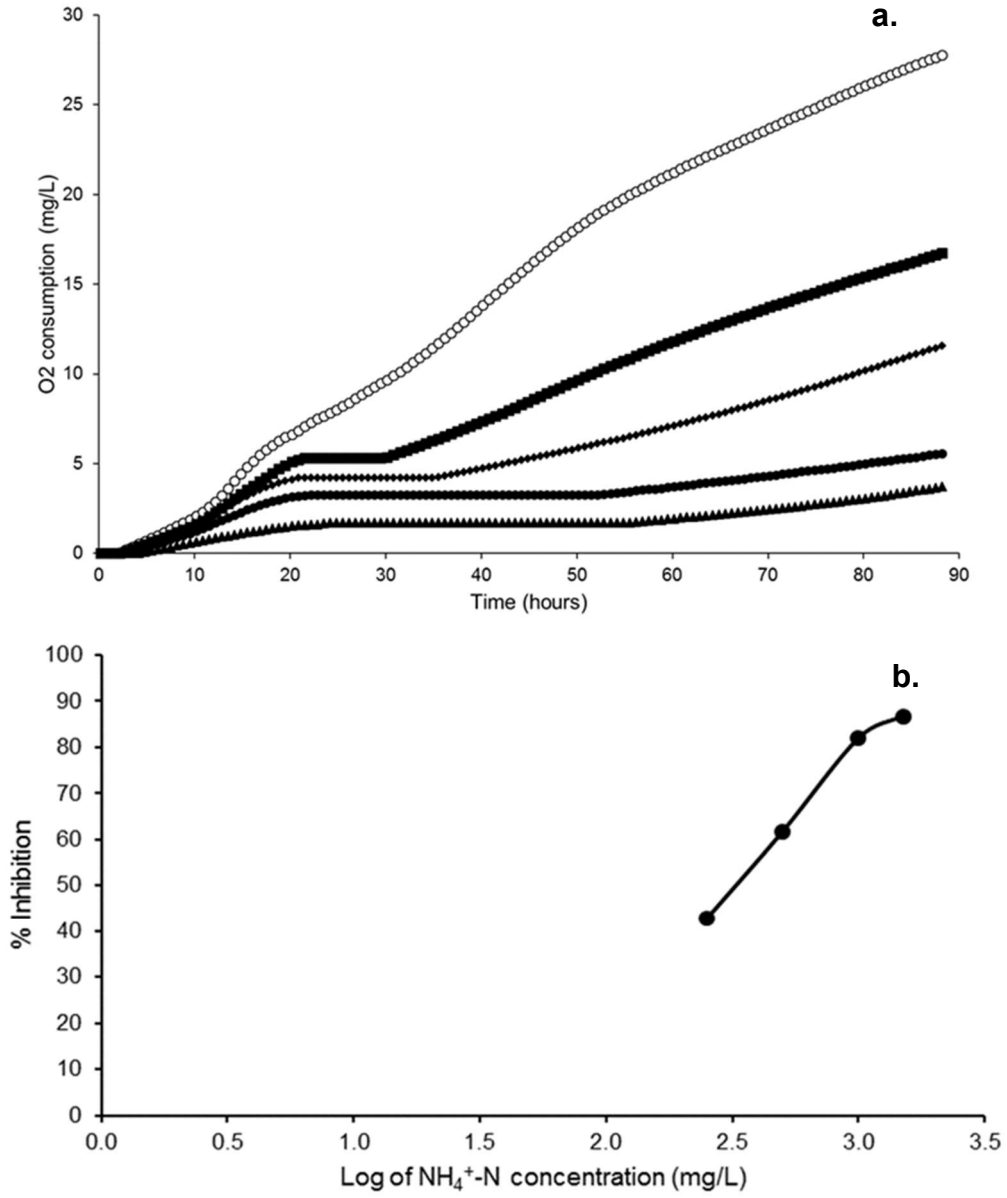

Figure 4-3: Impact of NH_4^+-N addition on the mixed culture over a 4 day duration in the respirometer a. Impact of NH_4^+-N addition on oxygen uptake ○ 82 mg/L (control) ■ 332 mg/L ♦ 582 mg/L ● 1082 mg/L ▲1582 mg/L NH_4^+-N b. Half maximal inhibitory concentration (IC50) of NH_4^+-N on SCN^- removal.

4.3.5 Temperature

The mixed culture was sensitive to process temperature (Figure 4-4). When the temperature was maintained at mesophilic conditions (25°C) SCN^- degradation was complete within 24 hours. When temperature was not maintained and it fluctuated between 8 - 21°C (psychrophilic range of temperatures), the SCN^- degradation was strongly impacted and decreased to 2 - 26%. The optimal temperature for the mixed culture was therefore within the mesophilic range of temperatures, fitting into the typical reported optimal temperature range of 25 - 35°C. (Robertson and Gijs Kuenen, 2006). Previous modelling of autotrophic thiocyanate degradation also suggested high temperature sensitivity (Kim et al. 2013b). It is therefore crucial that temperatures are controlled in treatment processes, such as activated sludge, to ensure effective degradation. This is particularly important in temperate climates, such as the UK, where the natural wastewater temperature is between 8 - 21°C.

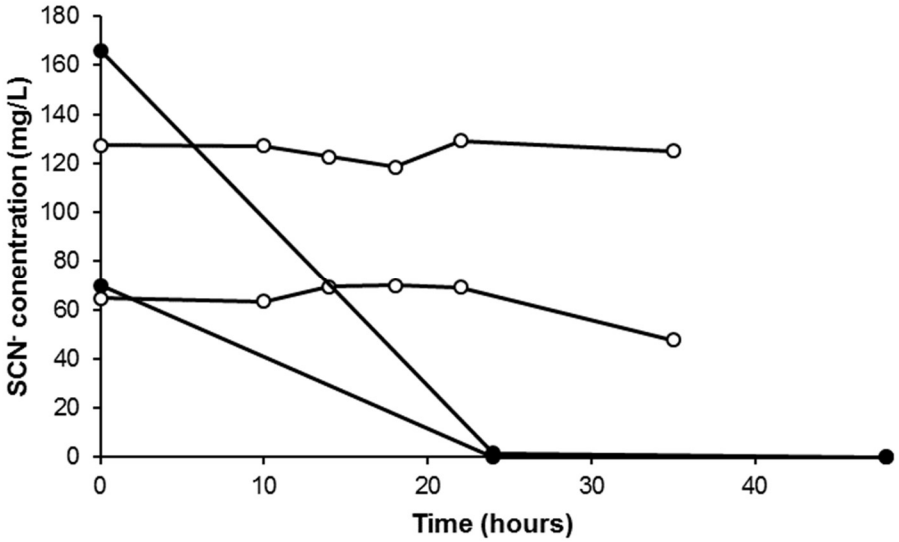

Figure 4-4: Impact of temperature on SCN^- degradation in mixed culture batch tests ● temperature maintained at 25 ± 1°C ○ temperature subject to diurnal temperature variation (8 - 21°C).

4.3.6 Molecular microbial ecology

Deoxyribonucleic acid sequencing analysis showed that the mixed culture was dominated by an uncultured species of *Thiobacillus* (26%) (Figure 4-5). The 16S sequence linked to SCN^- degradation in the mixed culture was previously identified by Bai et al. (2011), however, the sequence was not assigned to a species. The *Thiobacillus* genus was similarly the most abundant genus in a continuous flow bioreactor degrading SCN^- reported by Kantor et al. (2015). Furthermore, the activated sludge biomass contained a notable abundance of the genus *Mizugakiibacter* (13%), *Comamonas* (12%) and *Rhodanobacter* (11%) (Figure 4-1). *Mizugakiibacter* and *Rhodanobacter* are known for their iron-oxidising and nitrate reducing abilities (Wang *et al.*, 2017) whilst *Comamonas* has been associated with a wide range of abilities including the degradation of phenol (Zámocký *et al.*, 2001). Members of the *Rhodanobacter* and *Comamonas* genus were also observed in the bioreactors reported by Kantor et al. (2015).

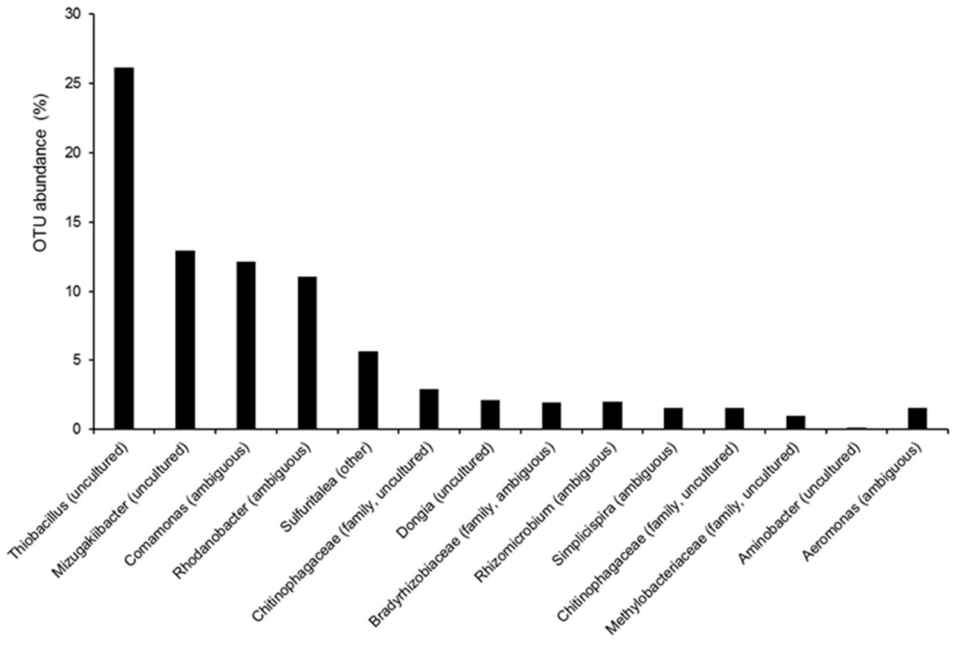

Figure 4-5: Molecular microbial analysis displaying operational taxonomic units (OTUs) and relative abundance in the activated sludge mixed culture determined through PCR gene sequencing.

The mixed culture was shown to effectively degrade SCN^- over a range of initial concentrations (Figure 4-1 and Table 4-3). The *Thiobacillus* genus has been recognised for SCN^- degradation for many years. Despite this, species within the *Thiobacillus* genus have been subjected to significant reclassification as from an original group of 17 species, only 3 species remain in the genus of *Thiobacillus* (*T. aquaesulis*, *T. thioparus* and *T. denitrificans*) (Kelly and Wood, 2000b). Of the *Thiobacillus* species, only *T. thioparus* and *T. denitrificans* have been documented to be capable of growing on thiocyanate as the sole source of energy (Kelly and Wood, 2000a) suggesting that the species present within the activated sludge were related to either *T. thioparus* or *T. denitrificans*. Whilst *T. thioparus* and *T. denitrificans* are genetically very similar (98% similarity) (Kelly and Wood, 2000a) *T. denitrificans* is distinguished from all other *Thiobacillus* species due to its ability to grow as a facultative anaerobe (Kelly and Wood, 2000a). *Thiobacillus thioparus* on the other hand is capable of reducing nitrate but not nitrite. Nitrite accumulation has been observed in the pre-denitrification ASP (Raper, Fisher, *et al.*, 2017) which could suggest a metabolic similarity to *T. thioparus*. Despite this, an ambiguous species of the genus *Rhodanobacter* was also identified in the mixed culture (Figure 4-5). As some species in this genus are capable of nitrate reduction but not nitrite reduction (Lee *et al.*, 2007) nitrite accumulation may also be attributed to other species in the mixed culture. As the SCN^- degradation investigation was focused on the properties of the mixed culture, it is therefore not possible to ascertain the full metabolic capability of SCN^- degraders. Further work is required to characterise the *Thiobacillus* species using pure cultures.

4.4 Conclusions

Thiocyanate degrading bacteria were the most abundant species in a mixed culture taken from an activated sludge process treating coke wastewater (26%). The mixed culture was capable of aerobic degradation whilst previous investigations demonstrated its capability to degrade SCN^- efficiently under

anoxic conditions. It was hypothesised that the mixed culture hydrolysed SCN^- to OCN^- and sulphide. Cyanate was subsequently hydrolysed to form NH_4^+ and HCO_3^- whilst sulphide was oxidised to form SO_4^{2-}. Ammonia was established to have an inhibitory action on SCN^- removal with an IC_{50} concentration of 316 mg/L. The addition of 180 mg/L of phenol also reduced SCN^- removal by 41%. The presence of NH_2OH was associated with a small inhibitory impact (9% at 16 mg/L). The mixed culture was able to degrade SCN^- at a range of concentrations, however, as the initial SCN^- concentration increased the end product formation changed. At SCN^- concentrations >110 mg/L SCN^- was hydrolysed but no ammonia production was observed. It is believed that the mixed culture degraded SCN^- to OCN^- and sulphide and that a rapid accumulation of OCN^- may have reduced the activity of cyanase. Additionally, SCN^-, degradation was sensitive to temperature changes with effective degradation being observed at mesophilic range. It is therefore important to control SCN^-, NH_4^+ and phenol concentrations in the influent of an ASP as well as temperature in order to achieve high SCN^- removal (>90%) and ensure compliance with the 4 mg/L SCN^- emission limit set by the Industrial Emissions Directive.

4.5 Acknowledgements

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Chapter 5 Enhancing the removal of pollutants in coke wastewater by bioaugmentation: A scoping study

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Abstract

BACKGROUND

Bioaugmentation and biostimulation were investigated for their ability to improve treatment efficiencies of thiocyanate (SCN^-), polycyclic aromatic hydrocarbons (PAHs), phenol and trace metals in coke wastewater. Additionally, the ability of the microorganisms supplemented with the bioaugmentation product to survive in a simulated river water discharge was evaluated.

RESULTS

A commercially available bioaugmentation product composed mainly of *Bacillus* sp. was mixed with activated sludge biomass. A dose of 0.5 g/L increased the removal of Σ6PAHs (sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene) by 51% and reduced SCN^- below 4 mg/L enabling compliance with the EU Industrial Emissions Directive (IED). Biostimulation (supplementing micronutrients and alkalinity) allowed compliance for both SCN^- and phenol (<0.5 mg/L).

Bacillus sp. accounted for 4.4% of the microbial population after 25 hours (1.5 g/L dose) which declined to 0.06% after exposure to river water (24 hours). Exposure of the activated sludge biomass to river water resulted in a 98.6% decline in viable cell counts.

CONCLUSION

To comply with the IED, bioaugmentation and biostimulation are recommended for the treatment of coke wastewater to enable an effluent Σ6PAHs of 6.6 µg/L, 0.3 mg/L phenol and 1.2 mg/L SCN^-. Such techniques are not anticipated to impact on downstream river water quality.

Keywords: Bioaugmentation; Biostimulation; Thiocyanate; Polycyclic aromatic hydrocarbons; Phenol; Trace metals

5.1 Introduction

Bioaugmentation involves the addition of microorganisms, selected for their specialised characteristics, to a treatment process in order to enhance removal of target pollutants whilst biostimulation involves the addition of supplements such as nutrients and micronutrients to improve microbial metabolism and consequently improve treatment efficiencies (Tyagi, da Fonseca and de Carvalho, 2011; Gerardi, 2015). Bioaugmentation and biostimulation, therefore, offer different routes by which an activated sludge process (ASP) can be upgraded to treat persistent pollutants. Bioaugmentation has been demonstrated to successfully improve the treatment of many industrial wastewaters whilst biostimulation has been reported to be important in nutrient limited industrial wastewaters (Burgess, Quarmby and Stephenson, 1999; Kim, Park, Lee, *et al.*, 2008; Bai *et al.*, 2010; Duque *et al.*, 2011; Martín-Hernández, Suárez-Ojeda and Carrera, 2012; Zhang *et al.*, 2014).

Coke wastewaters are formed in the production of coke, used in steel manufacturing, and originate from the quenching of hot coke masses, washing of ammonia stills and cooling and washing of coke oven gases (Kim, Park,

Jeon, *et al.*, 2008). Coke wastewaters contain a mixture of nitrogenous compounds and organic compounds, the concentrations of which are highly variable in response to the composition of the coals used in the coke ovens and the operational conditions (Marañón *et al.*, 2008). Coke wastewaters are typically characterised by ammonia concentrations of 50 - 500 mg/L, thiocyanate (SCN^-) concentrations of 100 - 400 mg/L and phenol concentrations of 60 – 400 mg/L (Vázquez *et al.*, 2006; Marañón *et al.*, 2008; Bai *et al.*, 2010). The sum of 6 polycyclic aromatic hydrocarbons (Σ6PAHs: sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene) was previously reported at 179 ±35 µg/L (Raper, Soares, *et al.*, 2017). Total trace metals (sum of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb) were also reported at a concentration of 4216 µg/L with individual trace metal concentrations ranging from 0.13 µg/L (Cd) to 3612 µg/L (Fe) (Raper, Soares, *et al.*, 2017). These wastewaters are regulated under the Industrial Emissions Directive (IED) and emission limits introduced in 2016 require that effluents are characterised by <4 mg/L SCN^-, <50 µg/L Σ6PAHs, <0.5 mg/L phenols and <1000 µg/L trace metals (sum of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and mercury (Hg)) (European Commission, 2013).

Coke wastewaters are typically treated through an ASP. Bioaugmentation has been shown to be successful in increasing the removal of phenol from industrial wastewaters. Duque et al. (2011) added a 2-fluorophenol degrading strain to a rotating biological contactor and demonstrated the ability of the strain to enable 2-fluorophenol degradation up to 50 mg/L. The strain was also able to cope with periods of substrate absence. Furthermore, the addition of phenol degrading bacteria to a biological contact oxidation reactor improved total phenol removal efficiencies from 66% to 80% (Fang *et al.*, 2013). Removal of phenol from coke wastewater was considered by Zhu, Tian and Chen (2012) who isolated two different strains of *Pseudomonas* (sp.PCT01 and PTS02). In synthetic solutions both strains performed similarly at phenol concentrations of ca. 235 and 460

mg/L, completely degrading the phenol within ca. 9 and 18 h respectively. In actual coke wastewater, with maximum phenol concentrations of 450 mg/L, degradation rates were reduced for both species. Although the investigation successfully isolated phenol degrading bacteria, the investigation used pure cells and did not investigate the ability of the added strains to survive in a mixed culture.

Biostimulation has been shown to have great potential for improved removal of PAHs. Sun et al. (2014) researched the impact of both bioaugmentation and biostimulation on the removal of PAHs from soil at a former coke work sites. Over 3 months total PAH concentrations dropped by 24% under control conditions, 35.9% with bioaugmentation and by 59% with biostimulation. Combined bioaugmentation and biostimulation resulted in only small improvements in total PAH removal compared to biostimulation alone, however, the combined action of biostimulation and bioaugmentation led to increased removal of heavy molecular weight PAHs. Bioaugmentation therefore had an important role in the removal of heavy molecular weight PAHs. Nutrient addition was also demonstrated to be important in the establishment of a cyanide degrading consortium treating effluent from a coke wastewater pre-denitrification ASP (Park *et al.*, 2008).

Whilst many studies have considered the ability of exogenous microorganisms to survive within a wastewater treatment process to be an important aspect of bioaugmentation, no study has considered the survivability in the receiving waterbody after treated effluent is discharged from an ASP. Survival of exogenous microorganisms in the receiving waterbody may result in ecological impacts which could potentially be detrimental to the balance of the ecosystem. Domestic wastewater treatment plants, for example, have been confirmed as sources of nitrifying bacteria in freshwater bodies (Brion and Billen, 2000). Whilst the seeding of nitrifying bacteria was typically beneficial in this study, introduction of other species could potentially have detrimental impacts to the

ecosystem as a result of changes in the composition of the bacterial population. The question therefore exists whether microorganisms supplemented through bioaugmentation are able to survive in ASP and subsequently receiving water bodies, opening up the possibility for negative impacts in the natural environment.

Although the full-scale coke wastewater treatment plant is capable of achieving high removal efficiencies for many pollutants, the high variability of the coke wastewater composition means that the ASP is not able to consistently meet the new IED emission limits, particularly for Σ6PAHs. Removal of Σ6PAHs to the emission limit of 50 µg/L is challenging, as coke making wastewaters are characterised by an abundance of heavy molecular weight PAHs which are characterised by high n-octanol/water partition coefficients (log K_{ow}) (Abu-salah *et al.*, 1996; Jeon and Madsen, 2013). Of the PAHs investigated, log K_{ow} values range from 5.12 - 7.66 (de Maagd *et al.*, 1998; Popp, Bauer and Wennrich, 2001). Additionally, although SCN^- removal efficiencies are high, typically 99%, the treatment of SCN^- has been recognised for its sensitivity (Staib and Lant, 2007). When instability occurs in the treatment process thiocyanate removal efficiencies are the first to decline after nitrification. Bioaugmentation and biostimulation may provide the answer. Despite this, bioaugmentation for the removal of PAHs has focussed on the treatment of contaminated soils and groundwater rather ASP applications (Straube *et al.*, 2003; Sun *et al.*, 2014).

This study is an initial investigation to understand the efficiency of bioaugmentation and biostimulation in the treatment of coke wastewater. The potential of bioaugmentation to remove SCN^- from coke wastewater was also investigated for the first time. Furthermore, the survivability of microorganisms supplemented through a bioaugmentation product in a simulated river water discharge was investigated, targeting a gap in knowledge within the field of bioaugmentation.

5.2 Experimental

5.2.1 Coke wastewater and activated sludge biomass

Coke wastewater was collected from a full-scale, wastewater treatment plant treating coke wastewater from a steel producing works. The wastewater had been subjected to tar separation and ammonia stripping and then combined with site drainage wastewater. Biomass used in batch tests was taken from the sites ASP operating under aerobic conditions and at a hydraulic retention time (HRT) of ca. 25 hours. Aeration was provided via a Vitox oxygen injection system. Temperature was maintained between 20 and 25^{o}C. Biomass was characterised by a sludge age of approximately 38 days.

5.2.2 Batch test to assess effectiveness of bioaugmentation towards pollutant removal and viability

Batch tests were conducted to assess the impact of bioaugmentation and biostimulation (micronutrient/alkalinity addition) on the treatment of SCN^-, Σ6PAHs, phenol and trace metals in the coke making wastewater. Coke wastewater and activated sludge biomass were combined to produce a mixed liquor suspended solids (MLSS) of 5400 mg/L replicating the full-scale ASP MLSS concentrations. Samples were placed on an incubated shaker plate (Grant-bio Orbital Shaker - Incubator ES-80) at 190 rpm and a temperature of 25^{o}C, for 25 hours to simulate the full-scale ASP. The impact of alkalinity addition, micronutrient addition and bioaugmentation was investigated by spiking the coke wastewater. Alkalinity was added at the previously optimised dose of 300 mg/L (as $CaCO_3$) through the addition of sodium carbonate (Na_2CO_3) (Raper, Fisher, *et al.*, 2017). A micronutrient solution was designed taking account of the coke wastewater characterisation and activated sludge nutrient requirements reported by Burgess, Quarmby and Stephenson (1999) (Table 5-1). A commercially available bioaugmentation product was added at doses of 0.1, 0.5 and 1.5 g/L. Reported bioaugmentation doses vary

significantly from 0.007 to 0.75 g/L, and do not appear to correlate to pollutant concentration. Therefore, doses tested in this study were selected to cover a broad range which would be feasible for full-scale applications (Jianlong *et al.*, 2002; Bai *et al.*, 2010). Samples were taken regularly. Thiocyanate was analysed at 0, 10, 13, 17, and 25 h. Phenol, sum nitrogen (sum of nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), ammonia-nitrogen (NH_4^+-N) and thiocyanate-nitrogen (SCN^--N)), soluble chemical oxygen demand (sCOD), PAHs and trace metals were analysed at 0 and 25 h.

Table 5-1: Composition of micronutrient solution used in batch tests.

Micronutrient	Concentration (mg/L)
Riboflavin (B2)	1
Pyroxidine hydrochloride (B6)	0.2
Vitamin B12	0.5
Niacin	1
Biotin	0.5
Phosphate	3
Calcium	1
Magnesium	3
Copper	0.03
Zinc	0.5
Molybdenum	0.4

5.2.3 Batch tests to investigate viability of the bioaugmentation product

Samples of mixed liquor were taken from the batch tests at 0 h and 25 h to assess the ability of the microorganisms supplemented through the bioaugmentation product to survive. Additionally, further batch tests were completed to mimic the effluent discharge into a receiving river water taking into

consideration an existing real scenario. Mixed liquor from the bioaugmentation batch tests was diluted with river water at a ratio of 1:1,690 to account for the dilution effect upon discharge according to typical river water volumes of the river receiving discharge from the full-scale treatment plant. The mixed liquor and river water batch tests were placed on an incubated shaker plate at 190 rpm and a temperature of 25^{o}C. Furthermore, a worst-case scenario approach was used and it was assumed that removal of activated sludge microorganisms from the effluent was low, due to poor settling in the secondary sedimentation tank in the wastewater treatment plant.

5.2.4 Chemical analysis

Samples were immediately filtered through 0.45 µm filters (VacuCap 90, Pall Corporation). Nitrite-nitrogen, NO_3^--N, NH_4^+-N and sCOD were analysed using Merck cell test kits according to the manufacturer's instructions. Thiocyanate was analysed colourmetrically by complex reaction with iron (III) at a wavelength of 465 nm (based on The Institute of Gas Engineers analytical method for thiocyanate) (The Institution of Gas Engineers, 1971). Phenol (mono) was analysed by complex reaction with 4-aminoantipyrene at a wavelength of 510 nm (based on ISO 6439:1990) (ISO, 1990). Both were analysed using a UNICAM spectrophotometer. pH was recorded using a Jenway 3540 pH meter (UK). Total nitrogen was calculated through the sum of NO_2^--N, NO_3^--N, NH_4^+-N and SCN^--N. Although TN is defined as containing organic nitrogen, coke wastewaters contain very little organic nitrogen and therefore the method used is a good approximation (Vázquez *et al.*, 2007). Total suspended solids were analysed according to standard methods (Eaton, 2005). Trace metals were analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) according to BS EN 14385:2004 (British Standards Institution, 2004). Polycyclic aromatic hydrocarbons were extracted using dichloromethane and then analysed by Gas Chromatography Mass Spectrometry according to US EPA Method 8270 (US EPA, 1996).

5.2.5 Bacterial speciation and viability

Flow cytometry was used to count the number of viable cells in the batch test samples based on the method described by Lipphaus et al. (2014). Samples were diluted with Evian water (Evian, Évian-les-Bains, France), filter-sterilized through a 0.2 µm filter and stained using SYBR® Green I (Life Technologies Ltd., Paisley, UK) and propidium iodide (Life Technologies Ltd., Paisley, UK) as staining agents. A vortex mixer was used to ensure cells were evenly distributed and to ensure effective staining. Samples were then incubated for 13 minutes at 37°C before being analysed on the flow cytometer, BD Accuri C6 with a 488nm solid-state laser (Becton Dickinson U.K. Ltd., Oxford, UK), using the standard gate method (Gatza, Hammes and Prest, 2013). In cells with membrane damage the propidium iodide partially replaces the SYBR® Green I, a change which is detected primarily in the FL3 detector (emission filter 670 LP). Viable cell counts can therefore be obtained by excluding propidium iodide fluorescent cells from the remaining SYBR® Green I stained cell count.

Polymerase chain reaction (PCR) was used to identify different microbial genera and species present in the bioaugmentation and river simulated batch tests. River simulation samples which were aqueous in nature were filtered through a sterile 0.2 µm membrane polycarbonate filter (GE Life Sciences, UK) and placed in a lysing matrix tube, whilst mixed liquor from the batch tests was placed directly in the lysing matrix tube. Deoxyribonucleic acid (DNA) was extracted using the MPBIO FastDNA Spin Kit for soil (Santa Ana, USA). The V4 and V5 regions of the 16S ribosomal RNA gene were targeted with the universal primers 515F and 926R (Quince *et al.*, 2011). Error correcting golay barcodes enabled sample multiplexing (Hamady *et al.*, 2012). HighPrep magnetic beads (Magbio, Gaithersburg USA) were used to purify the PCR products which were subsequently purified using QuantiFluor ONE (Promega, Madison USA). An equimolar pool of amplicons was sequenced using Illumina MiSeq with 2x300 v2 chemistry (Illumina, San Diego USA). The number of reads per sample varied from 338000 to 1.6M after quality filtering. The

sequences were analysed using QIIME 1.9 (Caporaso *et al.*, 2010) and were grouped at 97% similarity to create operational taxonomic units (OTUs). Representative sequences from each OTU were then taxonomically assigned using the SILVA 16S rRNA gene database v123.1 (Quast *et al.*, 2013). The identification of a sequence which matched unambiguously to a sequence in the database at a 97% similarity gave an exact species identification. An 16S sequence unambiguously matched to a database sequence but for which the taxonomy was unavailable resulted in a species being defined as “uncultured”. A 16S sequence which was identical to more than one sequence of the genus (at a 97% similarity) was defined as “ambiguous”. A 16S sequence for which the species data did not exist but was identified at genus level was defined as “other”.

5.3 Results and Discussion

5.3.1 Coke wastewater characterisation

The coke wastewater was characterised by a pH of 8.2 and a sCOD of 638 mg/L. The wastewater contained 128 mg/L and 74 mg/L of SCN^- and phenol (Table 5-2) respectively. Additionally, the wastewater contained NH_4^+-N, NO_3^--N and NO_2^--N at 89 mg/L, 9 mg/L and 13 mg/L respectively. Dissolved Σ6PAHs and trace metals were measured on the batch test influent wastewater at 3.5 µg/L and 47.2 µg/L respectively. Total Σ6PAHs and trace metals analysed on wastewater from the full-scale site averages at 170±30 µg/L and 132 ±23 µg/L respectively. It can be observed that there is a large difference between the total and dissolved PAHs and trace metals suggesting that they are associated with the suspended solids.

Table 5-2: Coke wastewater characterisation.

	Concentration (mg/L) and standard deviation
sCOD	638 ± 8
Phenol (mono)	74 ± 1
SCN^-	128 ± 11
NH_4^+-N	89 ± 1
NO_3^--N	9 ± 1
NO_2^--N	13 ± 1
PAHs (total) (µg/L)* †	170 ±30
PAHs (dissolved) (µg/L)*	3.5
Trace metals (total) (µg/L)** †	132 ±23
Total metals (dissolved) **	47.2
pH	8.2 ± 0

* Sum of fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene

** Sum of As, Cd, Cr, Cu, Pb, Ni and Zn.

† Average data taken from full-scale site

5.3.2 Impact of bioaugmentation and biostimulation on SCN^- removal

Thiocyanate degradation took place in 3 phases: acclimatisation, rapid degradation and reduced degradation. Under control conditions SCN^- declined by 58 mg/L over the first 13 hours as the biomass became acclimatised to the wastewater. After this acclimatisation, the SCN^- declined rapidly by 58 mg/L in 4 hours. The degradation then continued at a reduced rate with a further decline of 38 mg/L between 17 hours and 25 hours (Figure 5-1). All test conditions demonstrated this 3-phase removal trend.

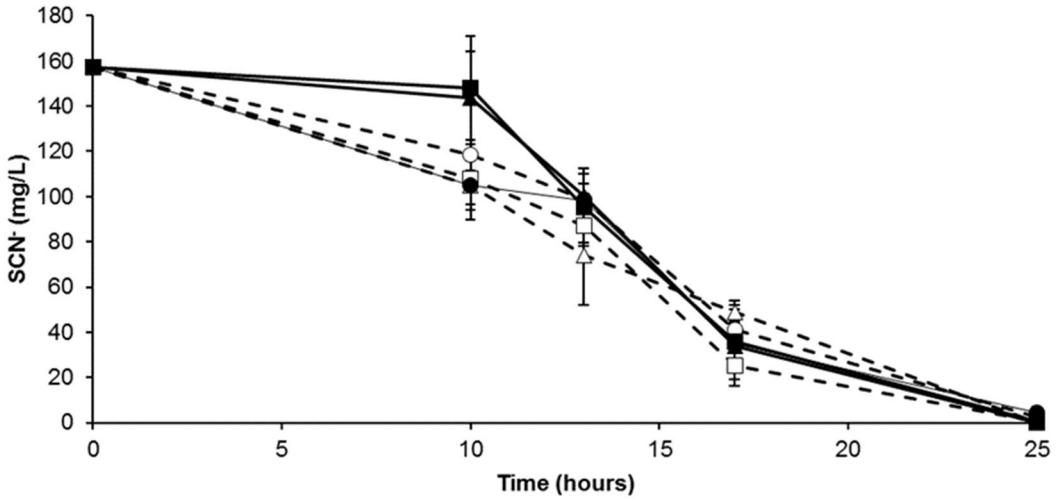

Figure 5-1: Impact of bioaugmentation and biostimulation on SCN^- degradation ○ - Control, □ -micronutrients, ∆ - alkalinity and bioaugmentation doses of ● - 0.1 g/L ■ - 0.5 g/L ▲- 1.5 g/L.

The addition of micronutrients led to a 9% increase in degradation, in the first 10 hours, compared to control conditions with SCN^- concentrations decreasing to 108 ± 11 mg/L. This increased degradation continued with a 12% improvement at 13 hours and a more substantial 39% improvement being observed at 17 hours. After 25 hours, SCN^- concentrations fell to 0.7 ± 1.2 mg/L compared to 2.7 ± 4.6 mg/L under control conditions allowing consistent compliance with the <4 mg/L SCN^- emission limit. The addition of micronutrients therefore had a marked benefit on SCN^- degradation kinetics and enabled a small but important improvement in SCN^- degradation after 25 hours, ensuring compliance with the IED. This suggests that coke wastewater does not contain the required micronutrients for the indigenous SCN^- degraders, which is a common occurrence in industrial wastewater (Burgess, Quarmby and Stephenson, 1999). Similar to micronutrient addition, the provision of alkalinity improved SCN^- removal. After 13 hours of incubation in batch tests, there was a 16% difference in SCN^- removal between the control and tests with added alkalinity. Whilst this difference declined to 1.7% at 25 hours, degradation of SCN^- was complete, therefore alkalinity addition could also ensure compliance with the 4

mg/L SCN^- emission limit. The activated sludge used in these tests had a high abundance of *Thiobacillus* (26%) (Figure 5-2), which are species known to be involved in SCN^- degradation. As SCN^- degraders are autotrophic in nature, the improved SCN^- removal through biostimulation may be associated with the increased concentrations of the required micronutrients or inorganic carbon associated with alkalinity addition which may be utilised by autotrophic thiocyanate degraders present in the biomass (Staib and Lant, 2007; Raper, Stephenson, *et al.*, 2017).

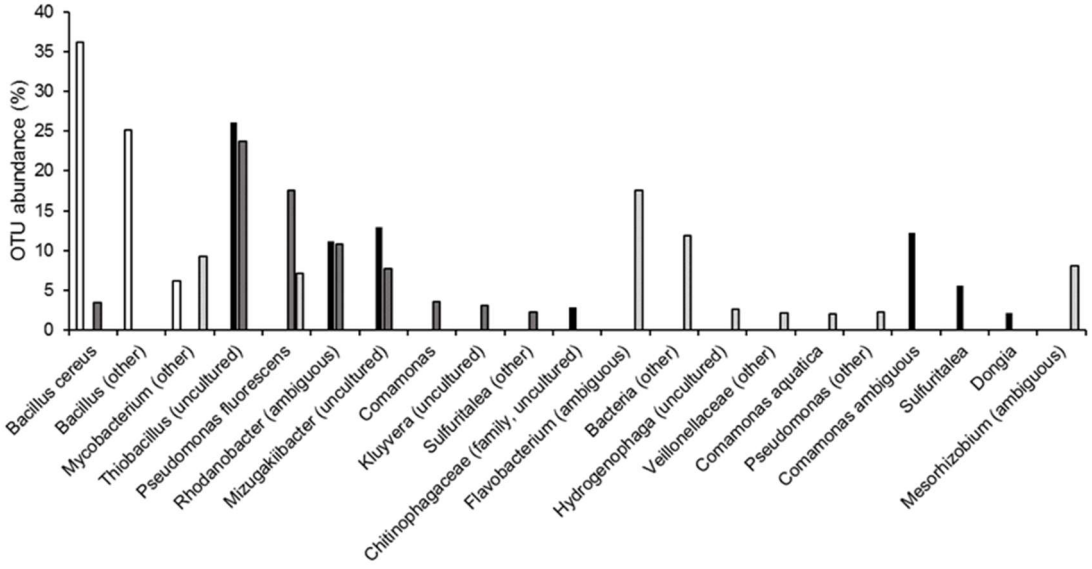

Figure 5-2: Operational taxonomic unit (OTU) abundance □ Bioaugmentation product ■ Inoculum biomass ■ Bioaugmentation batch test effluent (1.5 g/L) ■ Bioaugmentation batch test effluent (1.5 g/L) after 25 hours contact with river water. Uncultured: 16S sequence unambiguously matched to a database sequence but taxonomy was unavailable. Ambiguous: 16S sequence was identical to more than one sequence of the species (at a 97% similarity). Other: 16S sequence belongs to the genus but species data did not exist in the database.

A bioaugmentation dose of 0.1 g/L resulted in a similar degradation trend to control conditions in the first 13 hours. However, final SCN^- concentrations at 25 hours were higher than under control conditions at 4.3 ± 2 mg/L. The addition of bioaugmentation product at 0.1 g/L therefore offered no benefit to SCN^- degradation over a 25 hour period. At an increased dose of 0.5 g/L there was a notable delay in the time required for degradation to proceed. This delay may be associated with the acclimatisation required for the bioaugmented bacteria to adapt to the wastewater conditions or increased competition between species (Bouchez *et al.*, 2000). Despite the initial delay in degradation, by 13 hours the average SCN^- concentration was comparable to control conditions at 95 ± 17 mg/L. At 25 hours the final SCN^- concentration was 0.7 ± 1.2. This therefore offered a small improvement compared with control tests and ensured compliance with the emission limit. A dose of 1.5 g/L resulted in complete degradation of SCN^-. Figure 5-3 shows the bacterial speciation of the bioaugmentation product which was dominated by *Bacillus* sp. (91%) and *Mycobacterium* (other) (9%). Improved degradation may therefore be associated with the bioaugmentation product as some *Bacillus* sp. are associated with SCN^- removal (Lee *et al.*, 2003).

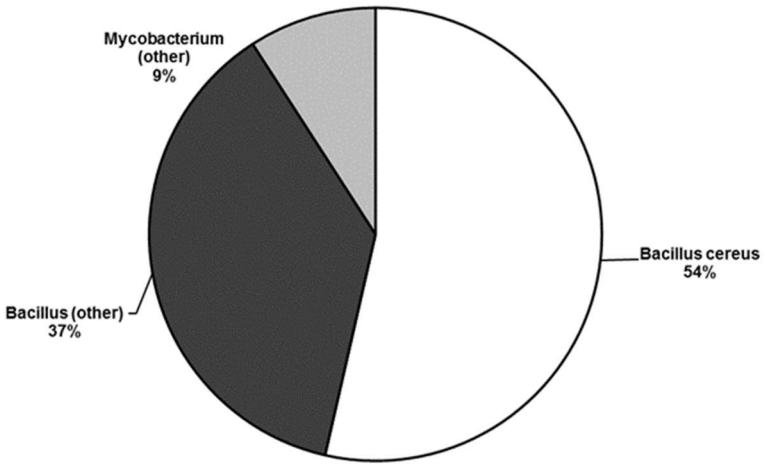

Figure 5-3: Bioaugmentation product composition according to PCR analysis and operational taxonomic unit assignment using the SILVA 16S rRNA gene database.

5.3.3 Impact of bioaugmentation and biostimulation on Σ6PAH removal

The IED applies to total PAHs and compliance with the 50 µg/L emission limit is challenging. As the PAHs need to be solubilised in order for microbial degradation to occur a focus was therefore given on the ability of bioaugmentation and biostimulation to improve the removal of dissolved PAHs (Table 5-3). The concentration of Σ6PAHs, under control conditions, increased from 3.5 µg/L to 13.4 µg/L after 25 hours. This increase is believed to result from desorption of PAHs from the suspended biomass solids. After 25 hours, under control conditions, benzo[a]pyrene and benzo[b/k]fluoranthene, both characterised by a molecular weight of 252 g/mol accounted for 66% of the Σ6PAHs at a concentration of 5.2 and 3.6 µg/L respectively. The higher the number of fused rings in a PAH compound the higher the molecular weight and greater the persistence of the compound. Indeno[1,2,3-cd)perylene and benzo[g,h,i]perylene, both with a molecular weight of 276 g/mol, had lower concentrations of 1.7 and 1.9 µg/L respectively. As they are characterised by higher molecular weights their lower concentration may be explained by lower rates of desorption. Fluoranthene, characterised by the lowest molecular weight of 202 g/mol and therefore most easily degraded was present at 1.0 µg/L.

The addition of micronutrients led to no improvement in Σ6PAHs removal. At 12.7 µg/L Σ6PAHs were comparable to control conditions (13.4 µg/L). Micronutrient limitations have previously noted as a possible cause behind the failure of benzo[a]pyrene degradation (Juhasz and Naidu, 2000). Despite this, the current investigation suggests that the availability of micronutrients is not a limiting factor for the removal of PAHs in coke wastewater. Bioaugmentation, on the other hand, was beneficial to the removal of Σ6PAHs at a dose of 0.5 g/L. A notable improvement in PAH removal was observed with Σ6PAHs declining to 6.6 µg/L, representing a 51% decrease in Σ6PAH concentrations. Lower molecular weight PAHs are more prone to microbial attack and degradation which explains the high removal of fluoranthene which declined by 60% to 0.4

µg/L. A 50% reduction was observed for both benzo[b/k]fluoranthene and benzo[a]pyrene. For the highest molecular weight compounds, indeno[1,2,3-cd)perylene and benzo[g,h,i]perylene, a 53% reduction was also observed with concentrations falling to 0.8 and 0.9 µg/L respectively. The improved removals were noteworthy due to the abundance of heavy molecular weight PAHs. At a lower dose (0.1 g/L) there was no improvement to Σ6PAH removal (16.4 µg/L). Additionally, tripling the bioaugmentation dose only led to a further 10% improvement in Σ6PAH removal.

The bioaugmentation product was dominated by *Bacillus cereus* (54%), *Bacillus* (other) (37%) and *Mycobacterium* (other) (9%) (Figure 5-3) which have been associated with the degradation of a range of PAHs (Samanta, Singh and Jain, 2002; Haritash and Kaushik, 2009). For instance, *Mycobacterium* are known for their good catabolic capabilities for PAHs with 5-benzene rings as they have mycolic acids which aid in the uptake of hydrophobic PAHs (Johnsen, Wick and Harms, 2005; Haritash and Kaushik, 2009; García-Díaz *et al.*, 2013; Mueller-Spitz and Crawford, 2013). The failure of bioaugmentation at 0.1 g/L suggests that the exogenous microorganisms were unable to establish themselves within the activated sludge mixed liquor. As higher doses had a positive impact on Σ6PAH removal, it is likely that the inoculum size at the 0.1 g/L dose was simply insufficient to over-come pressures such as grazing by protozoa and or insufficient numbers to be able to compete with the indigenous population (Ramadan, El-Tayeb and Alexander, 1990). The increased removal efficiency of Σ6PAHs at a dose of 0.5 g/L suggests that the exogenous microorganisms quickly acclimatised to the wastewater and laboratory conditions and enhanced the indigenous population of PAH degrading bacteria. Increased doses did not result in substantial improvements in the removal of Σ6PAHs suggesting that another factor became limiting in the system such as nutrients and carbon. Furthermore, the microbial degradation of PAHs can also be limited by the rate at which PAHs can be transferred to the microbial cells (Johnsen, Wick and Harms, 2005).

5.3.4 Impact of bioaugmentation and biostimulation on trace metal removal

The impact of bioaugmentation and biostimulation was subsequently investigated for trace metal removal (Table 5-4). Under control conditions the sum trace metals increased from 47.2 to 56.4 µg/L. This increase was believed to be the result of desorption from the suspended solids which can be impacted by changes to the pH and mass flux balances (Du Laing *et al.*, 2009). The addition of micronutrients and bioaugmentation led to little impact to the sum trace metal concentration (Table 5-4). Although small improvements were observed through bioaugmentation the percentage improvements were within the method uncertainty range and therefore no clear conclusions could be drawn. The main improvement seen was for Zn which was reduced from 13 µg/L (control conditions) to 1 µg/L at a dose of 0.1 g/L and 1.5 g/L. Despite this, at 0.5 g/L Zn removal was lower (9 µg/L) giving an unclear correlation between bioaugmentation dose and removal efficiency.

5.3.5 Impact of bioaugmentation and biostimulation on sCOD and nitrogen removal

The removal efficiency for sCOD under control conditions was 97%. Similarly, sCOD remained at 97% in all the biostimulation tests and bioaugmentation tests with doses of 0.1 and 0.5 g/L. However, at the higher dose of 1.5 g/L, the sCOD removal efficiency dropped to 88%, with 53 mg/L sCOD remaining. This may have resulted from bacterial degradation through endogenous metabolism due increased competition and reduced survival (Bouchez *et al.*, 2000).

Table 5-3: Impact of bioaugmentation and biostimulation on effluent PAH concentrations. Removal efficiencies in relation to the control are reported in brackets e.g. (21%).

	µg/L					
	Fluoranthene	**Benzo(b/k) fluoranthene**	**Benzo(a)pyrene**	**Indeno (1,2,3-cd) pyrene**	**Benzo(g,h,i)perylene**	**Σ6PAHs**
Molecular weight (g/mol)	202	252	252	276	276	
Start conditions	0.6	1.3	0.8	0.4	0.4	3.5
Control	1.0	5.2	3.6	1.7	1.9	13.4
Biostimulation:						
Micronutrient addition	0.8 (21%)	5.1 (1.9%)	3.5 (2.8%)	1.6 (5.9%)	1.7 (10.5%)	12.7 (5.3%)
Bioaugmentation dose:						
0.1 g/L	0.9 (13%)	6.4 (-)	4.6 (-)	2.2 (-)	2.3 (-)	16.4 (-)
0.5 g/L	0.4 (56%)	2.6 (50%)	1.8 (50%)	0.8 (50.6%)	0.9 (53.2%)	6.6 (51%)
1.5 g/L	0.3 (68%)	2.1 (59.6%)	1.5 (58.3%)	0.7 (60%)	0.7 (61.1%)	5.3 (60.1%)

Relative standard deviation of method: fluoranthene 14%, benzo(b/k)fluoranthene 14.9%, benzo(a)pyrene 8.1%, indeno(1,2,3-cd)pyrene 13.8%, benzo(g,h,i)perylene 14.2%.

Table 5-4: Impact of bioaugmentation and biostimulation on trace metal concentration. Removal efficiencies in relation to the control are reported in brackets e.g. (25%).

	µg/L							
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**	**Sum**
Start	9.5	0.01	15	0.3	0.2	17	5	47.2
Control	7.1	0.01	16	5.9	1.3	13	13	56.4
Biostimulation:								
Micronutrients	7.7 (25%)	nd	15 (6.3%)	4.2 (29%)	0.9 (30.8%)	10 (23%)	16 (-)	54.5 (4.5%)
Bioaugmentation dose:								
0.1 g/L	6.7 (-)	0.01 (-)	20 (-)	3.2 (46%)	1.4 (-)	12 (7%)	1 (92.3%)	44.4 (17.6%)
0.5 g/L	6.9 (-)	0.01 (-)	17 (-)	3.8 (36%)	1.3 (-)	13 (-)	9 (30.8%)	51.1 (-)
1.5 g/L	7.3 (8.3%)	0.01 (-)	17 (-)	4.5(24%)	1.3 (-)	15 (-)	1 (92.3%)	46.2 (9.6%)

Relative standard deviation of method: As 11.9%, Cd 9.7%, Cr 15.5%, Cu 20.1%, Pb 16.9%, Ni 10.6%, Zn 26.2%

Phenol removal efficiencies were unaffected by bioaugmentation with all tests showing a removal of >98%. Under all conditions phenol was reduced from 74 mg/L to 1 mg/L and below. Alkalinity addition led to a reduction in phenol concentration to 0.3 mg/L allowing compliance with the <0.5 mg/L emission limit. Ammonia-nitrogen concentrations were expected to increase as a result of SCN^- degradation (Kim, Park, Jeon, *et al.*, 2008; Raper, Stephenson, *et al.*, 2017). Alkalinity addition, however, led to ammonia-nitrogen removal (Table 5-5) which was consistent with the stimulation of autotrophic nitrifying bacteria.

5.3.6 Bacterial speciation, abundance and viable cell counts

It was important to understand whether the addition of exogenous microorganisms through bioaugmentation would impact microorganism speciation in the receiving river waterbody and or the viable cell counts present after exposure to river water. A Special Area of Protection (SPA) exists downstream of the discharge point for effluent from the full-scale coke wastewater treatment ASP which then subsequently flows into an estuary which is designated as a Special Area of Conservation (SAC), under the EU Habitats Directive (Joint Nature Conservation Committee, 2005, no date). As such it is important to understand whether the exogenous microorganisms hold the potential to have negative consequences within the receiving waterbody as a result of interactions with the native microorganisms such as competition and predation which may have further impacts higher up the food chain resulting in further impacts to the ecosystem.

Table 5-5: Impact of bioaugmentation and biostimulation on sCOD, phenol and ammonia-nitrogen in batch tests after 25 h of incubation.

	pH		sCOD			Phenol			Ammonia-nitrogen		
	0 h	25 h	0 h (mg/L)	25 h (mg/L)	Removal efficiency (%)	0 h (mg/L)	25 h (mg/L)	Removal efficiency (%)	0 h (mg/L)	25 h (mg/L)	Removal efficiency (%)
Control	7.2	7.6 ±0.3	422 ±50	12	97	74 ± 7	0.9 ± 0.5	99 ± 0.5	69 ± 4	93 ± 2	0
Biostimulation:											
Micronutrients	7.2	7.7 ± 0.1	″	″	″	″	1.2 ± 0.8	98 ± 1.1	″	89 ± 1	6
Alkalinity	8.5	8.2 ±0.1	″	″	″	″	0.3 ± 0.3	100 ±0.3	″	68 ± 3	0
Bioaugmentation dose:											
0.1 g/L	7.2	7.6 ± 0	″	″	″	″	0.9 ± 0.3	99 ± 0.3	″	95 ± 1	0
0.5 g/L	7.2	7.6 ± 0.1	″	″	″	″	1.0 ± 0.2	99 ± 0.2	″	95 ± 2	0
1.5 g/L	7.2	7.5 ± 0.1	″	52 ± 2	88 ± 3	″	1.0 ± 0.1	99 ± 0.1	″	94 ±2	0

Figure 5-2 shows the OTU abundance for the bioaugmentation product, indigenous biomass, batch test effluent combined with river water and batch test effluent combined with river water after 24 hours. It can be observed that the indigenous biomass was characterised by a high abundance of an uncultured species of *Thiobacillus* (26%), an uncultured species of *Mizugakiibacter* (13%), an ambiguous species of *Rhodanobacter* (11%) and an ambiguous species of *Comamonas* (12%). *Thiobacillus* is associated with the degradation of SCN^- whilst *Mizugakiibacter* and *Rhodanobacter* have been associated with their iron-oxidising and nitrate reducing abilities (Raper, Stephenson, *et al.*, 2017; Wang *et al.*, 2017). *Comamonas* bacteria have been associated with a wide range of abilities including the degradation of phenol (Zámocký *et al.*, 2001).

The abundance of OTUs was tracked in the batch test with the addition of 1.5 g/L of the bioaugmentation product. After 25 hours under batch test conditions *Bacillus cereus* was detected, at an abundance of 3.4% whilst *Bacillus* (other) was detected at an abundance of 0.96% (Figure 5-2). This suggests that some of the inoculated *Bacillus* bacteria were maintained in the activated sludge. A total abundance of 4.4% *Bacillus* species suggests that the population was potentially still able to play a role in the activated sludge like other species present in relatively low abundances such as nitrifying bacterial populations which account for 3-10% of the bacterial population in an ASP (Gerardi, 2002). Long-term studies would be required, however, to assess whether the population was sustained or whether maintenance dosing would be required (Ramadan, El-Tayeb and Alexander, 1990; Boon *et al.*, 2003). *Mycobacterium* on the other hand decreased to an abundance of just 0.03%. It is possible that this species was outcompeted by indigenous bacteria or was unable to survive in the coke wastewater as a result of toxic compounds such as phenol and SCN^- (Goldstein, Mallory and Alexander, 1985; Martín-Hernández, Suárez-Ojeda and Carrera, 2012). The OTU abundance data can give an indication of the causes behind the failure of a dose of 0.1 g/L to impact PAHs removal. At

this lower dose, the species abundance may have been too low within the activated sludge to play an important degradative role.

Of particular interest was the ability of the exogenous bacteria to survive simulated river water discharge. When effluent from the batch test was exposed to river water for 24 hours *Bacillus* sp. were detected at just 0.06%. *Mycobacterium* (found in the bioaugmentation product) was detected, however, this was identified as an indigenous species to the river water (abundance of 4%) and as the abundance from the activated sludge was very low the increased abundance is believed to be associated with their presence in the river water (Figure 5-2). Operational taxonomic units associated with the bioaugmentation product did not therefore become abundant in the river water suggesting that they may have succumbed to predatory pressures.

Viable cell counts increased in line with the bioaugmentation dose from 2.07 x 10^8 (control) to 2.17 x 10^8 (dose of 0.1 g/L), 2.30 x 10^8 (dose of 0.5 g/L) and 2.52 x 10^8 (dose of 1.5 g/L) (Figure 5-4). It is possible that this was attributed to the synergistic activities of the exogenous and indigenous bacteria. The number of viable cells declined firstly as a result of dilution. This decline was lower than expected according to the theoretical according to the dilution ratio (1:1,690) which was associated with the large dilution ratio which applied and the variability of the cell count within the river water. The dilution represented an average viable cell count decline of 93%.

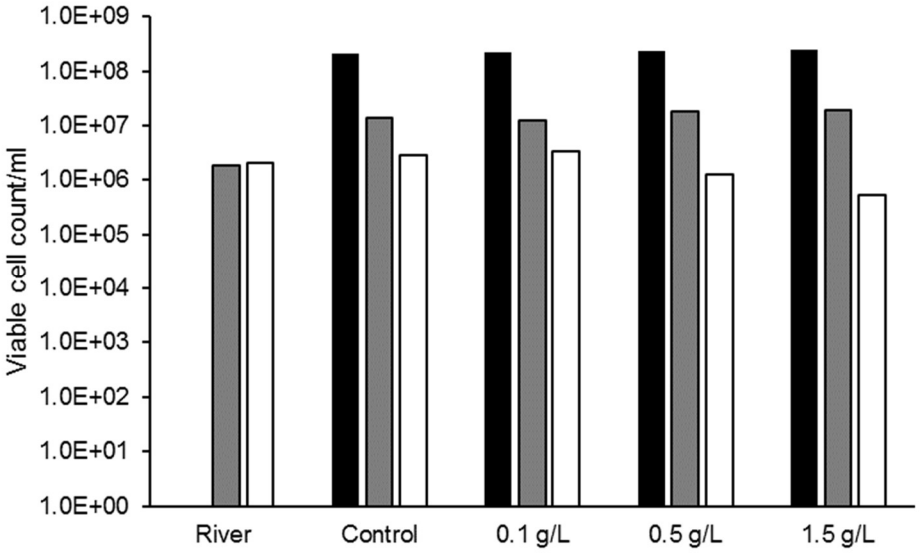

Figure 5-4: Impact of bioaugmentation on viable cell counts ■ - Bioaugmentation batch test effluent, ■ - Bioaugmentation batch test effluent after 0 hours contact with river water and □ - Bioaugmentation batch test effluent after 25 hours contact with river water.

After 24 hours exposure to the river water under typical treatment conditions (control) the viable cell count declined to 2.9 x 10^6 representing a further reduction in the viable cell count of 89%. The reduction in the number of viable cells may have resulted from competition between for the limited resources available in the receiving waterbody or due to the inability of the activated sludge bacteria to survive under the environmental conditions associated with the river water. The number of viable cells was therefore 98.6% lower than in the original activated sludge biomass. As a worst-case scenario was modelled, assuming no settling in the clarifier, it would be expected that viable cell count reductions would in fact be higher under normal operational conditions. Similar reductions were observed when bioaugmentation was applied.

5.4 Conclusion

Bioaugmentation using a commercially available product rich in *Bacillus* and *Mycobacterium* sp. at a dose of 0.5 g/L resulted in a 51% improvement in the removal of Σ6PAHs and enabled compliance with the SCN^- emission limit of <4 mg/L. Thiocyanate removal was also improved by both micronutrient and alkalinity addition ensuring compliance with the emission limit. Phenol removal was improved by alkalinity addition typically enabling compliance with the 0.5 mg/L emission limit. Biostimulation should be optimised for the removal of SCN^- and phenol. Operational taxonomic unit abundance data showed that the exogenous bacteria added through bioaugmentation at a dose of 1.5 g/L, accounted for 4.4% of the activated sludge biomass after 25 hours. After the activated sludge biomass was exposed to river water for 24 hours *Bacillus* sp. associated with the bioaugmentation product were detected at 0.06% therefore showing a significant reduction in OTU abundance. The viable cell count for the activated sludge biomass declined by 98.6% after 24 hours exposure to river water suggesting low survival of bacterial cells in the river water. Bioaugmentation and biostimulation are recommended for their application to coke wastewater having been demonstrated to be capable of producing an effluent characterised by an effluent Σ6PAHs of 6.6 µg/L, 0.3 mg/L phenol and 1.2 mg/L SCN^- which complies with the IED emission limit. Bioaugmentation is not anticipated to impact on downstream river water quality.

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Chapter 6 Nitrification and Denitrification of Coke wastewaters

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Abstract

The Industrial Emissions Directive (IED) requires that coke wastewater is treated to reach an effluent with <50 mg/L total nitrogen (TN). A shortage of alkalinity (3.6 mg as $CaCO_3$/mg NH_4^+-N) in the wastewater limited nitrification to 45%. Various compounds were tested as a source of additional alkalinity, with optimal results being found for sodium carbonate, which enabled 95% nitrification at 300 mg/L (as $CaCO_3$). Sodium bicarbonate led to incomplete ammonia oxidation (76%) whilst soda ash prevented nitrite oxidation. Addition of sodium hydroxide enabled 98% nitrification but was associated with NO_2^--N accumulation. Ammonia and nitrite oxidation had optimal pH ranges of 7.0 - 8.3 and 5.5 - 6.8, respectively. Due to the variable composition of coke wastewater, external organic carbon was also considered to enhance denitrification. A laboratory-scale anoxic-aerobic activated sludge process (ASP) was used to investigate glycerol and acetic acid as carbon sources. Glycerol was associated with a low biomass production (0.18 mg of biomass produced per 1 mg of glycerol) and mixed liquor suspended solids (MLSS) declined from 2235 mg/L to 750 mg/L leading to incomplete nitrification (<30%) and an effluent TN of 59 mg/L. Acetic acid had a higher biomass production (0.31 mg of biomass produced per 1 mg of acetic acid) maintaining stable MLSS concentrations (3137 mg/L). Overall, a denitrification-nitrification process with alkalinity (Na_2CO_3 at 300 mg/L) and acetic acid dosing enabled an effluent TN of 24 mg/L.

Keywords: Nitrification; denitrification; external carbon; industrial wastewater

6.1 Introduction

The production of coke within the steel industry produces significant volumes of wastewater which contain high concentrations of nitrogen (Staib and Lant, 2007; Marañón *et al.*, 2008; Pal and Kumar, 2014). Within Europe, coke wastewaters are regulated under the Industrial Emissions Directive (IED) which now requires the removal of total nitrogen (TN), consisting of the sum of ammonia-nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite-nitrogen (NO_2^--N) to <50 mg/L (European Commission, 2013). Further pollutants found in coke wastewater include phenol (60 - 400 mg/L) and thiocyanate (SCN^-) (100 - 400 mg/L) which increase the toxicity of the wastewater (Staib and Lant, 2007; Marañón *et al.*, 2008; Raper, Soares, *et al.*, 2017). Moreover, for each mole of SCN^- degraded, 0.24 moles of NH_4^+-N are produced which leads to increasing ammonia concentrations during the treatment process (Kim *et al.*, 2008). Treatment of coke wastewater has commonly consisted of treatment using an activated sludge process (ASP) although other pre or post-treatment chemical treatment methods may be included as part of the process (Pal and Kumar, 2014). Due to new requirements for TN removal, treatment plants must include both nitrification and denitrification with the pre-denitrification approach having been highlighted as the best available technique (BAT) in the best available techniques reference (BREF) document (European Commission, 2013) which consists of an anoxic-aerobic ASP.

Nitrification involves the biological oxidation of ammonia to nitrite by ammonia-oxidising bacteria (AOB) (typically *Nitrosomonas)* and subsequently to nitrate by nitrite-oxidising bacteria (NOB) (typically *Nitrobacter*). Recently, however, some species have been identified which can carry out complete oxidation of ammonia (comammox) to nitrate (van Kessel *et al.*, 2015). Nitrifying bacteria are notably sensitive to pH with the optimum pH for AOB and NOB being reported as 7.9 - 8.2 and 7.2 - 7.6 respectively (Anthonisen *et al.*, 1976). Despite this, it is well recognised that the optimum pH can be influenced by

different environmental conditions and the different genera of AOB and NOB which are present (Cho *et al.*, 2014). In the treatment of coke wastewater, Kim (2013) reported that the optimum pH for AOB fluctuated between 7.5 and 8 and altered in response to the temperature. Furthermore, the availability of inorganic carbon is critical for stable nitrification in order to provide sufficient alkalinity to buffer the acidification resulting from the production of hydrogen ions during ammonia oxidation. Additionally, inorganic carbon is used as a carbon source for growth by the autotrophic nitrifying bacteria (Wett and Rauch, 2003). Accounting for biomass growth, alkalinity is required at a stoichiometric rate of 7.07 mg as $CaCO_3$/mg NH_4^+-N.

Kim et al. (2009) reported the sudden loss of nitrogen removal during the operation of a full-scale anoxic-aerobic ASP and conducted laboratory-scale investigations into the causes behind the loss of treatment. By feeding the laboratory-scale reactor with the abnormal influent collected during the full-scale treatment loss they replicated the reduced nitrogen removal. Effluent NH_4^+-N increased from 2 mg/L up to 36, 60 and finally 80 mg/L suggesting the inhibition of nitrification in the aerobic reactor. It was established that there was insufficient inorganic carbon for nitrifying bacteria as the effluent inorganic carbon was observed to decline in line with the loss of nitrification from 15 mg/L to 5 mg/L. Furthermore, the loss of nitrification resulted in a decrease in nitrate concentrations being recycled back to the anoxic reactor which led to reduced inorganic carbon formation through denitrification, further exacerbating the shortage of inorganic carbon in the aerobic cell. Kim et al. (2009) also demonstrated that shock loadings of SCN^- (influent concentration increased from 400 to 900 mg/L) led to a small decrease in the nitrification efficiency from 98% to 94%. This was associated with increased concentrations of ammonia in the aerobic cell, due it its formation in the degradation of SCN^-, and therefore an inadequate supply of inorganic carbon. Additionally, the limited availability of inorganic carbon would result in increased competition between the thiocyanate degrading bacteria (which are typically autotrophic (Raper, Stephenson, *et al.*,

2017)) and nitrifying bacteria. Kim et al. (2009) reported that after a period of insufficient alkalinity the pre-denitrification process required a substantial recovery time (data not reported) in comparison to the recovery time required as a result of shock loads of other pollutants. The provision of adequate alkalinity for nitrification is therefore essential for stable treatment. The shortage of alkalinity in coke wastewater has previously been reported (Li *et al.*, 2003; Vázquez *et al.*, 2006). Through the use of batch tests, dosing sodium bicarbonate, Vázquez et al. (2006) reported an alkalinity requirement for coke wastewater of 6.5 mg $CaCO_3$/mg NH_4^+-N for a 98% removal efficiency. Although alkalinity has been identified as a limiting factor the optimal dose rate requires further investigation and there is no knowledge on the optimal dosing compound.

An adequate supply of organic carbon has also been established as critical for the removal of nitrogen through denitrification (Raper, Fisher, *et al.*, 2017). Variation in coke wastewater composition results in continually fluctuating soluble chemical oxygen demand: total nitrogen ratios (sCOD:TN) and consequently denitrification efficiencies can be limited by an inadequate supply of organic carbon (Raper, Fisher, *et al.*, 2017). In a pilot-scale anoxic aerobic ASP an influent sCOD:TN ratio of 5.7 enabled nitrogen to be removed to <50 mg/L whilst an influent sCOD:TN ratio of 5.2 resulted in significant increases in effluent TN to 89 mg/L. The removal of 1mg of nitrate-nitrogen (NO_3^--N) theoretically requires 2.86 mg COD although actual requirements vary in response to the type of wastewater, carbon source, microorganisms present and the operational conditions (Carrera, Vicent and Lafuente, 2004; Chakraborty and Veeramani, 2006). Consequently, as TN emission limits continue to be reduced, the use of external carbon addition is required to ensure effluent emission compliance. A range of alternative carbon compounds have been considered for additional carbon dosing including glucose, glycerol, acetic acid, methanol, ethanol and alternative waste products (Peng, Ma and Wang, 2007; Cherchi *et al.*, 2009; Paulo da Silva, Mack and Contiero, 2009;

Fernández-Nava *et al.*, 2010; Soares *et al.*, 2010; Yang, Wang and Zhou, 2012). The choice of carbon source must consider a range of factors including nitrogen-removal performance, cost, biomass production, non-toxic nature, denitrification ability, biomass adaptation requirements, effluent quality and the operational requirements of storage and handling, (Lee and Welander, 1996; Cherchi *et al.*, 2009).

The aim of this investigation was to identify suitable sources of both inorganic and organic carbon for coke wastewater in order to enhance TN removal through nitrification and denitrification. The type of inorganic carbon provided to coke wastewater has not been considered despite its addition having been highlighted. Thus, one objective of this investigation was to complete batch tests to investigate the impact of inorganic carbon provision on nitrification efficiencies through different sources and doses. The roles of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) were considered for their ability to promote stable nitrification. Soda ash (site grade Na_2CO_3) was also considered as a cheaper alternative which was already readily available on site. Additionally, the role of sodium hydroxide (NaOH) addition was also considered as this increases the availability of carbonate by altering the pH and associated carbonate equilibrium. Provision of organic carbon was investigated in a continuous laboratory-scale anoxic-aerobic ASP. Addition of external carbon in the form of phenol (the major source of carbon in coke wastewater) was compared with glycerol and acetic acid as alternative carbon sources. As glycerol is formed as a by-product from bio-diesel production it can typically be sourced cheaply and is non-toxic (Paulo da Silva, Mack and Contiero, 2009). Acetic acid, on the other hand, is easily degradable by many bacteria although it requires additional health and safety requirements for handling and storage (Peng, Ma and Wang, 2007; Cherchi *et al.*, 2009; Fernández-Nava *et al.*, 2010). The suitability of the different carbon sources was considered in relation to denitrification efficiency, process stability and sludge yield.

6.2 Experimental

6.2.1 Coke wastewater and activated sludge biomass

Coke wastewater was collected weekly from a full-scale steelworks in the UK. Due to the long-term nature of the denitrification optimisation tests, the wastewater was sourced weekly and was therefore subject to the same variability as the full-scale site. Activated sludge biomass was taken from the return activated sludge (RAS) feed of a pilot-scale ($1m^3$) anoxic-aerobic ASP which operated with an influent flow rate of 0.41 m^3/d, a hydraulic retention time (HRT) of 60 hours and a sludge age of ~80 days.

6.2.2 Nitrification batch tests

To investigate the impact of the type of inorganic carbon and optimise the chemical dose batch tests were completed in 1L bottles with a working volume of 0.95L. Aeration was provided through aquarium pumps maintaining the dissolved oxygen (DO) at ca. 3 mg/L. Temperature was maintained at 25^oC through the use of a water bath. Coke wastewater was combined with activated sludge biomass to produce a mixed liquor suspended solids (MLSS) of 4500 mg/L. Due to the variable nature of coke wastewater composition, nitrification batch tests were completed using the same wastewater i.e. collected on a single occasion, in order to allow comparability between conditions. Analytical grade sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$) and soda ash (technical grade Na_2CO_3) were dosed at 400 mg/L (as $CaCO_3$) in order to investigate the impact of the type of inorganic carbon on nitrification. Additionally, the impact of pH control on nitrification was investigated. Sodium hydroxide (5M) was added (200 - 300 µl) at 48 hour intervals to maintain the pH at ca. 7.8. Tests were run for 168 h with samples taken every 24 hours to monitor concentrations of NO_2^--N, NO_3^--N, NH_4^+-N, SCN^--N and pH. Upon selection of the optimal form of inorganic carbon (Na_2CO_3) the chemical dose was investigated following the same operational and sampling methods. Taking

account of the typical alkalinity of the raw coke wastewater and the theoretical alkalinity requirement for nitrification an alkalinity shortfall of ca. 300 mg/L was calculated. The impact of alkalinity addition (Na_2CO_3) was therefore investigated at a dose of 200, 300, 400 and 500 mg/L (as $CaCO_3$). Such doses were therefore selected to investigate the impact of a range of doses above and below the theoretical requirement.

6.2.3 Anoxic-aerobic laboratory-scale reactor

A 30L (working volume) laboratory-scale anoxic-aerobic ASP was designed with an anoxic reactor (10L), aerobic reactor (20L) and a clarifier (5L) (Figure 6-1). Coke wastewater was fed into the ASP at a flow rate of 11.5 L/d. Mixed liquor was recycled at 23 L/d between the aerobic and anoxic reactor (Cole-Parmer L/S variable speed peristaltic pump model no. HL-07528-30). A recycle ratio of 2 was selected according to the study of Chakraborty and Veeramani (2006). Suspended solids were kept in suspension through the use of mechanical stirrers. Temperature was maintained at 25^{o}C in both reactors through heaters (RENA, Smartheater 50, France). Dissolved oxygen was monitored in the anoxic reactor through the use of a DO probe (Multimeter 3420, VWR International) with average DO readings of <0.05 mg/L. Dissolved oxygen was maintained at 3 - 4 mg/L in the aerobic reactor through the use of air pumps. A dilute stock solution of sodium carbonate (251 mg/L as $CaCO_3$) (Na_2CO_3, AnalaR NORMAPUR, VWR International) was linked to an automated dosing system in the aerobic reactor to provide the alkalinity required for nitrification. Alkalinity was provided whenever the system pH fell below a pH of 7.6. Automated dosing allowed constant adaptation to the alkalinity requirement of the coke wastewater which was subject to a variable nitrogen load. Coke wastewater fed into the laboratory-scale anoxic-aerobic ASP was supplied with either phenol (control), acetic acid or glycerol as a carbon source to maintain a soluble chemical oxygen demand:nitrogen (sCOD:N) ratio of 8. The anoxic-aerobic ASP was operated under stable control conditions before subsequently changing the carbon source to glycerol or acetic acid.

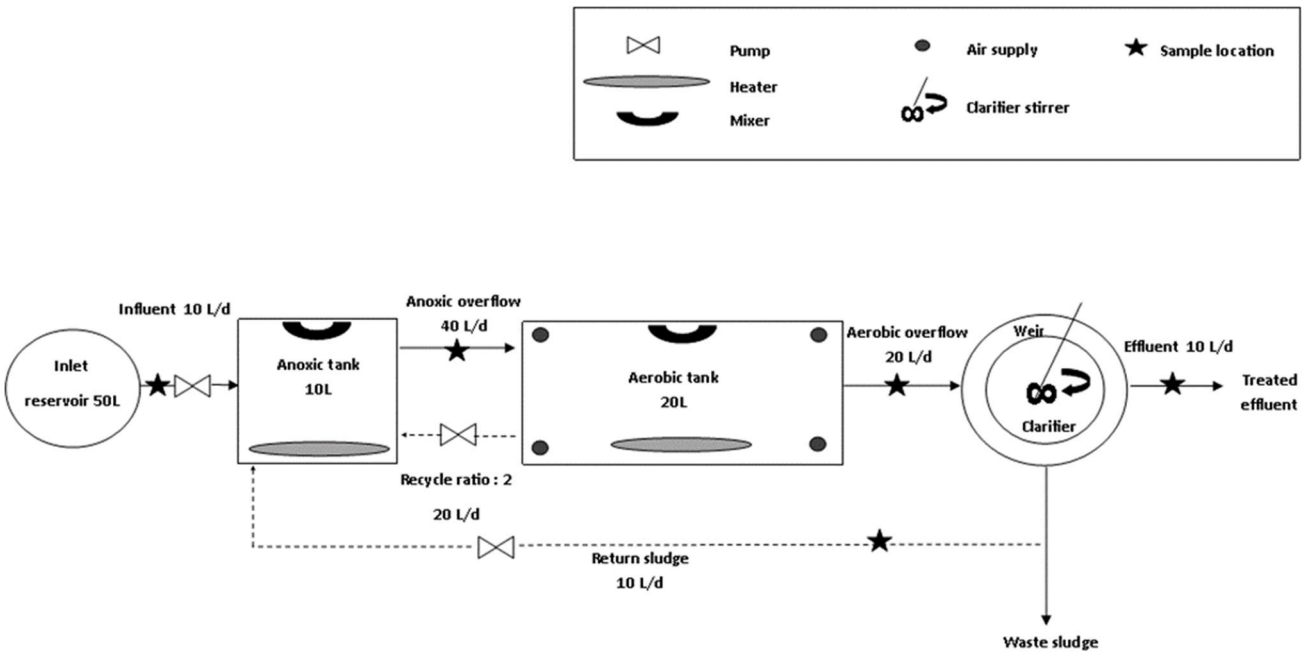

Figure 6-1: Schematic of anoxic-aerobic laboratory-scale ASP, operational conditions and sampling locations.

6.2.4 Analytical methods

Filtered samples (0.45 µm filters, VacuCap 90, Pall Corporation) taken from the inlet, anoxic and aerobic reactor, return sludge feed and outlet (Figure 6-1) were refrigerated at 2 - 5°C. Nitrogen compounds (NO_2^--N, NO_3^--N, NH_4^+-N) and sCOD were analysed using Merck cell test kits according to the manufacturer's instructions. Thiocyanate was analysed using a Jenway 6300 spectrophotometer (Staffordshire, UK) at a wavelength of 465 nm, being complexed with iron (III) to produce colour on an orange to red spectrum based on The Institute of Gas Engineers analytical method for thiocyanate (The Institution of Gas Engineers, 1971). Phenol (mono) was complexed with 4-aminoantipyrene producing a red solution measured at a wavelength of 510 nm (Jenway 6300 spectrophotometer, Staffordshire, UK) , based on ISO 6439:1990 (ISO, 1990). pH was recorded using a Jenway 3540 pH meter (UK). Mixed liquor suspended solids and alkalinity were analysed according to standard methods (Eaton, 2005). Total nitrogen (TN) was calculated as the sum of NO_2^--N, NO_3^--N, NH_4^+-N and SCN^--N. Organic nitrogen was excluded as these are not included in the Industrial Emission Directives (IED) (European Commission, 2013).

6.3 Results and Discussion

6.3.1 Coke wastewater composition

Coke wastewater composition is presented in Table 6-1. Coke wastewater used for the nitrification batch tests was characterised by a TN concentration of 118 mg/L consisting of 14 mg/L of NO_2^--N, 13 mg/L of NO_3^--N and 74 mg/L of NH_4^+-N. An SCN^- concentration of 72 mg/L contributed a further 17 mg/L of NH_4^+-N through its degradation. Phenol concentrations averaged at just 8 mg/L. The wastewater pH was 7.8. Over the 4 month operational period of the laboratory-

scale anoxic-aerobic reactor the coke wastewater was characterised by an average NH_4^+-N, NO_3^--N and NO_2^--N of 40 mg/L, 2 mg/L and 4 mg/L, respectively. Thiocyanate concentrations averaged at 79 mg/L contributing an additional 19 mg/L of NH_4^+-N leading to a TN concentration of 65 mg/L. Phenol concentrations averaged at 34 mg/L. The wastewater was characterised by an average alkalinity of 345 mg/L as $CaCO_3$ (Table 6-1).

Table 6-1: Coke wastewater characterisation during organic and inorganic carbon investigations.

	Inorganic carbon batch tests	Organic carbon anoxic-aerobic laboratory-scale ASP reactors
NO_2^--N	14 ± 3	4 ± 2
NO_3^--N	13 ± 11	2 ± 1
NH_4^+-N	74 ± 7	40 ± 6
SCN^-	72 ± 2	79 ± 23
SCN^--N	17 ±0.5	19 ± 5
TN [a]	118 ± 14	65 ± 9
sCOD	296 ± 48	510 ± 90 [b]
Phenol (mono)	8 ± 10	34 ± 10 [c]
pH	7.8 ± 0.1 [d]	6.9 ± 0.5 [e]
Alkalinity	345 ± 50 [f]	345 ± 50

[a] Sum of NO_2^--N, NO_3^--N, NH_4^+-N and SCN^--N.
[b] After dosing with carbon source under investigation
[c] Phenol prior to additional dosing during organic carbon trials
[d] pH prior to dosing with Na_2CO_3 dosing during inorganic carbon trials
[e] pH after external carbon addition
[f] of raw wastewater expressed as $CaCO_3$

6.3.2 Nitrification batch tests: Impact of different inorganic carbon sources

Without addition of inorganic carbon (control conditions), SCN^- degradation was complete in the first 24 hours (Figure 6-2) resulting in the production of an additional 17 mg/L of NH_4^+-N. Ammonia-nitrogen concentrations declined from

72 to 49 mg/L, indicating that nitrification was limited (45%). The pH declined rapidly from 7.8 to 7.3 at 24 hours, 6.5 at 48 hours and finally to 6 at 72 hours. When the pH fell to <6.5 no further ammonia oxidation was observed.

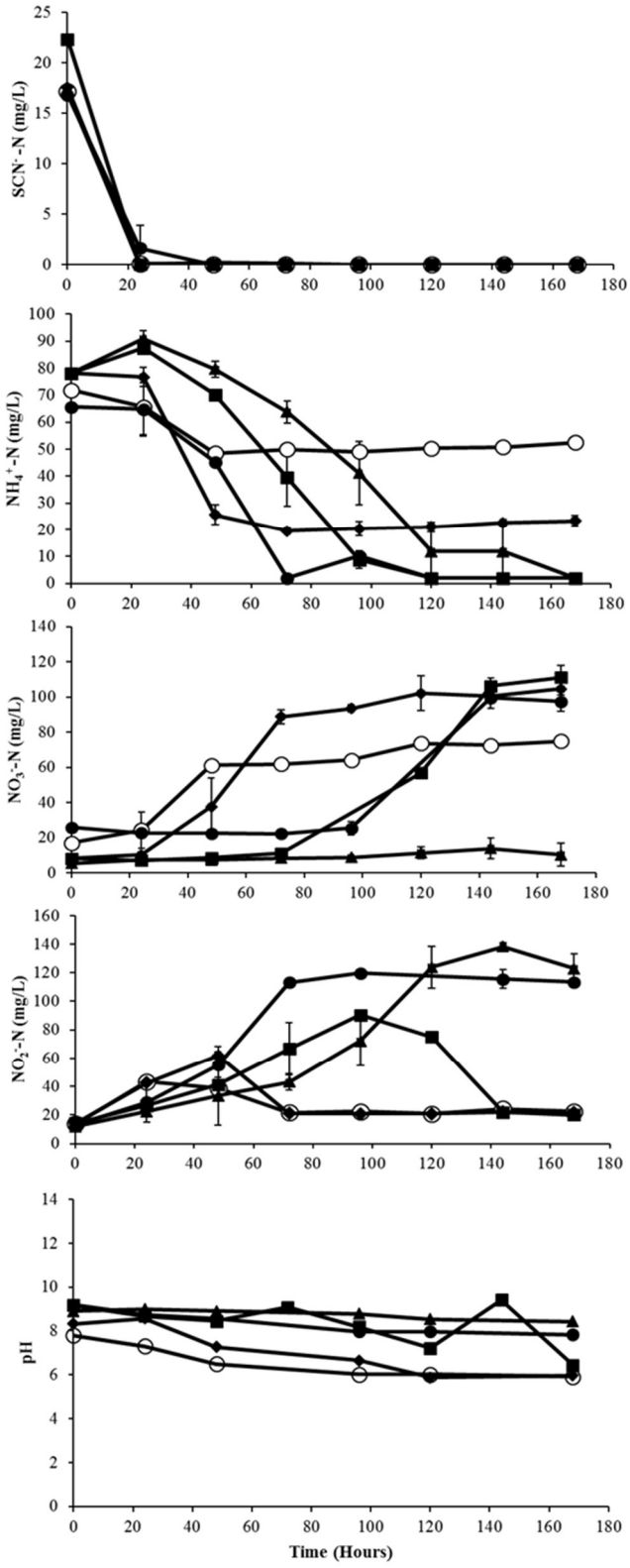

Figure 6-2: Impact of inorganic carbon on SCN^--N degradation, ammonia oxidation, production of NO_2^--N and NO_3^--N and pH ○ control ●Na_2CO_3 (400 mg/L) ▲ Soda ash (400 mg/L) ♦ $NaHCO_3$ (400 mg/L) ■ NaOH (5M, 200 - 300 µl).

The addition of Na_2CO_3 increased the nitrification efficiency to 98%, with NH_4^+-N being removed to below the detection limit of 4 mg/L. The increased nitrification efficiency led to the expected increase in NO_3^--N and NO_2^--N to concentrations of 99 mg/L and 115 mg/L, respectively (Figure 6-2). The pH declined from 9.2 - 7.8 at 120 hours. Similarly, the addition of soda ash led to overall nitrification efficiencies of 98% although complete removal of NH_4^+-N was not reached until 168 hours compared to 78 hours with Na_2CO_3. However, soda ash led to significant differences to the final form of oxidised nitrogen. Soda ash led to increased NO_2^--N concentrations of 123 mg/L but no increase in NO_3^--N concentrations (Figure 6-2). The pH was observed to decline gradually from 8.9 to 8.4 at 120 hours the accumulation of NO_2^--N without any subsequent formation of NO_3^--N indicated inhibition to NOB. Consequently, it was suspected that impurities associated with the soda ash could potentially decrease the activity of *Nitrobacter* resulting in a breakdown in the 2-step nitrification process.

The addition of $NaHCO_3$ was considered due to the higher contribution of carbonate per unit of pH. As the coke wastewater pH can at times exceed 8.5, it was desirable that pH increase as a result of alkalinity addition, was minimised. High pH values are associated with higher concentrations of free ammonia and toxicity to nitrifying bacteria, specifically the nitrite-oxidising bacteria (Anthonisen *et al.*, 1976). Addition of $NaHCO_3$ led an initial pH of 8.3, close to the optimum range associated with *Nitrosomonas* (Kim, 2013). Despite an initial optimal pH, the addition of $NaHCO_3$ led to lower nitrification efficiencies of 76% compared to Na_2CO_3 (98%) as a result of the incomplete NH_4^+-N oxidation. Concentrations of NH_4^+-N reduced from 78 mg/L to 25 mg/L in 48 hours, but ammonia oxidation then stabilised from 68 - 168 hours at ca 21 mg/L. By 72 hours, the pH (7.3) was below the optimum pH associated with AOB. Nitrite-nitrogen accumulated up to 61 mg/L at 48 hours. As a result of no further ammonia oxidation, NO_2^--N concentrations declined at 72 hours to 21 mg/L

whilst NO_3^--N concentrations continued to increase as a result of the conversion of NO_2^- to NO_3^- (Figure 6-2).

Improvements in nitrification were seen as a result of pH control via NaOH addition. Increasing the pH results in a shift in the carbonate equilibrium from carbonic acid to bicarbonate increasing the availability of carbon in the wastewater (Lind, 1985). Carbon dioxide originating from the degradation of SCN^- would be converted to carbonate. Nitrification efficiencies of 98% were observed with complete oxidation of NH_4^+-N in 120 hours. Nitrite-nitrogen and NO_3^--N accumulated to 90 mg/L and 106 mg/L respectively. Nitrite-nitrogen concentrations reduced after 72 hours falling to 22 mg/L at 144 hours (Figure 6-2). Improvements to the nitrification efficiency through pH control highlights the impact of pH on CO_2/carbonate equilibrium.

Sodium carbonate was selected as the best option for the provision of the additional alkalinity required for the nitrification of coke wastewaters. Sodium bicarbonate was not considered appropriate due to the incomplete degradation of NH_4^+-N whilst NO_2^--N accumulation through soda ash addition was undesirable due to its higher relative toxicity. Although NaOH resulted in similar efficiencies as Na_2CO_3, concerns were expressed over the storage and handling requirements for its on-site application and NO_2^--N accumulation and consequently Na_2CO_3 was considered more appropriate. Additionally, Na_2CO_3 provided additional carbonate directly to nitrifying bacteria rather than relying on the conversion of carbon compounds via pH control. Dose optimisation was therefore completed using Na_2CO_3 addition.

6.3.3 Nitrification batch tests: Impact of inorganic carbon dose

In order to optimise alkalinity addition different doses of Na_2CO_3 (200 - 500 mg/L as $CaCO_3$) were considered for their impact on nitrification efficiency and nitrogen speciation. Under control conditions the nitrification efficiency was comparable to those observed in previous experiments and was limited to 40% (Figure 6-3). Thiocyanate-nitrogen removal was complete within 24 hours under all Na_2CO_3 doses. Therefore, alkalinity addition was concluded to have no negative impacts to SCN^--N degradation.

The addition of 200 mg/L Na_2CO_3 (as $CaCO_3$) resulted in a 78% nitrification efficiency with concentrations of NH_4^+-N falling from 66 mg/L to 18 mg/L. Nitrite-nitrogen concentrations increased to 70 mg/L at 48 hours declining back to 24 mg/L at 72 hours. Nitrate-nitrogen increased rapidly from 25 mg/L at 0 hours to 85 mg/L at 72 hours. Accumulation then slowed down in response to no further NH_4^+-N oxidation (Figure 6-3). At 300 mg/L Na_2CO_3, NH_4^+-N removal efficiencies increased to 95% with concentrations falling from 66 mg/L to 4 mg/L.

At 400 mg/L Na_2CO_3, NH_4^+-N was removed to below the detection limit (98% nitrification efficiency). Nitrite-nitrogen concentrations increased to 115 mg/L at 72 hours but in contrast to lower doses, NO_2^--N persisted in the system and the production of NO_3^--N was delayed until 96 hours (Figure 6-3). A further increase in the dose to 500 mg/L Na_2CO_3 had a negative impact on nitrification. Although the nitrification efficiency remained at 98% the final end product was NO_2^--N with no NO_3^--N formation (Figure 6-3).

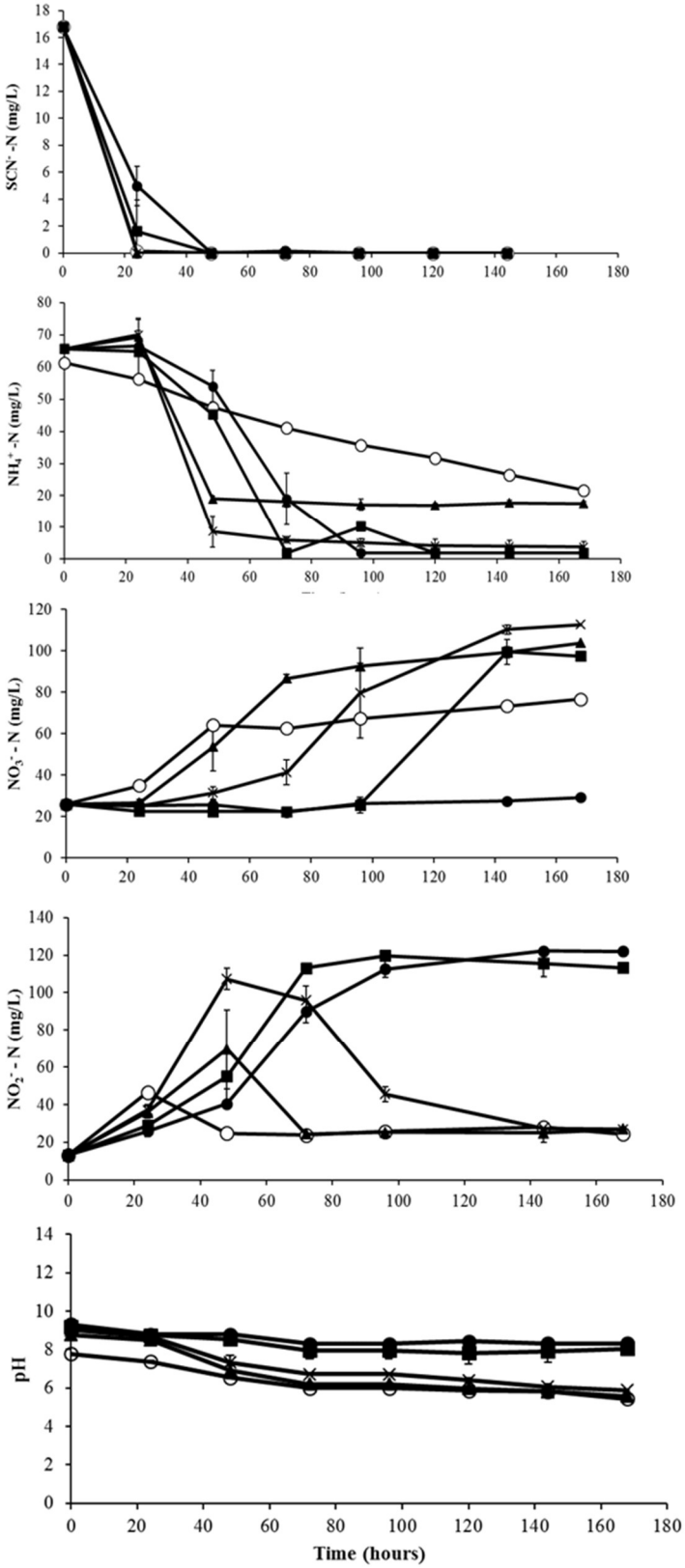

Figure 6-3: Impact of Na_2CO_3 dose on nitrogen speciation and pH ○ control ▲200 mg/L × 300 mg/L ■ 400 mg/L ●500 mg/L.

The optimal dose of Na_2CO_3 requires consideration of the potential NH_4^+-N removal, NO_2^--N accumulation and pH stability. Both NO_2^--N and NO_3^--N concentrations were impacted significantly by pH. Figure 6-4 shows the impact of pH on normalised NO_3^--N and NO_2^--N concentrations observed throughout the investigation. Above a pH of 8.8 there was no formation of NO_2^--N or NO_3^--N due to limited ammonia oxidation. Below pH 8.8, ammonia-oxidation was able to take place and NO_2^--N formation was observed. Between a pH of 6.8 and 8.3 there was a rapid accumulation of NO_2^--N. The formation of NO_3^--N was observed below a pH of 7.0. The optimum pH range, under batch conditions, for AOB was therefore observed to be between 7.0 and 8.3 whilst NOB bacteria were active at a pH of 5.5 - 6.8. Despite this, under continuous operational conditions in a pre-denitrification activated sludge process the biomass was observed to operate at a pH of 7.6 (Raper, Fisher, *et al.*, 2017) demonstrating the adaptability of the biomass to different conditions. This range was wider than other reported values suggesting a biomass more adapted to changing wastewater conditions. The optimum pH identified for NOB was below the commonly reported optimum pH value of 7.2 – 7.6 (Anthonisen *et al.*, 1976). Despite this, other NOB bacteria have been observed to be have lower optimum pH ranges from slightly acidic conditions to pH's of 4 (Gieseke *et al.*, 2006; Hüpeden *et al.*, 2016). *Nitrotoga sp*. HW29, for example, was reported to have an optimal pH of 6.8 and an optimal temperature of 22°C, which would be within the operational conditions tolerated by the NOB in this investigation (Hüpeden *et al.*, 2016). A dose of 300 mg/L was considered to be optimal allowing a nitrification efficiency of 95%, close to the maximum observed of 98%. This dose was considered optimal as it enabled the pH to remain in the optimal range for nitrification for the greatest time duration allowing effective ammonia oxidation and conversion of NO_2^--N to NO_3^--N.

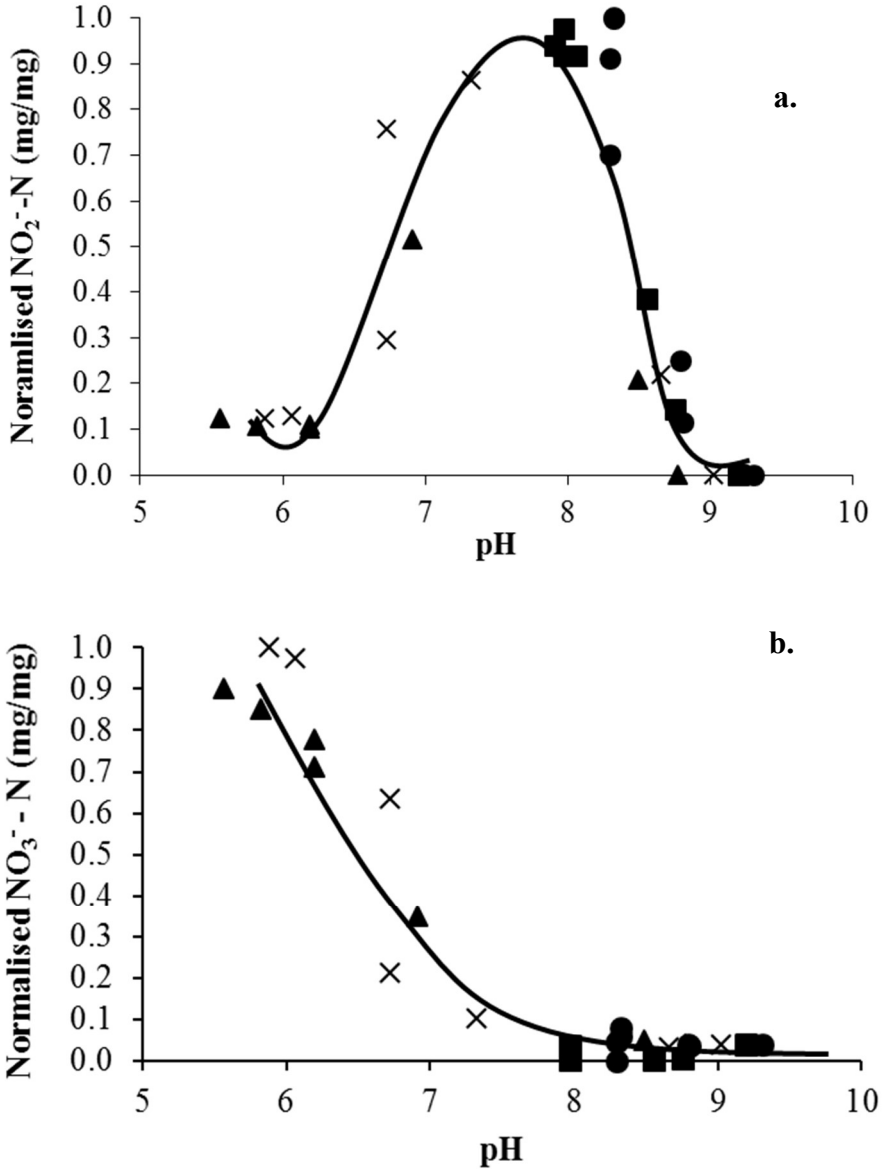

Figure 6-4: Impact of Na_2CO_3 dose ▲200 mg/L × 300 mg/L ■ 400 mg/L ●500 mg/L on pH and a) NO_2^--N concentration b) NO_3^--N concentration

6.3.4 Anoxic-aerobic laboratory-scale reactors: Impact of organic carbon source for denitrification

The impact of organic carbon source on denitrification efficiency and resulting effluent TN was investigated by comparing treatment efficiencies of the anoxic-aerobic ASP when dosed with phenol, glycerol and acetic acid at a constant sCOD:TN ratio of 8. Conditions were stabilised in the anoxic-aerobic ASP before comparisons were made (day 0 - 19). Under control conditions, where phenol was the primary form of organic carbon available, nitrification was maintained at an efficiency of 97% whilst denitrification averaged at 62% (Table 6-2 and Figure 6-5). Whilst nitrification efficiencies were stable, denitrification showed a high degree of variation with a standard deviation of 12.7 (Table 6-2). The effluent had a TN concentration of 34 mg/L on average, consisting of 20 mg/L NO_2^--N and 8 mg/L NO_3^--N. Ammonia-nitrogen was removed to below the detection limit of 4 mg/L. Both phenol and SCN^- degradation were complete.

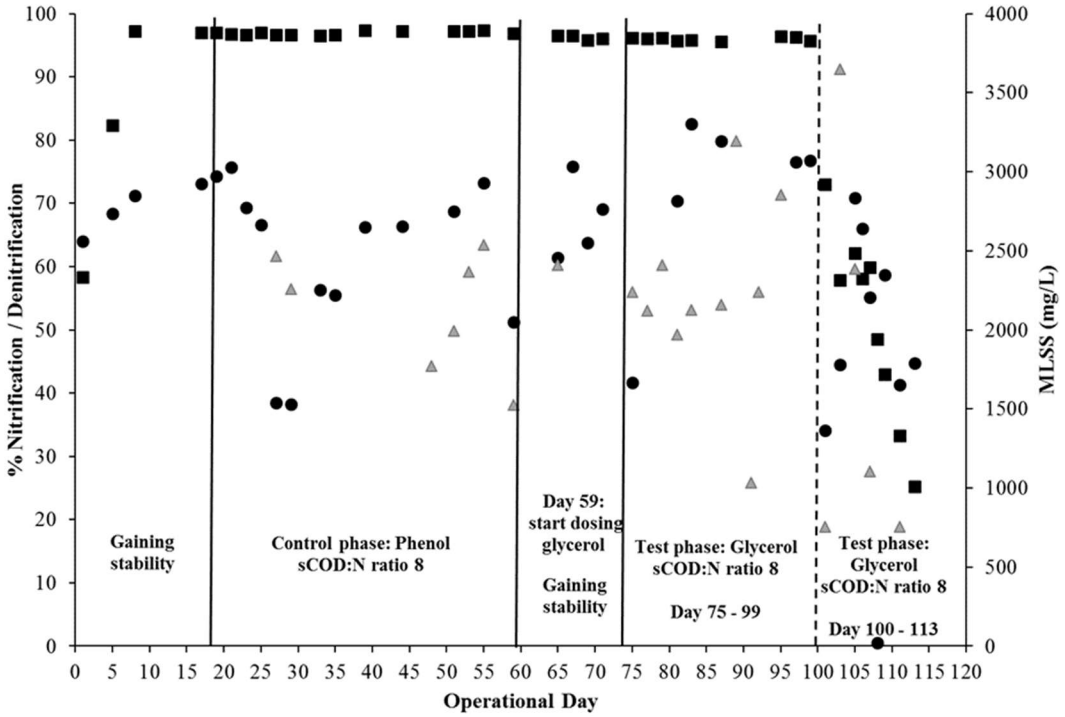

Figure 6-5: Nitrification and denitrification treatment efficiencies in the anoxic-aerobic laboratory ASP with phenol and glycerol as external carbon sources ■Nitrification ●Denitrification ▲MLSS --- Time of treatment efficiency decline (from day 100).

Table 6-2: Impact of glycerol dosing on denitrification, including removal efficiencies and effluent nitrogen emissions.

		Control (Phenol): Day 19 - 59		Glycerol: Day 75 - 99		Glycerol: Day 100 - 113	
		Av	SD	Av	SD	Av	SD
SCN^- removal efficiency	%	100	0	100	1	98	4
Nitrification	%	97	0	96	0	51	15
Phenol removal efficiency	%	100	0	98		98	
Inlet sCOD:TN ratio		8	1	8	0	8	1
Anoxic sCOD:TN ratio		5	1	5	1	4	1
NO_x experimental removal rate*	g NO_x/gVSS.d	5	1	3	0	4	1
Denitrification experimental biomass production**	g sCOD/gVSS.d	22	nd	14	nd	15	nd
Denitrification efficiency	%	62	13	71	15	46	21
Effluent TN	mg/L	34	5	27	6	45	10
Effluent NO_2^--N	mg/L	20	5	11	2	14	3
Effluent NO_3^--N	mg/L	8	2	13	6	2	1
Effluent NH_4^+-N	mg/L	2	0	2	0	27	9
MLSS	mg/L	2165	365	2235	566	1730	1266

* ((Anoxic NO_x loading rate (kg/m^3.d) - Aerobic NO_x loading rate (kg/m^3.d)) / ((0.8 x MLSS (mg/L)) x Reactor volume (m^3))) x 1000

**((Influent flow (m^3/d) x (Influent sCOD (g sCOD/m^3) - Effluent sCOD (g sCOD/m^3)) / (Reactor volume (m^3) x (0.8 x MLSS (g/m^3)))

Table 6-3: Impact of acetic acid dosing on denitrification, removal efficiencies and effluent nitrogen emissions.

		Control (Phenol): Day 23 - 59		**Acetic Acid:** Day 76 - 115	
		Average	SD	Average	SD
SCN^- removal efficiency	%	100	1	100	0
Nitrification	%	97	1	96	2
Phenol removal efficiency	%	100	0	98	2
Inlet sCOD:TN ratio		8	1	8	1
Anoxic sCOD:TN ratio		5	1	5	1
NO_x experimental removal rate*	g NO_x/g-VSS.d	4	1	3	1
Denitrification experimental biomass production**	g sCOD/g-VSS.d	19	nd	21	nd
Denitrification efficiency	%	75	11	76	10
Effluent TN	mg/L	30	3	24	4
Effluent NO_2^--N	mg/L	20	2	12	5
Effluent NO_3^--N	mg/L	7	2	9	5
Effluent NH_4^+- N	mg/L	2	0	2	1
MLSS	mg/L	5129	663	3137	687

* ((Anoxic NO_x loading rate (kg/m^3.d) - Aerobic NO_x loading rate (kg/m^3.d)) / ((0.8 x MLSS (mg/L)) x Reactor volume (m^3))) x 1000

**((Influent flow (m^3/d) x (Influent sCOD (g sCOD/m^3) - Effluent sCOD (g sCOD/m^3)) / (Reactor volume (m^3) x (0.8 x MLSS (g/m^3)))

When glycerol was added to the ASP, initially both nitrification and denitrification remained stable at 96% and 71%, respectively (Figure 6-5). Denitrification treatment efficiencies improved from 62%, with phenol as the carbon source, to 71% and consequently TN in the effluent declined from 34 to 27 mg/L (Table 6-2). After 24 days with glycerol as the main carbon source, the trends in treatment efficiencies changed with an observed rapid decline in the nitrification efficiency to 51% (day 100 -113) (Figure 6-5). Denitrification efficiencies also reduced to 46% (Table 6-2). In response to this effluent TN increased to 45 mg/L. Ammonia-nitrogen was observed in the effluent at an average of 27 mg/L reflecting the incomplete nitrification (Table 6-2). By day 108 the effluent was characterised by an effluent TN of 52 mg/L, in exceedance of the 50 mg/L emission limit, increasing to 59 mg/L on day 113. The MLSS under control conditions (phenol) was maintained at an average of 2165 mg/L. Initially, when glycerol was the main available carbon source the MLSS concentration remained stable at 2235 mg/L (day 75 - 99), however, after 25 days MLSS concentrations rapidly declined with the average MLSS declining to 1730 mg/L (day 100-113). The final MLSS concentration recorded was just 750 mg/L representing a 65% decrease in MLSS (Table 6-2). Changes in the utilisation of carbon were also notable in the denitrification experimental biomass production with a decline in sCOD consumption in the anoxic tank from 22 g sCOD/g-VSS.d to 14 g sCOD/g-VSS.d (day 75 - 99) and 15 g sCOD/g-VSS.d (day 100 - 113) (Table 6-2, Figure 6-5). As a result of the lower carbon utilisation there was an increase in the sCOD loading rate to the aerobic reactor from an average of 0.31 kg/m^3.d to 0.44 kg/m^3.d (day 100 - 113).

The impact of acetic acid was likewise compared against the treatment efficiency of the anoxic-aerobic ASP with phenol as the carbon source. Conditions in the anoxic-aerobic ASP were stabilised (day 0-22). With phenol as the carbon source (day 23 - 59) nitrification was maintained at 97% whilst denitrification averaged at 75% (Table 6-3 and Figure 6-6). Effluent TN averaged at 30 mg/L. Nitrite-nitrogen was the main contributor to the effluent TN

at 20 mg/L whilst NO_2^--N averaged at 7 mg/L. With acetic acid as the main carbon source the anoxic-aerobic ASP continued to both nitrify and denitrify effectively at average efficiencies of 96% and 76 % respectively (day 76 - 115- Table 6-3 and Figure 6-6). Moreover, acetic acid as a carbon source led to an improved removal of TN with the average effluent TN decreasing from 30 mg/L (phenol) to 24 mg/L (acetic acid). As with glycerol as the carbon source, a decline in the MLSS concentration was also observed with acetic acid, declining from 5129 mg/L under control conditions (phenol) to 3137 mg/L. In contrast, however, the MLSS subsequently stabilised (Table 6-3).

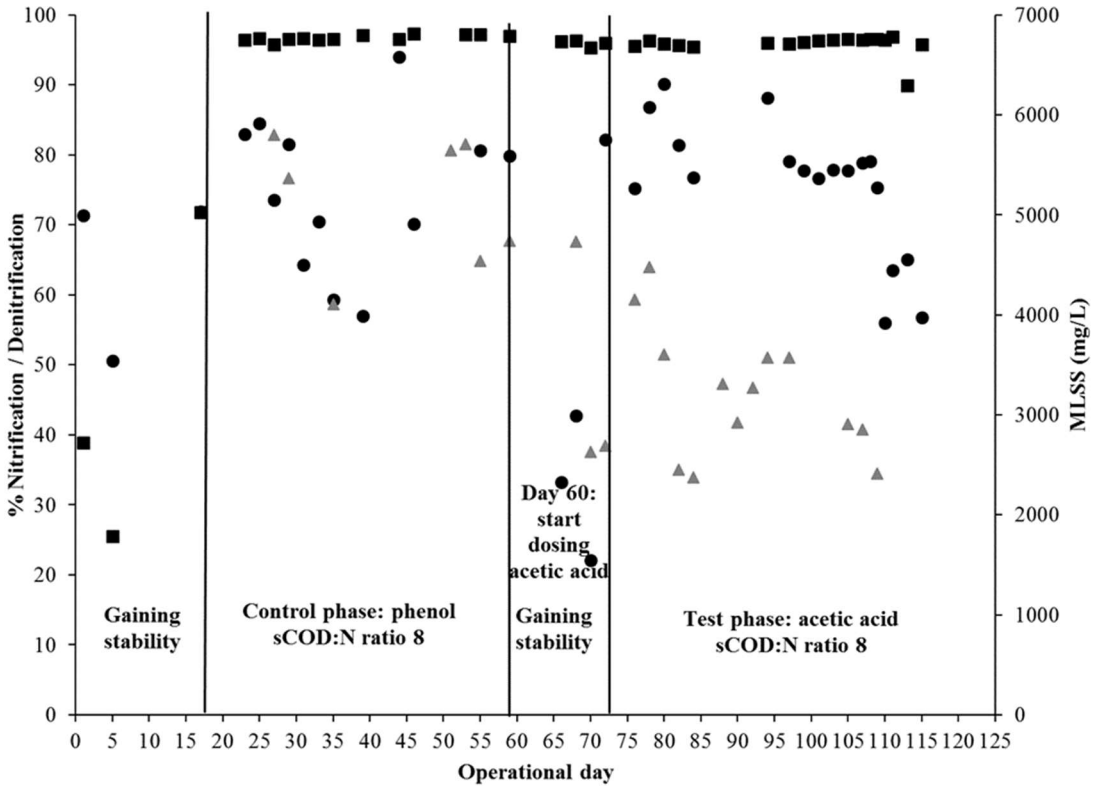

Figure 6-6: Nitrification and denitrification treatment efficiencies in the anoxic-aerobic laboratory-scale ASP with phenol and acetic acid as external carbon sources ■Nitrification ●Denitrification ▲MLSS.

The poor removal efficiencies associated with glycerol as a carbon source were associated with declining MLSS in the anoxic-aerobic ASP. When looking at the stoichiometric reaction between biomass production and substrate consumed it can be observed from the stoichiometry in **Equation 6-1** that 0.20 mg of

biomass is formed for every mg of phenol whilst 0.18 mg of biomass was produced from each mg of glycerol (**Equation 6-2**). As the MLSS declined there was an increased sCOD loading to the aerobic reactor which was believed to be responsible for the rapid decline in nitrification. Increased loading of sCOD has been associated with competition between heterotrophic and nitrifying bacteria as the carbon supply supports the growth of faster growing heterotrophic bacteria which may outcompete the slower growing nitrifying bacteria (Jenni *et al.*, 2014). Furthermore, Hanaki, Wantawin and Ohgaki (1990) demonstrated that increased organic loading, through glucose addition, reduced ammonia oxidation as a result of a lowered affinity between the ammonia oxidisers and ammonia.

$C_6H_5OH + 4.83\ NO_3^- \rightarrow 0.16\ C_5H_7NO_2 + 4.83\ HCO_3^- + 0.33CO_2 + 2.3\ N_2$ **Equation 6-1**

$C_3H_8O_3 + 2.14\ NO_3^- \rightarrow 0.14\ C_5H_7NO_2 + 2.14\ HCO_3^- + 0.14\ CO_2 + N_2 + 2.43\ H_2O$ **Equation 6-2**

$CH_3COOH + 0.83\ NO_3^- \rightarrow 0.17\ C_5H_7NO_2 + 0.83\ HCO_3^- + 0.33\ CO_2 + 0.33\ N_2 + H_2O$ **Equation 6-3**

It is possible therefore that the decline in suspended solids was associated with both the lower biomass production and the time required for the denitrifying bacteria to acclimatise to glycerol. Heterotrophic bacteria may be generalists or specialists. The provision of an alternative carbon supply has been associated with the move away from a generalist heterotrophic community towards a more specialist community requiring acclimatisation (Uprety *et al.*, 2012). A specialist community can use the substrate more effectively and produce a higher yield (Uprety *et al.*, 2012). This phenomenon has been reported for glycerol with the yield increasing over time as the biomass adapted and started to grow on the new carbon source (Uprety *et al.*, 2012). Whilst glycerol has previously been

associated with nitrite accumulation (Uprety *et al.*, 2012; Cyplik *et al.*, 2013) this was not observed in the current application (Table 6-2). From day 75 - 99 when the ASP was fed with glycerol, nitrification remained stable and no accumulation of NO_2^--N was observed. However, further research would be required to establish whether this was a true representation or as a result of the ineffective utilisation of glycerol as a carbon source.

According to the stoichiometry in **Equation 6-3**, biomass production for acetic acid is much higher at 0.31 mg of biomass production per mg of acetic acid. This aligns with the observed changes in MLSS which were seen when acetic acid was supplied to the anoxic-aerobic ASP. The initial decline in MLSS may be associated with the acclimatisation period. As the biomass became more acclimatised the MLSS stabilised at MLSS concentrations comparable to when phenol was the main carbon source. Due to its aromatic nature, phenol is a complex molecule and must firstly be converted to catechol and then subsequently degraded via ortho or met-fission to intermediates which can be used in central metabolic processes (Nair, Jayachandran and Shashidhar, 2008). Acetic acid, on the other hand, is a simple molecule which is readily available for microbial uptake. Although acetic acid is an easily assimilated molecule the overall carbon utilisation efficiencies were similar at 19 g sCOD/g-VSS.d and 21 g-sCOD/ g-VSS.d with phenol and acetic acid respectively.

6.4 Conclusions

Coke wastewater was typically characterised by an alkalinity that was insufficient for complete nitrification (3.6 mg as $CaCO_3$/ mg NH_4^+-N). Sodium carbonate was identified as a suitable chemical for the provision of additional alkalinity with an optimal dose of 300 mg/L (as $CaCO_3$). Sodium bicarbonate led to incomplete ammonia oxidation (76%) whilst soda ash resulted in NO_2^--N accumulation. Control of pH through NaOH addition allowed comparable

treatment efficiencies to Na_2CO_3 but was associated with increased health and safety requirements and NO_2^--N accumulation. Both NO_2^--N and NO_3^--N concentrations were impacted by pH with an optimal pH of 7.0 – 8.3 for ammonia oxidation whilst nitrite-oxidation was observed at a pH of 5.5 - 6.8. External carbon addition was required to maintain denitrification of coke wastewater. Glycerol was an ineffective carbon source resulting in declining MLSS concentrations (<1750) which was likely associated with the low carbon availability for biomass production from glycerol (0.18 mg of biomass produced per 1 mg of glycerol). Declining MLSS concentrations led to incomplete nitrification and reduced TN removal, finally producing an effluent characterised by 59 mg/L TN. Acetic acid on the other hand allowed continued stable nitrification and denitrification likely due to the higher carbon available for biomass production (0.31 mg of biomass produced per 1 mg of acetic acid) producing an effluent TN of 24 mg/L in compliance with the IED emission limit of <50 mg/L. To comply with emission limits Na_2CO_3 should be provided at a 300 mg/L (as $CaCO_3$) dose whilst acetic acid is suitable as an external carbon source.

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6.6 References

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Chapter 7 Nitrogen removal from coke making wastewater through a pre-denitrification activated sludge process

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Abstract

Under the Industrial Emissions Directive (IED), coke production wastewater must be treated to produce an effluent characterised by a total nitrogen (TN) <50 mg/L. An anoxic-aerobic activated sludge pilot-plant (1 m^3) fed with coke production wastewater was used to investigate the optimal operational requirements to achieve such an effluent. The loading rates applied to the pilot-plant varied between 0.198 - 0.418 kg COD/m^3.day and 0.029 - 0.081 kg TN/m^3.day, respectively. The ammonia (NH_4^+-N) removals were maintained at 96%, after alkalinity addition. Under all conditions, phenol and SCN^- remained stable at 96% and 100%, respectively with both being utilised as carbon sources during denitrification. The obtained results showed that the influent soluble chemical oxygen demand (sCOD) to TN ratio should be maintained at >5.7 to produce an effluent TN <50 mg/L. Furthermore, nitrite accumulation was observed under all conditions indicating a disturbance to the denitrification pathway. Overall, the anoxic-aerobic activated sludge process was shown to be a robust and reliable technology to treat coke making wastewater and achieve the IED requirements. Nevertheless, the influent to the anoxic tank should be monitored to ensure a sCOD:TN ratio >5.7 or, alternately, the addition of an external carbon source should be considered.

Highlights:

- Pre-denitrification allows effective nitrogen removal from coke making wastewater.
- Carbon availability limits denitrification efficiencies.
- Influent to the anoxic tank should have an sCOD:TN ratio >5.7 to obtain TN <50 mg/L.
- Nitrite accumulation is exacerbated by carbon limitation.
- Nitrification and thiocyanate/phenol removal were stable under all conditions.

Keywords: Coke making wastewater; nitrification; denitrification; sCOD:TN ratio; nitrate-respiring bacteria.

7.1 Introduction

Coke making wastewater is a by-product of the steel industry produced from the quenching of hot coke masses, washing of ammonia stills, cooling and washing of coke oven gases and the processing and purification of coke (Pal and Kumar, 2014). World crude steel production reached 1,620 million tons for the year 2015 (World Steel Association, 2016). Coke making wastewater is produced in substantial quantities with 1000 tons of coke typically producing 1000 m^3 of wastewater (Pal and Kumar, 2014). Such wastewater is hazardous, containing a complex mix of harmful and toxic compounds which require treatment prior to their discharge (Vázquez *et al.*, 2006b; Kim *et al.*, 2008; Marañón *et al.*, 2008). Quantities of the individual compounds vary over both time and space in response to the composition of the coals used and variations in plant operational conditions and production levels (Marañón *et al.*, 2008). Coke making wastewater is characterised by high concentrations of ammonia (NH_4^+-N) (50 - 500 mg/L) and thiocyanate (SCN^-) (100 - 400 mg/L) (Vázquez *et al.*,

2006a; Staib and Lant, 2007; Marañón *et al.*, 2008). Breakdown of SCN^- results in the formation of NH_4^+-N (0.24 g NH_4^+/g SCN^-) and therefore increased NH_4^+-N loading is observed during the treatment process (Kim *et al.*, 2008). Additional to the high nitrogen loading, the wastewater also contains other pollutant compounds such as phenol and polycyclic aromatic hydrocarbons (PAHs). Phenol has been reported at concentrations between 60 - 400 mg/L (Vázquez *et al.*, 2006a; Staib and Lant, 2007; Marañón *et al.*, 2008; Bai *et al.*, 2010). Concentrations of polycyclic aromatic hydrocarbons (PAHs) are less commonly reported and vary substantially. Burmistrz and Burmistrz (2013) reported 255-312 µg/L for the sum of 16 PAHs whilst Zhang et al. (2012) reported much higher concentrations of 5470 ± 907 µg/L for the sum of 18 PAHs. Raper, Soares et al. (2017) reported the sum of 6 PAHs at 179 ± 35 µg/L therefore being more comparable to the values reported by Burmistrz and Burmistrz (2013). Trace metals have been reported at 4216 µg/L (Raper, Soares, *et al.*, 2017).

Coke making wastewaters are regulated under the Industrial Emissions Directive (IED). Emission limits, introduced in 2016, require the reduction of total nitrogen (TN), the sum of ammonia-nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite-nitrogen (NO_2^--N), to <50 mg/L (European Commission, 2013). Due to the high nitrogen content of coke making wastewaters it is therefore essential for current treatment processes to combine both nitrification and denitrification processes. Coke making wastewaters are typically treated through an activated sludge process (ASP) (European Commission, 2013). A pre-denitrification configuration i.e. anoxic-aerobic ASP, enables the use of organic matter in the wastewater, reducing the need for an external carbon source, as well as lowering the aeration demand in the nitrification reactor, both resulting in operational savings (Kim *et al.*, 2008; Soares *et al.*, 2010). The treatment of shock-loadings of pollutants in coke wastewater was previously investigated through a laboratory-scale anoxic-aerobic ASP to determine the

cause of an observed treatment loss (Kim *et al.*, 2009). Despite this, there has been no consideration of the optimal conditions for TN removal.

The toxic influence of compounds such as SCN^- and phenol on nitrification and denitrification of coke wastewater treatment has previously been investigated. Vázquez et al. (2006b) reported that SCN^- negatively impacted the removal of ammonia, in a laboratory-scale ASP, which decreased from 0.081 kg NH_4^+-N/m^3/d in the absence of SCN^- to ca. 0.04 kg NH_4^+-N/m^3/d when SCN^- was increased to 80 mg/L. In contrast, Kim et al. (2011) reported that shock-loading of SCN^- did not directly impact nitrification, rather, it impacted total nitrogen removal. Increased nitrogen loading to the system resulted in higher concentrations of nitrate and carry over of nitrate into the effluent. Increasing the recycling of nitrified effluent to the anoxic tank, however, improved removal efficiencies of nitrate and in turn total nitrogen removal. The impact of SCN^- is therefore controversial. Furthermore, Kim et al. (2009) highlighted that the supply of inorganic carbon was critical in maintaining nitrogen removal as a result of the autotrophic nature of the nitrifying bacteria. When nitrification treatment efficiency declined the residual inorganic carbon in the treated effluent decreased from 15 to 5 mg/L, suggesting a shortage. Inlet alkalinity values were not reported. Vázquez et al. (2006a) reported an alkalinity addition requirement for coke wastewater of at least 6.5 mg $CaCO_3$/mg NH_4^+-N for a 90% removal efficiency.

In batch tests phenol was demonstrated to negatively impact nitrification efficiencies, decreasing oxygen uptake rates and increasing the required retention time for nitrification (Amor *et al.*, 2005). Despite this, under continuous treatment conditions, phenol concentrations of 35 - 2800 mg/L were shown to have no negative impact on nitrification (Amor *et al.*, 2005). Additionally Kim et al. (2009) reported that a pre-denitrification treatment system showed robustness to fluctuating phenol concentrations as a result of dilution in the anoxic tank where it was also rapidly degraded as a carbon source and so

consequently nitrifying bacteria were protected from exposure to high phenol concentrations.

Denitrification is impacted by the supply of organic carbon with the removal of 1g of NO_3^--N theoretically requiring 2.86 g of COD (Chakraborty and Veeramani, 2006). The actual requirement, nevertheless, varies in response to both the carbon compound, microorganisms present and the operational conditions (Carrera, Vicent and Lafuente, 2004; Chakraborty and Veeramani, 2006; Yang, Wang and Zhou, 2012; Metcalf & Eddy Inc, 2014). The requirements for acetic acid, ethanol and methanol have been reported to vary between 3 and 4.2 g/g NO_3^--N (Carrera, Vicent and Lafuente, 2004; Soares *et al.*, 2010; Metcalf & Eddy Inc, 2014). In a post-denitrification configuration, Vázquez et al. (2006b) reported an optimum dose of 1.2 L of methanol/m^3 corresponding to a COD:N ratio of 3.5. Kim et al. (2008) reported the removal of 3.5 - 5.0 g COD/g NO_3^--N in a laboratory-scale pre-denitrification reactor. Although phenol was utilised as a form of carbon in the anoxic reactor, as the loading rates increased removal efficiencies in the anoxic reactor declined due to the insufficient contact time and supply of nitrate. A loading rate of 0.06 g-phenol/L.d was associated with a 69.5% removal efficiency compared to a removal efficiency of 32.1% at a loading rate of 0.25 g-phenol/L.d. Residual phenol was, however, degraded in the aerobic reactor. Despite this, it was highlighted that sudden increases in phenol in the aerobic reactor may result in a sudden proliferation of heterotrophic bacteria which could potentially out-number slower growing nitrifying bacteria.

Treatment of coke wastewater through an anoxic-aerobic ASP has previously been investigated by Kim et al. (2009, 2011). The focus of the research was understanding the cause behind treatment failures on a full-scale anoxic-aerobic ASP (Kim *et al.*, 2009) and understanding the impact of SCN^- shock loading on nitrification (Kim *et al.*, 2011). Sudden failure of a full-scale anoxic-aerobic ASP was linked to a shortage of alkalinity which was previously

discussed (Kim *et al.*, 2009). Thiocyanate concentrations were shown to directly impact TN removals (Kim *et al.*, 2011). As influent SCN^- concentrations were increased in steps from 200 - 800 mg/L effluent TN concentrations increased from 64 mg/L to 123 mg/L. This resulted from increased concentrations of nitrate in the effluent from the aerobic reactor. Increasing the internal recycle of nitrified effluent was suggested as a way in which to improve nitrate removals.

Many laboratory-scale investigations have considered the inhibitory roles of pollutants associated with coke making wastewater (Kwon, Woo and Park, 2002; Amor *et al.*, 2005; Chakraborty and Veeramani, 2006; Vázquez *et al.*, 2006b; Kim *et al.*, 2009, 2011). Ammonia removal has been investigated through air stripping but this is unable to reduce ammonia sufficiently to enable compliance with a TN emission limit of 50 mg/L (Marañón *et al.*, 2008). Alkalinity supply has been suggested as important in maintaining nitrification efficiencies in a laboratory-scale anoxic-aerobic ASP whilst carbon requirements have been investigated in a laboratory-scale post-denitrification ASP (Lee and Park, 1998; Kim *et al.*, 2009). Optimal operational conditions and loading rates for TN removal in an anoxic-aerobic ASP have not been reported. The aim of this investigation was therefore to investigate TN removal at a larger scale through a pilot-scale anoxic-aerobic ASP and to identify the operational conditions required to consistently achieve an effluent TN of <50 mg/L.

7.2 Experimental

7.2.1 Coke making wastewater and activated sludge

The coke making wastewater used in this study was produced from a full-scale steelworks in the UK. During the long-term operation of the pilot-plant, the coke making wastewater was subject to the same variability as the full-scale site. The pilot-plant was seeded with activated sludge biomass taken from the return

activated sludge (RAS) line of the full-scale wastewater treatment system that operated an aerobic ASP with a hydraulic retention time (HRT) of 21 hours and a sludge retention time (SRT) of approximately 38 days.

7.2.2 Anoxic-aerobic pilot-plant configuration

A pilot-scale anoxic-aerobic ASP was designed with a 340 L (working volume) anoxic reactor, a 680 L (working volume) aerobic reactor and a 55 L (working volume) clarifier (Figure 7-1). Return activated sludge was fed back into the anoxic reactor at a flow rate of 0.32 - 0.55 m^3/d and was characterised by a mixed liquor suspended solids (MLSS) of 7150 mg/L. The sludge age was maintained at approximately 80 days through sludge wasting. Other studies, have investigated nitrate recycle ratios (between the aerobic and anoxic reactors) from 1 to 5 (Chakraborty and Veeramani, 2006; Kim *et al.*, 2008). Chakraborty and Veeramani (2006) reported a 75% removal of total nitrogen at a recycle ratio of 3. In this study, the recycle ratio was therefore kept at 3, to promote high removals whilst maintaining reasonable costs for the pumping requirement. A recycle ratio of 3 corresponded to a flow rate of 0.97- 1.64 m^3/d m^3/d.

Temperature was maintained at ~28^{o}C through the use of heaters in both the anoxic and aerobic reactors. Dissolved oxygen (DO) in the anoxic reactor was maintained below 0.3 mg/L whilst Vitox oxygen injection maintained the DO in the aeration reactor between the set points of 2 - 4 mg/L. Mixed liquor suspended solids were maintained at 4800±1600 mg/L and were kept in suspension through the use of a submersible pump in the anoxic reactor and through the action of the Vitox oxygen injection in the aeration reactor.

The addition of sodium carbonate was previously established as critical for successful nitrification of coke making wastewater through the provision of

inorganic carbon for nitrifying bacteria and pH buffering (Raper, Fisher, *et al.*, 2017). The influent wastewater was therefore dosed with the optimised concentration of sodium carbonate of 523 mg/L as $CaCO_3$. The pH averaged at 7.9 and 7.2 in the anoxic and aerobic reactors, respectively. The higher pH in the anoxic reactor was associated with the alkalinity dosing of the influent.

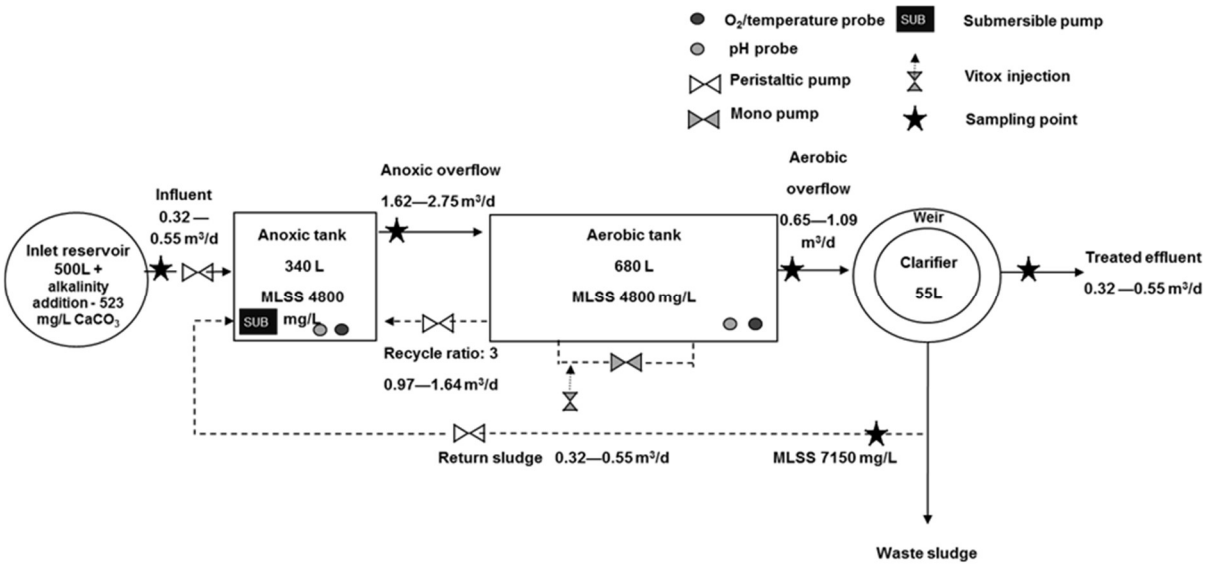


Figure 7-1: Pilot-scale anoxic-aerobic ASP configuration, operational conditions and sampling locations.

The pollutant loading rates applied to the pilot-plant varied between 0.198 - 0.418 kg COD/m^3.day and 0.029 - 0.081 kg TN/m^3.day, respectively, due to adjustments in the feeding rates (0.32 - 0.55 m^3/day) as well as the natural variability of pollutants concentration in the wastewater coming from the full-scale site. Consequently, the total hydraulic retention time (HRT) also varied from 76.5 - 45 h, which was significantly higher than the HRT of 16.7 h Kim et al. (2008) reported to be necessary to achieve stable nitrification and denitrification.

7.2.3 Analytical methods

Samples were taken from the inlet reactor, anoxic reactor overflow, aerobic reactor overflow, return sludge feed and system outlet (Figure 7-1). Samples were filtered through 0.45 µm filters and refrigerated at 2 - 5^{o}C before analysis. Total nitrogen (TN) was calculated through the measurement of NO_2^--N, NO_3^--N, NH_4^+-N and SCN^--N (included due to its degradation to NH_4^+-N). Although TN, is defined as containing organic nitrogen, coke making wastewaters contain very little organic nitrogen other than SCN^- (Vázquez *et al.*, 2007) and therefore the method used is a good approximation of TN. Nitrite-nitrogen, NO_3^--N, NH_4^+-N and soluble chemical oxygen demand (sCOD) were analysed using Merck cell test kits according to the manufacturer's instructions. Thiocyanate was analysed colourmetrically by complex reaction with thiocyanate and iron oxide at a wavelength of 465 nm, based on The Institute of Gas Engineers analytical method (The Institution of Gas Engineers, 1971). Mono phenols were analysed by complex reaction with 4-aminoantipyrene at a wavelength of 510 nm, based on ISO 6439:1990 (ISO, 1990). Both were analysed using a Jenway 6300 spectrophotometer (Staffordshire, UK). pH was recorded using a Jenway 3540 pH meter (UK). Total suspended solids (TSS), alkalinity and BOD_5 were analysed according to standard methods (Eaton, 2005). Data was analysed using a Grubbs' test to identify outliers. Outliers were subsequently excluded from the data set.

7.3 Results and Discussion

7.3.1 Coke making wastewater composition and pilot-plant operation

Over the 5 month period the pilot-plant was operated, the feed coke making wastewater was subject to the same variation as the full-scale treatment plant (Table 7-1). Total nitrogen concentrations averaged at 110 mg/L, varying from 87 to 152 mg/L. Ammonia-nitrogen averaged at 59 mg/L, varying from 43 to 75 mg/L. Nitrite-nitrogen levels ranged from 6 to 29 mg/L averaging at 13 mg/L

whilst NO_3^- -N levels were low, at an average of 3.3 mg/L and a maximum of 6.2 mg/L. Phenol concentrations averaged at 99 mg/L during the pilot-plant operation, however, concentrations were initially at ca. 166 mg/L, declining to 52 mg/L at the end. Phenol concentrations were more variable than previous reports (Raper, Soares, *et al.*, 2017). Thiocyanate was comparable to previous reports averaging at 154 mg/L (Raper, Soares, *et al.*, 2017). Soluble chemical oxygen demand averaged at 710 mg/L, ranging from 528 to 906 mg/L. Pollutant concentrations observed in the wastewater were therefore within the lower end of the ranges reported in the literature and lower than those observed by Kim et al. (2008).

Table 7-1: Characterisation of coke wastewater composition feed to the pilot-plant.

	Average and standard deviation (mg/L)
BOD	390 ± 60
sCOD	710 ± 110
NO_2^--N	13 ± 6
NO_3^--N	3 ± 1
NH_4^+-N	59 ± 9
TN	113 ± 15
SCN	154 ± 22
SCN^--N	37 ± 5
Phenol (mono)	99 ± 31
SS	40 ± 20
pH*	9.4 ± 0.2
Trace metals (µg/L)**	149 ± 21

*After alkalinity dosing (523 mg/L as $CaCO_3$)

** Sum of Cr, Ni, Cu, Zn, As, Cd and Pb.

During this study, the wastewater was fed to the pilot-plant at a rate between 0.32 - 0.55 m^3/day corresponding to HRTs from 45 - 76.5 h. Due to the high natural variation of the wastewater composition, the correlation between HRT and SCN^-, phenol and TN loading rates did not follow a linear relationship (Figure 7-2). In the current investigation, the lack of correlation between the HRT and loading rate was particularly noticeable for the phenol loading which varied from 0.026 to 0.039 kg/m^3.d at HRTs 76.5 – 52.5 h. At the shortest considered HRT, of 45 h, phenol loading rates were at their lowest (0.007 – 0.016 kg/m^3.d). Similarly, SCN^- loading rates did not increase linearly with the decreased HRTs with observed SCN^- loading rates of 0.046 – 0.057 kg/m^3.d, 0.038 – 0.059 kg/m^3.d and 0.044 – 0.067 kg/m^3.d at 76.5, 67.5 and 60 h HRT, respectively. Although TN loading rates showed an overall increase with declining HRT there was still considerable overlaps between the different HRTs (Figure 7-2). Traditionally, HRT has been used as an operational parameter, however, it is not useful for the treatment of industrial wastewaters which have a variable composition. When influent concentrations vary significantly setting a fixed HRT cannot guarantee consistent treatment efficiencies as pollutant loading rates can vary significantly which may result in an insufficient contact time between the degrading microorganisms and pollutant.

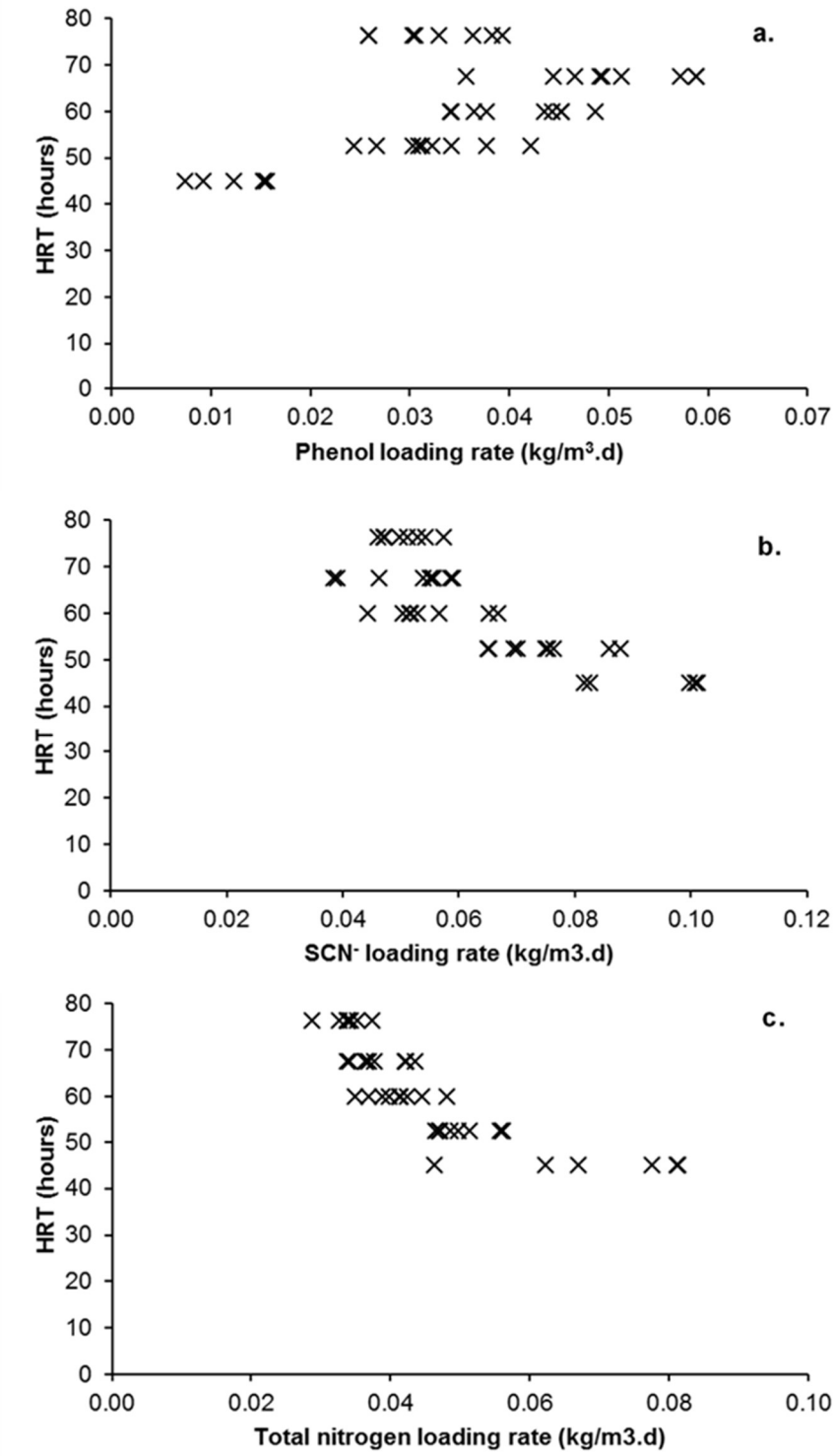


Figure 7-2: Correlation between HRT and a. phenol loading rate b. thiocyanate loading rate c. total nitrogen loading rate.

7.3.2 Biodegradation of phenol and thiocyanate

Influent to the anoxic-aerobic pilot-plant was characterised by phenol concentrations of 52 to 166 mg/L corresponding to a loading rate of 0.007 to 0.059 kg/m^3.d. The mass balances completed, demonstrate that phenol was removed primarily in the anoxic reactor with removal efficiencies of 82 - 99% (Figure 7-3 and Figure 7-4). These results indicate that phenol was consumed as a carbon source by denitrifying bacteria. Under high loading conditions (0.059 kg/m^3.d) some residual phenol passed into the aerobic reactor, but there was enough capacity in the aerobic reactor to ensure that the remaining phenol was degraded to 0 - 1.7 mg/L, averaging at 0.8 mg/L. Phenol removal efficiencies were consistently greater than 96%, averaging at 99%, similar to those observed by Kim et al. (2009) in laboratory-scale investigations. Nevertheless, further process optimisation would be required to ensure total compliance, as phenol emissions exceeded the 0.5 mg/L limit set by the IED. Increasing the sludge age may allow improved phenol degradation rates whilst the incorporation of other technologies such as bioaugmentation and activated carbon have also been shown to be effective at improving phenol removal efficiencies and resilience to shock-loads (Vinitnantharat *et al.*, 2001; Fang *et al.*, 2013).

Thiocyanate loading rates varied from 0.038 - 0.101 kg/m^3.d and removal efficiencies averaged at 99% resulting in average effluent concentrations of 1.1 mg/L (Figure 7-3 and Figure 7-4) complying with the 4 mg/L emission limit set by the IED (European Commission, 2013). Removal efficiencies of SCN^- were comparable to those reported by Kim et al. (2008, 2011) in a laboratory-scale anoxic-aerobic ASP. Degradation of SCN^- has been reported to be possible by a wide variety of bacteria under a wide range of conditions (Raper, Stephenson, *et al.*, 2017). Removal of SCN^- was complete in the anoxic reactor of the pilot-scale anoxic-aerobic ASP (Figure 7-3 and Figure 7-4) showing capability of the bacteria to degrade SCN^- under anoxic conditions. This contrasts the findings of

Kim et al. (2008, 2011) who reported that SCN^- degradation took place in the aerobic reactor of the laboratory-scale anoxic-aerobic ASP.

7.3.3 Nitrogen removal

7.3.4 Effluent TN <50 mg/L

Mass balances were produced for the anoxic-aerobic ASP pilot-plant to understand the conditions required to achieve an effluent characterised by TN of <50 mg/L (Figure 7-3 and Figure 7-4). When the effluent had a TN <50 mg/L the wastewater influent concentrations were characterised by: 60 mg/L NH_4^+-N, 10 mg/L NO_2^--N and 4 mg/L NO_3^--N (Figure 7-3). This corresponded to loading rates of 0.027 kg/m^3.d NH_4^+-N, 0.004 kg/m^3.d NO_2^--N and 0.002 kg/m^3.d NO_3^--N. Ammonia-nitrogen was therefore the largest contributor to TN in the influent. Thiocyanate was present at 163 mg/L in the influent and through its degradation it produced a further 39 mg/L of NH_4^+-N corresponding to an additional NH_4^+-N loading rate of 0.018 kg/m^3.d. Total nitrogen concentrations in the influent were 112 mg/L giving a loading rate of 0.051 kg/m^3.d. Nitrification was stable at 98% with ammonia being removed to below the detection limit of 2 mg/L comparable to other reported nitrification efficiencies (Vázquez *et al.*, 2006b; Kim *et al.*, 2008). As a result of nitrification, NO_3^--N concentrations increased substantially from an influent of 4 mg/L to a concentration of 25 mg/L in the aerobic reactor.

The concentrations of sCOD and phenol were 638 mg/L and 71 mg/L, respectively. The feed wastewater was therefore characterised by a sCOD:TN ratio of 5.7. Combining the influent, RAS and nitrate recycle, resulted in a sCOD loading rate of 1.429 kg/m^3.d and a TN loading rate of 0.408 kg/m^3.d resulting in a sCOD:TN ratio of 3.9 fed to the anoxic reactor. Nitrate was reduced in the anoxic reactor resulting in a 46% TN removal efficiency. The consumption of carbon for denitrification led to a 43% removal of sCOD and a 92% removal efficiency for phenol. As a result of the nitrogen removal, effluent from the

anoxic-aerobic ASP was characterised by a TN of 48 mg/L meeting the IED 50 mg/L emission limit.

7.3.5 Effluent TN >50 mg/L

A mass balance was subsequently produced for the anoxic-aerobic reactor when effluent TN concentrations exceeded the 50 mg/L emission limit (Figure 7-4). The influent concentrations were characterised by: 68 mg/L of NH_4^+-N, 23 mg/L NO_3^--N and 5 mg/L NO_2^--N. This corresponded to loading rates of 0.036 kg/m^3.d NH_4^+-N, 0.003 kg/m^3.d NO_3^--N and 0.013 kg/m^3.d of NO_2^--N. The influent contained 175 mg/L of SCN^- giving a loading rate of 0.093 kg/m^3.d. The degradation of SCN^- in the anoxic reactor supplied the system with a further 42 mg/L of NH_4^+-N to the system. Ammonia-nitrogen was the main contributor to TN. Nitrification removal efficiencies were also comparable at 96% with ammonia being removed to below the detection limit of 2 mg/L. In contrast, NO_3^--N concentrations increased, as a result of nitrification, from an influent of 5 mg/L to a concentration of 71 mg/L in the aerobic reactor.

Overall, the system was characterized by a TN loading rate of 0.069 kg/m^3.d, an increase on that observed when the anoxic-aerobic reactor was operating within the TN 50 mg/L emission limit (0.051 kg/m^3.d). Influent sCOD concentrations were 670 mg/L giving a higher loading rate of 0.357 kg/m^3.d. Phenol concentrations of 67 mg/L contributed to a much lower loading rate of just 0.013 kg/m^3.d. As a result of higher TN concentrations, the sCOD:TN ratio of the pilot-plant feed decreased to 5.2.

	Influent		Return sludge	Aerobic-Anoxic Recycle	Influent to Anoxic tank	Anoxic tank			Aerobic tank			Clarifier	Effluent	Whole system removal efficiency (%)
	mg/L	Loading rate (kg/m^3.d)	mg/L	mg/L	mg/L	mg/L	Loading rate (kg/m^3.d)	Removal efficiency (%)	mg/L	Loading rate (kg/m^3.d)	Removal efficiency (%)	Loading rate (kg/m^3.d)	mg/L	
sCOD	638	0.291	94	115	215	124	1.429	43	115	0.405	7	1.953	109	83
Phenol	71	0.032	0.8	2	15	1.3	0.100	92	1.7	0.004	0	0.055	1.2	98
NH_4^+-N	60	0.027	2	2	14	16	0.092	0	2	0.054	87	0.034	2	97
SCN^-	163	0.074	0	0	33	0.1	0.219	100	0.3	0.001	0	0.005	0.6	100
SCN^--N	39	0.018	0	0	8	0	0.053	100	0.1	0.000	0	0.001	0.1	100
NH_4^+-N + SCN^--N	99	0.045	2	2	21	16	0.144	26	2	0.054	87	0.035	2	98
NO_2^--N	10	0.004	16	19	17	16	0.104	7	19	0.053	0	0.326	16	0
NO_3^--N	4	0.002	26	25	21	1	0.139	93	25	0.005	0	0.420	27	0
NO_x-N	13	0.006	42	44	38	17	0.235	55	44	0.058	0	0.754	43	0
TN	112	0.051	46	48	61	33	0.408	46	48	0.113	0	0.820	48	57
sCOD:N	5.7					3.9								

Recycle ratio: 3

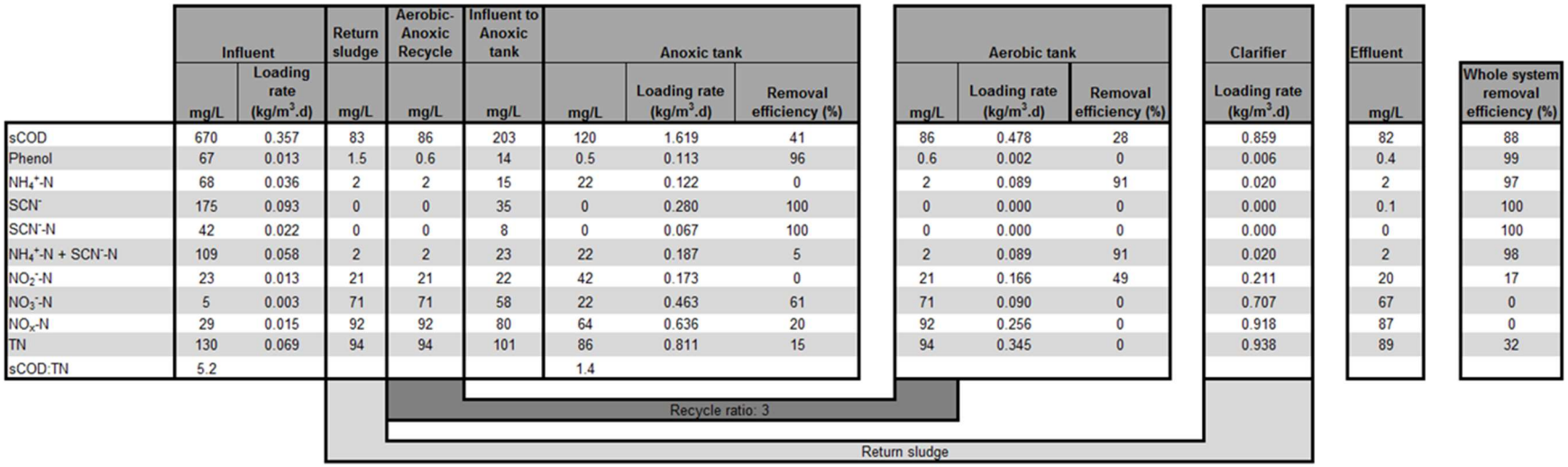
Return sludge

Figure 7-3: Mass balance for anoxic-aerobic pilot-plant characterised by an effluent <50 mg/L TN.

Influent loading rate = (influent flow (m^3/d) x concentration of pollutant in influent (kg/m^3) / total reactor volume (1.02 m^3)

Anoxic loading rate = (anoxic influent flow (m^3/d) x concentration of pollutant at influent of anoxic reactor (kg/m^3) / volume of anoxic reactor (0.34 m^3)

Aerobic loading rate = (aerobic influent flow (m^3/d) x concentration of pollutant at influent of aerobic reactor (kg/m^3) / volume of aerobic reactor (0.68 m^3)



	Influent		Return sludge	Aerobic-Anoxic Recycle	Influent to Anoxic tank	Anoxic tank			Aerobic tank			Clarifier	Effluent	Whole system removal efficiency (%)
	mg/L	Loading rate (kg/m^3.d)	mg/L	mg/L	mg/L	mg/L	Loading rate (kg/m^3.d)	Removal efficiency (%)	mg/L	Loading rate (kg/m^3.d)	Removal efficiency (%)	Loading rate (kg/m^3.d)	mg/L	
sCOD	670	0.357	83	86	203	120	1.619	41	86	0.478	28	0.859	82	88
Phenol	67	0.013	1.5	0.6	14	0.5	0.113	96	0.6	0.002	0	0.006	0.4	99
NH_4^+-N	68	0.036	2	2	15	22	0.122	0	2	0.089	91	0.020	2	97
SCN^-	175	0.093	0	0	35	0	0.280	100	0	0.000	0	0.000	0.1	100
SCN^--N	42	0.022	0	0	8	0	0.067	100	0	0.000	0	0.000	0	100
NH_4^+-N + SCN^--N	109	0.058	2	2	23	22	0.187	5	2	0.089	91	0.020	2	98
NO_2^--N	23	0.013	21	21	22	42	0.173	0	21	0.166	49	0.211	20	17
NO_3^--N	5	0.003	71	71	58	22	0.463	61	71	0.090	0	0.707	67	0
NO_x-N	29	0.015	92	92	80	64	0.636	20	92	0.256	0	0.918	87	0
TN	130	0.069	94	94	101	86	0.811	15	94	0.345	0	0.938	89	32
sCOD:TN	5.2					1.4								

Figure 7-4: Mass balance for anoxic-aerobic pilot-plant characterised by an effluent >50 mg/L TN.

Influent loading rate = (influent flow (m^3/d) x concentration of pollutant in influent (kg/m^3) / total reactor volume (1.02 m^3)

Anoxic loading rate = (anoxic influent flow (m^3/d) x concentration of pollutant at influent of anoxic reactor (kg/m^3) / volume of anoxic reactor (0.34 m^3)

Aerobic loading rate = (aerobic influent flow (m^3/d) x concentration of pollutant at influent of aerobic reactor (kg/m^3) / volume of aerobic reactor (0.68 m^3)

Significant differences in treatment efficiencies occurred in the anoxic reactor. Due to the increased production of NO_3^--N in the aerobic reactor, from 25 mg/L to 71 mg/L, the loading rate of NO_3^--N to the anoxic reactor more than tripled from 0.139 to 0.463 kg/m^3.d. The NO_3^--N specific removal rate increased from 0.081 g/g VSS.d (TN <50 mg/L) to 0.186 g/g VSS.d (TN >50 mg/L) in response to the increased loading. The sCOD loading increased from 1.429 kg/m^3.d (TN <50 mg/L) to just 1.619 kg/m^3.d. Nitrate-nitrogen concentrations in the anoxic reactor increased from 1 mg/L (TN <50 mg/L) to 22 mg/L. The concentration of NO_2^--N was more than double, at 42 mg/L, with no removal being observed. Consequently, the denitrification efficiency decreased from 93% to 61%. Total nitrogen removal in the anoxic reactor declined from 46% to 15%. Decreased nitrogen removals were associated with the decreased sCOD:TN ratio of influent to the anoxic reactor of 1.4 which was insufficient for the complete removal of NO_3^--N and NO_2^--N. Figure 7-5 shows the impact of the anoxic sCOD:TN ratio on NO_2^--N, NO_3^--N, oxidised nitrogen (NO_x -N) and TN concentrations in the anoxic reactor. When the anoxic sCOD:TN ratio declined below a ratio of 4, NO_2^--N concentrations started to increase. Below an sCOD:TN ratio of 2 concentrations of NO_2^--N and NO_3^--N increased rapidly.

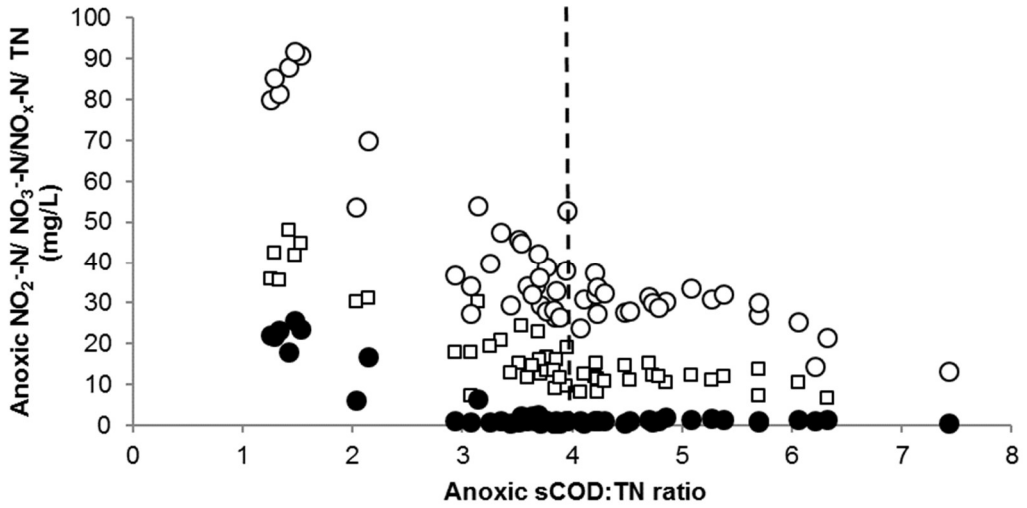


Figure 7-5: Changing concentrations of □ NO_2^--N, ● NO_3^--N and ○ TN in the anoxic reactor in response to changes in the anoxic sCOD:TN ratio. Dashed line marks anoxic sCOD:TN ratio below which nitrogen rapidly accumulates.

As a result of the poor performance of the anoxic reactor under carbon limited conditions the anoxic-aerobic ASP effluent TN increased substantially to 89 mg/L greatly exceeding the 50 mg/L IED emission limit. With effluent concentrations of 67 mg/L of NO_3^--N the emission limit was exceeded by NO_3^--N concentrations alone. Whilst the decline in the sCOD:TN ratio of the influent appeared relatively small, declining from 5.7 to 5.2, the increased TN loading had a much more significant impact on the sCOD:TN ratio observed in the anoxic reactor which declined from 3.9 to 1.4. The sCOD:TN ratio of the anoxic reactor is therefore a more accurate indication of TN removal potential. Liu et al. (1996) reported that the carbon requirement was best represented by the influent COD:TN ratio during the treatment of coal gasification and coke plant wastewater, however, comparability needs to recognise the different treatment configuration which consisted of the use of a submerged biofilm in the anoxic cell and also the use of total COD rather than soluble COD. Furthermore, an influent sCOD:TN ratio of 5.7 in the current investigation resulted in TN removal efficiencies of 57%, much lower than the 83% removal efficiency observed by Liu et al. (1996) at a COD:TN ratio of 5.

7.3.6 Nitrite removal in the anoxic-aerobic reactor

A notable characteristic of the anoxic-aerobic ASP was the poor removal of NO_2^--N (Figure 7-6). The presence of NO_2^--N can be indicative of a disturbance or limitation within the nitrification and or denitrification step process (Philips, Laanbroek and Verstraete, 2002). Several factors have been associated with the accumulation of NO_2^--N during the denitrification process including the type of carbon (Rocher *et al.*, 2015), reactor pH (Glass and Silverstein, 1998; Cao, Qian and Meng, 2013), the rate of NO_3^--N and NO_2^--N reduction (Wilderer *et al.*, 1987; Philips, Laanbroek and Verstraete, 2002) and the abundance of species present (Philips, Laanbroek and Verstraete, 2002). Carbohydrates and organic acids have been reported to result in the accumulation of 0.2 - 0.3 g NO_2^--N/g NO_3^--N whilst alcohols such as methanol, ethanol or glycerol resulted in lower accumulations of 0.05 - 0.1 g NO_2^--N/g NO_3^--N (Rocher *et al.*, 2015). A high pH

may result in the accumulation of NO_2^--N as the NO_2^--N reduction rate decreases with increased pH (Cao, Qian and Meng, 2013). The abundance of species present in the mixed liquor can also impact NO_2^--N accumulation due to the relative numbers of true denitrifying bacteria (complete both NO_3^--N and NO_2^--N reduction) and incomplete denitrifiers/nitrate-respiring bacteria (complete NO_3^--N reduction but are unable to reduce NO_2^--N) (Philips, Laanbroek and Verstraete, 2002).

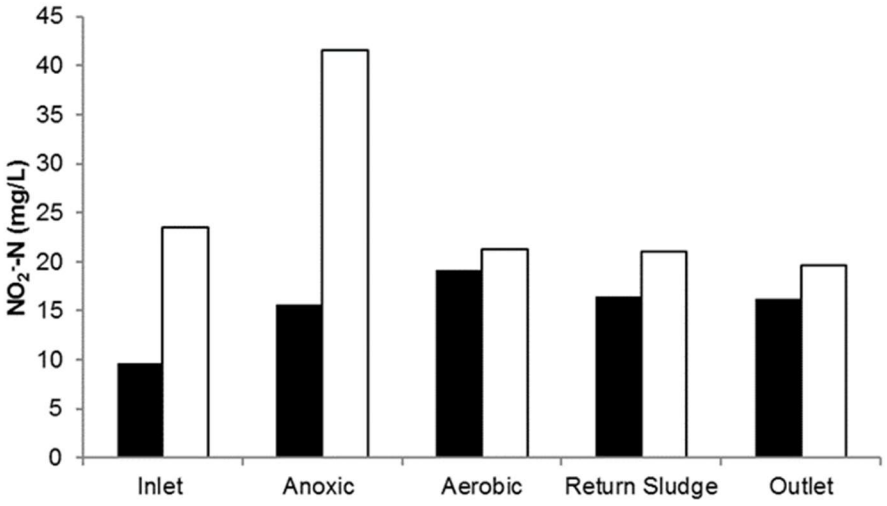


Figure 7-6: Variation of nitrite-nitrogen (NO_2^--N) concentration at different stages of the anoxic-aerobic ASP pilot-plant when effluent concentration was TN <50 mg/L - ■ and at TN >50 mg/L - □.

When effluent TN concentrations were <50 mg/L, NO_2^--N accumulated from 10 mg/L in the influent to 16 mg/L in the effluent. Under carbon-limiting conditions when the effluent TN exceeded 50 mg/L, NO_2^--N concentrations were observed to increase significantly in the anoxic reactor suggesting that the main disturbance was associated with the denitrification and the reduction of NO_2^--N to nitrogen gas. Nitrite-nitrogen entered the anoxic reactor at 22 mg/L whilst effluent from the anoxic reactor contained 42 mg/L of NO_2^--N, representing a 55% increase in NO_2^--N concentrations. Under carbon limited conditions, the pH was suitable for denitrification and therefore species abundance may be

responsible for the increased NO_2^--N accumulation. Species of *Rhodanobacter* genus have previously been identified as representing a significant abundance (11%) in the bacterial composition of the activated sludge used in this study (Raper, Stephenson, *et al.*, 2017). Whilst some species of *Rhodanobacter* (*Rhodanobacter denitrificans*) have been associated with complete denitrification (Prakash *et al.*, 2012) others (*Rhodanobacter thiooxidans*) have been characterised as capable of NO_3^--N reduction but not NO_2^--N reduction (Lee *et al.*, 2007). Under carbon limited conditions, competition for electron donors becomes more intense which has been reported to favour NO_3^--N reduction (Oh and Silverstein, 1999). The competitive conditions can therefore lead to a further increase the numbers of incomplete denitrifiers/nitrate-respiring bacteria. Consequently, the high loading of NO_3^--N to the anoxic reactor observed when the effluent TN >50 mg/L, would result in reduced NO_2^--N removal efficiencies further exacerbating NO_2^--N accumulation. Any accumulation of NO_2^--N is undesirable due to the resulting impact on the ability to reach ever tightening nitrogen emission limits and its higher toxicity relative to other nitrogen compounds. Consequently, a clear indication of the pathways leading to NO_2^--N accumulation requires more investigation.

7.4 Conclusion

The anoxic-aerobic ASP pilot-plant was capable of removing SCN^- and phenol under all loading rates to 100% and 96% respectively. Nitrification remained stable at >96% under all conditions. Both phenol and SCN^- were utilised as carbon sources during denitrification. Organic carbon availability was a critical parameter in nitrogen removal. Influent to the anoxic-aerobic ASP required an sCOD:TN ratio of 5.7 to enable an effluent characterised by a TN concentration <50 mg/L. At an sCOD:TN ratio of 5.2 the emission limit was exceeded (89 mg/L) as NO_3^--N removal efficiencies in the anoxic reactor decreased from 93% to 61%. The presence of NO_2^--N in the anoxic reactor under all conditions

indicates a disturbance to the denitrification process which may be attributed to the bacterial speciation and was exacerbated under carbon limited conditions.

7.5 Acknowledgements

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Chapter 8 Discussion

In order to comply with the IED emission limits it is necessary to update current coke wastewater treatment processes to include nitrogen removal. An emission limit of <50 mg/L total nitrogen (TN) requires current treatment works to be upgraded to include nitrification and denitrification. Additionally, it is important to have a better understanding of the thiocyanate (SCN^-) degradation process in order to improve process stability and enable compliance with the emission limit of <4 mg/L. Furthermore, techniques to increase the removal of PAHs and trace metals require investigation as reduced emission limits are becoming ever more challenging to meet.

8.1 Total nitrogen removal from coke wastewater

Increased knowledge on the effects of inorganic pollution in the natural environment such as eutrophication, have led to increasingly stringent nitrogen emission limits (Camargo and Alonso, 2006). Consequently, there is now a need for coke wastewater treatment to include both nitrification and denitrification to produce an effluent characterised by <50 mg/L total nitrogen.

A number of batch tests were completed and a continuous laboratory-scale anoxic-aerobic reactor was operated to investigate nitrification and the impact of organic carbon source. Nitrification was limited by the availability of inorganic carbon (Chapter 6) which was required to buffer the acidification associated with nitrification and also acted as a substrate for autotrophic nitrifying bacteria (Wett and Rauch, 2003). The coke wastewater under investigation was characterised by a typical alkalinity of 3.6 mg as $CaCO_3$/mg NH_4^+-N representing a shortage compared to the stoichiometric requirement of 7.07 mg as $CaCO_3$/mg NH_4^+-N (accounting for biomass growth). Nitrification was

therefore limited to 40% (Chapter 6). The addition of inorganic carbon via sodium carbonate (Na_2CO_3) addition at 300 mg/L enabled a nitrification efficiency of 95%. Despite inorganic carbon provision having been highlighted as important, there were no studies on suitable compounds for the provision of additional inorganic carbon and/or the impact of different compounds on overall treatment performance. Sodium carbonate was considered the most effective source of alkalinity allowing complete ammonia oxidation. Sodium bicarbonate on the other hand led to incomplete ammonia oxidation (76%) whilst soda ash prevented nitrite oxidation. Sodium hydroxide was also considered, as a method by which to increase carbonate availability through alteration of the carbonate equilibrium. Ammonia oxidation had an optimal pH range of 7.0 – 8.3 whilst nitrite oxidation required a pH 5.5 - 6.8.

In relation to denitrification, the removal of 1g of NO_3^--N theoretically requires 2.86 g of COD. To maintain an effluent TN <50 mg/L, external carbon addition was necessary to ensure suitable organic carbon availability (Soares *et al.*, 2010). Glycerol and acetic acid were investigated as alternative carbon sources (Chapter 6). Glycerol was determined to be an ineffective carbon source resulting in low biomass production. According to the stoichiometry, 0.18 mg of biomass is produced for each mg of glycerol. This was lower than the 0.2 mg of biomass produced for each mg of phenol and 0.31 mg of biomass produced for each mg of acetic acid. This resulted in a decrease in the MLSS when glycerol was used. As a result of the declining MLSS, the sCOD loading rate to the aerobic tank increased from an average of 0.31 kg/m^3.d to 0.44 kg/m^3.d and nitrification efficiencies declined rapidly. Increased sCOD availability in the aerobic cell would enhance the growth of heterotrophic bacteria which could then out-compete nitrifying bacteria (Jenni *et al.*, 2014). Concerns had been raised around nitrite accumulation associated with glycerol usage (Uprety *et al.*, 2012), however, a nitrite accumulation was not observed in the current investigation. The higher biomass production for acetic acid enabled stable nitrification and denitrification. Moreover, acetic acid as a carbon source led to

an improved removal of TN with the average effluent TN decreasing from 30 mg/L (control conditions) to 24 mg/L (acetic acid).

A pilot-scale anoxic-aerobic ASP (1.02 m^3) was operated to investigate the impact of carbon and nitrogen loading on treatment efficiencies (Chapter 8). The inlet feed was subject to the temporal variations seen on the full-scale site allowing comparability to future operational conditions. As a result of the influent variability, the loading rates varied between 0.198 - 0.418 kg COD/m^3.day and 0.029 - 0.081 kg TN/m^3.day, respectively. Inlet wastewater was dosed with Na_2CO_3 in order to support nitrification. Under all conditions nitrification remained stable with ammonia being removed to below the detection limit, comparable to other reported nitrification efficiencies (Vázquez *et al.*, 2006; Kim *et al.*, 2008).

Both phenol and SCN^- were utilised as carbon sources during denitrification (Chapter 8). Removal of SCN^- in other coke wastewater treatment plants had previously been reported to occur under aerobic conditions. Denitrification efficiencies were limited by carbon availability. Effluent from the pilot plant was characterised by a TN of <50 mg/L when the influent to the anoxic-aerobic ASP was maintained at a sCOD:TN ratio of >5.7. When the sCOD:TN ratio declined to 5.2 NO_3^--N removal efficiencies in the anoxic reactor declined from 93% to 61% in response to the limited carbon availability. This resulted in an effluent characterised by 89 mg/L TN, far in excess of the 50 mg/L emission limit.

Nitrite-nitrogen was present in the anoxic reactor under all operational conditions. Accumulation of nitrite as a result of factors such as pH and dissolved oxygen were ruled out. This suggested that there was a disturbance to the denitrification process which was attributed to the bacterial species present, for example, the genus *Rhodanobacter* was identified in the activated sludge biomass of which some species are associated with nitrate reduction but

not nitrite reduction (Wang *et al.*, 2017) (Chapter 4). Under carbon limited conditions increased NO_2^--N concentrations were observed (an increase from 16 mg/L to 42 mg/L).

8.2 Thiocyanate degradation

Through both literature reports (Staib and Lant, 2007) and the experience of practitioners and operators on the full-scale coke wastewater treatment plant the mechanism of SCN^- degradation has been identified as subject to controversy. Increasing the understanding of SCN^- degradation was therefore considered to be important, firstly to allow the <4 mg/L emission limit to be reached but also due to the contribution of ammonia-nitrogen from the SCN^- degradation process which would in turn impact compliance with TN emission limits. Thiocyanate degradation was stable in an aerobic ASP with a 99% removal efficiency (Chapter 3). Similarly, in the anoxic-aerobic configuration SCN^- removal was complete in the anoxic tank (Chapter 6 and 7). This contradicted other studies on coke wastewater where SCN^- degradation was associated with aerobic treatment (Park *et al.*, 2008; Kim *et al.*, 2011).

Literature reports that SCN^- degrading bacteria may be heterotrophic or autotrophic in nature (Chapter 4). The identification of the requirement for either organic or inorganic carbon was therefore essential to enable the provision of a suitable carbon source. DNA sequencing analysis identified *Thiobacillus* as a dominant species. It was therefore believed that SCN^- degradation was associated with the activity of *Thiobacillus*. (Chapter 4). This suggested that the SCN^- degrading bacteria were autotrophic as species of the *Thiobacillus* genus are characterised as autotrophic. Within the *Thiobacillus* genus 3 species have been identified, *T. aquaesulis*, *T. thioparus* and *T. denitrificans* of which only *T. thioparus* and *T. denitrificans* are capable of growth on thiocyanate. The two species have a 98% similarity and are distinguished in their nitrite reduction

capabilities (Kelly and Wood, 2000). *Thiobacillus thioparus* is unable to reduce nitrite whilst *T. denitrificans* is capable of complete reduction of nitrate to nitrite and subsequently nitrogen gas. Due to the presence of nitrite accumulation in the anoxic cell (Chapter 7) under all conditions and the identification of other species in the biomass that are capable only of nitrate reduction rather than coupled nitrate and nitrite reduction (Chapter 4) it was not possible to determine in the present investigation whether the uncultured species possessed a greater metabolic similarity to *T. thioparus* or *T. denitrificans*.

Several different pathways have been reported for SCN^- degradation (Chapter 4), however, these can be categorised into two main pathways: 1) SCN^- undergoes hydrolysis to form NH_4^+ and SO_4^{2-} or 2) SCN^- is hydrolysed following the carbonyl sulphide pathway to form NH_4^+ and carbonyl sulphide (COS). It is likely, however, that the mixed culture degrades SCN^- forming NH_4^+ and SO_4^{2-} as sulphate has been observed to accumulate during the full-scale treatment process (data not reported). The suspected pathway for SCN^- degradation under both anoxic and aerobic conditions can be seen in Figure 8-1 along with the subsequent nitrification/denitrification of SCN^--N. Under anoxic conditions SCN^- is degraded forming cyanate (OCN^-), nitrogen gas and sulphate using NO_3^- as the electron acceptor. The cyanate is subsequently hydrolysed to form ammonia and bicarbonate. Under aerobic conditions SCN^- is hydrolysed to OCN^- and sulphide. Cyanate is subsequently hydrolysed to form ammonia and bicarbonate and sulphide oxidised to form sulphate.

Degradation of SCN^- was shown to require mesophilic temperatures (Chapter 4). Respirometry investigations highlighted that SCN^- degradation was impacted by the initial SCN^- concentration. When the initial SCN^- concentration exceeded 100 mg/L SCN^- hydrolysis took place but no NH_4^+ production was observed. This was believed to be the result of a rapid accumulation of OCN^- which reduced the activity of cyanase. Thiocyanate removal was inhibited by NH_4^+ with an IC_{50} value of 316 mg/L (Chapter 4). Furthermore, phenol also had an

inhibitory role with 180 mg/L reducing SCN^- degradation by 41% (Chapter 4). It is therefore important to control both NH_4^+ and phenol concentrations entering the ASP in order to maintain effective SCN^- degradation. Nitrite, which had previously been suspected of causing inhibition to SCN^- degradation was demonstrated to have no inhibitory role (Chapter 4). Additionally, SCN^- degradation was observed to take place before nitrification (Chapter 4) and therefore SCN^- degradation was unlikely to be impacted by nitrification. Thiocyanate removal was improved via alkalinity addition (Chapter 5) supporting the autotrophic nature of SCN^- degrading bacteria whilst micronutrient addition also ensured compliance with the 4 mg/L emission limit (Chapter 4).

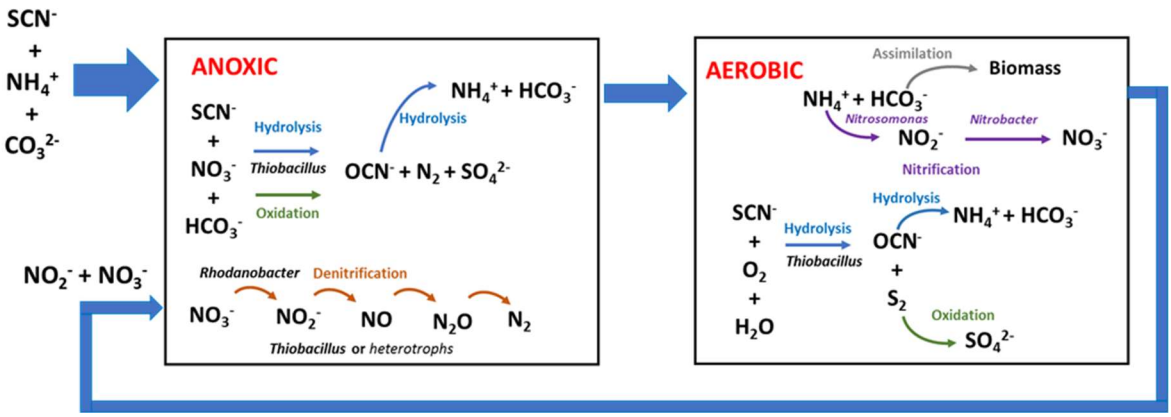

Figure 8-1: Schematic representation of the likely pathway of SCN^- degradation and associated nitrogen removal in the anoxic-aerobic ASP.

8.3 Phenol degradation

Phenol (total) degradation was observed to occur in aerobic conditions through an aerobic ASP process with removal efficiencies of 97% (Chapter 3). Phenol (mono) removal efficiencies averaged at 99% in the anoxic-aerobic pilot-plant and laboratory-scale reactors (Chapter 6 and 7) occurring under both anoxic

and aerobic conditions. Removal efficiencies were similar to those observed by Kim et al. (2009). Phenol (mono) removal occurred primarily in the anoxic tank, however, under high loading conditions some residual phenol (mono) passed into the aerobic tank. Phenol (mono) degradation was completed in the aerobic tank under these conditions. The influence of phenol on nitrification is controversial, with some reporting inhibition (Inglezakis *et al.*, 2016) whilst other studies have shown no inhibition (Amor *et al.*, 2005; Kim *et al.*, 2009). It is suspected therefore that high loading of phenol could potentially disrupt nitrification through excessive growth of heterotrophic bacteria in the aerobic tank (Kim *et al.*, 2009). Despite high treatment efficiencies phenol (mono) was degraded to 0 – 1.7 mg/L averaging at 0.8 mg/L (Chapter 7). Therefore, further work is required to ensure that phenol can be maintained at <0.5 mg/L to comply with the IED emission. One possibility, is biostimulation. The addition of alkalinity improved phenol removals in batch tests producing an effluent with an average concentration of 0.3±0.3 mg/L (Chapter 5), however, further work would be required to elucidate the cause behind this improved removal.

8.4 Treatment options for PAHs and Trace metals

Both PAHs and trace metals were removed during the ASP. Under typical operational conditions removal efficiencies for Σ6PAHs and trace metals were 58% and 35% respectively. However, with reduced emission limits for PAHs and both PAHs and trace metals being listed as priority substances (European Commission, 2014) the treatment process needs to be resilient and able to cope with the high variability in wastewater composition. Both activated carbon and bioaugmentation were identified to be capable of increasing the resilience of the ASP by enhancing pollutant removal.

8.4.1 Activated carbon (AC) - Lignite

A pilot-scale (0.64 m^3) aerobic ASP was operated to investigate the removal of trace metals and PAHs. Previously, few investigations considered the benefits of AC under continuous operational conditions or for the treatment of coke wastewater (Chao, Yeh and Shieh, 1986). The application of AC to PAHs and trace metals in coke wastewater had not previously been investigated. Addition of 400 mg/L powdered activated carbon (PAC) resulted in improved removals of Σ6PAHs with effluent emissions falling from 54 ± 21 µg/L to 34 ±11 µg/L. Improved removals were seen for all of the PAHs investigated demonstrating the applicability of PAC to high molecular weight PAHs (202 - 276 g/mol) which are associated with increased persistence in the environment (Juhasz and Naidu, 2000). The improved removal was associated with the adsorption of PAHs on to the PAC. Trace metal removals were variable. Addition of PAC improved the removal of Ni, Cr and Cd by 22.6%, 20.5% and 12.4 % respectively. On the other hand, trace metal removals for Al, Mn, Fe, Co, Cu, As and Pb were shown to decline. Both pH and competition for active sites were believed to have been responsible for declines in treatment efficiencies. Powdered activated carbon addition had no impact on removal efficiencies of SCN^- or phenol.

Economic considerations of AC have not been described in any detail and therefore the economic feasibility of PAC was estimated for the treatment of coke wastewater. To treat 2000 m^3/d of coke wastewater an initial CAPEX of £0.38 per m^3 wastewater and an OPEX of <£0.01 per m^3 of wastewater would be required. The cost of PAC addition was compared to the use of membranes and ozone treatment as comparisons against other AC applications were not available. Ozonation and membrane filtration are typically applied to domestic wastewater and are associated with high CAPEX and OPEX costs e.g. £1,000 per m^3 CAPEX and £0.25 per m^3 OPEX. Addition of PAC was associated with an estimated CAPEX of £0.38 per m^3 and an OPEX of <£0.01 per m^3. The use of PAC may therefore offer an economically viable treatment method especially

as pollutants requiring treatment in domestic wastewater are typically 10 - 100 times lower than concentrations observed in coke making wastewater whilst consent limits are typically higher than those required by the IED for coke making wastewater.

8.4.2 Bioaugmentation

Bioaugmentation has been criticised over recent years as an ineffective treatment method due to numerous reports of treatment failures. Despite this, literature reported that many advances have been made in the approach allowing a better understanding of the impacts of bioaugmentation (Chapter 2). The application of bioaugmentation to coke wastewater was reported by Park et al. (2008), however, bioaugmentation was applied to effluent of a pre-denitrification process and was only maintained through significant nutrient addition and long-term augmentation. Addition of bacteria directly into the activated sludge biomass treating coke wastewater was therefore required. Bioaugmentation resulted in a 51% improvement in the removal of dissolved Σ6PAHs from coke wastewater at a bioaugmentation dose of 0.5 g/L (Chapter 5). The improved removals were significant due to the abundance of heavy molecular weight PAHs. *Bacillus* and *Mycobacterium* species were dominant in the bioaugmentation product and were associated with the improved removal of Σ6PAHs at a dose of 0.5 and 1.5 g/L. At a lower dose of 0.1 g/L no improvement was observed which was associated with the inability of the added bacteria to become established in the activated sludge biomass which was likely to have resulted from predation (Ramadan, El-Tayeb and Alexander, 1990). Tripling the dose to 1.5 g/L, led to a further 10% increase in Σ6PAHs removal efficiency compared to removals seen at a dose of 0.5 g/L (Chapter 5). This suggested that another factor became limiting such as the mass transfer of PAHs to the bacterial cells (Johnsen, Wick and Harms, 2005). Biostimulation (addition of supplements such as micronutrients) was also considered as nutrient limitations have been highlighted in industrial wastewaters (Burgess,

Quarmby and Stephenson, 1999), however, micronutrient addition had no impact on PAH removal or trace metal removal.

Species abundance data concluded that bacteria added through bioaugmentation, at a dose of 1.5 g/L, accounted for 4.4% of the operational taxonomic unit (OTU) abundance in the activated sludge biomass after 25 hours. Furthermore, whilst some studies have considered the survival of inoculated bacteria within treatment processes there has been a paucity of information surrounding the survival of inoculated bacteria after discharge of the treated effluent. After the activated sludge biomass was exposed to river water for 24 hours *Bacillus* OTUs associated with the bioaugmentation product were detected at 0.06% therefore showing a significant reduction in abundance (Chapter 5). Flow cytometry revealed that after the sludge biomass was exposed to river water for 24 hours the viable cell count declined by 97.3 % suggesting low survival of bacterial cells associated with the activated sludge.

8.5 Requirements for the ASP to treat coke wastewater to achieve the TN emission limit of the IED whilst maintaining and, or improving the removal of priority pollutants including PAHs, phenol, SCN^- and trace metals

Figure 8-2 highlights the operational conditions which are required to produce an effluent compliant with the IED emission limits:

- Nitrification - requires addition of inorganic carbon (300 mg/L Na_2CO_3).
- Denitrification - requires an sCOD:N ratio >5.7. Assuming a phenol concentration of 70 mg/L, influent TN should be maintained at 0.051 $kg/m^3.d$. External carbon addition may be required, if phenol concentrations are low, due to the variable composition of the influent wastewater. Acetic acid is a suitable form of carbon. Nitrite accumulation may also be limited by ensuring sufficient carbon supply.

- Thiocyanate removal - can take place under anoxic and aerobic conditions. High removal efficiencies require mesophilic temperatures (ca. 25^{o}C) and control of ammonia (IC_{50}: 316 mg/L) and phenol concentrations (ca <100 mg/L).
- Phenol - can be removed under anoxic and aerobic conditions, however, further work is required to ensure that phenol is consistently removed to the emission limit of 0.5 mg/L.
- Σ6PAHs - improved removal through activated carbon addition (20% increase in removal at an AC dose of 400 mg/L) and bioaugmentation (51% increase in removal at a dose of 0.5 g/L). Further optimisation is required to identify optimal activated carbon dose.
- Trace metals - Activated carbon (400 mg/L) increased removal of Ni, Cr and Cd by 22.6%, 20.5% and 12.4 % respectively.

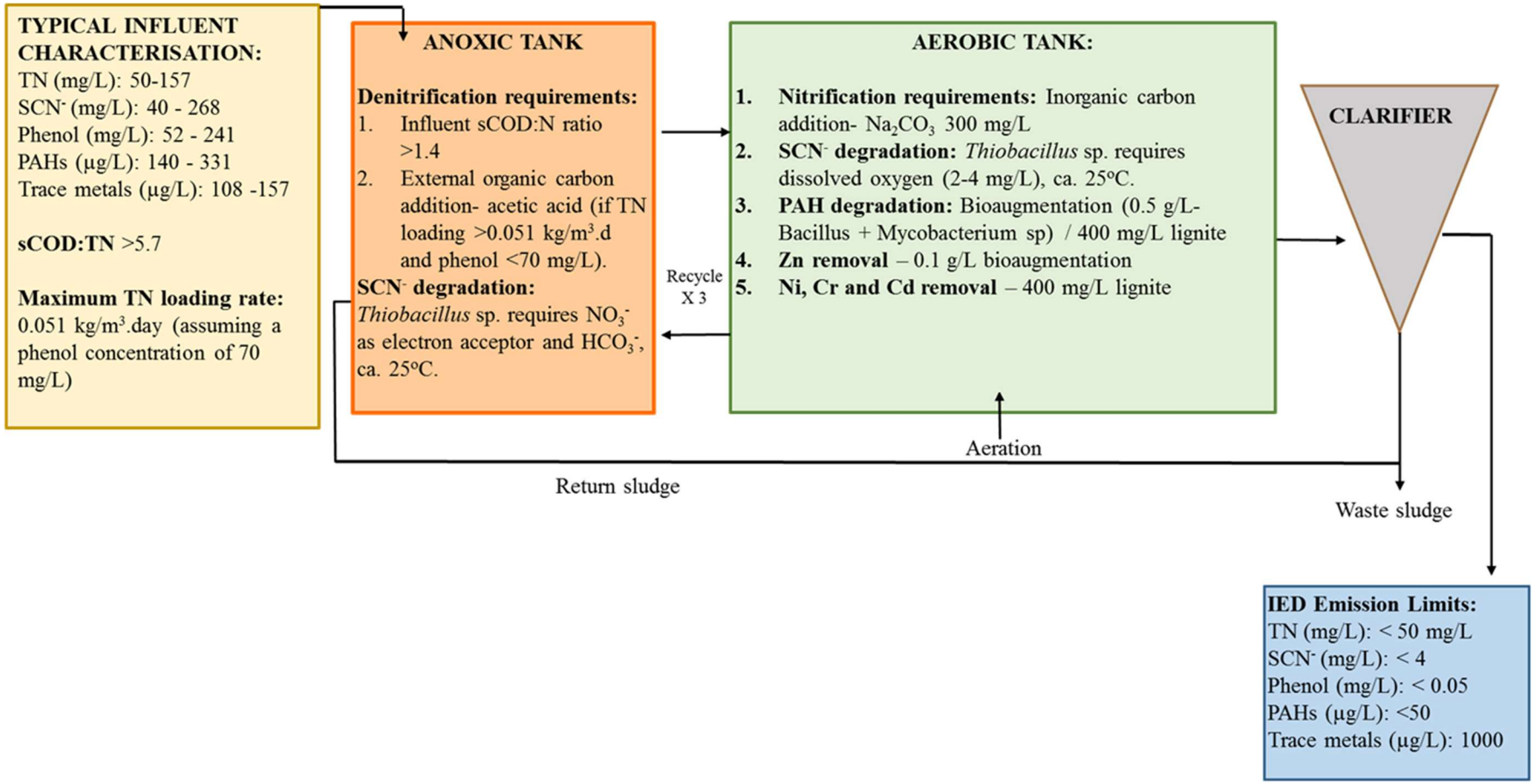

Figure 8-2: A schematic representation of the requirements for an ASP treating coke wastewater in order to comply with IED emission limits.

8.6 Contribution to knowledge

This research has contributed to the understanding of the factors required to allow total nitrogen (TN) removal from coke wastewater whilst maintaining and/or improving the removal of priority pollutants including polycyclic aromatic hydrocarbons (PAHs), phenol, thiocyanate (SCN^-) and trace metals. The main contributions to knowledge are highlighted in Table 8-1.

Activated carbon (AC) was investigated for its ability to improve the removal of Σ6PAHs and trace metals. Only one example of AC addition to coke wastewater treatment has previously been reported (Chao, Yeh and Shieh, 1986). Additionally, this was the first time that PAH removal from a wastewater was considered and the first-time trace metal removal was studied in a continuous ASP treatment process. Activated carbon was shown to be improve the removal of PAHs, nickel, cadmium and chromium through adsorption. The economic viability of AC addition was also demonstrated.

A scoping study was carried out to investigate the potential benefits to the removal of priority pollutants that could result from bioaugmentation. The study investigated the application of bioaugmentation to the removal of PAHs in water for the first time with previous investigations having focussed on PAH removal from soils. It was also the first time that bioaugmentation was considered for improving SCN^- removal. Additionally, biostimulation was considered for the treatment of coke wastewater concluding that biostimulation can improve removal efficiencies for both SCN^- and phenol. An assessment of the survivability of the exogenous microorganisms in the ASP offered more insight into the successful establishment of bioaugmentation in an ASP treating coke wastewater. Furthermore, it was the first time that the survival of exogenous microorganisms was considered in the discharge from a treatment plant offering

an understanding of the potential risks that could arise downstream of a treatment plant applying bioaugmentation.

Understanding the mechanism behind SCN^- degradation was important as not only are SCN^- emissions regulated but its degradation produces NH_4^+ which contributes to TN loading. An understanding of the SCN^- degradation mechanism within coke wastewater was developed. An uncultured *Thiobacillus* species was identified as responsible for SCN^- degradation in the mixed culture. Empirical evidence has demonstrated the capability of the mixed culture to degrade SCN^- in both anoxic and aerobic conditions. This was the first time that the degradation of SCN^- in coke wastewater was reported to occur under anoxic conditions. Batch tests, respirometry tests and the operation of an anoxic-aerobic ASP have increased understanding of the mechanisms behind SCN^- degradation and inhibitory compounds. Increased understanding of the degradation mechanisms enabled the identification of operational parameters which should be controlled to promote effective SCN^- degradation in an anoxic-aerobic ASP. Research into compounds inhibitory to SCN^- degradation has been limited and therefore data provided through respirometry investigations adds important information into the field. Testing highlighted that nitrification is unlikely to impact SCN^- degradation as SCN^- degradation proceeds more rapidly than nitrification. Control of influent phenol and ammonia concentrations were identified as important in maintaining treatment efficiencies.

An understanding of the requirements to meet TN emission limits of <50 mg/L was developed. The provision of alkalinity was confirmed as critical to nitrification. It was identified that the intrinsic alkalinity of the coke wastewater was insufficient to meet the requirements for ammonia oxidation. For the first time, suitable compounds for alkalinity provision were assessed and the dose was optimised. It was demonstrated that, as a result of the high variability of the wastewater composition, nitrogen loading can exceed carbon supply resulting in sub-optimal conditions for denitrification. The impact of the sCOD:TN ratio on

TN emissions was reported. Different carbon sources were evaluated for their ability to promote denitrification with acetic acid being highlighted as a suitable compound. Glycerol was investigated for its application to coke wastewater for the first time but was shown to be a poor carbon source. Furthermore, nitrite accumulation in the ASP was previously not understood but results from the investigation highlighted that nitrite accumulation was likely to be related to bacterial speciation as other factors such as pH were ruled out.

8.7 Impacts of the research

Findings from this investigation are being implemented on a full-scale coke wastewater treatment plant in order for the plant to meet the new emission limits. Results from the investigation have been used as the driving force for modifying the full-scale treatment plant which currently operates with aerobic treatment alone. Information regarding the loading conditions and required recycle ratios are being used to scale modifications accordingly. Investigations into appropriate carbon provision (both inorganic and organic) has focussed attention on understanding the key principles behind degradation mechanisms. The investigation has highlighted that intrinsic inorganic and organic carbon are both limited and are not available in sufficient concentrations to maintain stable TN removal. This is of notable importance as historically carbon availability was higher and the study has highlighted that changes to the wastewater composition over recent years has had a significant impact on carbon to nitrogen ratios. This has demonstrated that the provision of carbon now needs to be included in future planning to account for storage facilities and process economics. Consideration of suitable carbon sources has also re-focussed attention on suitable compounds as sources previously favoured on cost and health and safety grounds have been shown to be unsuitable. An increased understanding of the requirements of SCN^- degrading bacteria have enabled operators to manage the influent to the treatment plant to prevent treatment losses through inhibition. Investigations into AC and bioaugmentation have

identified polishing techniques which can be implemented as required in the second round of plant modifications to improve the removal of priority pollutants such as PAHs and trace metals. The knowledge gained from this investigation has the potential to be applied to further coke wastewater treatment sites working closely with the sponsors in the UK, Europe and further afield.

Wider impacts of the research include the identification of an uncultured species of *Thiobacillus* which opens up the potential for further work and characterisation of this SCN^- degrading species. Further work on pure studies will enable the full metabolic capabilities of the species to be determined. The success of AC addition for PAH and trace metal removal in an ASP has demonstrated the potential suitability of AC addition to other ASP treatment plants where such pollutants are a concern. Scoping investigations into bioaugmentation have highlighted the applicability of bioaugmentation to coke wastewater, particularly for PAH removal, and further work at full-scale can be conducted. Additionally, the removal of PAHs in the ASP has proven the applicability of the technique to a wider range of wastewaters which are contaminated with PAHs creating many future research avenues. The investigation into the survivability of exogenous bacteria adds valuable research into the field of bioaugmentation of coke wastewaters. Furthermore, as survival of exogenous bacteria has been shown to be limited under conditions mimicking a river receiving effluent from a treatment plant concerns surrounding full-scale applications have been addressed making an important contribution towards encouraging full-scale trials.

Table 8-1: Contributions to knowledge arising from the research project.

	What has been confirmed?	What has been developed?	What has been found which is brand new?
Theoretical knowledge		Chapter 2 - Bioaugmentation has a range of applications suitable for the treatment of industrial wastewater. Pilot and full-scale investigations are required to advance knowledge and understanding. Chapter 4: Understanding of SCN^- degradation pathways.	Chapter 6– denitrification stoichiometry for glycerol and phenol
Empirical evidence	Chapter 6: Nitrification requires inorganic carbon and denitrification requires organic carbon. Chapter 6 – Organic carbon source impacts biomass growth and stability.	Chapter 5: Survival rates of exogenous bacteria added to an ASP Chapter 4: *Thiobacillus* bacteria require mesophilic temperatures Chapter 4: SCN^- degradation proceeds faster than nitrification therefore SCN^- degradation is unlikely to be impacted by nitrification. Chapter 6: Alkalinity/inorganic carbon is required at 300 mg/L to support nitrification. pH control through NaOH addition also promotes nitrification. Ammonia oxidation had an optimal pH range of 7.0 – 8.3 whilst nitrite oxidation	Chapter 3- PAC (400 mg/L) improves the removal of heavy weight PAHs from coke wastewater. PAC improves removal of Ni, Cr and Cd. Chapter 5: Bioaugmentation (0.5g/L) improves the removal of PAHs from coke wastewater. Chapter 5: Biostimulation (alkalinity dosing) and micronutrient addition improves SCN^- degradation. Alkalinity addition improves phenol removal efficiencies. Chapter 5: Viable cell counts decline rapidly after ASP effluent is exposed to river water.

		required a pH of 5.5 - 6.8. Chapter 6: Acetic acid is a suitable form of organic carbon for external carbon addition to coke wastewater. Chapter 7: SCN^- degradation occurs under anoxic conditions	Chapter 4: SCN^- degrading bacteria in coke wastewater ASP belong to an uncultured species of the genus *Thiobacillus*. Metabolic capabilities include anoxic degradation. SCN^- degradation is inhibited by high concentrations of ammonia (IC_{50} 316 mg/L) and phenol (ca. 200 mg/L). Chapter 6: Soda ash prevents nitrite oxidation. Chapter 6: Glycerol is unsuitable for external carbon addition to an ASP treating coke wastewater due to low biomass production. Chapter 7: sCOD:N ratio >5.7 is required to enable an effluent TN concentration of <50 mg/L. External carbon addition is therefore required to cope with the variability in coke wastewater composition.
Methodology		Chapter 3: Application of PAC to an ASP. Chapter 5: Application of bioaugmentation to coke wastewater treatment. Chapter 7: A pre-denitrification ASP is suitable for the treatment	Chapter 5: Use of flow cytometry and PCR to investigate survival of augmented bacteria in an ASP and receiving waterbody.

		of coke wastewater.	

8.8 Future work

8.8.1 Optimise the dose rate of AC for PAH removal in an ASP.

Chapter 2 demonstrated that AC addition to an ASP improved the removal of Σ6PAHs reducing effluent Σ6PAH emissions from 54 ± 21 µg/L to 34 ±11 µg/L and hence allowing compliance with the 50 µg/L IED emission limit. Only one dose rate of 400 mg/L was investigated and therefore further work should be completed in order to optimise the dose rate. Further work may also be completed to investigate the impact of different AC types as competition for suitably sized pore spaces may have limited removal efficiencies for some trace metals (Al, Mn, Fe, Co, Cu, As and Pb).

8.8.2 To investigate bioaugmentation and biostimulation in a continuous ASP process to enhance removal efficiencies.

Chapter 3 concluded that in batch tests bioaugmentation was able to improve the removal of Σ6PAHs by 51% through bioaugmentation at a dose of 0.5 g/L. Further tests should therefore be completed in a continuous flow ASP to compare removal efficiencies. Biostimulation through alkalinity addition was able to ensure effluent SCN^- concentrations complied with the IED emission limit. Investigations should assess the impact of dose rate under continuous treatment conditions. Chapter 3 indicated that inoculated *Bacillus* species were able to survive in batch tests. It is important to assess the ability of the *Bacillus* species to maintain an active population under continuous flow conditions and or optimise required maintenance dose rates. Further analysis of viable cell counts is required to confirm viable cell reduction rates. Finally, an economic consideration of bioaugmentation needs to be completed.

8.8.3 Investigate speciation of SCN^- degrading bacteria

Chapter 4 concluded that SCN^- degradation was completed by an uncultured *Thiobacillus* species with metabolic capabilities similar to *T. thioparus* and *T.*

denitrificans. Further work on pure cultures is required for a detailed understanding of the metabolic capabilities of the species.

8.8.4 Investigate techniques to improve phenol removal

Phenol removal efficiencies have not been improved by most of the investigated techniques including AC, bioaugmentation and conversion to an anoxic-aerobic ASP. Alkalinity addition led to a reduction in average effluent concentrations to 0.3±0.3 mg/L. Due to the standard deviation it is still therefore possible that exceedances would be observed. Despite excess alkalinity in the anoxic-aerobic ASP effluent phenol concentrations were still typically in exceedance of the 0.5 mg/L emission limit. Further work is therefore required to investigate improvements for example considering a bioaugmentation product containing specialised phenol degrading bacteria.

8.9 References

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Chapter 9 Conclusions

The conclusions are presented in respect to the original objectives stated in section 1.4.

Objective 1: To critically review the application of bioaugmentation to activated sludge processes treating industrial wastewaters to understand current knowledge and enabling method development for its application to coke wastewater.

- The field of bioaugmentation has developed significantly with increased understanding of strain selection and inoculum introduction strategies.
- Bioaugmentation has been demonstrated for a range of wastewater types and has proven to successfully remove contaminants including aromatic and nitrogen compounds.
- There is a lack of studies with representative control conditions against bioaugmentation trials. Many investigations have failed to report control conditions or have reported before and after data rather than parallel studies.
- The use of genetically engineered microorganisms (GEM) may assist in improving removals of xenobiotic compounds and compounds with complex degradation processes which may require the action of multiple bacteria. Legislation currently limits the application of GEM. Future research must both comply with and inform legislation.
- Full-scale investigations are limited with further research being required at this scale.
- Economics of bioaugmentation requires considerably more investigation.

Objective 2: To evaluate the role of activated carbon (AC) in the removal of PAHs and trace metals from coke wastewater effluent in an ASP treatment system.

- Addition of powdered activated carbon (PAC) improved the removal of Σ6PAHs with effluent emissions falling from 54 ± 21 µg/L to 34 ±11 µg/L. The improved removal was associated with the adsorption of PAHs on to the PAC. Improved removal allowed compliance with the IED emission limit of 50 µg/L.
- Addition of PAC improved the removal of Ni, Cr and Cd by 22.6 %, 20.5 % and 12.4 % respectively. Trace metal removals for Al, Mn, Fe, Co, Cu, As and Pb were shown to decline. Both pH and competition for active sites were believed to have been responsible for declines in treatment efficiencies.
- The application of PAC to treat 2000 m^3/d of coke wastewater would require an estimated CAPEX of £0.38 per m^3 wastewater and an OPEX of <£0.01 per m^3 of wastewater. PAC therefore has the potential to be an economically favourable method by which to improve PAH removal.

Objective 3: To elucidate the degradation pathway of SCN^- in order to understand the requirements of SCN^- degrading bacteria.

- Thiocyanate degrading bacteria are the most abundant species in activated sludge from a coke wastewater ASP (26%). Deoxyribonucleic sequencing analysis identified an uncultured SCN^- degrading bacteria which belonged to the genus *Thiobacillus*.
- Thiocyanate removal was limited by temperature requiring mesophilic temperatures.
- Thiocyanate degradation rates increased with increased SCN^- concentrations, however, at concentrations above 110 mg/L the expected ammonia production was not observed which suggested a breakdown in the degradation pathway believed to be due to reduced activities of cyanase.
- Thiocyanate was removed under both anoxic and aerobic conditions. It is suspected that under anoxic conditions SCN^- was degraded to form cyanate (OCN^-) nitrogen gas and sulphate, using NO_3^- as the electron

acceptor. The cyanate was subsequently hydrolysed to form ammonia and bicarbonate. Under aerobic conditions SCN^- was hydrolysed to OCN^- and sulphide. Cyanate was hydrolysed further to form ammonia and bicarbonate and sulphide was oxidised to form sulphate.

- Ammonia was shown to be inhibitory to SCN^- degradation (IC_{50} value = 316 mg/L).
- Phenol was also shown to have an inhibitory role resulting in a 41% decline in the removal efficiency at 180 mg/L.

Objective 4: To evaluate the ability of bioaugmentation and biostimulation to enhance the removal of SCN^-, PAHs and trace metals. Furthermore, to investigate the ability of exogenous bacteria to survive in a simulated river water discharge system.

- Biostimulation via micronutrient addition resulted in improved removals of SCN^- with average effluent concentrations of 0.7 ± 1.2 mg/L compared to 2.7 ± 4.6 mg/L under control conditions. Biostimulation in the form of alkalinity addition allowed complete removal of SCN^-.
- Bioaugmentation at a dose of 0.5 g/L allowed a 12% improvement in SCN^- degradation. A dose of 0.1 g/L brought about no benefits whilst a higher dose of 1.5 g/L resulted in minimal further improvements.
- The removal of ∑6PAHs increased by 51% through bioaugmentation at a dose of 0.5 g/L. The removal of ∑6PAHs did not increase further at increased doses.
- Inoculated *Bacillus* species accounted for 4.4% of the activated sludge biomass after 25 hours (1.5 g/L dose). After the activated sludge biomass was exposed to river water (24 hours) the abundance of *Bacillus* species was 0.06%.
- Overall bacteria from the ASP showed low levels of survivability when released to the receiving water environment with viable cell counts decreasing by an average of 89%.

- The viable cell count (at bioaugmentation dose 1.5 g/L) declined by 97.3 %, compared to 98.6 % under normal treatment conditions, suggesting low survival of bacterial cells associated with the activated sludge.
- Survival of ASP and exogenous bacteria in the receiving water environment is therefore expected to be limited.

Objective 5: To identify suitable carbon sources for stable nitrification and denitrification.

- The alkalinity typically available in coke wastewater (3.6 mg as $CaCO_3$/mg NH_4^+-N) is insufficient to enable complete nitrification. Sodium carbonate allowed complete ammonia oxidation and production of nitrate with the optimum dose being established as 300 mg/L (as $CaCO_3$).
- Nitrite-nitrogen and NO_3^--N formation were impacted by pH. Ammonia oxidising bacteria had an optimal pH range of 7.0 - 8.3 whilst nitrite oxidising bacteria had an optimal pH range of 5.5 - 6.8.
- Glycerol was identified as an ineffective external carbon source for denitrification due to low biomass production (0.18 mg biomass per mg of glycerol) which was linked to declining mixed liquor suspended solids and loss of nitrification and denitrification. Acetic acid provided a sustainable source of external carbon (0.31 mg biomass produced per mg of acetic acid).

Objective 6: To characterise the optimal operational conditions for TN removal in an anoxic-aerobic ASP.

- The sCOD:TN ratio is critical to enable consistent compliance with the IED emission limit of 50 mg/L TN due its influence on denitrification

efficiencies. An influent sCOD:TN ratio of 5.7 allowed effluent TN to remain below 50 mg/L whilst a ratio of 5.2 resulted in the emission limit being exceeded (89 mg/L).

- Nitrification remained stable at >96% under all conditions.
- Removal of SCN^- and phenol averaged at 100% and 96% respectively. Both SCN^- and phenol were utilised as carbon sources in the anoxic reactor.
- The presence of NO_2^--N in the anoxic cell under all conditions indicated a disturbance to the denitrification process and was exacerbated under carbon limited conditions. This was associated with species abundance in the activated sludge biomass. It is suggested that there was an imbalance between true denitrifying bacteria and nitrate-respiring bacteria. A species of the *Rhodanobacter* genus, some of which are known for their nitrate but not nitrite-respiring behaviour, was identified within the sludge biomass (11%).