

Hybrid Remediation Strategies for Industrial Effluents



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Abstract

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Many industrial effluents are toxic, difficult to treat and contain potentially valuable resources. Current treatment methods are expensive and unsustainable. Bioprocessing is a promising alternative, but poses multiple challenges due to the chemically complex nature and high variability of different industrial effluents. As a result, it benefits from being combined with advanced oxidation processes. In this study, the potential benefit of coupling bioprocessing with nanoscale zero-valent iron and electron beam treatment was examined. Both processes have shown great promise individually in environmental applications but have not previously been applied in a hybrid system. This triple combination proved to be an effective approach when tested against two recalcitrant metalworking fluids, a textile dyeing effluent and a distillery wastewater. Strategies to remediate the different industrial effluents included a selection of key performance indicators and their amalgamation into a novel remediation index to assess treatment effectiveness. The different treatments were optimised both individually and in combination. Hybrid sequential e-beam, bioprocessing and nanoscale zero-valent iron treatment followed by bioprocessing synergistically detoxified both metalworking fluids and reduced their chemical oxygen demand (COD) by 92.8 ± 1.4 % and 68.4 ± 1.6 % respectively. E-beam pre-treatment of the metalworking fluids enabled high-COD bioprocessing at the expense of residence time. Hybrid treatment also remediated the textile dyeing wastewater achieving a 99.4 ± 0.4 % reduction in colour, a 54.6 ± 6.1 % reduction in COD, a 89.0 ± 3.5 % reduction in sulphate and a 67.7 ± 4.2 % reduction in total nitrogen. E-beam treatment also enabled the recovery of copper from a distillery wastewater, whilst proving to have significant potential for the disinfection of industrial effluents post-bioprocessing. Overall process economics, footprint and sustainability were evaluated on a lab-scale and estimated for an industrial-scale implementation. The scope for improvements in the hybrid treatment system is discussed and suggests that future implementations of novel hybrid processes could provide tailor-made solutions for sustainable *in situ* treatment of a wide range of industrial effluents.

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Nomenclature

A	Absorbance
AAS	Atomic Absorption Spectroscopy
AOP	Advanced Oxidation Process
ATP	Adenosine Triphosphate
BET	Brunauer–Emmett–Teller Surface Analysis
BOD	Biological Oxygen Demand [mg L ⁻¹]
CCD	Charge Coupled Device
CFU	Colony-forming Unit
Chromophore	Part of a molecule that is responsible for its colour
COD	Chemical Oxygen Demand [mg L ⁻¹]
COSHH	Control of Substances Hazardous to Health
DI water	De-ionised water
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen [mg L ⁻¹]
DWW	Distillery Wastewater
E-beam	Electron Beam
EDX	Energy-dispersive X-ray Spectroscopy
FCI	Fixed Capital Investment

G	Glucose
Gy	Gray (unit of ionising radiation dose) [1 Gy = 1 J kg ⁻¹]
IRR	Internal Rate of Return
kGy	Kilo Gray (unit of ionising radiation dose) [1 kGy = 1 kJ kg ⁻¹]
KPI	Key Performance Indicator
kWh	Kilowatt-hour (unit of energy) [1 kWh = 3.6 MJ]
LB	Lysogeny broth
LC	Liquid Chromatography
MM	Minimal Medium
MWF	Metalworking Fluid
NDIR	Nondispersive Infrared Detector
NZVI	Nanoscale Zero-Valent Iron
OD	Optical Density
PBS	Phosphate Buffered Saline
PER	Post-Exposure Cell Count Recovery
pH	Potential of Hydrogen
PI	Propidium Iodide (stain)
PRR	Post-Respiration Cell Count Recovery
Recalcitrant	Resistant to biological degradation

RI	Remediation Index
RLU	Relative Light Unit
RM	Rich Medium
rpm	Revolutions per minute
SEM	Scanning Electron Microscopy
SG	SYBR Green (dye)
STEM	Scanning Transmission Electron Microscopy
TC	Total Carbon [mg L ⁻¹]
TCE	Trichloroethylene
TDIC	Total Direct and Indirect Costs
TDW	Textile Dyeing Wastewater
TEM	Transmission Electron Microscopy
TIC	Total Inorganic Carbon [mg L ⁻¹]
TOC	Total Organic Carbon [mg L ⁻¹]
UV	Ultraviolet
μ	Mean
n	Number of replicates
p	p-value in null hypothesis significance testing
σ	Standard Deviation

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1 Introduction

1.1 The Importance of Industrial Effluent Treatment

In a global framework of a Fourth Industrial Revolution, long-term economic growth is still primarily driven by industry and the cutting edge technological advances associated with it (Schwab 2017). Advances in many fields, among which artificial intelligence, robotics, autonomous vehicles, nanotechnology and biotechnology are transforming global production, transportation and consumption systems. Historically, disruptive industrial advances have been accompanied by increasing pollution, new types of contamination and the depletion of natural resources (UNIDO 2015).

As industries require a workforce living locally, they are typically found near high population areas, which are directly exposed to industrial emissions and effluents if left untreated. For example, areas close to Spanish industries have been associated with increased cancer risk (Fernandez-Navarro et al. 2012; Garcia-Perez et al. 2015) and death rates near chemical, pharmaceutical and power plants are reported to be elevated in so-called ‘cancer villages’ in China (Guardian 2013). Combined with an increasingly urban, ageing and growing population worldwide (UN 2015), more people than ever before will therefore find themselves in closer proximity to industry and its concomitant industrial pollution.

The world is being driven by market and regulatory pressures towards cleaner production processes, e.g. zero waste, cradle-to-cradle lifecycle design in the automotive, consumer appliances and electronic sectors (Kumar and Putnam 2008). However, the inertia of the

system and the vast, complex and ever-changing industrial landscape will continue to worsen current levels of contamination.

This study focuses on pollution caused by industrial effluents. The estimated total volume of wastewater generated in the world is currently 350 km³ per year, of which up to 20 % can be directly attributed to industry (UNESCO 2009). Many industrial processes, such as the complex organic chemicals industry, producing pesticides, pharmaceuticals, paints, dyes and petrochemicals, will continue to consume large quantities of water and produce corresponding vast amounts of toxic industrial effluents. Global water demand is projected to increase by 55 % by 2050 (UN 2014) mainly due to growing manufacturing and industrial use. Industrial effluent treatment remains a major concern directly relevant to ecological health and economic stability and as a consequence, innovative wastewater treatment technologies will be required for the foreseeable future.

The main issues of industrial wastewaters are organic pollutants (including non-biodegradable organics and pathogenic microorganisms), excess nutrients (in particular nitrates and phosphates, which cause eutrophication), heavy metal contamination and novel emerging contaminants such as medicines, e.g. antibiotics, endocrine disruptors and micro-plastics from cosmetic products (Kuznetsov et al. 2015; Bolong et al. 2009). In their latest ‘world water development report’, the UN estimate that 300-500 millions of tons of heavy metals, solvents and toxic sludge are generated per year, which are traditionally disposed of to landfill or the sea, i.e. unsustainably dumped into the environment (UN 2014).

In the developed world up to 70 % of generated wastewater is currently treated before being

returned to the ecosystem, whereas in less economically developed countries this figure can be as low as 8 % (Sato et al. 2013). Treatment in the developed world is largely motivated by stringent effluent discharge regulations motivated by the toxic nature of many effluents and their proximity to humans, but more frequently by economic and financial incentives (Sato et al. 2013). There is therefore an increasing requirement to develop and apply technologies that are efficient, scalable, financially viable and sustainable. Any successful novel treatment process would also have the potential to be self-contained and compatible with current engineered infrastructures while treating industrial effluents and simultaneously recycling a large fraction of the organics, metals and waters on-site.

1.2 Current State of Industrial Effluent Treatment Systems

The key challenge for industrial effluent treatment is two-fold: decontamination, to prevent further damage to the earth's ecosystem and human health; and resource recovery of water, energy and metals. Resource cycles need to be closed in order to make human activities sustainable (Nelson et al. 2003). A successful treatment must detoxify the waste, by making it more bioavailable while keeping energy and capital costs at a minimum and enabling recovery of as many of the valuable fractions, i.e. water, organics and valuable metals, as possible. This would minimise the carbon footprint of the industrial process, maximise profits and minimise the impact of the final treated effluent in the environment.

Conventional treatment is often expensive and energy intensive (Oller et al. 2011). Current treatment technologies such as reverse osmosis and ultrafiltration are effective, but have

undesirable process economics and environmental footprints (Petrinic et al. 2015). They are particularly cost and capital intensive due to the complex nature of industrial waste streams, their high pollutant loads and filtration membranes being prone to fouling (Barakat 2011) with treatment cost found to vary exponentially with increasing contaminant removal (Feng and Chu 2004). Moreover, they often simply concentrate the problem rather than treat it, which means that even though the waste is sequestered, it starts accumulating and will require further processing.

1.3 Bioprocessing – Low-Cost and Sustainable?

Biological treatment of complex wastewaters is an excellent alternative as it requires little energy to run and is effective at removing high loads (Eckenfelder et al. 1989). Biological treatment is universally used for municipal wastewater treatment and has been widely investigated in industrial waste streams due to its low running and operating costs and ecological footprint (Eiroa et al. 2008; El Hajjoui et al. 2008a; Jagadevan et al. 2011; Kornaros and Lyberatos 2006; Muszynski et al. 2007).

Biological treatment is based on exploiting the enormous diversity of microorganisms that can degrade toxic compounds and use them as energy or nutrient sources. Moreover, it addresses issues of scalability and could potentially be employed at small scale, *in situ*. For instance, a highly adapted and particularly resistant microbial consortium, developed for treating metalworking fluids (van der Gast et al. 2003) has proven to have potential to be part of a hybrid treatment process centred around bioprocessing.

Chemical Oxygen Demand (COD) is an established parameter commonly employed to characterise decontamination in industrial effluents. Various studies have investigated biological COD removal for metalworking fluids for CODs ranging from 560 mg L^{-1} to $230,000 \text{ mg L}^{-1}$ whilst successfully removing between 26 % and > 99 % of the organic load, which is typically diluted prior to bioprocessing (Cheng et al. 2005, 2006). However, few studies have considered how the initial COD concentrations of the metalworking fluids affect COD removal during bioprocessing, even though toxicity has been shown to vary with initial concentration of industrial wastewaters (Borrely et al. 2000).

Different configurations of biological systems have proven to be very effective for particular classes of wastewaters (Kornaros and Lyberatos 2006; van der Gast et al. 2003), but they have their limits in terms of metabolic ability and vulnerability to toxicity. They are therefore particularly susceptible to toxic shock loads, which can cause inhibition of key metabolic processes and considerably affect their effectiveness (Leifheit and Mohr 2001). Biological systems have evolved to be energetically parsimonious but they are not perfect. Shock loads can in extreme cases lead to cell death. Moreover, some contaminants in industrial effluents are intrinsically resistant to biological degradation, i.e. recalcitrant, and are largely resistant to rapid biotransformation and require long (sometimes geological) time scales to achieve adequate remediation (Amaral-Silva et al. 2016).

To overcome this limitation of bioprocessing for industrial wastewater treatment, a potential approach is to complement it with more vigorous chemical and physical treatment methods, most notably advanced oxidation processes (AOPs), resulting in more complete treatment

and offering scope for resource recovery (Jagadevan et al. 2011). Ideally, a hybrid treatment would consist of a combination of AOPs that have different mechanisms of action, e.g. adsorption and degradation. The most promising industrial effluent treatment technologies today in terms of sustainability are therefore bioprocessing coupled with more energy-intensive advanced oxidation processes (Amaral-Silva et al. 2016).

1.4 Advanced Oxidation Processes as Complementary Treatments to Bioprocessing

In general, advanced oxidation processes (AOPs) are chemical or physical processes that generate highly reactive hydroxyl radicals that remove organics from wastewaters by oxidation (Csay et al. 2014). The radicals are usually produced *in situ* from one or more primary oxidants such as ozone, hydrogen peroxide, oxygen or water and energy sources such as UV light, irradiation or the use of catalysts, like titanium dioxide (Glaze et al. 1987). AOPs are particularly efficient for treating secondary wastewaters to enhance the biodegradability of recalcitrant organics by converting them into more biodegradable compounds (Rizzo 2011).

One of the most widely used and promising AOPs is the Fenton process, which uses Fe^{2+} and hydrogen peroxide (H_2O_2) to generate hydroxyl radicals *in situ*, which can oxidise complex organic molecules (N. Wang et al. 2016). However, the required addition of H_2O_2 can cause toxic by-products to form in the case of sub-stoichiometric additions (Munoz et al. 2011). A wide array of AOPs exists (Horáková et al. 2014; Merayo et al. 2013) and the novelty lies

in their complementary use. A key issue, which must be considered when exploiting hybrid technologies, which combine biological treatment with AOPs, is the harmonisation of the different technologies to ensure that they are complementary with maximal efficiency. AOPs for instance are usually very effective at killing exposed cells, so the application of AOPs as a complement needs to be carefully considered when combining it with bioprocessing.

Nanoscale zero-valent iron (NZVI) has been frequently employed as an AOP (Hansson et al. 2012; Fard et al. 2013) and is known to be highly effective for degrading organics in industrial waste streams (Crane and Scott 2012). The main mechanisms of action are adsorption and degradation on the surface of NZVI (Noubactep 2008). Electron beam irradiation (e-beam) treatment is another promising AOP and has been widely studied on several complex industrial effluents (Han et al. 2012; Kurucz et al. 1995; Pikaev et al. 1996; Sampa et al. 1998). However, there have been few if any studies on how NZVI and e-beam treatment can be exploited conjointly as complements to bioprocessing.

1.5 Nanoscale Zero-Valent Iron (NZVI) - The Future of Environmental Remediation?

Nanomaterials are used globally in a large range of different consumer and industrial products and processes in what is a rapidly growing field (Crane and Scott 2012). This is due to the high efficacy and versatility of nano-scale materials (Qu et al. 2013). In particular, a large theoretical and empirical body of evidence has demonstrated that NZVI plays a

potentially significant role in environmental remediation applications (Crane and Scott 2012; Dickinson and Scott 2010; Klimkova et al. 2011; Noubactep 2008; Qu et al. 2013; Scott et al. 2011; C. Wang and Zhang 1997) and is the most widely studied reductant in environmental applications (Tratnyek et al. 2003). In addition, with advances in the field, and decreasing production costs at higher efficiencies (Miehr et al. 2004), it is becoming a competitive and lucrative alternative to traditional approaches.

Zero-valent iron has been widely employed in permeable reactive barriers for groundwater remediation (Scherer et al. 2000), as it generates reactive oxidants from oxygen in a Fenton-like reaction (Lee et al. 2014). Nanoscale zero-valent iron (NZVI) has a higher reactivity due to its smaller particle size leading to a greater surface area to volume ratio (W. Zhang 2003), which results in more vigorous reduction reactions (C. Wang and Zhang 1997). As a strong reducing agent, NZVI is able to degrade a wide range of organic and inorganic pollutants (Boparai et al. 2011; Tang and Lo 2013) and is particularly effective for the immobilisation of heavy metals and their subsequent removal from aqueous systems (Dickinson and Scott 2010). In particular, in one study, a real acid mine wastewater stream that was particularly recalcitrant to biological degradation was treated using NZVI leading to a reduction of all monitored pollutant constituents including a high concentration of sulphate and a cocktail of metals, which was also accompanied by an increase in pH (Klimkova et al. 2011).

NZVI is a source for the formation of corrosion products for contaminant adsorption, incorporation or co-precipitation (Noubactep et al. 2006; Scott et al. 2011) and generates Fenton-like radicals for possible catalytic contaminant reduction (Noubactep 2008) through

various redox reactions, resulting in oxidation and reduction on the surface (Crane et al. 2011). As the typical method of deployment of NZVI is via colloidal suspensions, a key feature is that NZVI is magnetic and thus the spent NZVI and its corrosion products are in general also magnetic. Reacted NZVI can therefore be easily and cheaply removed from wastewaters by magnetic separation (Qu et al. 2013), which is the most widely adopted and efficient method of recovering NZVI from aqueous solutions (Yavuz et al. 2009). The time that elapses between synthesis of NZVI and its application in treatment is important, since contaminant removal is strongly dependent on the extent of iron corrosion, i.e. NZVI depletion, which is larger and faster for nano-scale iron than for micro-scale or bulk iron (Noubactep and Care 2010). However, some NZVI particles have the potential to remain reactive in air or water for extended periods of time up to eight weeks allowing for flexible storage, handling and treatment (W. Zhang 2003).

Dissolved oxygen (DO) is not reported to pose any significant problems, as the electron-consuming effect from DO is overcome by the electron donating capacity of NZVI (Yang and Lee 2005). The depletion of oxygen leads to negative charge, such as chloride ions, building up on the surface of the NZVI (Eglal and Ramamurthy 2014). It has also been reported that an increase in temperature can affect metal adsorption rates onto NZVI (Boparai et al. 2011). The efficiency of NZVI treatment is pH dependent, as it is an acid-driven surface mediated process most efficient at low pHs. In one study from a petroleum refinery, the optimal wastewater treatment occurred at a pH of 5 (Rasheed et al. 2011), whereas at higher pH iron precipitated due to the high hydroxide concentration (Yang and Lee 2005).

It remains unclear how toxic NZVI is when it enters the ecosystem (Crane and Scott 2012). Thus, any new findings on its environmental impact and potential toxicity will advance the field and mature this technology. When tested on river water, NZVI temporarily caused DO depletion and a decrease in pH (Barnes et al. 2010b). However, in the same study no long-term changes to the bacterial community structure were observed. A potential issue to be addressed could be the generation of toxic intermediates as by-products from the NZVI treatment, which might lead to an overall increase in toxicity and reduction in biological degradation process efficiency (Hashim et al. 2011). In one study involving a hybrid system of NZVI coupled with biology, it was found that increasing NZVI concentration slowed biological degradation of TCE and sulphate to the point where it stopped completely (Barnes et al. 2010a). Hence, in a sequential treatment system, all NZVI should be separated out before any subsequent biological treatment step.

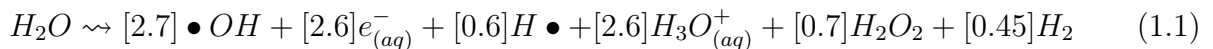
An increasing feature of modern industry is the drive towards conservation and maximal recycling of resources (WERF 2010). An important feature to consider is the potential to recycle and reuse the NZVI. NZVI particles are known to be difficult to regenerate and this is due to irreversible reduction reactions and the formation of iron oxide layers around the reacted immobilised molecules, which passivates and exhausts the NZVI (Tang and Lo 2013). However, recent studies have shown that it might be possible to reuse NZVI a certain number of times before it is fully exhausted (Jagadevan et al. 2012; Sohn et al. 2006). Other approaches reuse the NZVI by acid-treating it to reactivate its surface (Sun et al. 2011).

1.6 Electron Beam Irradiation Treatment – A Silver Bullet?

Electron beam irradiation (e-beam) treatment is thought to add over £800 billion in value to a variety of commercial products and processes (Berejka et al. 2014). These include the discovery of radiation crosslinking of the world’s largest volume commodity plastic; low-energy e-beam surface curing of inks, coatings and adhesives; and numerous applications for surface decontamination of packaging materials such as medical equipment, devices and food eliminating the need for terminal product sterilisation (Berejka et al. 2014).

E-beam treatment is currently being employed for environmental remediation purposes at four major industrial-scale sites around the world on different industrial effluents (Hanna 2008), due to its very high dose rate, efficiency, throughput and promising economics (Getoff 1996). Irradiation treatment is recognised as being effective when it comes to treating refractory organic wastewaters (Cooper et al. 1992; Ting and Jamaludin 2008), even in the presence of high salinity (Duarte et al. 2004b), very opaque waters full of particulates (Kurucz et al. 1990) and generally complex effluent conditions (Duarte et al. 2004b). Gamma irradiation, the main alternative to e-beam treatment, requires a radioactive source such as cobalt-60, which is not required for e-beam treatment (J. Wang and Wang 2007) offering advantages in terms of maintenance and safety (Berejka 1995). Moreover, e-beam treatment achieves greater decolouration and degradation of dyes than gamma irradiation (Abdou et al. 2011) and can achieve similar positive results to activated carbon in wastewater treatment (Sampa et al. 2004). Pulse radiolysis has been previously reported to be optimal for the degradation of organics (Getoff 2002).

E-beam treatment relies on the formation of reactive radicals and species that interact with any organic molecules in the water. The radiolysis of water can be presented by the following equation (Spinks and Woods 1990) showing the generation of primary, oxidising ($\bullet OH$) and reducing ($e_{(aq)}^-$ & $H\bullet$) radicals, which are pH independent (Cooper et al. 1992):



Values in brackets are the radiation chemical yields, i.e. the number of radicals, excited states or other products formed in water absorbing energy of 100 eV.

The effectiveness of the e-beam is largely attributable to the hydroxyl ($\bullet OH$) radicals generated during the radiolysis of water. The $\bullet OH$ radicals are able to attack electron rich sites such as aromatic rings, amidogen groups and azo-compounds (Rauf and Ashraf 2009) and are therefore particularly effective at destroying the dye chromophore of textile dyeing wastewaters (Ghaly et al. 2014). Textile dyeing wastewaters are typically highly coloured, highly recalcitrant and hard to treat. Effluents from the dyeing process are hardly biodegradable due to their high concentration of refractory organic compounds, which also make them highly coloured (Al-Momani and Touraud 2002).

The main issue with radicals and radiolytically generated species is that they have a poor selectivity (Fang and Wu 1999). Radical generation can be enhanced by adding oxygen, hydrogen peroxide or ozone to improve the overall process efficiency, but requires the constant, costly addition of chemicals and raises issues in terms of the health and safety of operatives

(Gehring et al. 1995). Combined e-beam treatment with oxidants, as mentioned above, has however proven to improve removal efficiency of pollutants in industrial waste streams and lower e-beam doses (Sampa et al. 2007).

Moreover, many ubiquitous molecules are radical scavengers, such as nitrates, carbonates and humic substances (Jankowska et al. 2004), which affect radiolytical degradation of organics by oxidising its radicals before they can degrade the problematic toxic compounds. This can increase the dose required to treat real-life industrial waste streams by several orders of magnitude with one study on coking wastewater reporting 20-fold dose increases required for treating a toxic compound in a real industrial effluent when compared to the pure compound in water (Fang and Wu 1999). Adding specific chemicals can help prevent radical scavenging (Şolpan et al. 2003), but once again raises the footprint and cost of the process and so is less desirable.

The organic destruction efficiency of the e-beam is also affected by the presence of metals, and the higher the metal content the greater the required dose for equivalent degradation (Duarte et al. 2004a). This is of particular concern as industrial effluents typically are complex chemical mixes consisting of various organics and metals. On average, to destroy over 90 % of the organics in simulated wastewaters a dose of 20 kGy is required (Duarte et al. 2002), whereas for certain industrial waste streams doses up to 100 kGy are required to reduce toxicity significantly (Borrely et al. 2000).

It has previously been reported that incorporating e-beam treatment into a conventional treatment method for a paper-mill waste stream led to a considerable reduction of organics

in the waste stream, enabling up to 80 % of the load being treated, thus meeting the standard to be reused in the same process (Shin et al. 2002).

A recent study showed that the e-beam could potentially be used to disperse NZVI particles and prevent aggregation, making them remain stable in suspension for up to two months (J. Zhang et al. 2013) and thereby potentially increasing their reductive capabilities suggesting simultaneous e-beam and NZVI treatment might have some merit as well.

While e-beam treatment on its own does not reduce COD in general (Duarte et al. 2000), e-beam pre-treatment of a textile dyeing wastewater resulted in a decrease of toxicity, a decrease in retention time and a subsequent 30-40 % improvement in biological COD removal (Han et al. 2012). The increase in biodegradability was attributed to the radiolytical conversion of biologically recalcitrant compounds into more bio-available lower molecular weights compounds such as organic acids or aldehydes (Getoff 1986).

pH has been found to be effective at tracking the extent of mineralisation of pollutants during irradiation. For a textile dyeing wastewater, an initial decrease in pH was reported to be linked to the radiolytical breakdown of the dye chromophore (Vahdat et al. 2012). A subsequent increase in pH may suggest that some of the breakdown products were being mineralised and removed from the system (Ting and Jamaludin 2008).

E-beam treatment has been reported not to result in any subsequent mutagenic activity in subsequent biological treatment (Gehring et al. 1992), which is promising for its integration with bioprocessing at any stage. Due to its particular advantages, it is possible that the e-beam has its place in both pre-treatment of industrial effluents as well as in post-treatment

(Duarte et al. 2000), hence it should be investigated at all stages of a hybrid treatment process at different irradiation doses.

Another advantage of e-beam irradiation technology is its potential for disinfection. Disinfection of a municipal effluent has been successfully demonstrated with e-beam and gamma irradiation having comparable efficiencies at equivalent dose rates (Han et al. 2012). However, there have been few if any studies on disinfection of industrial effluents post-bioprocessing. Thus, e-beam irradiation has the potential to satisfy a key requirement of the Health & Safety Executive COSHH schedule, which states that “biological cells employed in industrial processes have to be deactivated prior to discharge or recycling” (HSE 2002).

1.7 Rationale for a Hybrid Bioprocessing, NZVI and E-Beam Remediation Strategy

It is clear that biological treatment has its limits and to exploit its full potential, it benefits from being integrated with more robust technologies, which have been shown to improve subsequent treatment of complex industrial wastewaters (Jagadevan et al. 2011; Jagadevan et al. 2012; Jagadevan et al. 2013), such as e-beam treatment (Han et al. 2012) and NZVI treatment (Crane and Scott 2012). The individual techniques all have their own relative merits, but they also have the potential to overcome their individual limitations and complement each other well. The key innovation lies in combining them synergistically. Although previous studies have combined various AOPs with biological treatment in a number

of different configurations, this is the first study combining and optimising NZVI, e-beam and bioprocessing in a novel hybrid process for a range of industrial effluents.

1.8 Remediation Assessment Strategies

It is often difficult to assess the impact of treated industrial effluent once released into the environment, which is why new strategies are needed to refine current methods of assessing remediation (Prasse et al. 2015). A robust assessment tool should include a variety of indicators, describing the changes in chemical composition, eco-toxicity and bioavailability of a treated effluent as well as evaluate the sustainability of the treatment in terms of resource recovery, energy and cost footprint.

As there is no universally accepted way of determining toxicity removal, a variety of established measures such as chemical oxygen demand (COD), biological oxygen demand, suspended solids, total organic carbon, pH and colour measured by optical density are employed for evaluating the toxicity of industrial effluents and determining their chemical state. COD is an established parameter commonly employed to evaluate waste streams and is used as a legal measure of pollution load. Albeit important, it is not sufficient on its own to describe the impact of wastewater on the environment and in general, the combination of several complementary methods allows for a more versatile and complete evaluation of toxicity from an environmental perspective (Muszynski et al. 2007).

A diverse range of biological assays and biosensors have been used successfully for investigat-

ing the impact of irradiation (Bojanowska-Czajka et al. 2011; Borrelly et al. 2000; Drzewicz et al. 2005) and NZVI (Jagadevan et al. 2012) on waste streams and specifically for the reduction of toxicity. However, to date, no standard toxicity model to quantitatively evaluate detoxification by amalgamating established measures with biological assays and biosensors has yet been developed. As toxicity removal efficiency also depends on the initial concentration and the nature of the stream (Borrelly et al. 2000; Peijnenburg and Jager 2003), the overall assessment of decontamination is enhanced with each additional parameter. Bacterial whole-cell biosensors, such as the commonly employed *E. coli* HB101 (pUCD607), are a rapid, cost-effective and easy way to assess acute toxicity of a waste stream and integrate exposure, accumulation and toxicity (Maderova and Paton 2013) and are best combined with additional methods to give a more comprehensive eco-toxicity profile (Farré and Barceló 2003).

The most significant explanatory proxies for detoxification can be used to build a multi-parameter model bringing well-established parameters together with pertinent biological assays. This allows for a holistic evaluation of remediation effectiveness for any hybrid treatment system, which can subsequently be used to identify optimal treatment protocols for specific classes of water.

1.9 Significance of Research

Research Hypothesis

The combination of three treatment technologies (bioprocessing, nanoscale zero-valent iron and electron beam treatment) has the potential to improve current industrial treatment for the remediation of a range of chemically distinctive industrial effluents.

Research Aim

The overall aim of this research was to investigate the effectiveness of combining complementary treatment technologies for the remediation of industrial effluents. Strategies to achieve this included an adequate selection of treatments for the hybrid system and a tactical choice of key performance indicators to assess remediation effectiveness. Bioprocessing, nanoscale zero-valent iron and e-beam treatment have each individually featured in different environmental remediation applications and have been successfully employed on various scales at promising economics. The main focus of this work was to investigate the potential for these technologies to be combined in a novel hybrid treatment and to reveal possible synergies between them. The most effective treatment combination and order for each industrial effluent was determined. The hybrid technologies were assessed for additional quality applications such as the potential for effective resource recovery and for disinfection using the e-beam. Lastly, overall process economics, energetics and sustainability were evaluated at lab-scale and compared to a hypothetical industrial-scale implementation of the novel hybrid process.

Objectives

- Identify and amalgamate suitable key performance indicators into a proprietary remediation index to assess treatment effectiveness
- Determine the optimal hybrid bioprocessing, e-beam and NZVI treatment modes for two metalworking fluids, a textile dyeing and a distillery wastewater
- Investigate the potential to recover copper from a distillery effluent using the e-beam
- Evaluate the disinfection potential of the e-beam
- Analyse overall process economics, energetics and sustainability

1.10 Thesis Outline

This thesis includes the introduction of a new remediation assessment tool, the design of a novel hybrid system, its optimisation for three classes of recalcitrant industrial effluents and an analysis of its financial viability. It also investigates additional benefits of the e-beam. The thesis has been divided into six chapters: Chapter 1 begins with a review on the treatment of recalcitrant industrial effluents and highlights the rationale behind a hybrid bioprocessing, nanoscale zero-valent iron and e-beam treatment system for their remediation. Chapter 2 presents the materials and methods employed in this study and establishes the method for assessing remediation effectiveness by designing a remediation index. Chapter 3 is the first experimental chapter and investigates a hybrid bioprocessing, nanoscale zero-valent iron and

e-beam treatment process for an oily waste stream in a full factorial experiment. In Chapter 4, a textile dyeing wastewater is exposed to the hybrid treatment process to investigate the system's versatility, robustness and effectiveness. Chapter 5 is the final experimental chapter and investigates the potential for copper recovery from a distillery waste stream and the potential for effluent disinfection using the e-beam. It also evaluates the overall footprint of the hybrid treatment system. Chapter 6 summarises the main conclusions of the thesis and suggestions for future work.

2 Materials and Methods

This chapter summarises the analytical techniques (statistical, chemical, biological and physical) employed in this study. The main key performance indicators considered are presented and a remediation index, with the aim of evaluating and comparing the remediation effectiveness across different treatments and different effluents, is introduced. A summary of the industrial effluents used in this thesis is presented and the experimental procedures for bioprocessing, nanoscale zero-valent iron treatment and e-beam treatment are set up. Some of this work is adapted from (Thill et al. 2016).

2.1 Statistical Analysis

Unpaired t-tests were performed throughout this study and differences were considered to be statistically significant at $p < 0.05$. It was assumed that data collected as part of this work followed a normal distribution, which allowed parametric t-tests to be applied. The uncertainty values are the standard deviations (σ) for the mean (μ) values of triplicate measurements ($n = 3$) unless otherwise indicated and given as $\mu \pm \sigma$.

2.2 Chemical Analysis

To quantify chemical composition and changes in composition before and after treatment, a range of different techniques was employed and presented below.

2.2.1 Chemical Oxygen Demand, Carbon and Nutrients

For wastewater treatment, a key parameter of concern was overall pollution load, which is typically measured using chemical oxygen demand (COD). Subsequently, the composition of COD was investigated in terms of elements such as carbon and nutrients, i.e. nitrogen, sulphur and phosphorus, which can be further broken down. In particular, a significant parameter was the total organic carbon (TOC) content of samples and their nitrogen and sulphur breakdowns in terms of complex nitrogen / ammonium / nitrate and sulphate / sulphide respectively. Total nitrogen is the sum of complex nitrogen, ammonium and nitrate, whereas total sulphur is the sum of sulphate and sulphide. Understanding the breakdown gives an insight into whether the system is carbon or nutrient limited and allows to identify the pinch points during bioprocessing.

Chemical Oxygen Demand (COD), Total Nitrogen (TN_b), Ammonium (NH_4-N), Nitrate (NO_3-N), sulphate (SO_4^{2-}), sulphide (S^{2-}) and phosphate (PO_4-P) concentrations were analysed using Hach Lange cuvette tests employing a Hach HT200S and a Hach DR 2800 spectrophotometer, both UK. Cuvette tests enable the accurate determination of pollutant concentrations for a wide spectrum of parameters and measuring ranges. They require a minimum amount of reagents, small sample volumes, i.e. a few millilitres, and are easy to recycle and dispose of after use as no chemicals are released into the environment. Different cuvettes have different *modi operandi*. For example, COD was analysed using potassium dichromate as the oxidant in a strongly acidic media (sulphuric acid) in the presence of a silver sulphate catalyst. The reduction in the yellow colour of chromium is evaluated by a spectrophotometer

to determine the COD of the sample.

Total organic carbon (TOC) concentration was measured using a Shimadzu TOC V-CPH Analyser, UK. The system was calibrated with a potassium hydrogen phthalate standard solution (Sigma-Aldrich). All solutions were prepared using 15 M Ω Milli-Q de-ionised (DI) water. A TOC analyser is able to oxidise all organic compounds, in particular insoluble macromolecular organic compounds that are typically hard-to-decompose as well as easy-to-decompose low molecular weight organic compounds. A sample is injected into a combustion tube heated to 680°C, filled with an oxidation catalyst. Upon injection, the total carbon (TC) in the sample is oxidised into CO₂, which is detected by a non-dispersive infrared detector (NDIR) after dehumidification of the combustion products. The software then integrates the area under the NDIR signal (a peak), which is then converted into a TC concentration using a six-point linear standard calibration curve. Total carbon consists of organic carbon and inorganic carbon (present as a combination of carbonates and bicarbonates). To determine total organic carbon (TOC), total inorganic carbon (TIC) needs to be subtracted from the TC measurement:

$$TOC = TC - TIC \quad (2.1)$$

This is achieved by acidifying part of the sample and sparging it with purified air to only convert its inorganic carbon into CO₂ and measuring it in the same way as described above.

2.2.2 Metal Concentrations

Metal concentrations were measured using Atomic Absorption Spectroscopy (AAS). AAS was performed using an Agilent 240FS AA, UK. AAS can be used to determine over 70 different elements in solution and is based on the absorption of light by free atoms in their gas phase (Welz et al. 2005). The sensitivity of the AAS comes from the fact that each element has only one particular wavelength for which its electrons can be excited (Becker-Ross et al. 1996). It requires standard solutions with known concentrations to establish the relationship between measured absorbance in the sample and the standard solution. For this study, the AAS was calibrated in the ranges of 0.02-3 mg L⁻¹ Cd, 0.06-15 mg L⁻¹ Cr, 0.03-10 mg L⁻¹ Cu, 0.06-15 mg L⁻¹ Fe, 0.1-20 mg L⁻¹ Ni, 0.1-30 mg L⁻¹ Pb, 0.01-30 mg L⁻¹ Zn. The industrial effluents were diluted with DI water to fall into the linear calibration ranges.

This spectrophotometer uses a mixture of air and acetylene gas to produce a flame into which the calibration standards are injected to obtain four-point linear calibration curves for the ranges given above. Thereafter, the samples are injected and the amount of absorbed energy recorded and compared to the calibration curves and unknown concentrations are determined. The absorbance (A) of a sample is proportional (constant of proportionality ε) to both the concentration of the attenuating species (c) as well as the length of the light path (l) corresponding to the cuvette thickness (Beer-Lambert Law):

$$A = \varepsilon cl \tag{2.2}$$

2.3 Spectroscopic Analysis

2.3.1 Optical Density

In this study, optical density (OD) was measured using a Shimadzu UV 1800 Spectrophotometer, UK. Absorbance is equal to the logarithm of the intensity of light transmitted through a blank, i.e. DI water, with respect to that of a sample, measured at its maximum absorption wavelength (Rauf and Ashraf 2009):

$$\text{Absorbance } A = \log_{10}\left(\frac{\text{Intensity of light transmitted through blank}}{\text{Intensity of light transmitted through sample}}\right) \quad (2.3)$$

The OD reduction, i.e. degree of colour removal, over the course of a particular treatment is equal to the associated change in absorbance of the sample divided by its initial absorbance (given in its percentage form):

$$OD = \frac{A_{initial} - A_{final}}{A_{initial}} \cdot 100 \quad (2.4)$$

2.3.2 Liquid Chromatography

The Liquid Chromatography instrument was an Agilent 1120 Compact System, UK. It comprised a gradient pump, a column oven, an auto sampler and a UV detector. Data was acquired and analysed using the Agilent EZ-Chrom Elite Compact software package. The mobile phase was composed of 60 % pure acetonitrile in water containing a volume fraction

of 0.03 % formic acid. The flow rate of the mobile phase was maintained at 1 mL min⁻¹ and the injection volume was 3 µL with the detection in the region of 210 nm.

2.4 Analytical Biological Techniques

2.4.1 Bacterial Media Preparation

Technical agar No. 3, nutrient agar, nutrient broth powders, M9 minimal medium salts 5x powder, anhydrous glucose and ampicillin were purchased from Sigma Aldrich, UK. Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$), at a purity of 99+ %, and magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$), at a purity of 99.5 %, were purchased from Fischer Scientific, UK. The media were made as per the manufacturer's instructions and glucose was diluted in DI water and filter sterilised using 0.2 µm PTFE syringe filters (Fisher Scientific, UK) before addition to samples or media. For M9 minimal medium, 56.4 g L⁻¹ powder was suspended in 1 L of DI water and autoclaved. 1 M CaCl_2 and 1 M MgSO_4 were filter sterilised before being added to the cooled solution. Stock solutions were stored at room temperature.

2.4.2 Growth and Enrichment Studies

Aerobic growth studies were conducted either in a 500 mL volume shake flask (50 mL working volume) at 120 rpm agitation or in 96-well plates, which were incubated at 25 °C in a shaking Biotek Synergy HT microplate reader, UK. Anaerobic growth studies were undertaken in 100 mL serum bottles (50 mL working volume), sparged with nitrogen gas for 15 mins after

addition of medium before incubation at 25 °C and 120 rpm agitation. Agars were made in batches of 500 mL as per the manufacturer’s instructions and diluted with DI water before being autoclaved. Agar was allowed to cool to 40 °C before being transferred aseptically to 90 mm polystyrene Petri dishes (Fisher Scientific, UK). Minimal medium agar was made at twice the indicated strength and diluted 1 : 1 with a 40 °C pre-heated M9 minimal medium solution prior to aseptic plate pouring. Concentration gradient plates were prepared by successively pouring two layers of agar into a propped-up Petri dish.

2.4.3 Bacterial Biosensor

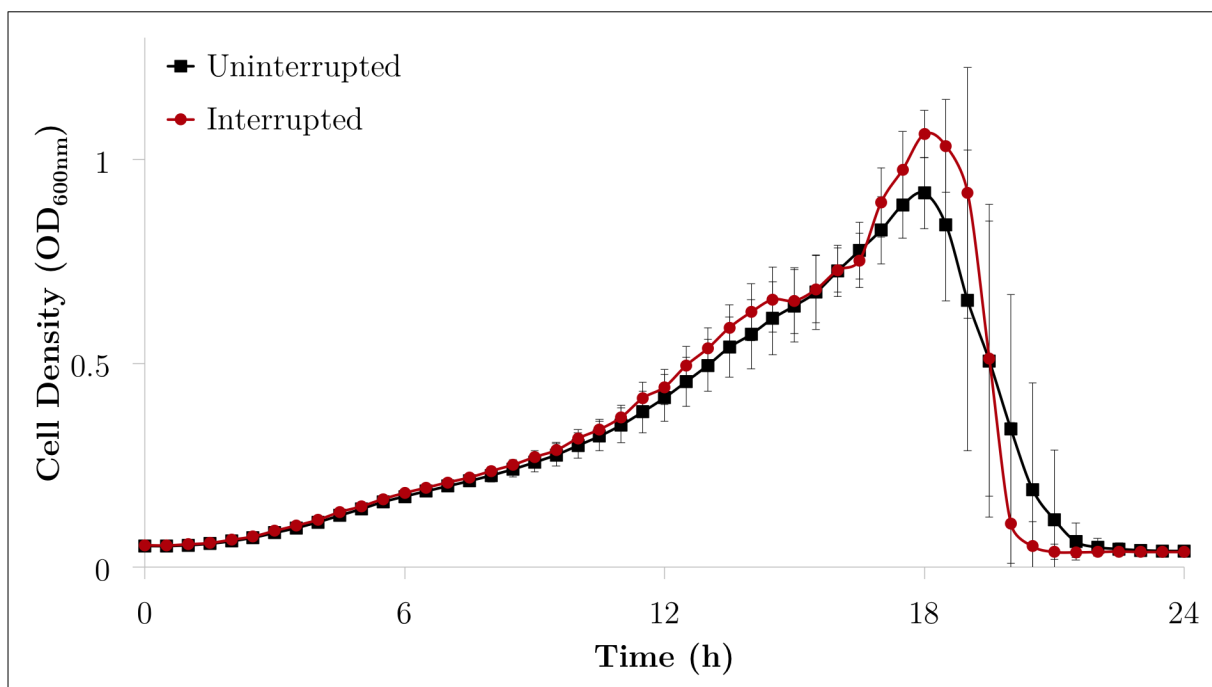
Microbial bioassays are an ideal choice in order to assess environmental remediation of industrial wastewaters (Bundy et al. 2001). The acute toxicity of the different samples was assayed by the metabolic *E.coli* HB101 (pUCD607) biosensor, which can act as a proxy for higher organisms for measuring the toxicity of wastewater samples (Trott et al. 2007). The *E.coli* HB101 has been transformed with a plasmid containing a *luxCDABE* gene cassette (Strachan et al. 2001). The pUCD607 multicopy plasmid vector of the biosensor is designed to be constitutively expressed and light output is proportional to the ATP concentration in the cell, i.e. proportional to metabolic activity in the cell (Paton et al. 1995). If something disrupts ATP production in the cell, bioluminescence is reduced. Thus, toxicity is inversely related to bioluminescence (Maderova and Paton 2013).

The *E.coli* HB101 (pUCD607) biosensor was streaked out from stock suspensions onto Lysogeny broth (LB) agar (also Luria broth, Lennox broth, Luria-Bertani) with 1 g L⁻¹

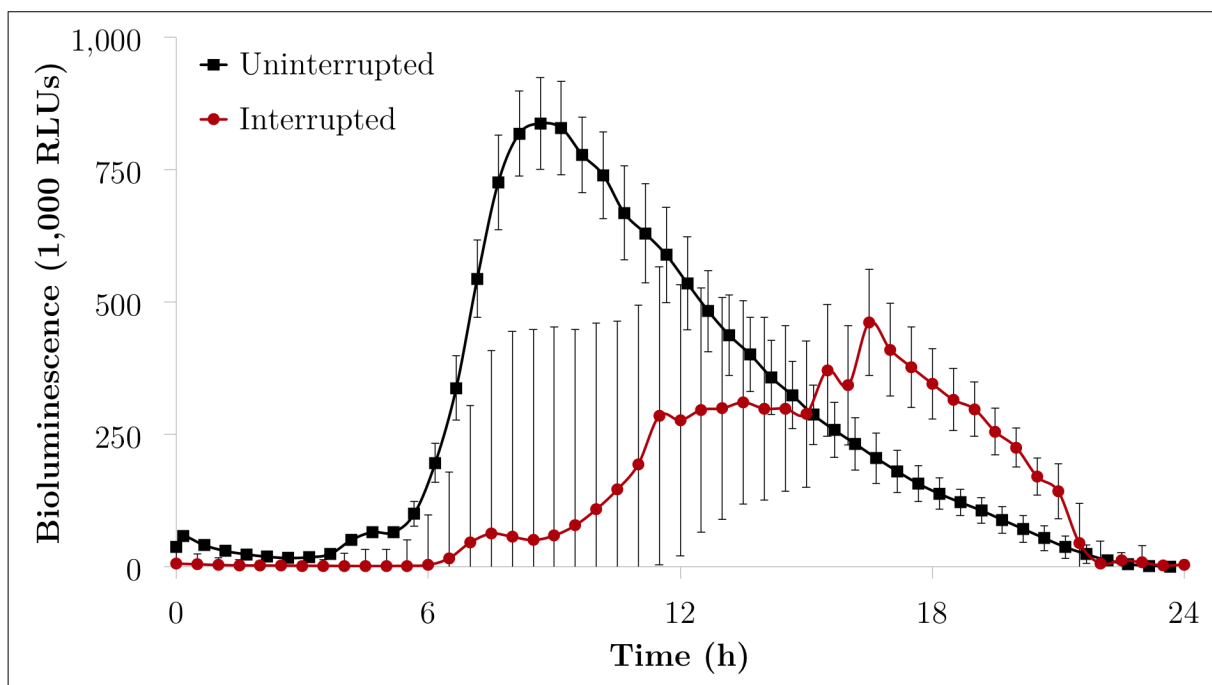
anhydrous glucose and $50 \mu\text{g mL}^{-1}$ ampicillin and incubated for 24 h at 37°C . An inoculation loop was used to transfer a single colony-forming unit (CFU) of the biosensor from the agar into a shake flask containing 100 mL of sterile LB with 1 g L^{-1} anhydrous glucose and $50 \mu\text{g mL}^{-1}$ ampicillin and put in a shaking incubator at 37°C and 150 rpm. The growth curves are shown in Figure 2.1a. The ampicillin antibiotic was added to select for cells containing the pUCD607 plasmid.

For maximum bioluminescence, the aim was to expose the biosensor to the sample in its mid-exponential phase, which was found to depend on the number and frequency of sampling interruptions. Figure 2.1b shows that the optimal time (mid-exponential) was 7 h for the biosensor without sampling interruptions and that this increased significantly in a simulated sampling mode (10 min shaking & 1 min sampling) in a microplate reader. Although sampling was found to affect the optimal time, intermittent sampling was necessary to track and identify the optimal time corresponding to maximum bioluminescence for the shake flask experiments. This observer effect (Heisenberg 1927) was circumvented by undertaking a series of parallel shake flask experiments with no intermittent sampling and assuming their growth and bioluminescence profiles were comparable. This set of experiments confirmed that the optimal time for maximum bioluminescence in the flasks was close to 7 h as found for the microplate reader.

After 7 h, the shake flask cell suspensions were centrifuged at $1,790g$ for 5 min, followed by the removal of the supernatant and re-suspension of the cells in 3 mL of phosphate buffered saline (PBS, Tablets, Fischer Scientific UK). A $20 \mu\text{L}$ cell suspension was subsequently added



(a) Growth Curves



(b) Bioluminescence Assays

Figure 2.1: *E.coli* HB101 (pUCD607) biosensor (a) growth curves and (b) bioluminescence assays for uninterrupted and interrupted (stopped every ten minutes for one minute) modes of the shaking incubator. Mean is shown and error bars represent standard deviation ($n = 84$).

to each well of a black 96-well microplate (Fischer Scientific, UK) containing 180 μL of sample using a multichannel pipette. The microplate contained 1 row ($n = 12$ wells) of PBS as control and 84 replicate samples. Bioluminescence was read after 20 min of exposure (corresponding to the maximum bioluminescence achieved in the PBS control) and measured in relative light units (RLUs). Acute toxicity was expressed as percentage of PBS control.

After exposure to the samples, serial 10-fold dilutions from the bioluminescence study were immediately prepared and sub-cultured in triplicates onto Lysogeny broth agar with 1 g L^{-1} anhydrous glucose and 50 $\mu\text{g mL}^{-1}$ ampicillin. Inoculated plates were incubated at 25 $^{\circ}\text{C}$ for 24 h and visible colonies counted to obtain post-exposure cell count recovery (PER) expressed as percentage of inoculum. The cell counts were thus determined using the Miles and Misra method (Miles et al. 1938) using 20 μL dispensed volumes in triplicates as shown in Figure 2.2a.

2.4.4 Respiration and Plate Counts

A chronic toxicity assay was evaluated by exposing a mixed bacterial community with a proven ability to biodegrade complex waste streams (van der Gast et al. 2003) to the different samples. These bacterial consortia were taken from the mature bioreactors and exposed to the samples over 24 h. Microbial respiration was monitored employing the MicrorespTM system (Dilly 2001; Wakelin et al. 2013) throughout test exposure. MicrorespTM is a 96-well microplate-based respiration system developed to measure CO_2 evolution over short periods of time mimicking natural environmental conditions. It is based on the use of an indicator

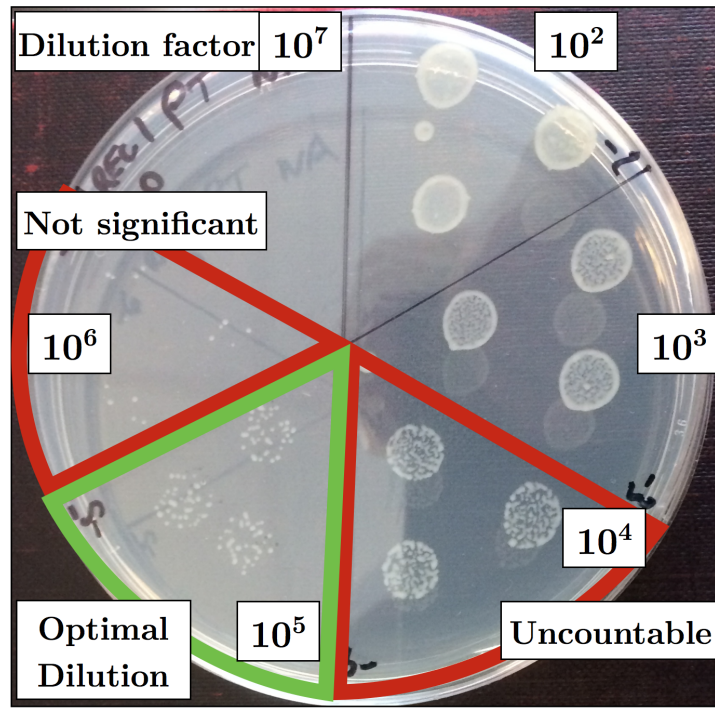
dye that changes colour with the change in pH when CO₂ reacts with bicarbonate (Rowell 1995) as shown in Figure 2.2b. The change in absorbance at 570 nm can be converted into %CO₂ using the following formula (Macaulay 2006):

$$\%CO_2 = -0.2265 - \frac{1.606}{1 - 6.771 \cdot A_n} \quad (2.5)$$

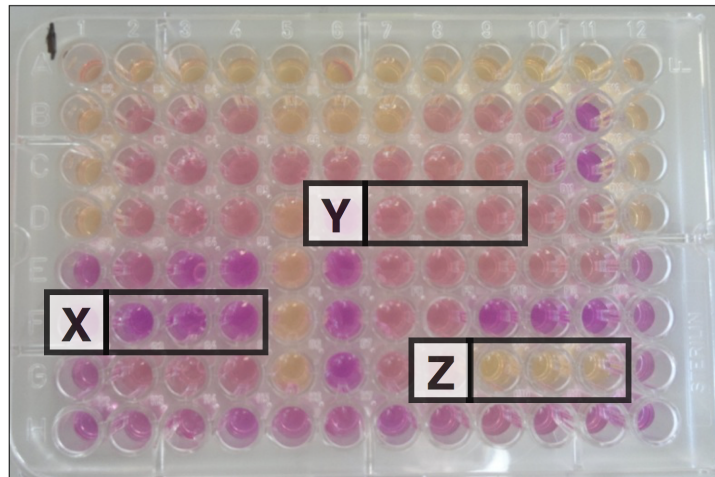
$$A_n = \frac{A_{final}}{A_{initial}} \cdot A_{mean} \quad (2.6)$$

The Microresp[™] detection plates were prepared from a 3 % Purified Agar and an indicator solution (made up from 18.75 mg Cresol Red, 16.77 g KCl and 0.315 g NaHCO₃ per liter and stored in the dark) using an agar : indicator ratio of 2 : 1. The plates were stored and regenerated after use in a humid soda lime desiccator.

After 24 h exposure, post-respiration cell count recovery (PRR) was determined by serial 10-fold dilution and sub-culturing in triplicates onto Nutrient Agar, incubated for 24 h at 25 °C. Cell counts were expressed as colony-forming units (CFUs) per mL. PRR consisted of plating out the culturable fraction of the bacterial consortium on agar plates for each sample. Results were quantified relative to the initial inoculum to determine whether cell proliferation or death had taken place. Respiration together with post-exposure recovery plate counts determines viability as a measure of chronic toxicity as shown in Table 2.1.



(a)



(b)

Figure 2.2: (a) shows typical results from a Miles & Misra plate counting experiment used for PER and PRR. Colony-forming units (CFUs) are counted in triplicates and then averaged for the dilution factor (10^5 in this case) at which the highest number of discrete colonies is visible (usually between 5 – 50) indicated in green. At a higher dilution (10^6) plate counts are not statistically significant whereas at a lower dilution (10^4), CFUs are uncountable both indicated in red. (b) shows the plate used in the MicroResp™ respiration system. If no CO_2 is produced, the pH sensitive gel remains purple (region X), whereas if CO_2 is produced, i.e. respiration occurs, the gel colour changes from purple to yellow (region Y shows low respiration and region Z shows high respiration).

	High PRR Counts	Low / No PRR Counts
High Respiration	Bioavailable	Toxic with stress respiration
Low / No Respiration	Inhibitory	Toxic and lethal

Table 2.1: Respiration and post-respiration cell count recovery (PRR) plate counts determine viability as a measure of chronic toxicity.

2.4.5 Flow Cytometry

Flow cytometry is a quick and reliable method to quantify cell viability in suspension and can be used as a complement to the Miles & Misra method. SYBR [®] green I (SG) and propidium iodide (PI) stains can be used together in a live / dead cell viability assay (Berney et al. 2007). SG is a membrane permeable nucleic acid stain that preferentially binds to double-stranded DNA, whereas PI is membrane impermeable to live, viable cells. PI can only bind to double-stranded DNA for non-viable (either moribund or dead) cells with damaged membranes.

To establish the proportions of live, dead and moribund cells after e-beam irradiation, SG / PI staining of cells was undertaken. Both stains were supplied by Invitrogen[™], UK. The samples containing the irradiated cells were incubated at 25 °C for 10 min after addition of SG in a 1 : 10⁴ dilution and PI at a concentration of 10 µg mL⁻¹. The samples were then analysed by a BD FACSCalibur flow cytometer, UK. The FL1 fluorescence channel at 530 nm was used to collect the SG stain (green) and the FL3 channel at 585 nm was used to collect the PI stain (red). Cells that are stained by both stains are said to be moribund or dying and have high FL1 and FL3 values.

2.5 Assessment of Remediation Effectiveness

2.5.1 Key Performance Indicators

In order to quantitatively evaluate the extent of remediation, which industrial effluents had undergone, established measures including COD were complemented with biological assays and a metabolic biosensor. The main key performance indicators (KPIs) for determining the effectiveness of treatments employed as proxies for remediation effectiveness were: chemical oxygen demand (COD), optical density (OD) and overall toxicity. Overall toxicity was evaluated using the following four biological assays, introduced in Sections 2.4.3 and 2.4.4 of this chapter, to assess acute and general toxicity. The acute toxicity of samples was evaluated using the *E.coli* HB101 (pUCD607) biosensor and post-exposure cell count recovery (PER) whilst their general toxicity was evaluated using respiration and post-respiration cell count recovery (PRR).

Results were normalised and presented in the form of radar graphs to show the extent that the individual KPIs had improved by. The centre point of the radar graphs represents the original untreated industrial wastewater, whereas the 100 % mark, corresponding to 100 % KPI improvement represents the tap water control, as shown in Figure 2.3. Tap water was chosen as negative control and to dilute effluents throughout this study, due to its industrial relevance, typically used during operation of industrial fluids for various purposes including dilution. In other words, if after treatment a KPI has improved by 100 %, the treated effluent is effectively equivalent to tap water for that particular KPI in terms of toxicity and

is considered to be fully detoxified and remediated against that KPI.

2.5.2 Remediation Index

The Mogden Formula (Walker 2000) is employed by water and sewage companies in the UK to charge companies for their industrial effluents. Similarly, it would be useful to design a remediation index that allows for comparison of remediation effectiveness between different classes of treated effluents in this study. A basic index would simply consider the aggregate improvement of the different KPIs. However, different waste streams present different challenges in terms of remediation with some KPIs not being relevant.

Effective treatment should address the main issues associated with a particular industrial effluent. For example, one stream might have a very high COD that needs treating above anything else, while another might be highly coloured, which has priority in that scenario. If a particular stream contains a particularly valuable resource, e.g. a metal that is desirable to recover, then this too can be added into the remediation index, with the normalised percentage recovery for that particular resource being just another KPI. The same is true for a particularly unwanted contaminant or nutrient in end-of-pipe effluents before discharge.

Moreover, according to the treatment priority required of the industrial effluents, the improvements in KPIs could be of different relative importance. In this case, a more adequate index would therefore be composed of a strategic selection of relevant KPIs, which are weighted and normalised with respect to the sum of the weighting factors and normalised by the index

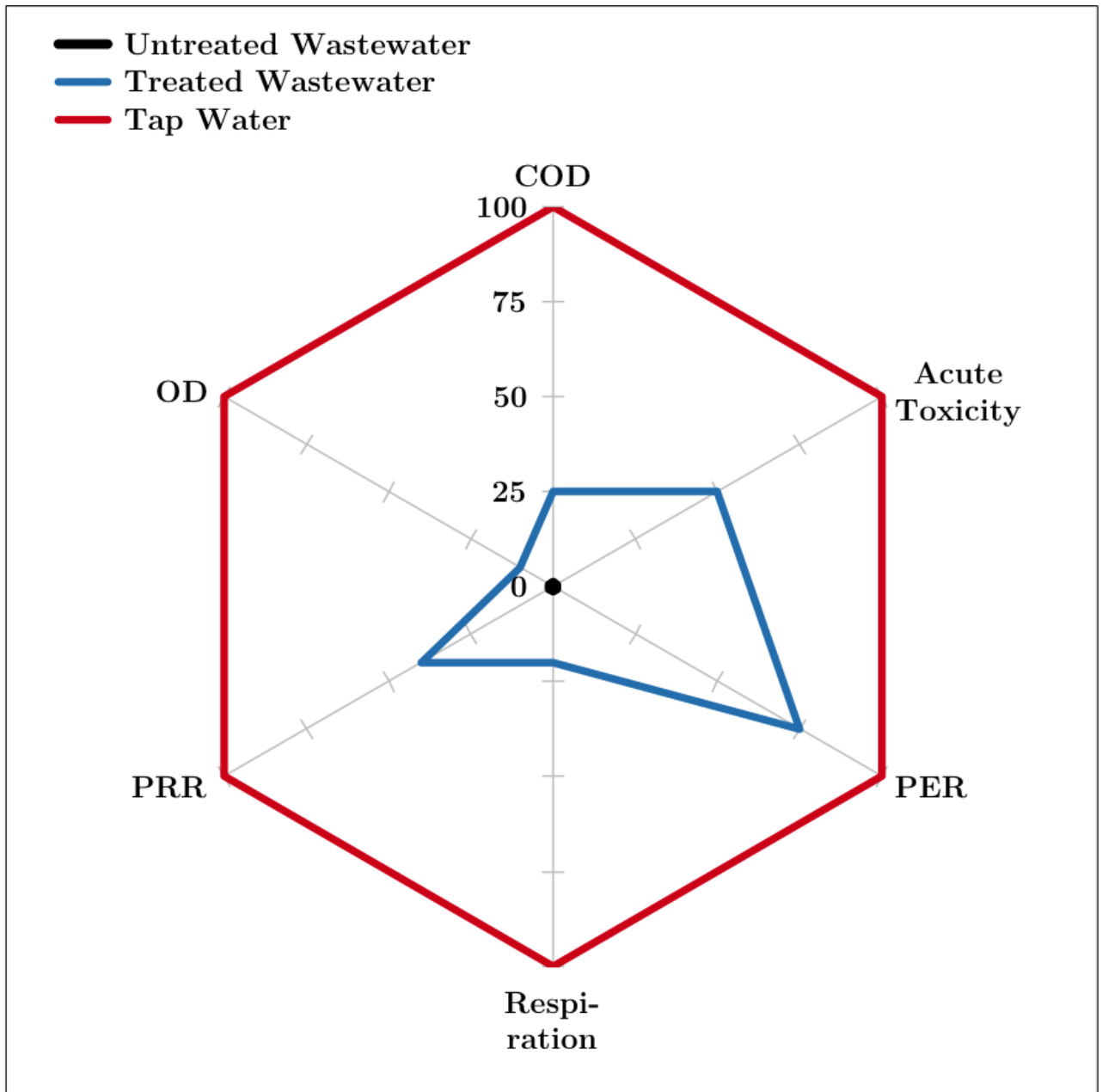


Figure 2.3: Radar graph showing normalisation procedure using a hypothetical untreated wastewater sample (black) as the centre of the graph (0 % remediation) and tap water (red) as the baseline aim (100 % remediation) and a hypothetical example of a wastewater after treatment (blue), which shows a 25 % improvement in COD, a 50 % improvement in acute toxicity, a 75 % improvement in PER, a 20 % improvement in respiration, a 40 % improvement in PRR and a 10 % improvement in OD.

for the tap water control as indicated in Equation 2.7. The index of the tap water control ($I_{control}$) is not necessarily one, e.g. one of the KPIs chosen could be a resource to be recovered, which would not be attainable from the tap water control.

$$Remediation\ Index = \frac{\sum_{i=1}^n (w_i \cdot KPI_i)}{I_{control} \cdot \sum_{i=1}^n w_i} \quad (2.7)$$

The remediation index (RI) designed in Equation 2.7 is used to evaluate and compare the remediation effectiveness of hybrid treatment on different classes of industrial effluents that would have been difficult to compare quantitatively without an index.

A note of caution, any novel weighted index runs the risk of resulting in cherry-picked KPIs and weighting factors that will represent the treatment results in a much more favourable way than true. To minimise this effect, the four standard toxicity assays (Acute Toxicity, PER, Respiration, PRR) are assigned unit weightings as they form the basic detoxification assessment of the RI and give a holistic overview of remediation effectiveness.

2.6 Particle and Precipitate Identification and Analysis

In addition to identifying the chemical composition of particles and precipitates, it is of paramount importance to identify other physical properties such as particle size, particle shape, surface properties, oxidation states and their changes over time as they all influence the overall material properties, e.g. reaction rates, surface areas and their reusability. A range of imaging and spectroscopic analyses are employed in this study.

High-resolution scanning electron microscopy (SEM) images were obtained using a Zeiss Sigma HD VPSE operating at 5 kV HV, with a 30 μm aperture and at a working distance between 3.1 mm and 4.2 mm. Scanning transmission electron microscopy (STEM) was performed using a Fei Helios Nanolab 600i at 30 kV and 86 pA. For STEM, the particulate was suspended in ethanol via sonication for 120 s and then attached onto a copper mesh support grid and dried under vacuum (10^{-2} mbar) for 2 h.

Energy dispersive x-ray spectroscopy (EDX) was carried out using a Bruker Quantax 400 EDS detector attached to the Zeiss SEM. EDX provides information about the surface composition of the samples. The samples were sputter-coated in gold before EDX analysis and attached onto a carbon mesh grid.

Specific surface area was measured by Brunauer-Emmett-Teller (BET) surface area analysis. The data was extrapolated using a five-point isotherm by measuring a known weight of the dried sample using a Quantachrome Nova1200e BET system following the nitrogen adsorption method. Prior to BET analysis, the samples were degassed under vacuum (10^{-2} mbar) for 12 h at a temperature of 75 ± 0.5 °C.

Transmission electron microscopy (TEM) was performed using a JEOL JEM-2100 microscope operating a LaB_6 source and equipped with an Oxford instrument 80 mm thin window EDX detector and a Gatan Orius CCD camera.

SEM, STEM, EDX and BET analysis were undertaken in collaboration with Dr. Sarah Tesh at the University of Bristol and TEM was undertaken in collaboration with Prof. Andrew Watt at the University of Oxford.

2.7 Industrial Effluents

The various recalcitrant chemically complex industrial wastewaters were provided by different industrial collaborators and diluted with tap water as described in the corresponding chapters. These industrial effluents were selected as representatives of a variety of recalcitrant industrial effluents presenting different challenges. The two semi-synthetic metalworking fluid waste streams (MWFs) featuring in Chapter 3 were provided by Ford¹ and BP². The pristine MWF served as a highly toxic model waste stream, and the other was an actual industrial operationally exhausted MWF. Both had characteristically high CODs and were opaque.

The highly coloured textile dyeing wastewater stream (TDW) in Chapter 4 was provided by the Hong Kong Productivity Council³. The main compounds of the TDW were ammonium sulphate (acid-forming salt and fixing agent), sodium dithionite (main reductive bleaching agent), glacial acetic acid (acid dye colouring agent), acid dye, a levelling agent (surfactant controlling uptake of dye and ensuring uniform dye migration and distribution) and sodium hydroxide. The physico-chemical parameters of the TDW are presented in Chapter 4.

The final industrial effluent considered was provided by the alcoholic beverages company Diageo⁴. It consisted of a mixed-waste distillery wastewater (DWW) originating from a distillery and contained a high concentration of copper.

¹<http://www.ford.co.uk> (accessed on 7 March 2017)

²http://www.bp.com/en_gb/united-kingdom.html (accessed on 7 March 2017)

³<https://www.hkpc.org/en/> (accessed on 7 March 2017)

⁴<http://www.diageo.com> (accessed on 7 March 2017)

2.8 Bioprocessing

Biological treatment experiments were carried out in different bioreactors (e.g. independent replicates) at room temperature in 15 mL fixed-film batch bioreactors, with a working volume of 10 mL, with airflow maintained at 4 mL min^{-1} for aerobic treatment using a 25 W Blagdon air pump rated at 25 L min^{-1} . The aerobic-anaerobic transition time had been previously established and found to be two hours, whereas anaerobic-aerobic transition occurred within seconds after switching the air back on (Thill 2013). The bioreactors, shown in Figure 2.4d, had been inoculated with a five-membered bacterial consortia previously reported to be effective for biological treatment of MWF streams (van der Gast et al. 2003) and started from a 3 L mature bioreactor (Figures 2.4a, 2.4b, 2.4c). The acclimatisation period in the 15 mL bioreactors was 21 days to allow the biomass to grow and acclimatise to the industrial effluent substrates (Figure 2.4e), which is in line with similar studies (Jagadevan et al. 2011).

Biofilms are formed in fixed-film reactors, when multiple microorganisms come out of their planktonic state and collectively attach onto solid surfaces, where they become enclosed in a self-secreted matrix of extracellular polymeric substance. Biofilms in general make the community more resilient to stresses due to the protective barrier formed and shared use of available resources (Davies 2003). The biofilms in the bioreactors are supported by polypropylene Bio-Blok® matrices, which were supplied by Expo-net Danmark A/S. These particular matrices ensure quick and effective biofilm growth due to their large specific surface area and suitable surface structure.

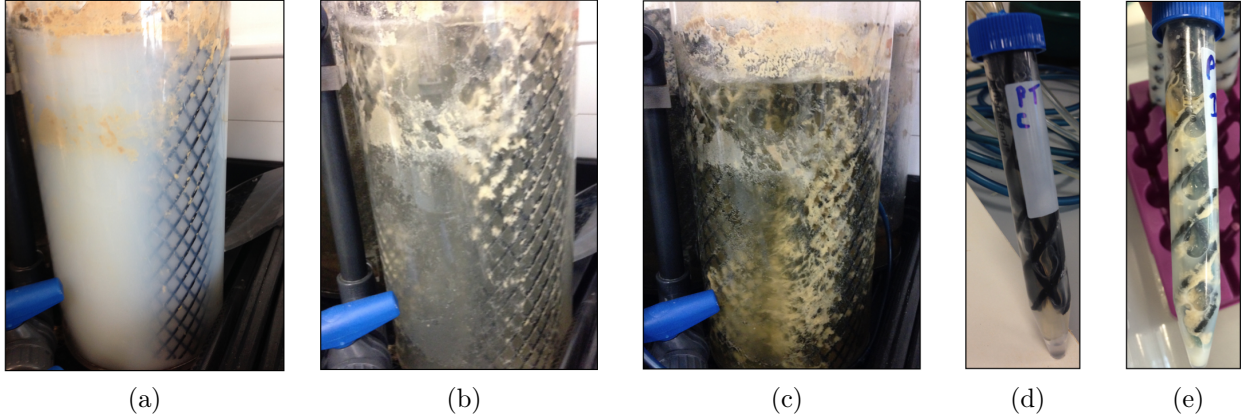
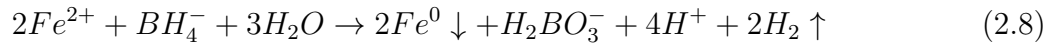


Figure 2.4: Mature 3 L bioreactor (a) at feed, (b) after 24 hours, (c) after 72 hours displaying the decolouration of a metalworking fluid during aerobic operation and (d) experimental 15 mL bioreactor set-up at start-up and (e) after 28 days inoculation under intermittent feeds of fresh MWF. The black matrix and the biofilms can be clearly seen.

2.9 Nanoscale Zero-Valent Iron Treatment

2.9.1 Synthesis

Nanoscale zero-valent iron (NZVI) particles were synthesised in batches using a borohydride reduction method, which has been previously reported (Dickinson and Scott 2010). Ferrous iron is reduced to a metallic state using sodium borohydride via the following reaction:



The beakers were washed overnight in 10 % nitric acid and rinsed with Milli-Q water (resistivity 15 MΩ cm) before use. After dissolving 7.65 g of $FeSO_4 \cdot 7 H_2O$ in 50 mL of Milli-Q water, the pH was adjusted to 6.8 using a 4 M NaOH solution before 3 g of $NaBH_4$ were slowly added. The precipitated product was isolated by centrifugation and then sequentially washed

twice with water, ethanol and acetone (20 mL at each wash). After drying the nanoparticles in a desiccator under low vacuum (approximately 10^{-2} mbar) for 48 h at 60 °C, they were stored until required. All chemicals (iron (II) sulphate ($\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$) 99+ %, sodium hydroxide (NaOH) pellets, sodium borohydride (NaBH_4) 98+ %, nitric acid (HNO_3) 68 %, and solvents (ethanol 70 %, acetone >95 %)) for the synthesis of NZVI were obtained from Fisher Scientific and employed without any further purification and solutions were prepared using Milli-Q water.

2.9.2 Particle Characterisation

SEM informs about the morphology of a sample and leads to a good depth of field due to the narrow e-beam employed and gives a 3D image. SEM imaging of NZVI particles after synthesis show primary NZVI aggregates of approximately 50-75 nm in diameter (Figure 2.5). This is in agreement with typical particle size ranges found with the borohydride reduction synthesis method with 90 % of the particles synthesised falling in the range of 1-100 nm (Wang and Zhang 1997). The individual NZVI particles aggregate in the form of rods due to their magnetic property. The surface area of the synthesised NZVI as characterised by BET was $17.63 \text{ m}^2 \text{ g}^{-1}$. STEM informs about the internal composition, crystallisation and atomic structure of a sample and results in a 2D image and can be used in conjunction with SEM to characterise NZVI particles in more detail. Figure 2.6 shows a STEM of the iron oxide layer of approximate 10-15 nm thickness around the NZVI particles after synthesis. This is typical for NZVI particles, which always have an oxide layer around them (Noubactep 2008),

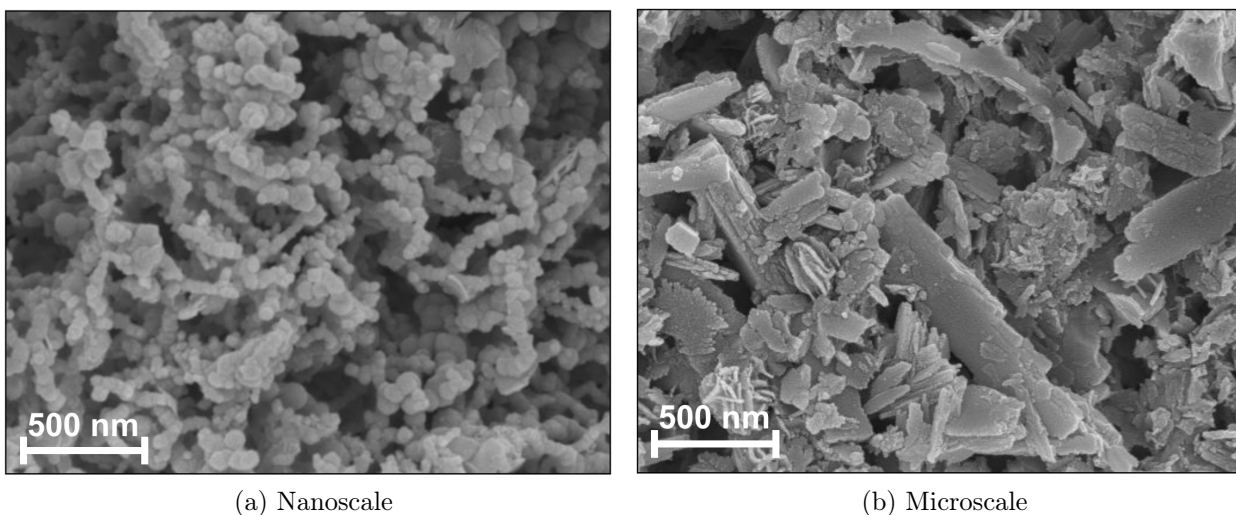


Figure 2.5: SEM images of NZVI particles (a) after synthesis showing primary aggregate of diameters less than 100 nm and (b) after usage showing crystallised aggregates, which are no longer nanoscale.

reported to vary between 1 and 25 nm (Martin et al. 2008).

2.9.3 Experimental Procedure

All NZVI experiments were carried out at room temperature in 15 mL test tubes. Due to the batch manufacturing process employed, NZVI treatment experiments resulted in pseudo replication. Before use, the nanoparticles were sonicated for 5 min using a probe sonicator at 80 W (VC - 50 2 mm Microtip, VWR, UK) before being added to the samples under constant stirring and left to react for 1 h before being separated out using centrifugation and magnetic separation and stored for subsequent analysis. Further sonication (> 5 min), stirring and reacting (> 1 h) did not lead to any further significant improvement in NZVI treatment.

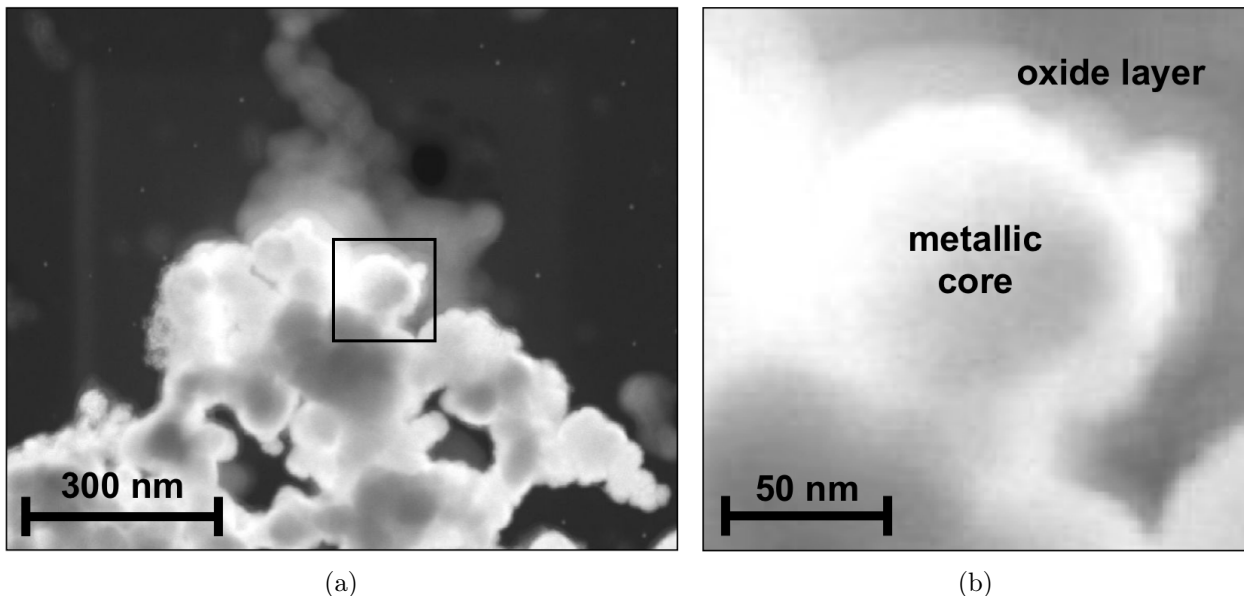


Figure 2.6: STEM images of NZVI particles at two different magnifications. The boxed area on (a) is further magnified in (b) and shows that the structure of NZVI consisted of an iron oxide layer of 10-15 nm thickness as a ring of lighter shade (grey) around the metallic core of 80-90 nm diameter (white).

2.10 E-Beam Treatment

2.10.1 Experimental Procedure

The main body of work for the electron beam irradiation (e-beam) treatment was undertaken on a 6 MeV electron single pulse linear accelerator at the Gray Institute, Department of Oncology, University of Oxford (shown in Figure 2.7). This particular e-beam was designed for medical applications and is not typical for industrial applications. The irradiation was performed in a batch system using synthetic quartz (QS) cuvettes, supplied by Hellma Analytics, UK. Similar to NZVI treatment experiments, due to the batch-based irradiation using a single e-beam, this resulted in pseudo-replication of the experimental results. The

cuvettes have a 1 mm wall thickness, which is equivalent to 2.2 mm of water as penetration thickness is approximately inversely proportional to material electron density. As e-beams typically have non-uniform irradiation dose distributions due to build-up effects during interaction of radiation and matter, the 2.2 mm water equivalent thickness at 6 MeV is ideal to eliminate this non-uniformity (Vojnovic 2011). The beam irradiation dose is then very flat throughout the cuvette (Getoff 1996). Figure 2.8 shows a cuvette containing a sample before and during e-beam irradiation as well as the beam profile and the effect of a misaligned beam on a coloured sample.

The 6 MeV electron pulses were delivered at a frequency of 50 Hz, at an average pulse current of 150 mA and nominal pulse length of 4 μ s, resulting in an average dose of 50.1 ± 2.4 Gy per pulse, determined by Fricke Dosimetry. Fricke Dosimetry is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) in an aerated acidic aqueous environment by ionising irradiation. The dose was determined by relating the change in absorbance at 304 nm and 25 °C for a 10 mm path length cell to absorbed dose. The Fricke solution was prepared using 10 mM ferrous ammonium sulphate, 0.4 M H_2SO_4 and 1 mM NaCl. Ammonium iron sulphate ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6 \text{H}_2\text{O}$), sodium chloride (NaCl) and sulphuric acid (H_2SO_4) were provided by Fischer Scientific, UK.

A temperature rise of 20.2 ± 1.7 °C was observed for every 1,000 pulses delivered and temperature control experiments (up to 80 °C) of all the samples throughout this thesis did not reveal any statistically significant impact of this temperature rise on any KPIs. High-dose samples were intercooled every 3,000 pulses to avoid the loss of samples due to evapora-

tion. Any changes in KPIs of the industrial effluents observed after e-beam treatment were therefore not attributable to thermal treatment.

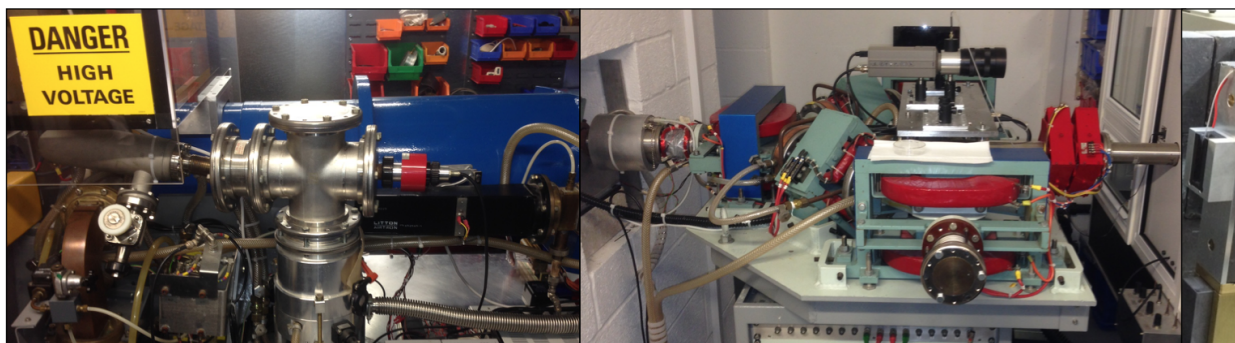


Figure 2.7: Side view of e-beam set-up at the Gray Institute, Department of Oncology, University of Oxford.

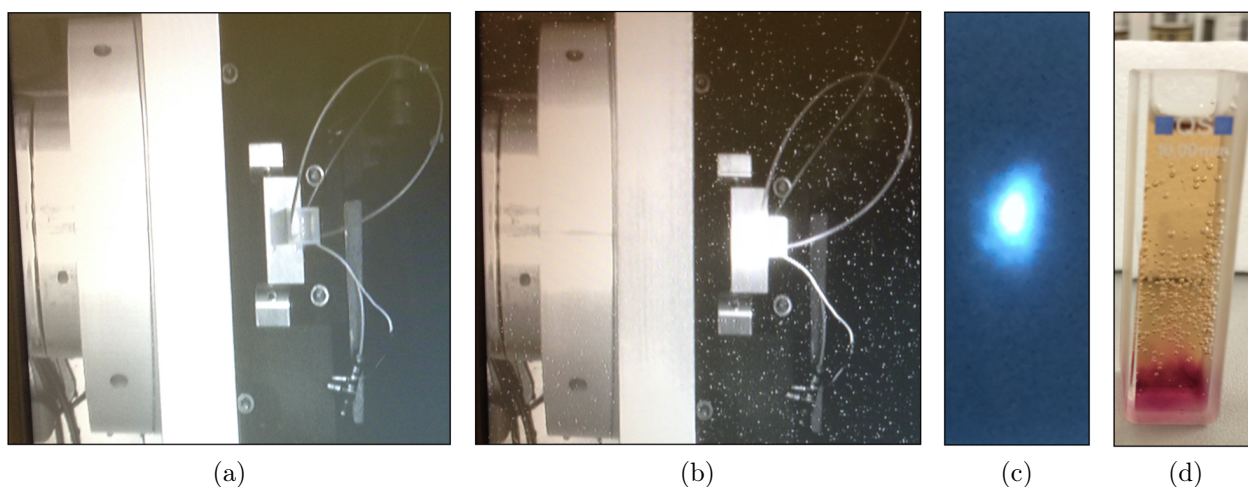


Figure 2.8: Top view of cuvette with sample (a) before irradiation and (b) during irradiation, where Cherenkov radiation is observed. (c) Beam profile on a phosphorus screen (d) Effects of misaligned beam visualised on an initially purple sample - where e-beam irradiation resulted in non-uniform decolouration as the bottom part of the cuvette received a lower irradiation dose (pink) compared to the top of the cuvette (yellow).

2.10.2 E-beam Operation

In an e-beam, the electrons are generated in an electron gun. The electron gun consists of a cathode and an anode. Electrons are generated by thermionic emission due to heating of an inner filament (the cathode). The anode is placed in front of the cathode and the electric field thus generated accelerates the electrons to energies up to 50 keV. A pulsed modulator supplies the energy to the electron gun and the 2 MW rated magnetron. The magnetron generates very high frequency radio waves (3 GHz), which are supplied to the cavity via a waveguide and are the main source of power generating the high-energy electron beam in the cavity. The cavity is a resonance chamber, where electrons are bundled up in sequential corrugations and accelerated. All these processes take place in a vacuum and the coils providing the magnetic field require cooling, so the process requires power for pumping and cooling, which is however negligible when compared to the power requirement for the high voltage power supply (Vojnovic 2011). Figure 2.9 shows the main components of the e-beam employed in this study.

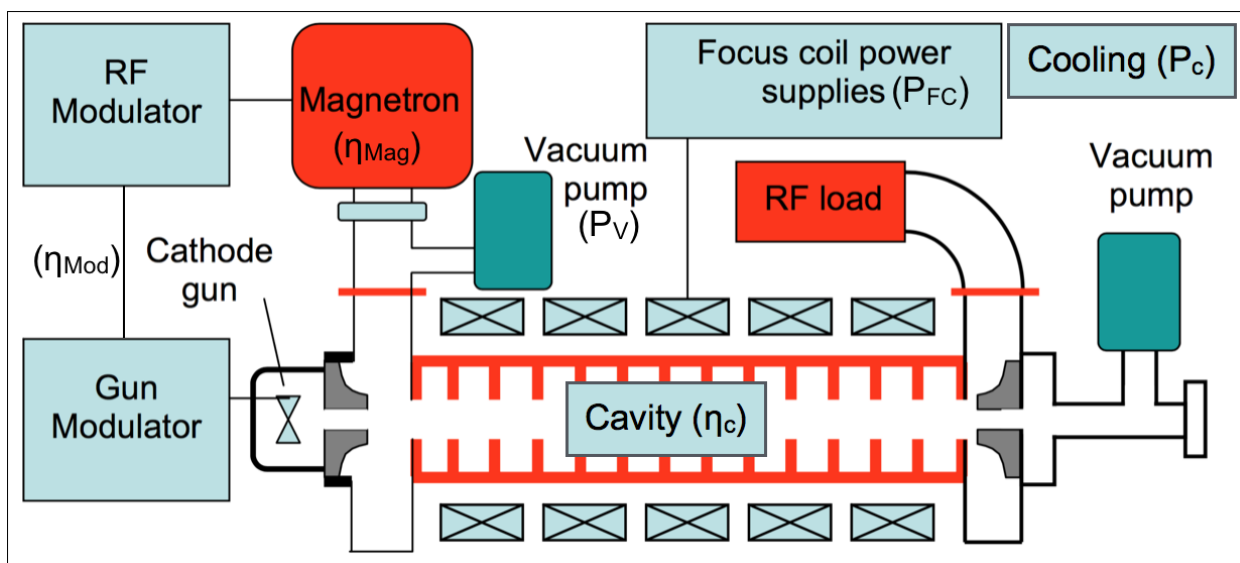


Figure 2.9: Diagram showing the main components of the e-beam employed in this study. This figure has been adapted from a Gray Institute schematic (Vojnovic 2011).

2.10.3 Continuous Flow Set-Up

The continuous flow experiments in Chapter 5 were undertaken on a smaller 1.1 MeV e-beam at Daresbury Laboratory at STFC's Accelerator Science and Technology Centre, UK. This e-beam is again not typical for environmental remediation applications, but due to its different configuration could be set up for continuous flow experiments.

3 Hybrid Treatment Process for Metalworking Fluids

3.1 Outline of Remediation Strategy

The aim of this chapter was to evaluate the remediation potential of bioprocessing, NZVI and e-beam treatment individually and in combination for treating two recalcitrant MWFs. The key performance indicators (KPIs) employed to determine the effectiveness of the treatments were COD, optical density (OD), acute toxicity, post-exposure recovery (PER), respiration and post-respiration recovery (PRR). In doing so, the individual treatments were optimised and the extent of synergy between the treatments in a hybrid system was determined. Some of this work is adapted from (Thill et al. 2016).

3.2 Metalworking Fluid Characteristics

Two semi-synthetic metalworking fluids with different application histories were investigated: a pristine, i.e. unused, MWF and an operationally exhausted MWF. The pristine MWF served as a highly recalcitrant model waste stream, because it contained fresh biocide and was specifically designed to be resistant to bio-deterioration. The operationally exhausted MWF corresponded to a real industrial effluent obtained from a metalworking workshop. The physico-chemical characteristics of both MWFs are shown in Table 3.1 and corresponded to a typical 5 % working concentration of the pristine MWF and the actual effluent concentration of the exhausted MWF as produced by industry.

Effluent	COD (mg L ⁻¹)	OD _{390 nm}	TOC (mg L ⁻¹)	TN _b (mg L ⁻¹)	pH
Pristine	117,180 (5,680)	>3	22,130 (4,570)	5,400 (850)	7.9 (0.5)
Exhausted	19,190 (150)	>3	3,330 (130)	705 (20)	7.5 (0.1)

Table 3.1: Physico-chemical characteristics of MWF industrial effluents. Mean of triplicates ($n = 3$) is shown with standard deviations given in parentheses.

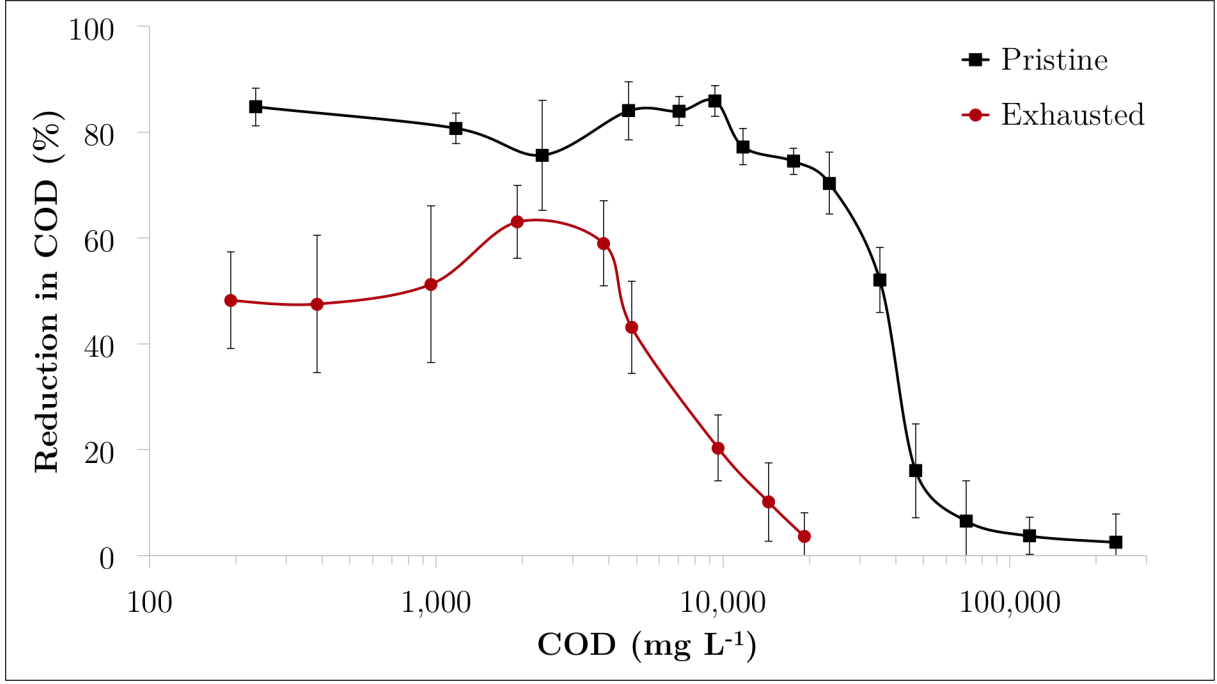


Figure 3.1: Effect of bioprocessing on COD removal for the pristine and exhausted MWFs expressed as percentage reduction of starting CODs given on a log-scale. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

3.3 Bioprocessing

Chapter 1 showed that few studies had considered how the initial COD concentrations of the MWFs affected COD removal during bioprocessing. In this study, the effect of varying the initial COD by prior dilution of both MWFs was investigated. The biological COD removal effectiveness over a range of dilutions for both MWFs is summarised in Figure 3.1. The initial COD concentrations of both MWFs affected COD removal efficiency. Figure 3.1 shows that

for the concentrated streams, as presented in Table 3.1, no significant reduction in COD was achieved. The optimal COD removals were found to occur at CODs of $9,375 \pm 454 \text{ mg L}^{-1}$ and $1,919 \pm 15 \text{ mg L}^{-1}$ achieving COD reductions of $85.9 \pm 3.4 \%$ and $63.0 \pm 7.1 \%$. At lower concentrations, COD removal was not reduced significantly.

At optimal treatment concentrations, steady-state conditions were achieved after three days, when most of the biodegradation had occurred as shown in Figure 3.2. No significant COD removal was observed to occur during the following eleven days. The remaining effluent and its corresponding COD were classified as recalcitrant, i.e. resistant to further biodegradation. Optical density (OD) at the optimal treatment concentration was reduced by $89.1 \pm 3.1 \%$ for the pristine MWF and by $23.1 \pm 4.9 \%$ for the exhausted MWF stream at 390 nm. This choice of wavelength was arbitrary and corresponded to the lower end of the visible spectrum. In fact, OD increased monotonically at lower wavelengths for both MWFs before and after treatment. Maximum absorbance could therefore not be defined. Below 250 nm, increased fluctuations in OD were observed corresponding to photo-dissociation effects in the samples.

3.4 Nanoscale Zero-Valent Iron Treatment

Nanoscale zero-valent iron (NZVI) treatment effectiveness of both MWFs was evaluated and contrasted with bioprocessing in terms of performance in this section. NZVI treatment of both MWFs was found to reduce COD up to 10 g L^{-1} and 5 g L^{-1} respectively for the pristine and exhausted MWF with no significant further reductions observed at higher NZVI concentrations as shown in Figure 3.3. Even at doses as high as 50 g L^{-1} (not shown in

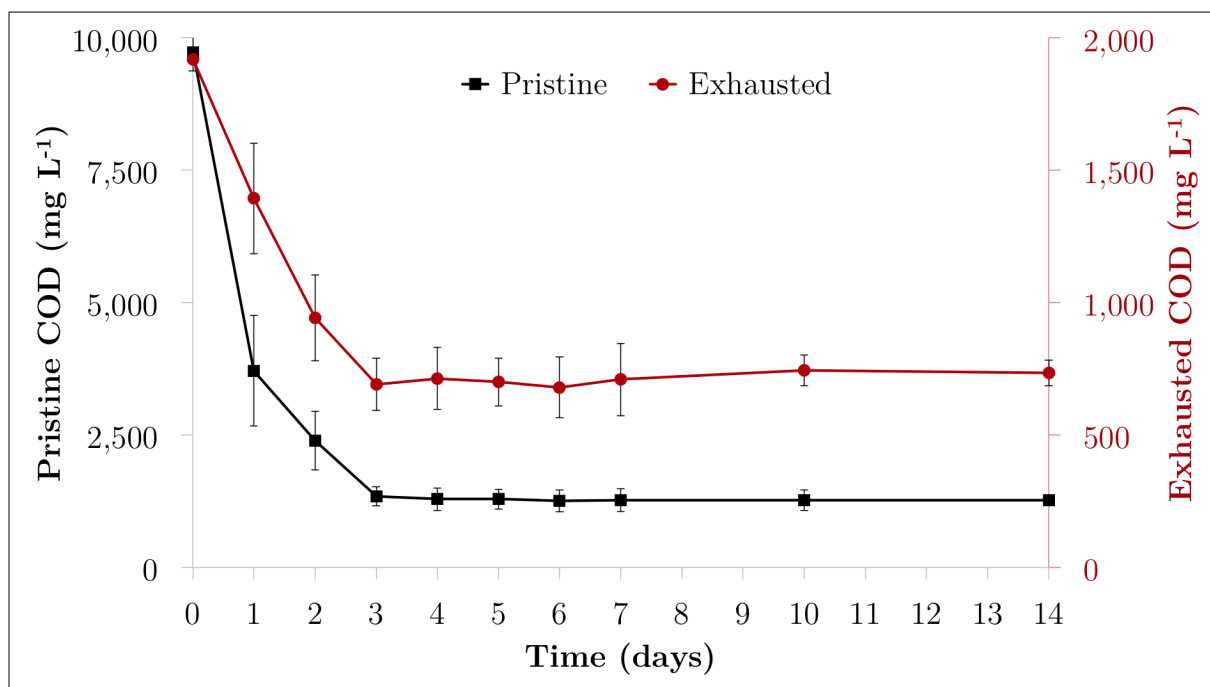


Figure 3.2: Temporal profile of COD removal for pristine and exhausted MWFs at their optimal treatment concentrations during bioprocessing. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

Figure 3.3), no significant improvement was observed. Removing reacted NZVI from the sample using centrifugation and magnetic separation and subsequently adding fresh NZVI (at 1 and 10 g L⁻¹) did not improve COD removal further either. The COD of pristine MWF was reduced by $90.1 \pm 2.0 \%$ at 10 g L⁻¹ and the COD of exhausted MWF was reduced by $54.0 \pm 4.8 \%$ at 5 g L⁻¹. The corresponding OD reductions were $89.9 \pm 2.7 \%$ for the pristine MWF and $56.2 \pm 3.8 \%$ for the exhausted MWF at 390 nm.

NZVI treatment also mitigated overall toxicity of both MWFs tested more effectively than bioprocessing. For the pristine MWF, all KPIs apart from COD were significantly greater for the NZVI treatment as shown by the improvements in KPIs in Figure 3.4. There was no statistically significant difference in COD removal between bioprocessing and NZVI treatment

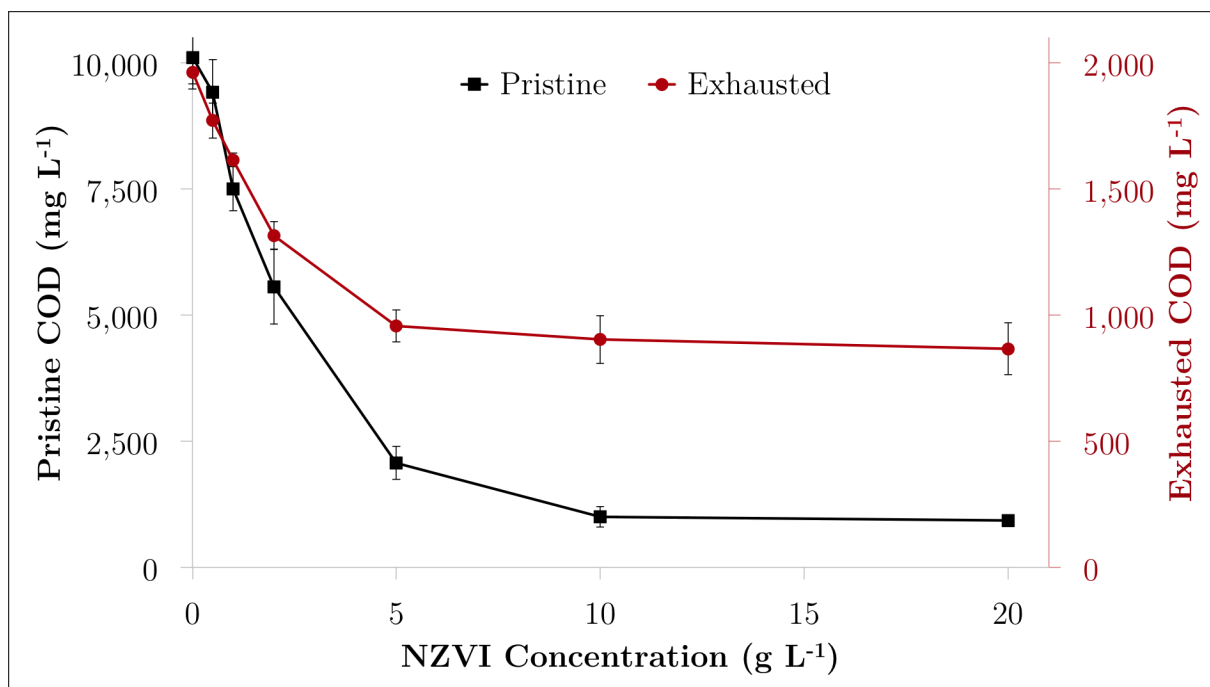


Figure 3.3: Reductions in COD for pristine and exhausted MWFs at NZVI concentrations between 0 g L⁻¹ and 20 g L⁻¹. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

for the pristine MWF. For the operationally exhausted MWF, acute toxicity, respiration and PRR cell counts were improved with respect to bioprocessing. However, COD removal was significantly lower ($p < 0.01$) for NZVI treatment when compared to bioprocessing for the exhausted MWF.

3.5 E-Beam Treatment

E-beam treatment of either MWF had no detectable effect on COD removal or OD reduction. For the exhausted MWF, none of the results were significant. However, there was a direct correlation between e-beam dose and acute toxicity reduction for the pristine MWF. Acute toxicity of the pristine MWF decreased with increasing dose for the pristine MWF as shown

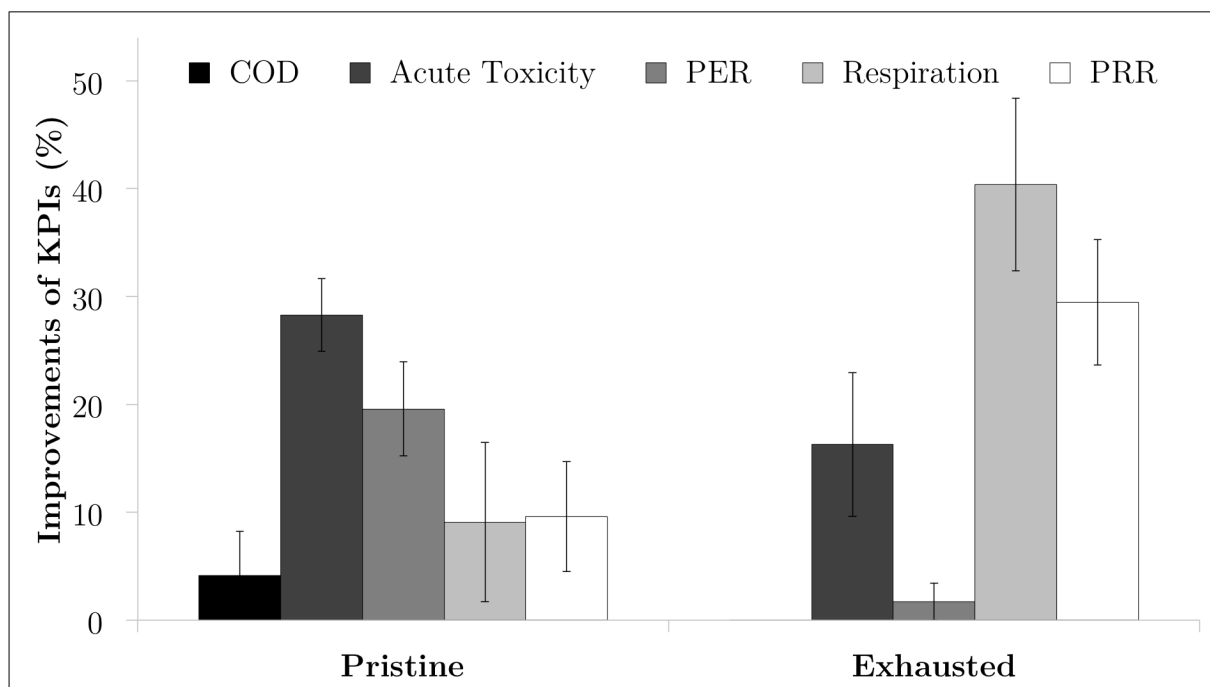


Figure 3.4: Percentage KPI Improvements of NZVI treatment with respect to bioprocessing of pristine and exhausted MWFs. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

in Figure 3.5. It was found that pre-irradiating the pristine MWF at 50 kGy led to a 92.7 ± 3.1 % reduction in acute toxicity, which was accompanied by the formation of an orange-brown precipitate. Higher doses, up to 500 kGy were tested, however they did not lead to any further detectable changes in KPIs for either stream.

Examination of a greater spread of irradiation doses around 50 kGy revealed that acute toxicity was reduced during two phases and that there was a stationary and a transition period in between for the pristine MWF as shown in Figure 3.6. Toxicity was found to decrease approximately linearly at lower doses up to 2 kGy, by 44.1 ± 4.2 %. Between 2 kGy and 25 kGy, a stationary period was reached, during which no significant improvements in toxicity were observed. This was then followed by a transition period from 25 kGy to 35

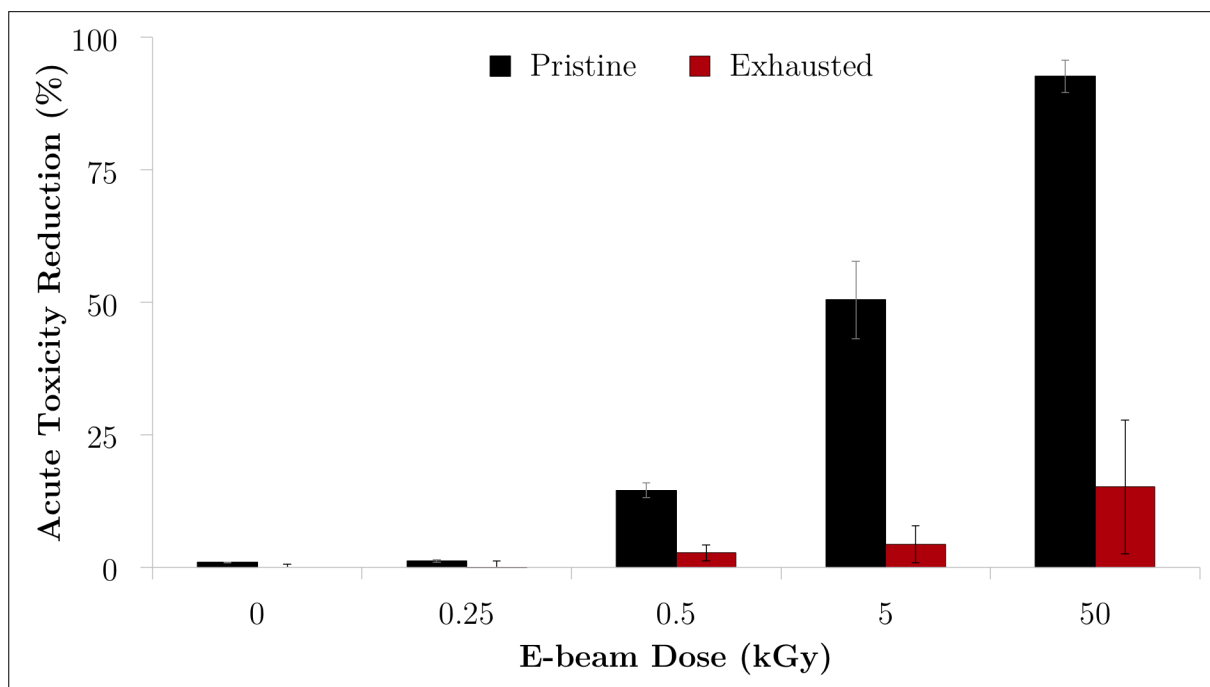


Figure 3.5: Percentage acute toxicity reduction as a function of e-beam dose. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

kGy, followed by a second plateau beyond 35 kGy, with no further improvements in acute toxicity observed at higher doses (tested up to 500 kGy but not shown in Figure 3.6).

EDX analysis of the SEM image, as shown in Figure 3.7, identified the precipitate as calcium carbonate (CaCO_3). The CaCO_3 precipitate started forming in the transition period between 27.5 kGy and 35 kGy. In this same range, standard deviations of acute toxicity measurements were found to increase, varying between 15.5 % and 24.0 %. Beyond 35 kGy, standard deviations became smaller and acute toxicity was reduced by 93.6 ± 4.3 % at 35 kGy with no significant further reductions or increases at higher doses. Control experiments on tap water did not result in any calcium carbonate precipitation, whereas diluting the MWF with DI water did result in precipitation.

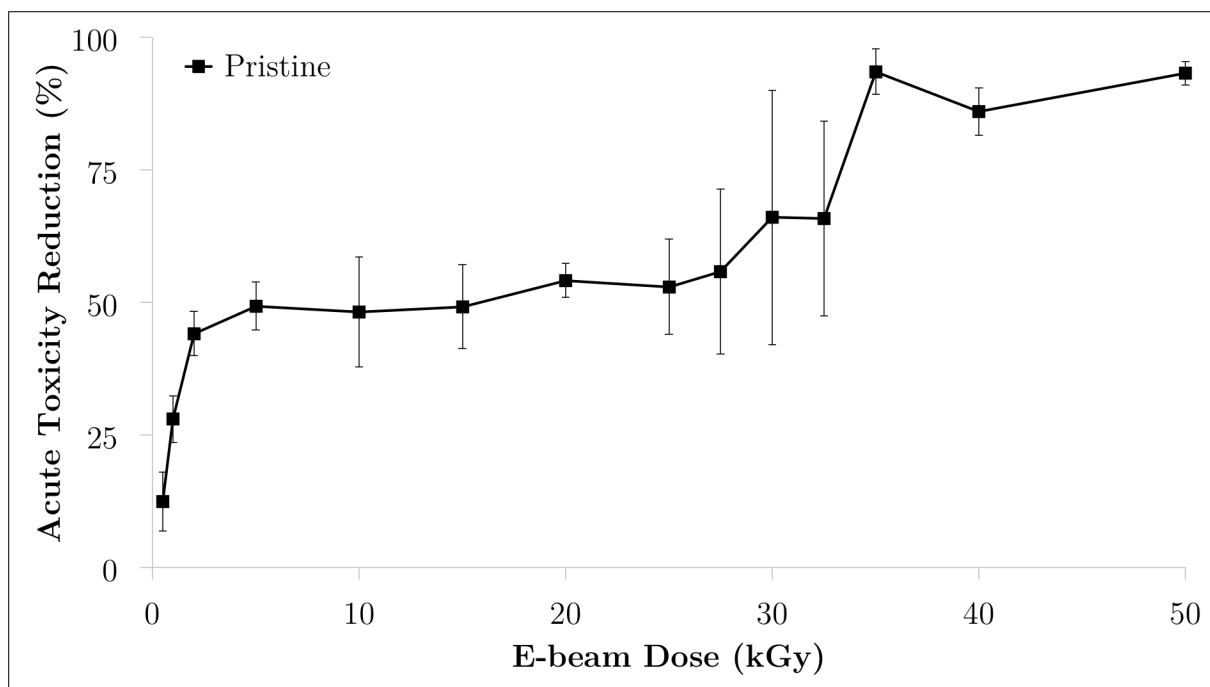


Figure 3.6: Acute toxicity reduction as a function of e-beam dose for the pristine MWF. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

3.6 Toxicity Reduction after NZVI and E-Beam Treatment

Both NZVI and e-beam treatment reduced the toxicity of the exhausted MWF. However, significantly greater colony-forming units (CFUs) of the bacterial consortium taken from mature bioreactors were determined after e-beam irradiation compared to the untreated MWF ($p = 0.0004$). In contrast, significantly lower CFUs were observed after NZVI treatment compared to the untreated MWF ($p = 0.0003$), as shown in Table 3.2, suggesting that e-beam treatment generated a less toxic environment for the consortium than the NZVI treatment.

Further examination of the plates, shown in Figure 3.8, revealed that there were community composition changes in addition to the change in the number of CFUs. Serendipitously, the mature bioreactor inoculum consisted of red and white CFUs, which gave a visual clue as to

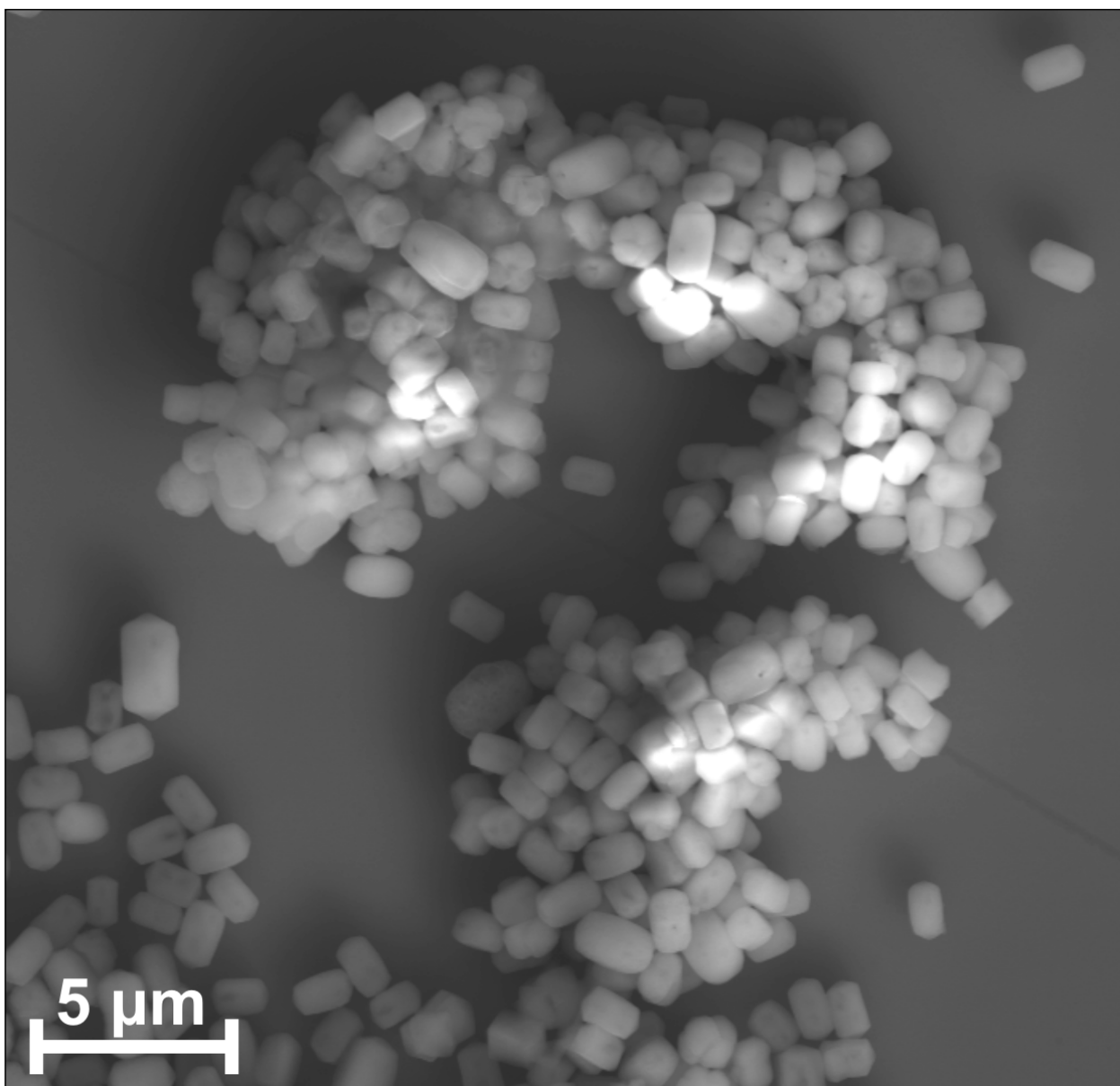


Figure 3.7: SEM image of calcium carbonate precipitate as characterised by EDX analysis.

Treatment Mode	10^3 CFUs per mL
Untreated	106.7 (15.3)
E-beam	703.3 (95.0)
NZVI	3.8 (1.8)

Table 3.2: Comparison in CFUs after exposure of inoculum to untreated, e-beam pre-treated and NZVI pre-treated exhausted MWF.

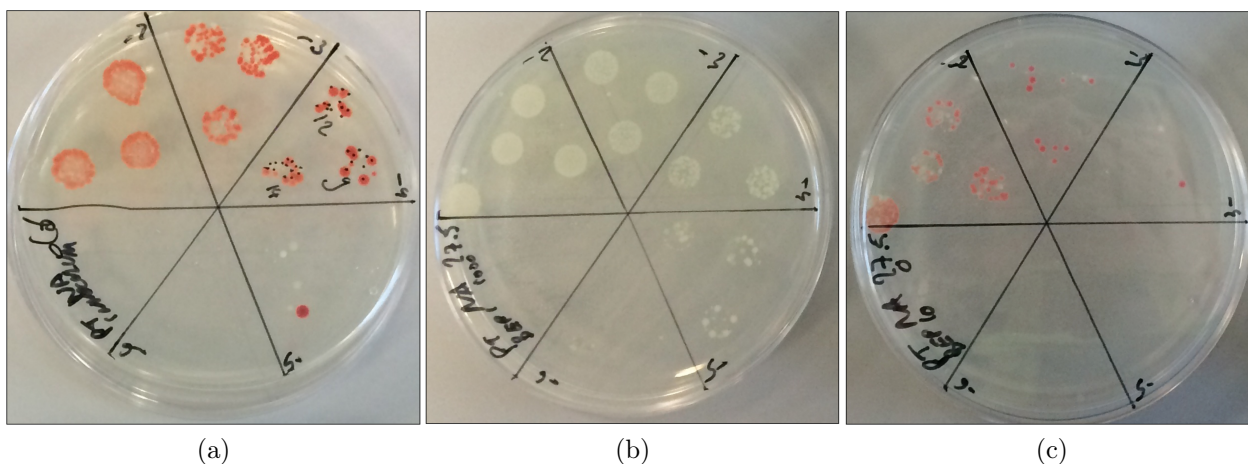


Figure 3.8: Miles and Misra plate counts after exposing mature bioreactor inoculum to (a) untreated exhausted MWF (red & white CFUs) (b) e-beam treated MWF (white CFUs only) (c) and NZVI treated MWF (red & white CFUs).

what had occurred. After 10 min exposure to untreated, e-beam treated and NZVI treated exhausted MWF, samples of the three streams were taken, vortexed for 10 s, plated out at serial 10-fold dilutions and incubated overnight on nutrient agar plates at 25 °C. It was found that both red and white colonies had survived in the untreated exhausted MWF. Under an optical microscope, the red / white inoculum was found to consist of both large red yeast cells and small white bacterial cells, differentiated by their size. E-beam treatment selectively killed of the red yeast whilst the bacteria survived. NZVI treatment, even though it reduced CFUs, enabled for both yeast and bacterial cells to survive.

3.7 Optimal Hybrid Treatment

3.7.1 Sequential Hybrid Treatment

A full factorial study investigating all possible sequential combinations of bioprocessing, NZVI and e-beam treatment was undertaken to identify the optimal order of sequential hybrid treatment. Optimal in this context consisted of achieving maximal remediation performance under minimal energy requirements (at the lowest effective e-beam dose) and minimal resource use (at the smallest effective NZVI concentration). It was expected that both NZVI and e-beam treatment could be employed after bioprocessing to breakdown some of the recalcitrant fraction and allow for a second round of bioprocessing improving overall COD removal and KPIs.

The study employed the optimal starting concentrations for bioprocessing, as determined in Section 3.3 of this chapter, an e-beam treatment dose of 50 kGy and two different NZVI concentrations of 1 g L⁻¹ and 10 g L⁻¹ respectively. The results of the study are shown in the exhaustive decision tree in Figure 3.9. The optimal sequential treatment train that led to synergistic remediation of both MWFs was found to be sequential e-beam, bioprocessing, nanoscale zero-valent iron treatment followed by another bioprocessing stage. No significant improvements were observed after the first day for the bioprocessing step. For the pristine MWF, there was an overall 92.8 ± 1.4 % COD reduction, which was significantly greater than COD removal for biological treatment ($p = 0.0363$). Figure 3.10 shows the pristine MWF before and after optimal hybrid treatment. For the exhausted MWF, COD removal

was 68.4 ± 1.6 %, which did not represent a significant improvement.

The effects of bioprocessing, NZVI treatment, e-beam treatment and optimal hybrid treatment were compared in terms of their individual KPI changes. The percentage KPI improvements indicated that all three treatments on their own improved at least some of the KPIs measured. Figure 3.11 shows that for the pristine MWF, bioprocessing mainly improved COD and OD, e-beam was able to improve acute toxicity and PRR, whereas NZVI was predominantly responsible for improvements in OD, COD, respiration and PER. Figure 3.12 shows that the contour of the hybrid treatment for the exhausted MWF largely followed that of the NZVI treatment, suggesting that the latter was the main contributing technique in terms of remediation.

3.7.2 Simultaneous Hybrid Treatment

The impact of simultaneous e-beam and NZVI treatment was investigated for both MWFs using NZVI jet sprayed cuvettes and by suspending NZVI in non-coated cuvettes during e-beam irradiation. Figure 3.13 visually confirmed that the irradiated NZVI did stay longer in suspension. However, for the MWFs tested in this study, simultaneous NZVI and e-beam treatment did not translate into any detectable improvements when compared to sequential e-beam and NZVI treatment. NZVI at a concentration of 1 g L^{-1} was added to one mature bioreactor for completion and as expected, the biofilm was killed.

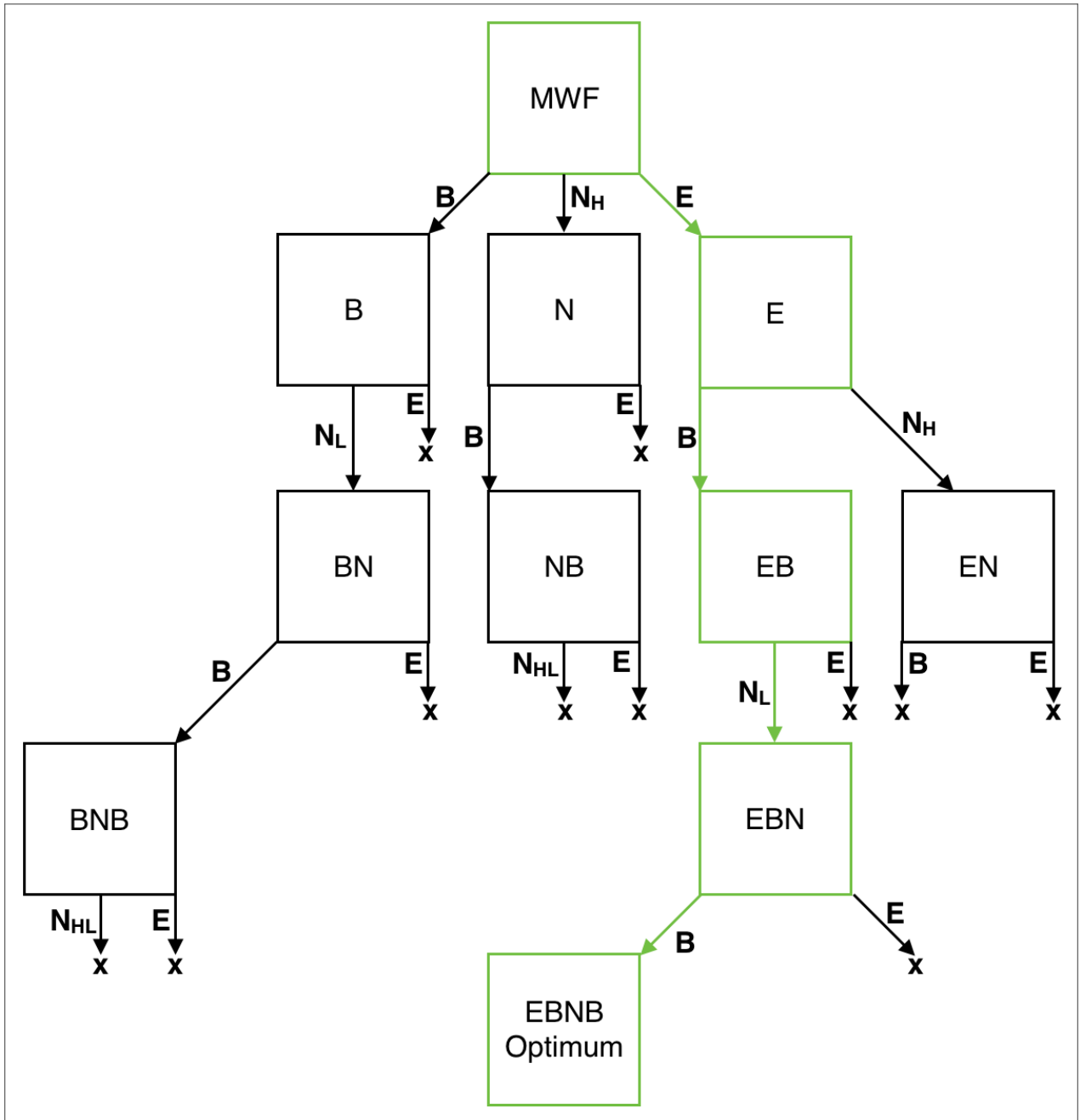


Figure 3.9: Visualisation of full factorial study decision tree: B (Biological), N (Nano Iron treatment at H (High Concentration of 10 g L^{-1}), L (Low Concentration of 1 g L^{-1}) and HL (No difference between high and low dose)), E (E-beam dose of 50 kGy), x (Dead end as no improvement in any KPIs). Optimal sequential treatment mode is shown in green.

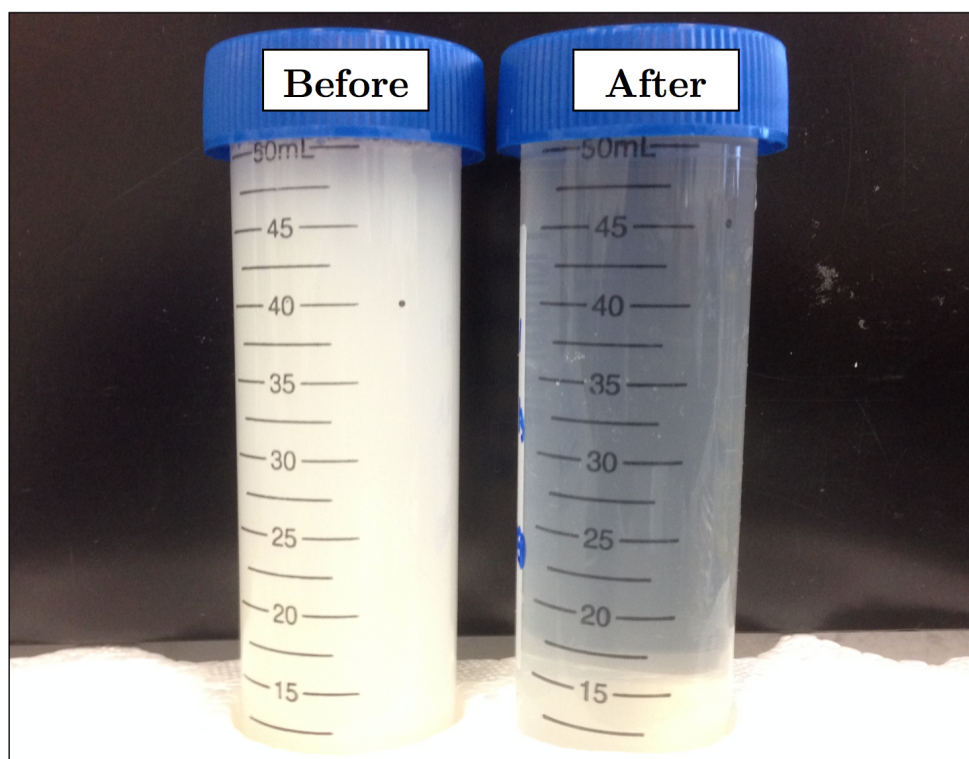


Figure 3.10: Pristine MWF before and after optimal sequential treatment.

3.7.3 E-Beam Pre-Treatment

In Section 3.7.1 of this chapter, e-beam pre-treatment was found to be beneficial to bioprocessing. However, for optimal performance, it was necessary to treat the MWFs at their optimal initial COD concentrations. Specifically, this meant that the concentrated pristine MWF required a 25-fold dilution prior to biotreatment, whereas the exhausted MWF had to be diluted 10-fold. To attempt to circumvent this dilution requirement, the impact of pre-treating the concentrated waste streams with the e-beam at 50 kGy prior to bioprocessing was investigated.

Pre-irradiation of both the pristine and the exhausted MWFs allowed for subsequent biologi-

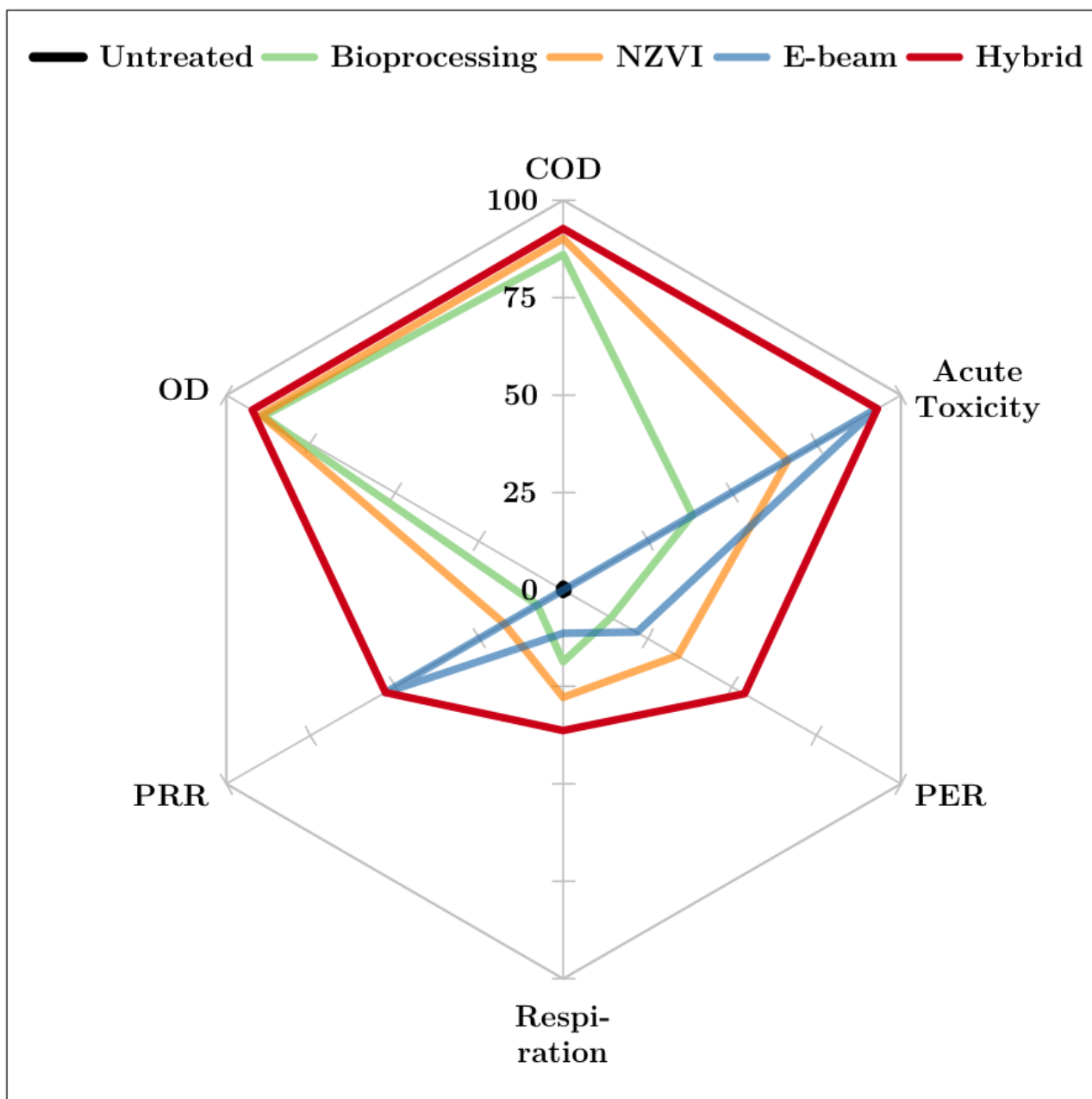


Figure 3.11: Percentage KPI improvements for the pristine MWF after individual bioprocessing, NZVI, e-beam treatment and optimal hybrid sequential treatment as determined in Figure 3.9.

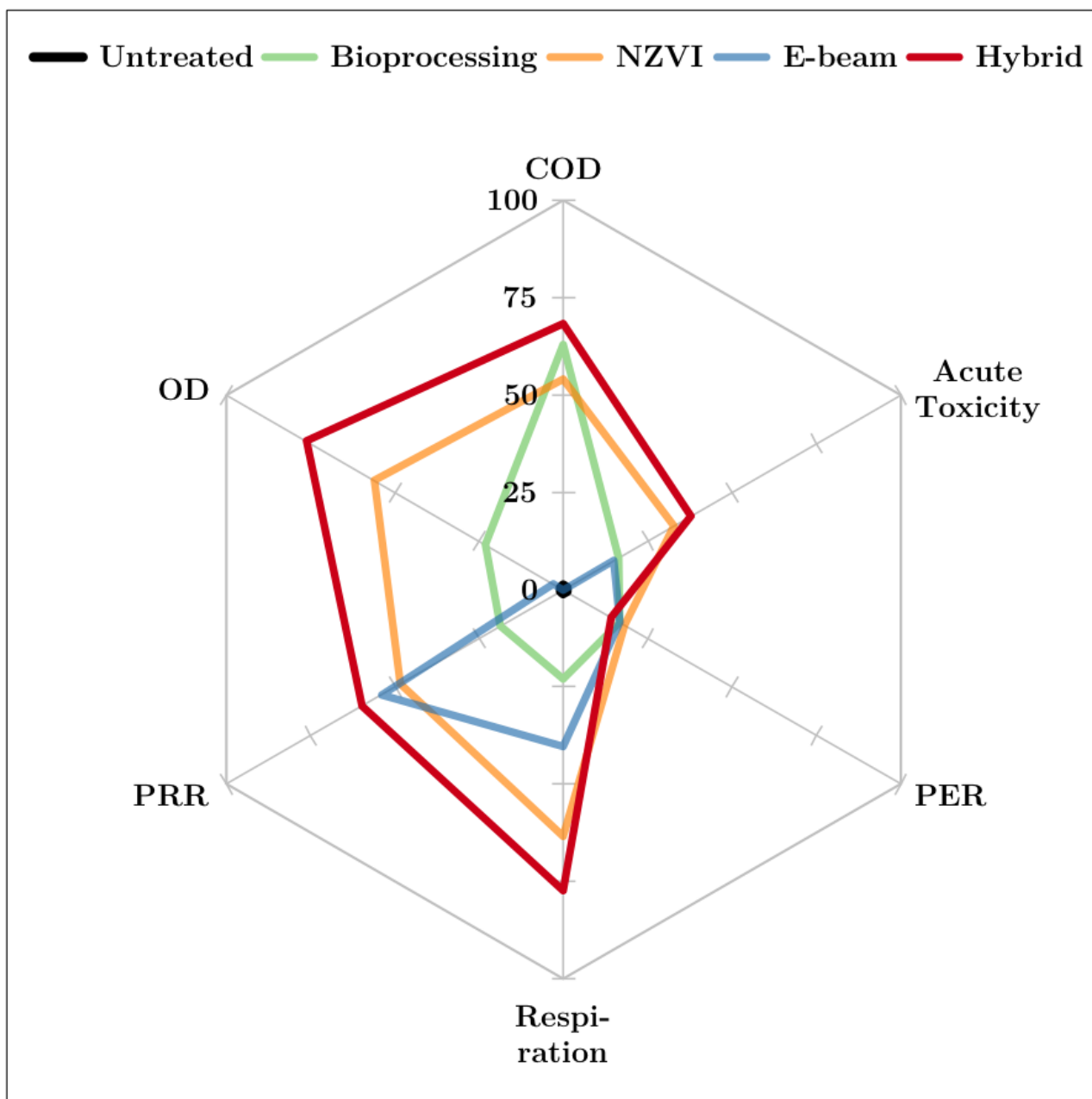


Figure 3.12: Percentage KPI improvements for the exhausted MWF after individual bioprocessing, NZVI, e-beam treatment and optimal hybrid sequential treatment as determined in Figure 3.9.

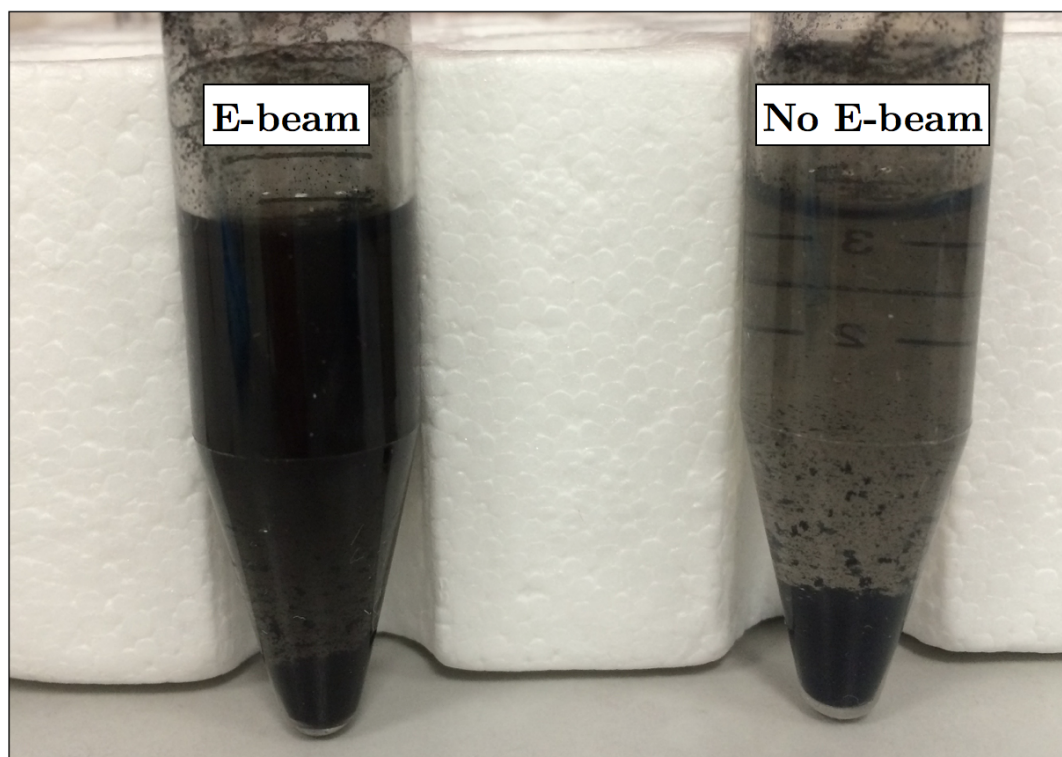


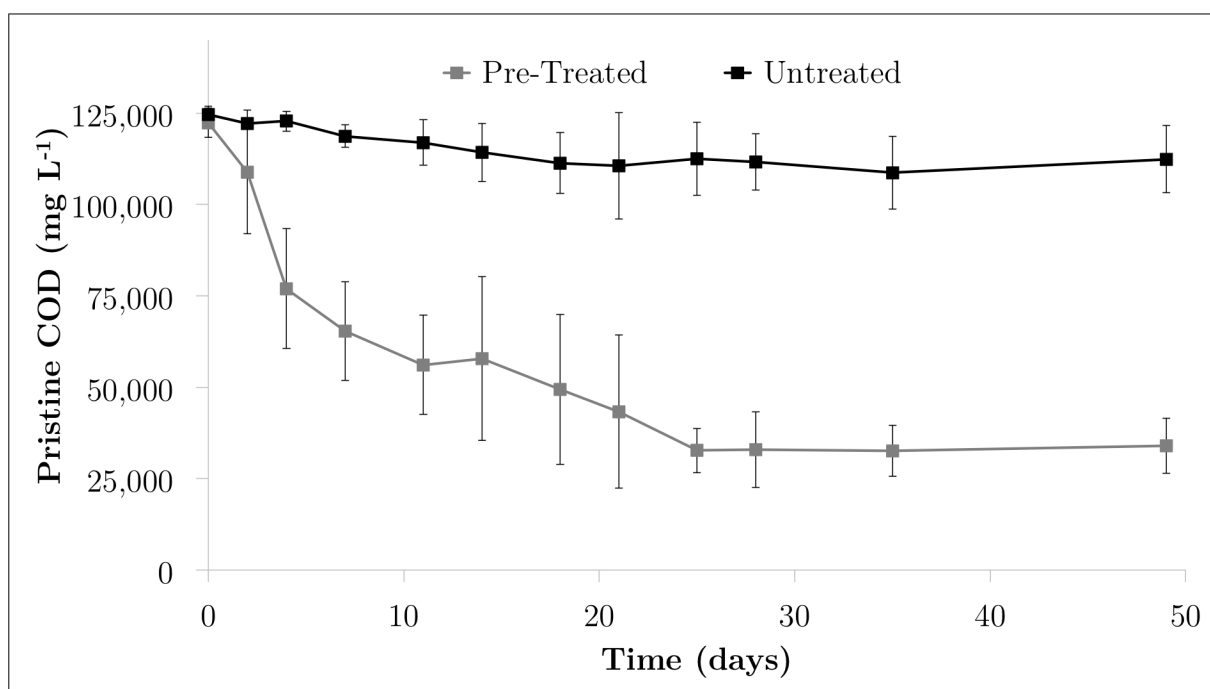
Figure 3.13: Irradiated and non-irradiated NZVI one week after e-beam irradiation.

cal degradation, which achieved $49.4 \pm 19.4 \%$ and $11.4 \pm 6.6 \%$ COD removal after 14 days and $69.5 \pm 8.0 \%$ and $24.6 \pm 4.8 \%$ after 49 and 91 days respectively as shown in Figure 3.14. Without e-beam pre-treatment, COD removals were significantly lower after 49 respectively 91 days at $9.9 \pm 6.2 \%$ ($p = 0.005$) and $12.8 \pm 5.2 \%$ ($p = 0.044$) respectively.

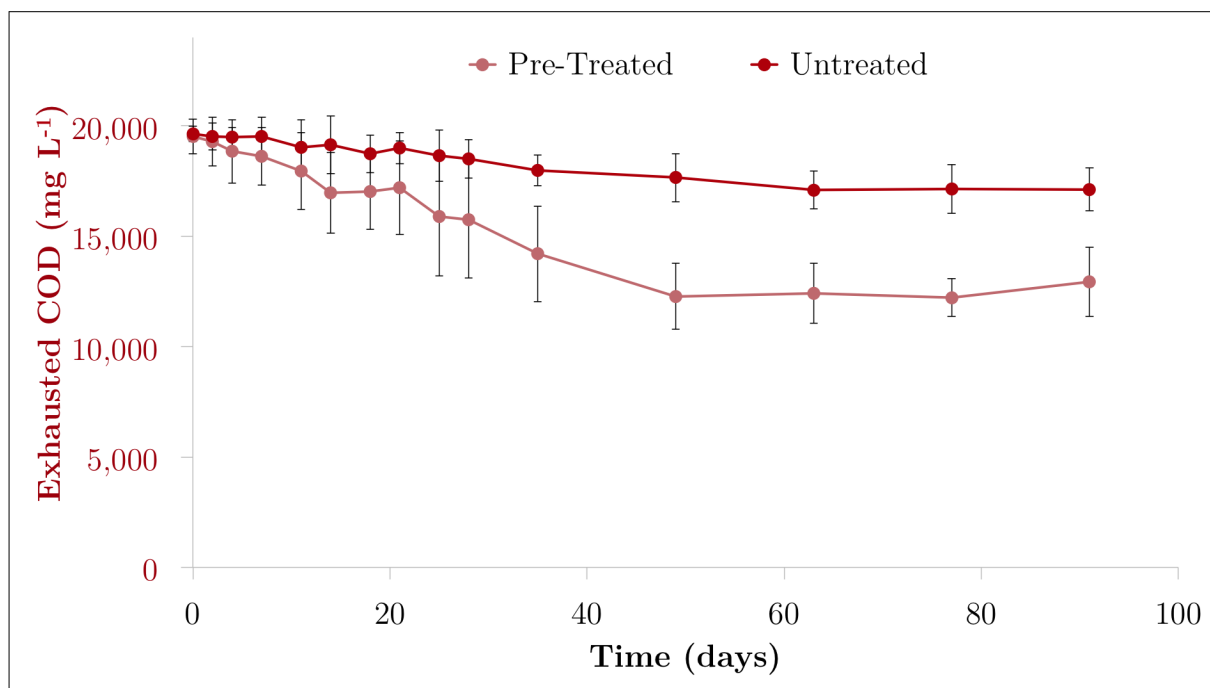
3.8 Assessment of Remediation

3.8.1 Bioprocessing

Bioprocessing studies over a range of CODs for both types of MWFs studied demonstrated that there was an optimal dilution, enabling maximum COD removal for both waste streams.



(a) Pristine MWF



(b) Exhausted MWF

Figure 3.14: Comparison of COD removal for bioprocessing of untreated and 50 kGy e-beam pre-treated (a) pristine and (b) exhausted MWFs. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

At their full concentrations, no COD removal was detected as expected. Whilst the pristine MWF was specifically designed to be bio-resistant at this concentration, i.e. its working concentration, the operationally exhausted MWF was chosen because of its intrinsic recalcitrance and toxic nature due to chemical changes induced during metal cutting applications. Unexpectedly, no further COD reductions were observed below the starting CODs of $9,375 \pm 454 \text{ mg L}^{-1}$ and $1,919 \pm 15 \text{ mg L}^{-1}$ for the pristine and exhausted MWFs respectively, suggesting all toxicity was mitigated by this point and the residual CODs of $1,342 \pm 181 \text{ mg L}^{-1}$ and $692 \pm 99 \text{ mg L}^{-1}$ were not bioavailable. This was unexpected, as toxicity has been previously shown to vary with initial concentration (Borrely et al. 2000), and it was hypothesised that equally COD removal efficiency would increase at lower COD starting concentrations, which was only observed to be the case down to a certain level. As a result, dilution beyond the optimal treatment ranges would not be economical for bioprocessing and provide no detectable benefit.

In terms of CODs, the exhausted MWF samples were found to be less susceptible to bioprocessing compared to the pristine samples. This may be attributed to the elevated biocide concentrations, which are typically added during operation as well as previous biological degradation and changes in their chemical profile during operation and subsequent storage. This resulted in recalcitrant end products accumulating as most of the biological oxidation had previously taken place. COD removal at optimal concentration for the pristine MWF was higher than for a comparable study, achieving 85.9 % COD removal at $9,375 \text{ mg L}^{-1}$ compared to 30 % achieved at starting CODs ranging between $9,980 \text{ mg L}^{-1}$ and $13,500 \text{ mg L}^{-1}$.

L^{-1} (Connolly et al. 2006). Moreover, acclimatised bioreactors were found to achieve most significant COD removal after 3 days, which was faster than comparable studies requiring hydraulic retention times of 5 (Cheng et al. 2006), 10 (Connolly et al. 2006) and 17 days (van der Gast et al. 2004). This could be related to the fact that reactor working volumes of 10 mL employed in this study were orders of magnitude smaller compared to working volumes of 5 L (Cheng et al. 2006), 4.8 L (Connolly et al. 2006) and 1.5 L (van der Gast et al. 2004) in comparable studies. This suggests that rapid bioprocessing might be related to biofilm density and that optimal bioreactor designs require dense-packing.

3.8.2 Nanoscale Zero-Valent Iron Treatment

NZVI treatment was found to reduce COD of pristine MWF by up to 90.1 % at 10 g L^{-1} , which was higher than in a similar study employing comparable NZVI particles of 10 nm diameter (Jagadevan et al. 2012), where a COD removal of 78 % was achieved. A similar study observed that increasing NZVI concentration beyond 5-10 g L^{-1} did not improve performance. This was probably related to scavenging effects caused by ferric hydroxides formed during NZVI treatment, as adding fresh NZVI did not improve performance, so reactant limitation was not the cause. Another explanation could have been that higher residues of toxic organic NZVI by-products might have formed after adding excess NZVI, which was however excluded on the basis of actual toxicity reductions observed in the different KPIs after NZVI treatment. Small differences in performance might stem from differences in the size of NZVI particles employed as well as due to a different thickness of their iron oxide layers and the

differences in synthesis methods.

It was also found that NZVI treatment mitigated toxicity more effectively than bioprocessing. A similar study found that toxicity, as measured by an *Acinetobacterbaylyi* ADP1_recA-lux biosensor, was reduced by 85 % after the application of NZVI (Jagadevan et al. 2012). This biosensor expresses bioluminescence as a function of genotoxicity and can be compared with the *E.coli* HB101 (pUCD607) biosensor expressing bioluminescence as a function of metabolic toxicity employed in this study. Acute toxicity, as measured by the biosensor, was significantly reduced after NZVI treatment for both MWFs. Toxicity reduction was measured in a different way in both studies, so actual percentage values could not be compared quantitatively but the trends in both studies showed that both types of NZVIs detoxified the different MWFs. This suggested that NZVI treatment might be best employed as a post-bioprocessing polishing step, especially as it appeared to further reduce toxicity.

3.8.3 E-Beam Treatment

It was found that pre-irradiating the pristine MWF at 50 kGy led to a 92.7 ± 3.1 % reduction in acute toxicity, which could be attributed to the radiolytical breakdown of key toxic molecules such as the biocides. The e-beam proved to be most effective at high doses of 50 kGy, which was found to be in line with a similar study on oily wastewater treatment (Duarte et al. 2004b) and another on swine wastewater employing optimal doses of 20-75 kGy (Lim et al. 2013). This was in contrast with a study on a cork wastewater, where a 50 kGy e-beam dose led to an increase in toxicity (Madureira et al. 2017).

At low doses up to 2 kGy, some radiolytical transformations improved the toxicity of the pristine MWF. To gain any further improvement, a higher irradiation dose was required with a performance plateau between 2 kGy and 32.5 kGy. A second phase in acute toxicity reduction coincided with the onset of CaCO_3 precipitation, as sudden increases in standard deviation were observed. These variations were associated with different onsets of precipitation between replicates. Above 35 kGy and up to 500 kGy the standard deviations became smaller again and acute toxicity settled to a level around 90 % and there was no further improvement in performance. This also suggested that subsequent complete mineralisation of the effluent did not take place. Mineralisation is defined as the process through which an organic substance, e.g. a carboxylic acid, becomes oxidised into an inorganic substance, i.e. carbon dioxide.

Calcium carbonate precipitation was unexpected and originated from the pristine MWF itself as confirmed by the control experiments at the end of Section 3.5 of this chapter. The orange-brown colour of the calcium precipitate observed was unusual and probably due to contaminants as calcium carbonate would normally expected to be white. The step improvement in toxicity due to the CaCO_3 precipitation was thought to be due to two effects. Firstly, it could have been due to carbonate (CO_3^{2-}) scavengers being removed from the system. Carbonates are known radical scavengers (Cooper et al. 1992) and are in competition with $\bullet\text{OH}$ radicals (Cooper et al. 2002). Secondly, it could have been due to calcium ions (Ca^{2+}) in the MWF effluents. Calcium ions have been documented to be inhibitory to growth for some bacteria such as *Ralstonia solanacearum* (He et al. 2014). No precipitation was observed for the exhausted MWF, but the fact that irradiation was less

effective on this stream suggested that it may also have contained a high concentration of contaminants that were effective as radical scavengers.

The plate count experiments offered an additional insight into the complexity of toxicity and the difficulties in assessing it. Whereas plate counts, measured as colony-forming units per milliliter, indicated that NZVI was more toxic to a mixed consortium than e-beam treatment, it was found that NZVI treatment allowed for greater diversity, as exposure of eukaryotic cells to e-beam treated effluent was lethal. This underlined the importance of strategically choosing an adequate selection of KPIs to assess detoxification of industrial effluents during any treatment approach. In particular, this was of importance in terms of choosing a selection of KPIs for the design of a remediation index. It also showed that whichever KPIs were selected, any selection or index will never fully reflect all aspects of toxicity. Any robust bioremediation assessment system would however benefit from a certain level of flexibility as non-quantitative indicators can sometimes give insight into a particular aspect of toxicity.

3.8.4 Hybrid Treatment

Simultaneous hybrid treatment did not work. In fact, NZVI added to a bioreactor completely abolished reactor function and simultaneous NZVI and e-beam treatment of either MWF did not result in improved remediation. E-beam irradiation of suspended NZVI resulted in particles being able to stay much longer in solution and potentially prevent aggregation. Though it did not improve performance in this scenario, as adequate suspension of NZVI was achieved after sonication, one could hypothesise scenarios where this could result in lower

NZVI doses being required for treatment in the first instance as well as be potentially useful to treat very dilute effluents.

Sequential e-beam, bioprocessing, nanoscale zero-valent iron and bioprocessing resulted in synergistic remediation of both MWFs. Surprisingly, it was not possible to improve post-bioprocessing MWF effluents using either e-beam or NZVI treatment so as to achieve subsequent significant improvements in bioprocessing performance. This was probably due to the high concentration of scavenger molecules in the MWF. However, e-beam pre-treatment led to synergistic hybrid treatment. It presumably broke down some of the non-bioavailable fractions of the recalcitrant, which were accompanied by an immediate reduction in toxicity and allowed bioprocessing to occur more efficiently. Moreover, it reduced hydraulic retention time for bioprocessing from 3 days to 1 day, which was in line with another study where e-beam pre-treatment reduced retention time by half (Han et al. 2002).

The hybrid treatment was more effective on the pristine MWF than on the exhausted MWF. Though the exhausted MWF did not exhibit a significant improvement in COD removal, the hybrid treatment train led to more uniform results, as evidenced by a decrease in standard deviation in the final COD, suggesting that hybrid treatment made the system more reliable than bioprocessing on its own.

E-beam pre-treatment also reduced dilution requirements of the MWF, by facilitating treatment of both MWFs in their concentrated form at high CODs of 125,000 mg L⁻¹ for the pristine MWF and 19,600 mg L⁻¹ for the exhausted MWF respectively. However, this came at the expense of an order of magnitude higher reaction time in the bioreactors. This brought

KPI	Acute Toxicity	PER	Respiration	PRR	COD	OD
Weighting Factor	1	1	1	1	2	0.5
Pristine Change (%)	93.0	53.6	36.2	53.0	92.8	92.2
Exhausted Change (%)	37.7	14.1	77.3	59.7	68.4	76.3

Table 3.3: Strategic selection of KPIs, assigned weighting factors and percentage changes of pristine and operationally exhausted MWF after optimal hybrid treatment.

to mind the golden rule of mechanics, which essentially states that what you gain in power, you lose in displacement – in this case: what you save in water, you pay for in time and energy.

3.8.5 Remediation Indices

Chapter 2, Section 2.5.2 established a Remediation Index (RI) (given by Equation 2.7) with the aim of comparing the treatment effectiveness between different industrial effluents. Acute toxicity, PER, respiration and PRR were assigned unit weightings as basic KPIs of the RI. The treatment priorities for the remediation of both MWFs were to reduce the COD, overall toxicity and to a lesser extent reduce OD. The corresponding KPI weightings are presented in Table 3.3. The tap water control had a unit-RI for this selection of KPIs. The RIs were evaluated at 71.9 % for the pristine MWF and 56.0 % for the exhausted MWF for optimal hybrid sequential treatment. The remediation index of the pristine MWF was thus higher than of the operationally exhausted MWF, suggesting the pristine MWF was more amenable to remediation.

3.8.6 Conclusion

The studies summarised in this chapter determined the optimal starting concentrations for bioprocessing, identified that there was a ceiling on maximal NZVI concentrations and identified an optimal e-beam treatment dose for the treatment of two MWFs. E-beam irradiation of the pristine MWF resulted in calcium carbonate precipitation above 35 kGy, which was associated with a decrease in acute toxicity. Five-stage hybrid e-beam, bioprocessing, nanoscale zero-valent iron and bioprocessing sequential treatment led to synergistic remediation of both MWFs, whereas hybrid simultaneous treatment was not found to be effective. E-beam pretreatment allowed for subsequent bioprocessing of high-COD MWF effluents at the expense of longer reaction times. Hybrid treatment of both pristine and exhausted MWFs resulted in remediation indices of 71.9 % and 56.0 %.

4 Strategies to Remediate a Textile Dyeing Wastewater

4.1 Outline of Remediation Strategy

The aim of this chapter was to apply the hybrid system designed in Chapter 3 to a highly coloured and recalcitrant real industrial textile dyeing wastewater (TDW) and establish its remediation potential for this particular industrial effluent. The main objectives for the remediation of the TDW were colour removal and nutrient removal together with toxicity reduction. Possible strategies to achieve this are presented in Figure 4.1 and consisted of:

- determining the scope for bioprocessing by undertaking a number of enrichment and growth studies to characterise the toxicity of the TDW
- assessing aerobic and anaerobic bioprocessing effectiveness
- applying NZVI and e-beam treatment individually and in hybrid configurations

COD (mg L⁻¹)	TOC (mg L⁻¹)	OD	pH	TN_b(mg L⁻¹)
223.9 (3.7)	74.9 (0.9)	>3	8.8 (0.1)	22.6 (1.4)
SO₄²⁻ (mg L⁻¹)	PO₄³⁻ (mg L⁻¹)	Cd (mg L⁻¹)	Cr (mg L⁻¹)	Cu (mg L⁻¹)
20.4 (1.6)	<0.5	<0.02	<0.06	<0.03
Fe (mg L⁻¹)	Ni (mg L⁻¹)	Pb (mg L⁻¹)	Zn (mg L⁻¹)	
0.1 (0.04)	<0.1	<0.1	0.2 (0.07)	

Table 4.1: Textile dyeing wastewater characteristics. TN_b stands for total bound nitrogen. Phosphate was measured rather than total phosphorous, as phosphate is the primary form of phosphorus in water. Means of triplicates ($n = 3$) are shown and standard deviations are shown in parentheses if within measuring range.

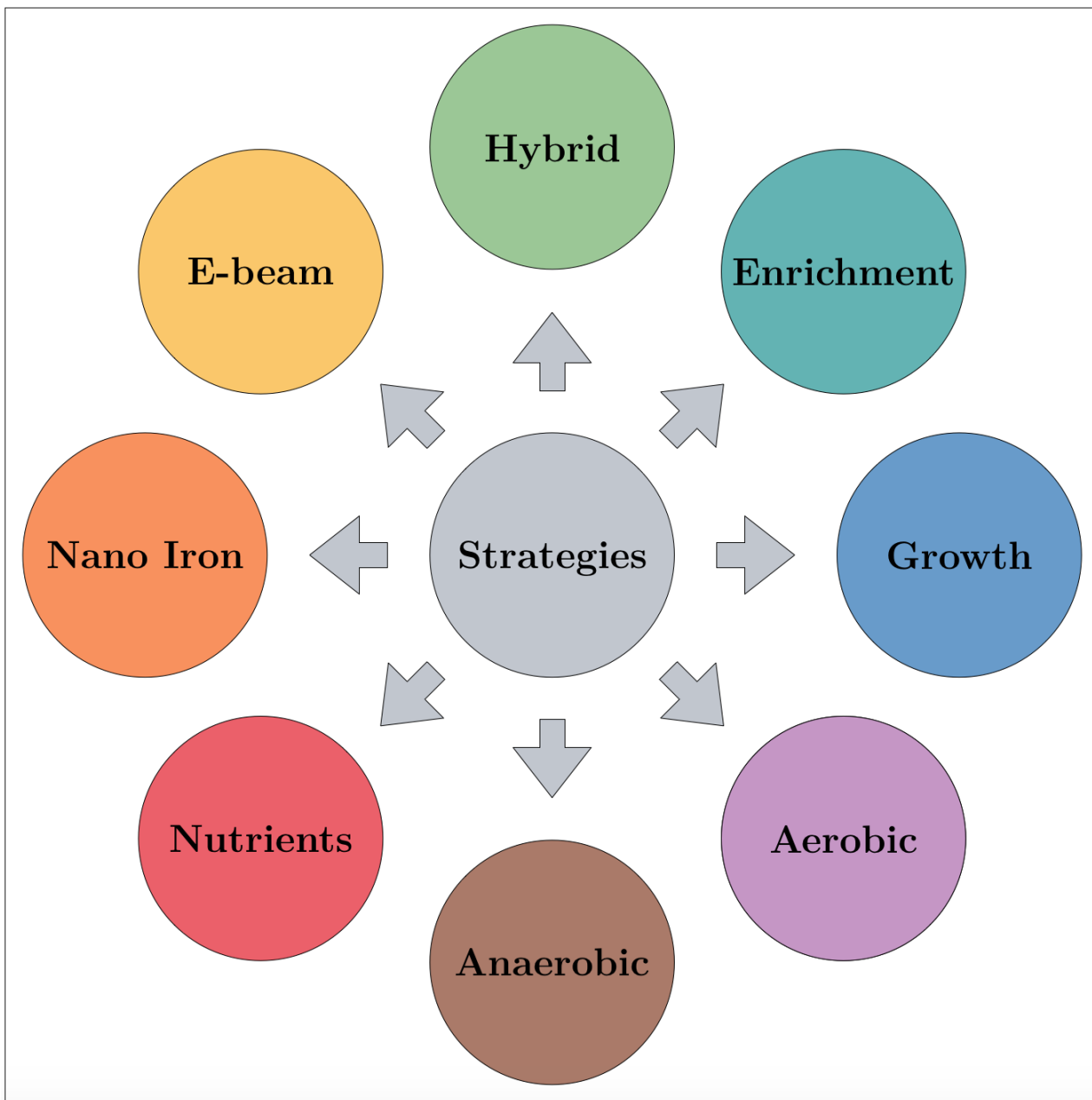


Figure 4.1: Hybrid remediation strategies for an industrial textile dyeing wastewater.

4.2 Textile Dyeing Wastewater Characteristics

The physico-chemical parameters of the industrial textile dyeing wastewater (TDW) are summarised in Table 4.1 for a concentration of 10 %. Initial screening experiments indicated that no measurable remediation was achieved at higher TDW concentrations and a 10-fold pre-dilution was required. The 10 % TDW concentration was used as base concentration throughout this chapter apart from the end of Section 4.9.6, which considers hybrid treatment of the concentrated 100 % TDW.

Apart from COD, other pertinent KPIs for the TDW were optical density (OD), pH and total organic carbon (TOC). One of the main treatment goals was to decolourise the TDW, which was monitored by tracking reductions in OD. Measuring pH could potentially provide insight into the mechanisms of removal or degradation of pollutants in the TDW. As described in Chapter 1 Section 1.6, pH has been found to be effective at tracking the extent of mineralisation of pollutants during irradiation. The fate of carbon was monitored by TOC analysis.

4.3 Enrichment

The best microorganisms to treat industrial effluents are often found in the effluents themselves, as any organism able to proliferate within or in that effluent must be adapted to it. Indigenous microorganisms were shown to benefit from enhancement by a resilient mixed consortium optimised for a MWF (van der Gast et al. 2004). However, no culturable fractions

were found to be present in this particular TDW. Likewise, attempting to enrich the TDW by incubating it for up to five days under varying concentrations of glucose addition, minimal (M9) or rich medium (Nutrient Broth) supplementations or any combinations thereof failed to recover culturable cells. Optical density (OD) of the incubated TDW did not change during growth studies in the shake flasks under any supplementation regime, while neither nutrient agar nor gradient agar plates (shown in Figure 4.2a) supported any growth.

As there were no culturable fractions present in the TDW, the next set of experiments set out to determine whether the TDW was lethal to different inocula and whether it would be possible to use and recondition some of the currently employed consortia for MWF remediation (van der Gast et al. 2004) on the TDW. Exposing an inoculum taken from the mature bioreactors to the TDW for 24 h at concentrations ranging from 0 % (water control) to 10 % TDW showed that in suspension, no significant differences between plate count inoculum recovery CFUs for a tap water control, PBS control or any concentrations of the TDW were observed. However, TDW did not support plate growth of the inoculum. Figure 4.2b shows that 10 % TDW technical agar was lethal to the mixed community as well as the biosensor, whose growth might also have been a good indicator for toxicity.

4.4 Growth Studies

In the previous section, neither inoculum was able to grow on a TDW agar plate, but mixed community bacteria in suspension were able to survive. Incubation of both inocula on a nutrient agar control plate resulted in visible colonies as shown in Figure 4.2c. TDWs some-

times require the addition of nutrients in order to initiate bioprocessing (Lotito et al. 2012; Turan-Ertas 2001). To discern whether the system was carbon-limited, nutrient-limited or limited in both, different agar plates were set up. Technical agar (containing no nutrients), Technical agar with glucose, Minimal medium (50 % M9) agar, minimal medium agar with glucose, rich medium (nutrient agar) and rich medium with glucose plates were set up. Some plates were poured in the form of concentration gradient plates, as shown in Figure 4.2d and described in Chapter 2 Section 2.4.2, to determine whether nutrient addition would allow for growth and whether there was a minimum inhibitory toxic concentration to growth under different conditions.

Glucose was chosen as the carbon source as it has previously been shown to be an effective carbon source for this particular consortium during anaerobic bioprocessing of a MWF (Thill 2013). Glucose is an easily available and commonly employed carbon source for bioreactors in aerobic and anaerobic conditions (Miqueleto et al. 2010). Different carbon sources were not considered as part of this study.

The mixed community was able to grow on the minimal medium with glucose and rich medium as shown in Figures 4.2e and 4.2f. No growth was observed for the technical agar, technical agar with glucose or minimal medium agar without glucose plates, suggesting the system was both carbon- and nutrient-limited and did not support bacterial growth.

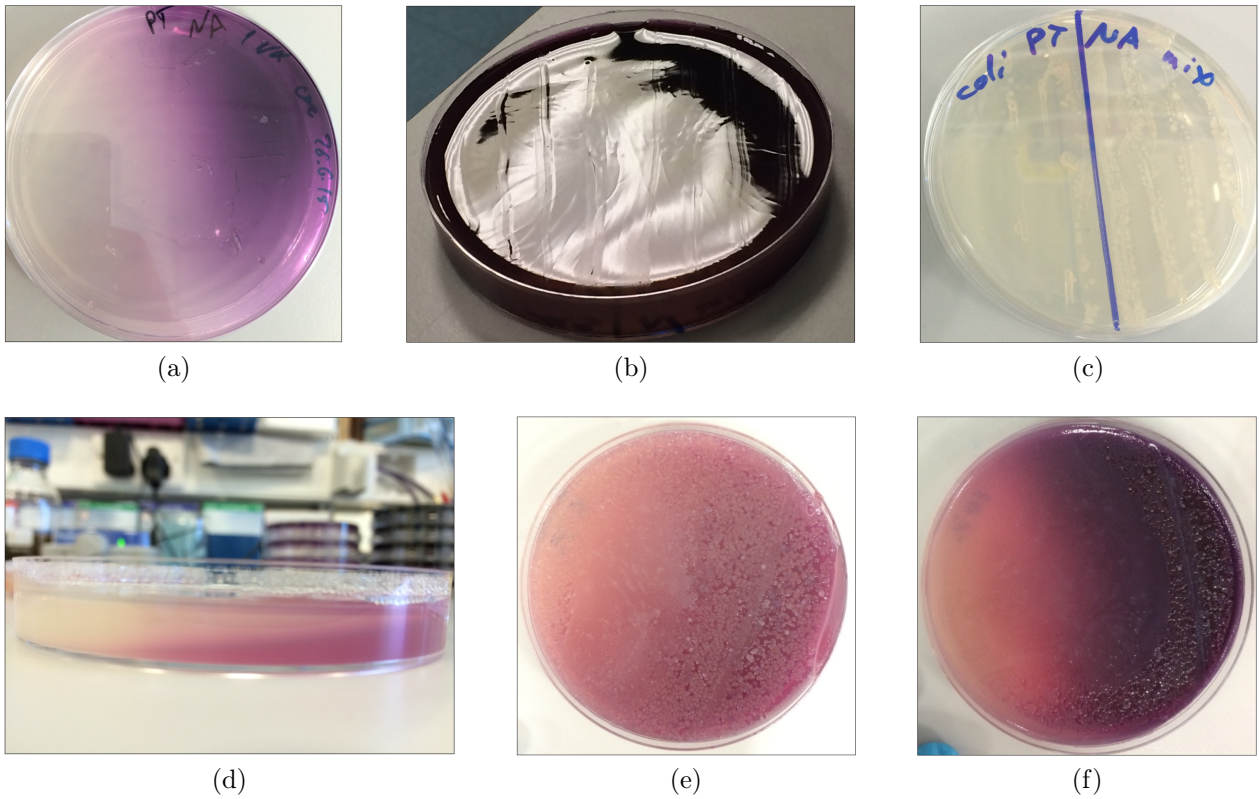


Figure 4.2: (a) No growth on enrichment gradient agar plate made from rich medium and 10 % TDW (b) No growth on 10 % TDW technical agar (c) Growth on 10 % nutrient agar control plate (d) Gradient plate side view (e) Growth on 10 % TDW & M9 & glucose (f) Growth on 10 % rich medium & glucose.

	Wells 1-3	Wells 4-6	Wells 7-9	Wells 10-12
A	Water Control	Water Control	Water Control	Water Control
B	Glucose 0.1 %	Glucose 0.2 %	Glucose 0.5 %	Glucose 1 %
C	Minimal Medium	MM	MM	MM
D	MM & G 0.1%	MM & G 0.2%	MM & G 0.5%	MM & G 1%
E	Rich Medium	RM	RM	RM
F	RM & G 0.1%	RM & G 0.2%	RM & G 0.5%	RM & G 1%

Table 4.2: Layout of 72 wells used during 96-well plate growth experiments in TDW under various glucose (G), minimal medium (MM) and rich medium (RM) additions.

Growth on agar concentration gradient plates suggested that there was a difference in culturability dependent on TDW concentration. At the low TDW concentration end of the Petri

dishes, swarming growth was observed, which altered into a single colony growth mode towards the high TDW concentration side as observable in Figures 4.2e and 4.2f. No minimum inhibitory concentration was observed up to 10 % TDW.

Similarly, growth studies were undertaken in 96-well plates, incubated at 25 °C, to determine the effect of glucose concentration and effect of different nutrient media, as shown in Table 4.2 and a selection of the most pertinent growth curves is displayed in Figure 4.3. Neither individual glucose (Growth curve shown in Figure 4.3) nor minimal medium additions were found to lead to any observable growth in the 10 % TDW. Under glucose addition, OD decreased approximately linearly over 24 h. A combination of nutrients and glucose was required to initiate a positive OD change as shown in Figure 4.3. Minimal medium with glucose allowed for growth, but OD was only observed to start increasing after a lag phase of 5.3 ± 0.3 hours, whereas OD increased from the start under rich medium addition. Glucose concentration was found to have no significant impact on the growth curves.

4.5 Aerobic and Anaerobic Bioprocessing

A study on aerobic and anaerobic bioprocessing of TDW was undertaken in parallel in bioreactors and shake flasks & serum bottles, with the corresponding flask & bottle experiments mimicking the exposure modes in the bioreactors.

When exposed to the bioreactors containing mature biofilms, neither aerobic nor anaerobic bioprocessing showed any significant COD or TOC removal. No significant change in COD

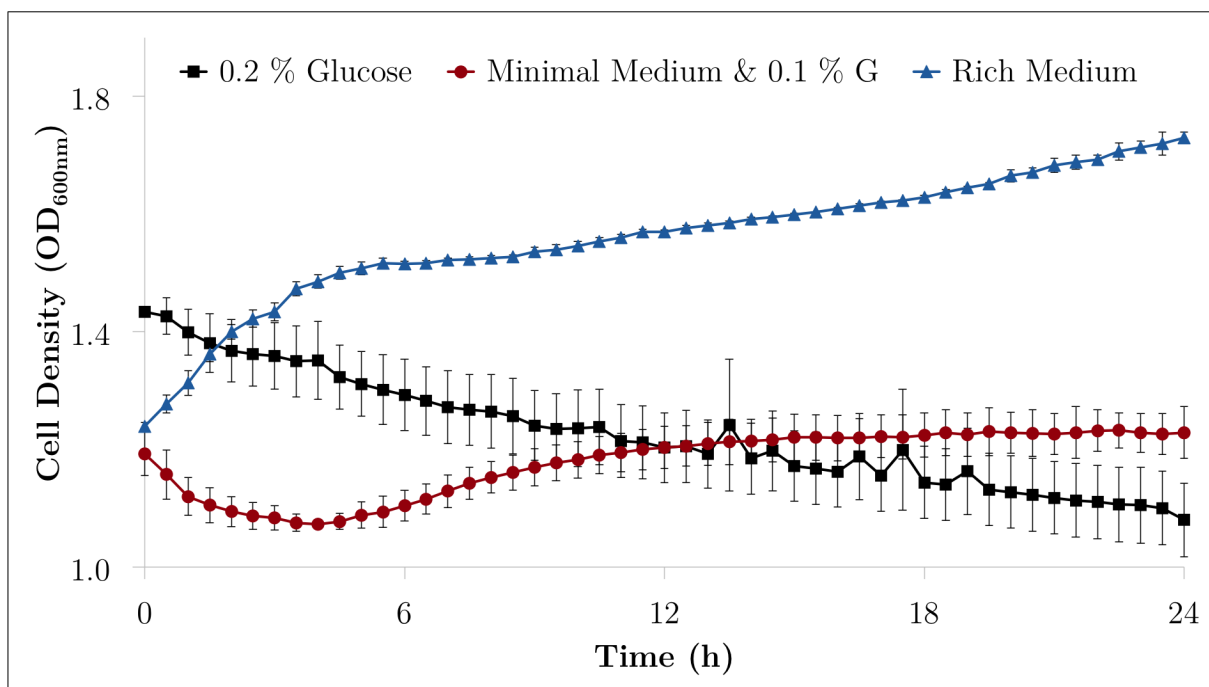


Figure 4.3: A selection of growth studies of the mixed community in 10 % TDW supplemented with 0.2 % glucose, minimal medium & 0.1 % glucose and rich medium without glucose. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

was observed after 7-day aerobic incubation followed by 28-day anaerobic exposure to the mature bioreactors as shown in Figure 4.4. Adding 0.1 % glucose on day 35 temporarily increased COD (and TOC) as expected. Although this added COD was removed after 24 h, it did not lead to any significant subsequent COD (or TOC) removal. Allowing the bioreactors to go anaerobic (as described in Chapter 2 Section 2.8) and adding a second 0.1 % dose of glucose to subsequent anaerobic treatment led to similar results. The added carbon was metabolised, but led to no further significant improvements in COD and TOC reduction. Neither acute toxicity nor general toxicity KPI analyses showed any significant mitigation of toxicity after either 7-day aerobic, 28-day anaerobic, 1-day aerobic with glucose addition or 7-day anaerobic bioprocessing with carbon addition.

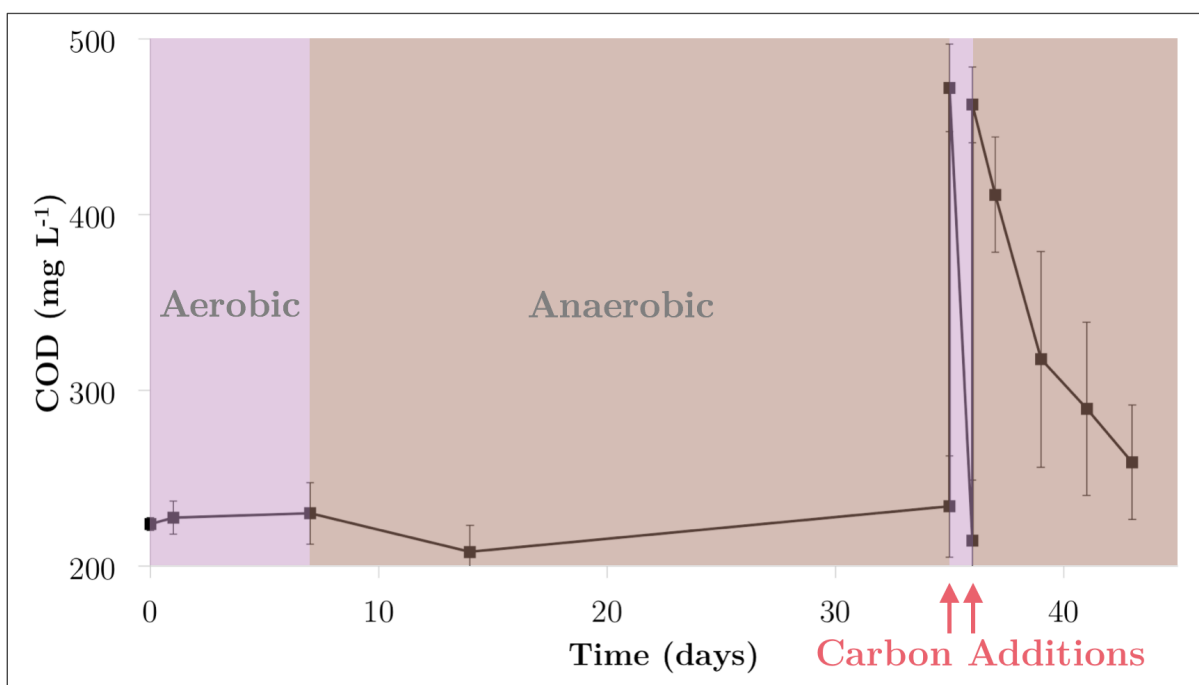


Figure 4.4: COD Dynamics of the TDW over the course of 43 days during sequential 7-day aerobic (purple), 28-day anaerobic (brown), 1-day aerobic (purple) and 7-day anaerobic (brown) modes with 0.1 % glucose additions at days 35 and 36 as indicated by the red arrows. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

After this 43-day experimental regime on the TDW, the bioreactors acclimatised again to the MWF without requiring new inoculation to achieve MWF degradation performances comparable to the ones described in Chapter 3 Section 3.3 after a 21-day acclimatisation period.

The shake flask experiments, run in parallel to the bioreactors, did not achieve any significant COD or TOC removals either. Figure 4.5 shows that 28-day anaerobic bioprocessing did gradually improve the OD of the TDW by 23.9 ± 6.0 % at its maximum absorbance of 560 nm. Biofilms were observed to have formed above the liquid level after anaerobic treatment. Moreover, non-culturable precipitations were detected at the bottom of the serum bottles.

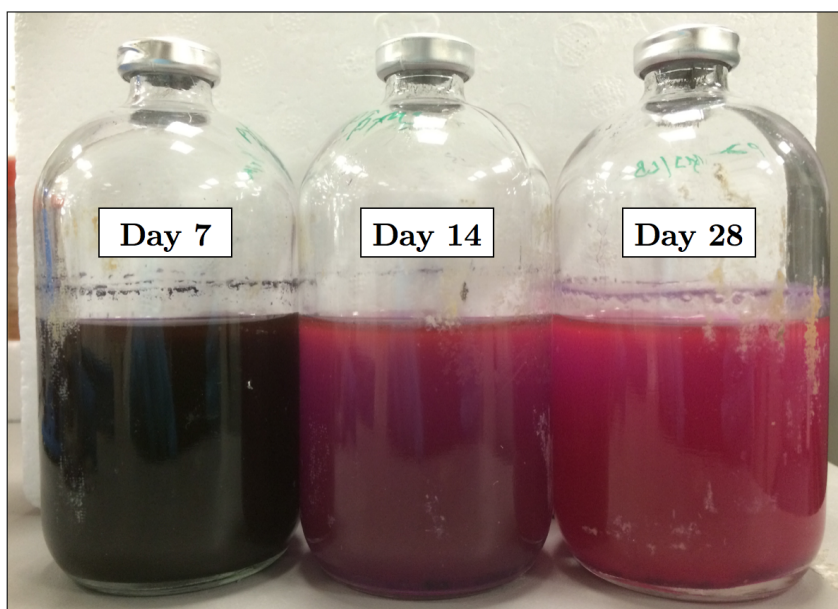


Figure 4.5: Optical Density Changes after 7, 14 and 28-day anaerobic treatment respectively from left to right.

4.6 Nitrogen and Sulphur Removal Studies

Although bioprocessing improved neither COD nor TOC removal and did not mitigate toxicity, it induced nitrogen and sulphur removal. After 24 h aerobic biological treatment, a total sulphate removal of $88.3 \pm 5.4 \%$ was recorded. Sulphate content was reduced significantly ($p = 0.0001$) from $22.6 \pm 1.4 \text{ mg L}^{-1}$ to $2.6 \pm 1.3 \text{ mg L}^{-1}$. This was comparable with sulphate removal efficiencies of over 90 % achieved after 24 h for bioprocessing of a MWF (Thill 2013). This did not further improve after prolonged bioreactor exposure, under anaerobic conditions or under carbon addition. Sulphide concentrations were measured and found to be negligible compared to the sulphate concentrations, never amounting to more than 0.2 mg L^{-1} . Phosphate concentrations were below detection limits throughout the experiment. The evolution of the nitrogen profile of the TDW during bioprocessing revealed that some of

the nitrogen present in the stream could also be removed. A sequential study, i.e. employing different modes one after the other, on the effect of aerobic and anaerobic bioprocessing under different glucose additions showed that the optimal nitrogen removal sequence was an initial anaerobic followed by an aerobic stage with glucose addition as shown in Figure 4.6. Initial aerobic treatment demonstrated no significant changes in nitrogen removal (not shown in Figure 4.6). Moreover, no significant reduction in total nitrogen was detected after anaerobic treatment, but the nitrogen profile changed during anaerobic pre-treatment. In fact, there was a significant conversion of complex nitrogen into ammonium with complex nitrogen declining from $11.0 \pm 1.2 \text{ mg L}^{-1}$ to $4.3 \pm 0.5 \text{ mg L}^{-1}$ and ammonium increasing from $9.6 \pm 0.2 \text{ mg L}^{-1}$ to $14.6 \pm 1.4 \text{ mg L}^{-1}$ ($p = 0.001$). Aerobic treatment on its own after anaerobic treatment improved total nitrogen removal significantly from $26.6 \pm 1.3 \text{ mg L}^{-1}$ to $22.4 \pm 1.5 \text{ mg L}^{-1}$ ($p = 0.026$) achieving an overall $59.6 \pm 11.4 \%$ reduction to $10.8 \pm 3.3 \text{ mg L}^{-1}$ ($p = 0.0011$) after glucose addition.

4.7 Nanoscale Zero-Valent Iron Treatment

Studies reported in Chapter 3 demonstrated that NZVI treatment was effective for COD removal of MWFs, so it was hypothesised that a similar improvement could be achieved for the TDW. NZVI treatment was effective at reducing the OD of the TDW as shown in Figure 4.7 at a range of dilutions of the TDW, achieving a $96.7 \pm 2.9 \%$ colour removal at 10 g L^{-1} for TDW at 10 %. Colour removal was found to improve at increased NZVI concentrations and decrease at increased TDW concentrations as can be clearly seen in Figure 4.7.

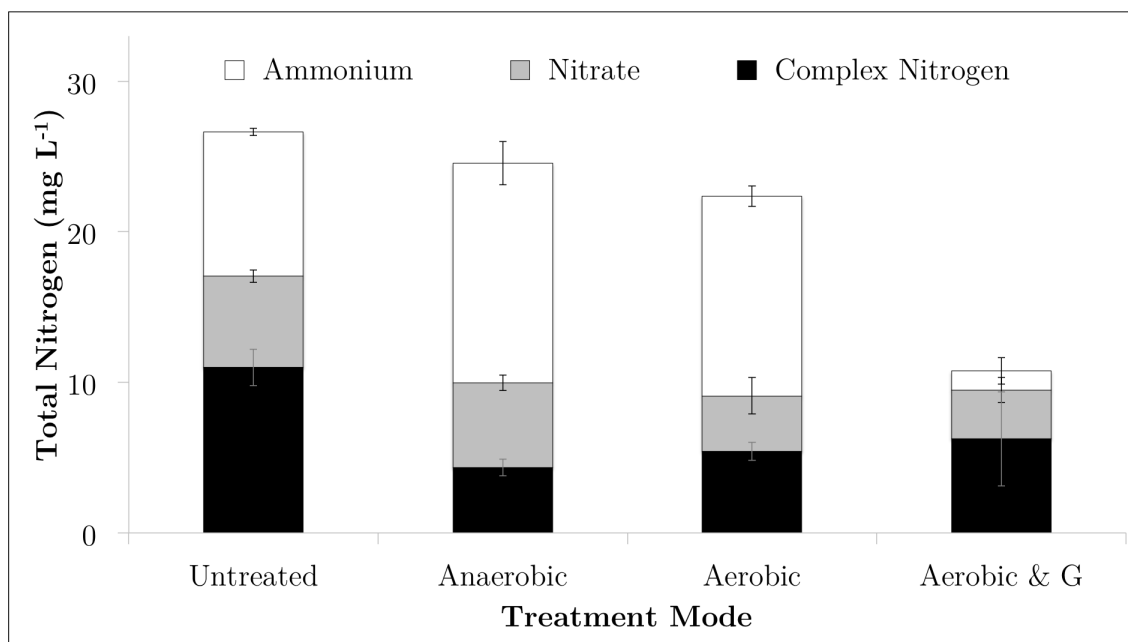


Figure 4.6: Nitrogen breakdown profile of the TDW before and after sequential anaerobic, aerobic and aerobic treatment with glucose (G) addition. The total column height represents the total amount of nitrogen present in the stream. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$) for complex nitrogen, nitrate and ammonium with their corresponding error bars.

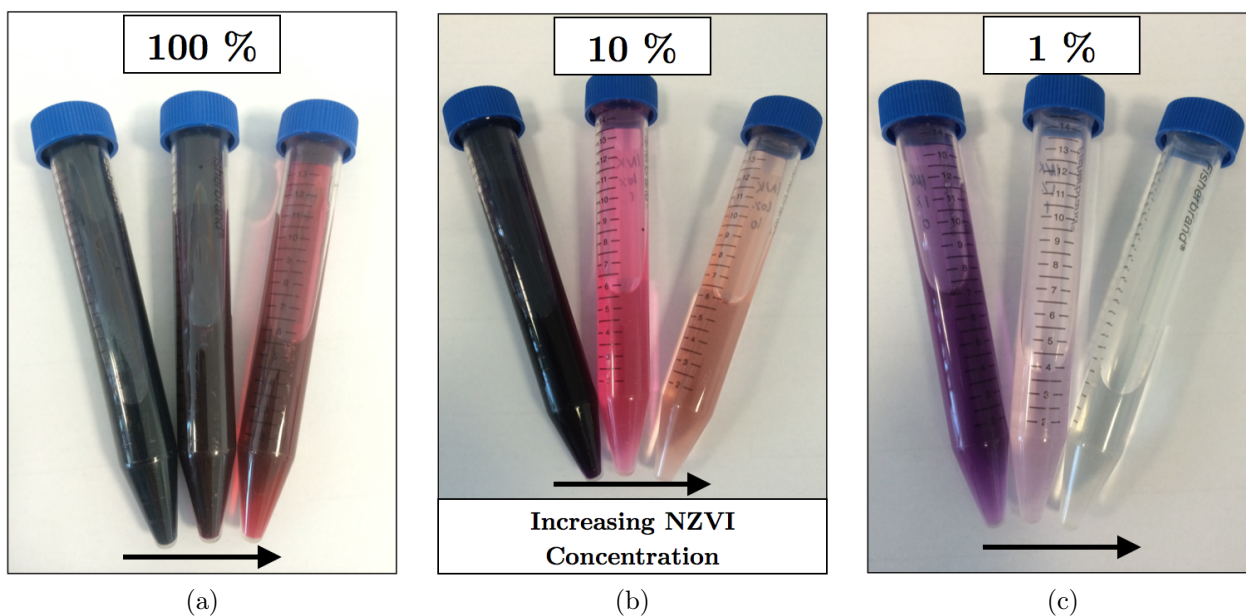


Figure 4.7: Visualisation of optical density reductions of (a) 100 % (b) 10 % (c) 1 % TDW at increasing concentrations of NZVI (0, 1 and 10 g L^{-1}) from left to right.

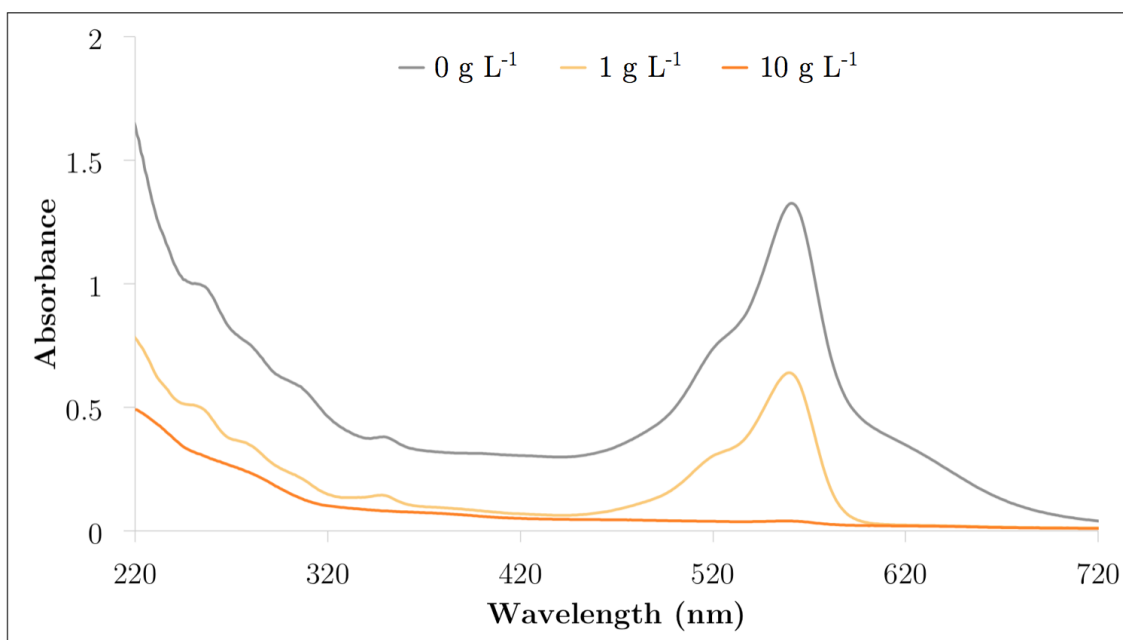


Figure 4.8: Optical density spectra of the 10 % TDW as a function of wavelength after 0 g L⁻¹, 1 g L⁻¹ and 10 g L⁻¹ NZVI treatment. Maximum absorbance occurs at 560 nm.

Figure 4.8 shows that the main decolouration peak measured at 560 nm was reduced with increasing NZVI concentrations and nearly completely disappeared at 10 g L⁻¹. At wavelengths below 350 nm, several small peaks in the spectrum were detected, still present at 1 g L⁻¹, presumed to correspond to different chemical compounds.

COD and TOC removal were both observed to increase above 0.1 g L⁻¹ and started to plateau above 1 g L⁻¹ as shown in Figure 4.9. At 10 g L⁻¹, COD removal was 42.5 ± 7.9 % and TOC removal was 30.8 ± 7.1 %. Adding more NZVI did not have any detectable impact on treatment efficiency. With increasing NZVI doses, a significant decrease in pH was observed varying from 8.8 ± 0.1 for the untreated TDW to 8.0 ± 0.2 for TDW treated at 1 g L⁻¹ ($p = 0.006$) as shown in Figure 4.9. After NZVI treatment at 10 g L⁻¹, pH was equal to 7.9 ± 0.1 . No further significant changes in KPIs were found to occur at NZVI concentrations

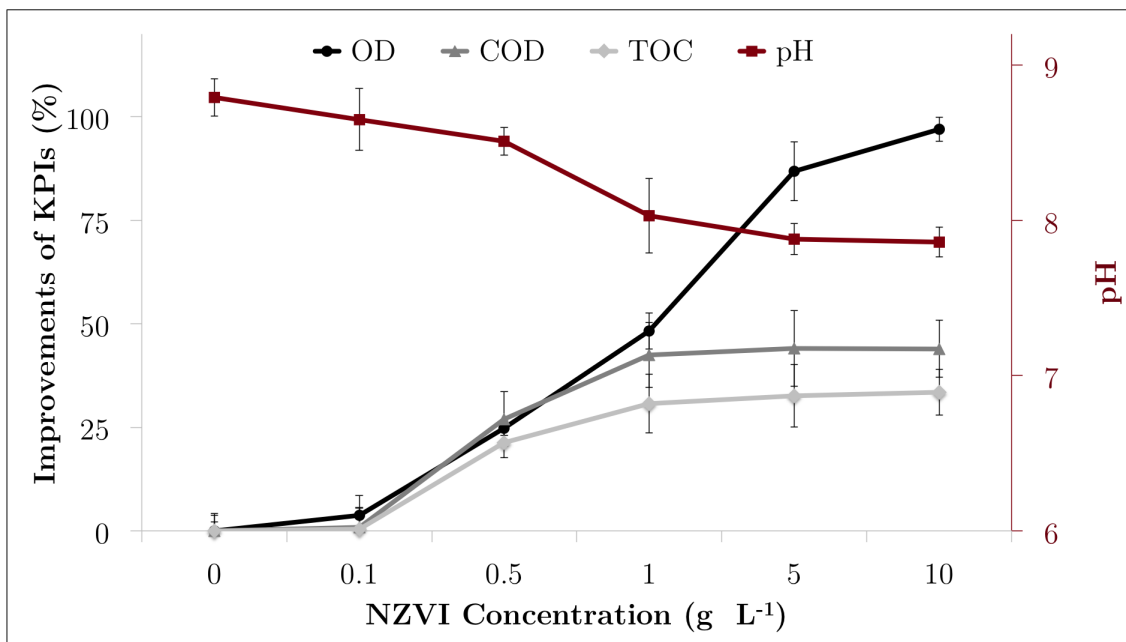


Figure 4.9: Variation of KPIs with increasing NZVI concentrations. Improvements in COD, TOC and OD are given on a grey-scale on the left hand side axis and are expressed as percentage improvements. pH is given in red on the right hand side axis. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

above 10 g L⁻¹, tested at 20 g L⁻¹ and 50 g L⁻¹ (not shown in Figure 4.9).

4.8 E-Beam Treatment

As with NZVI treatment, e-beam treatment was found to be effective at colour removal, albeit requiring a very high dose of 1,000 kGy to achieve a 97.6 ± 3.3 % improvement in OD, as demonstrated by the changes in OD in Figure 4.10. At increasing dose, the main peak in OD at 560 nm was found to disappear, leading to the treated TDW becoming clearer.

The initial colour removal during e-beam treatment was found to go in parallel with an initial COD increase by 11.8 ± 7.8 % at 50 kGy as shown in Figure 4.11. TOC was not

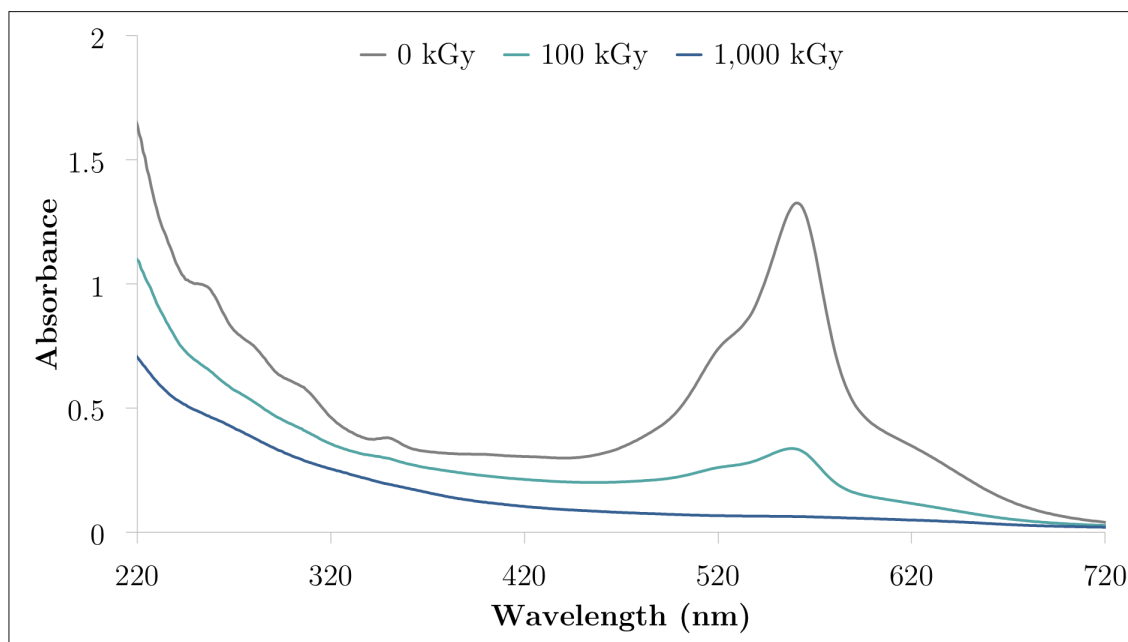


Figure 4.10: Optical density spectra of the 10 % TDW as a function of wavelength after 0 kGy, 100 kGy and 1,000 kGy e-beam doses.

significantly different at 50 kGy when compared to its value at 0 kGy. The COD increase was accompanied by a significant decrease in pH from 8.8 ± 0.2 to 6.5 ± 0.2 ($p = 0.0001$) at 50 kGy. At higher doses, COD and TOC were found to decrease up to 35.5 ± 9.1 % and 35.7 ± 6.7 % respectively at 1,000 kGy accompanied by an insignificant increase in pH ($p = 0.13$).

Liquid chromatography (LC) of the TDW before and after exposure to both NZVI and e-beam treatments, shown in Figure 4.12, showed that the initial two main peaks were still present after e-beam treatment. They were accompanied by several small and indistinct peaks, which appeared in the spectrum. NZVI treatment showed no visible breakdown products after LC analysis even at the lowest NZVI concentration tested of 0.1 g L^{-1} .

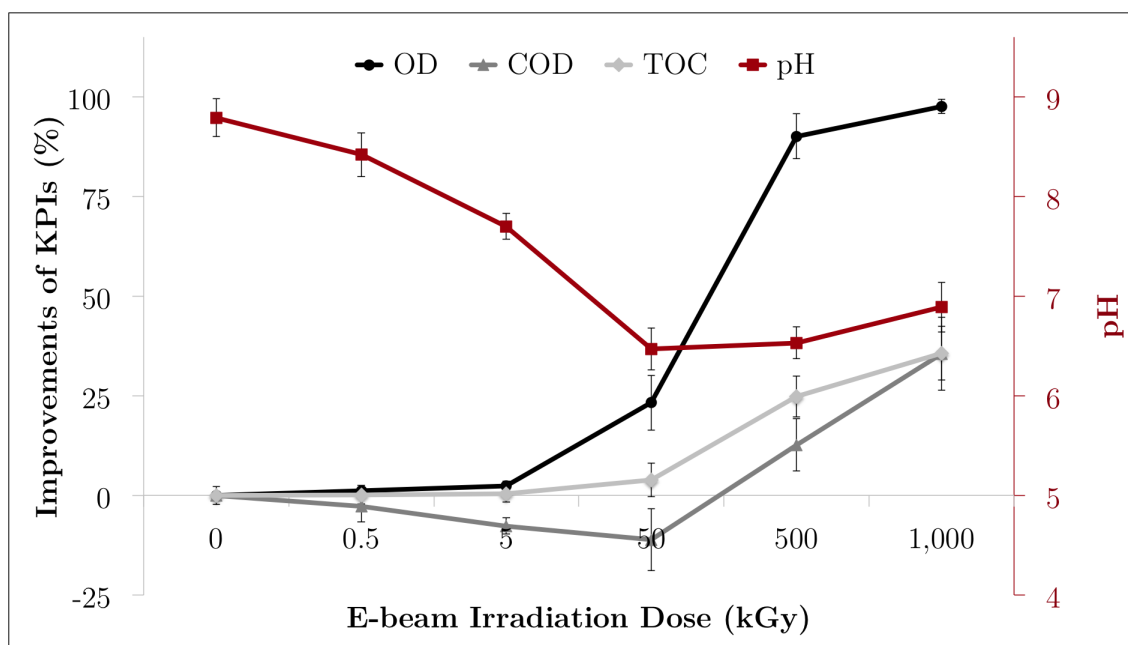


Figure 4.11: Variation of KPIs at increasing e-beam doses. Improvements in COD, TOC and OD are given on a grey-scale on the left hand side axis and are expressed as percentage improvements. pH is given in red on the right hand side axis. Mean is shown and error bars represent standard deviation for triplicates ($n = 3$).

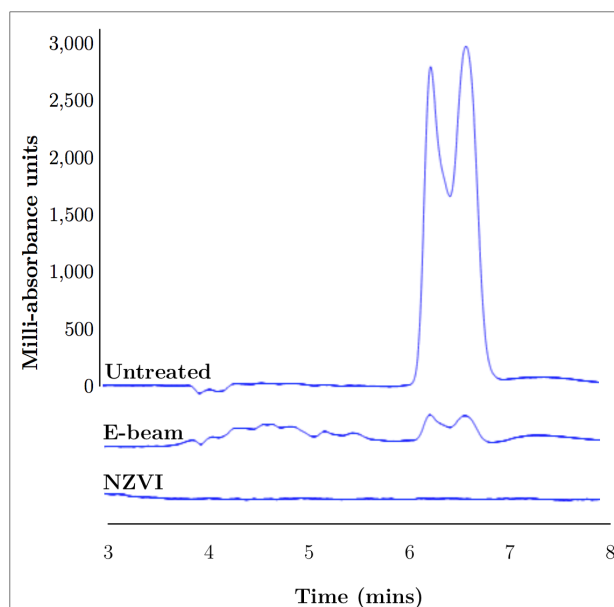


Figure 4.12: LC of untreated TDW, TDW after e-beam treatment at 50 kGy and after NZVI treatment of 10 g L⁻¹. Milli-absorbance units are arbitrary units that can be considered relative to each other. One unit corresponded to a signal strength of 1 mV. LC signals are given to scale with respect to the untreated TDW as reference signal.

4.9 Hybrid Treatment

4.9.1 Improvements in Key Performance Indicators

Regarding toxicity KPIs, e-beam treatment was the only one showing significant improvement. In fact, e-beam treatment on its own was statistically more effective than any other treatment individually and even hybrid treatment with regards to toxicity as shown in Figure 4.13.

4.9.2 Bioprocessing as Pre-Treatment?

The most effective bioprocessing sequence consisted of running the reactors aerobically for one day to remove the sulphate then anaerobically for 28 days to transform some of the complex nitrogen into ammonium before running it aerobically again for one day with carbon addition before employing NZVI and e-beam treatment. In terms of treatment sequence there are two logical places for bioprocessing in a hybrid system. The first is at the beginning, due to the effective biological sulphur and nitrogen removal achieved during bioprocessing. This could also potentially improve the treatment efficiency of e-beam treatment by removing some of the nitrogen and sulphur based radical scavengers and NZVI treatment by taking out some of the chemical load out of the solution leading to potentially lower e-beam doses and NZVI concentrations to achieve equivalent remediation efficiencies.

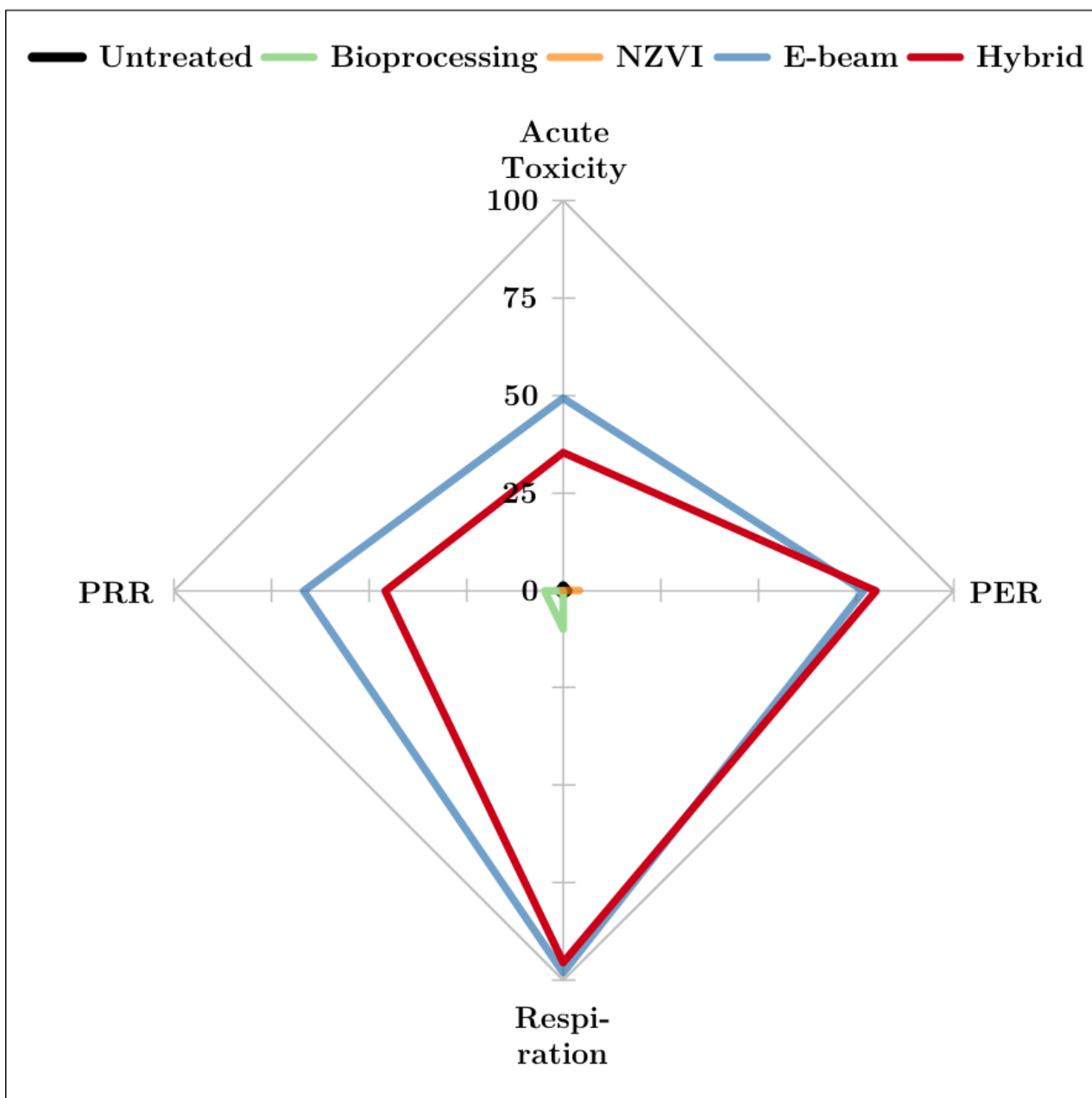


Figure 4.13: Percentage KPI improvements for the TDW after individual bioprocessing, NZVI, e-beam treatment and optimal hybrid sequential treatment.

4.9.3 E-Beam Pre-Treatment?

The second logical place employs bioprocessing after e-beam pre-treatment and prior to the onset of mineralisation, presupposed to be identifiable as the point of minimum pH. As determined in Figure 4.11, the minimum pH would lie between a dose of 5 and 50 kGy. Determining the exact dose corresponding to the minimum was not considered to be a crucial element of this study and it was decided to use a 50 kGy dose in the hybrid system. At this dose, some mineralisation may already have occurred and 50 kGy may be beyond optimum dose, but since the TOC profile beyond 50 kGy was found to increase and pH remained constant, the conditions at 50 kGy were presumed to be more representative of the minimum than the conditions at 5 kGy. A previous study contraindicated the use of e-beam as pre-treatment, as nitrogen removal was found to be adversely affected by e-beam pre-treatment for a swine wastewater (Lim et al. 2013).

4.9.4 Order of NZVI and E-Beam Treatment

It was difficult to predict the optimal order in which NZVI or e-beam treatment should be employed, as e-beam treatment was found to be more effective at detoxification (Figure 4.13), whereas NZVI treatment was found to be better at OD and COD removal for the TDW (Comparing Figure 4.9 to Figure 4.11). Therefore, both sequences were tested. There was no detectable difference on KPI improvements between using a 1 or 10 g L⁻¹ concentration of NZVI apart from changes in OD. However, this was overcome by bioprocessing and e-beam treatment in the hybrid system, which reduced any excess OD effectively.

Mode	OD	COD	Toxicity	Nitrogen	Sulphate
1 g L ⁻¹ 50 kGy	>3	Unchanged	Unchanged	Unchanged	Unchanged
10 g L ⁻¹ 500 kGy	32.0 % (7.7)	29.4 % (4.8)	30.1 % (9.8)	42.3 % (12.4)	57.1 % (7.5)

Table 4.3: Percentage KPI Changes after sequential bioprocessing, NZVI and e-beam treatments (concentrations and doses given under Mode) on concentrated TDW (100 %). Toxicity is expressed as the average percentage improvements in the four toxicity KPIs.

4.9.5 Optimal Sequential Treatment

The results from the sequential analysis demonstrated that bioprocessing led to better overall remediation as pre- than post-treatment in conjunction with the e-beam. It was also established that NZVI treatment followed by e-beam treatment yielded better results than employing them vice versa. The optimal hybrid sequence was therefore sequential 1-day aerobic, 28-day anaerobic, 1-day aerobic bioprocessing with carbon addition followed by 1 g L⁻¹ NZVI and 50 kGy e-beam treatment. This demonstrated that a combined hybrid treatment achieved a 99.4 ± 0.4 % reduction in colour, a 54.6 ± 6.1 % reduction in COD, a 89.0 ± 3.5 % reduction in sulphate and a 67.7 ± 4.2 % reduction in total nitrogen. Overall toxicity improvements are shown in Figure 4.13 and given in Table 4.4 in Section 4.10.4.

4.9.6 Concentrated Textile Dyeing Wastewater Treatment

The effect of exposing concentrated TDW (100 %) to the hybrid system described above was also investigated. An order of magnitude higher NZVI concentrations as well as e-beam irradiation dose were required to achieve any significant improvement as shown in Table 4.3.

4.10 Assessment of Remediation

4.10.1 Attempts at Bioprocessing

Section 4.3 of this chapter showed that even though there were no indigenous culturable populations in the TDW, an added mature bioreactor consortium survived in suspension in the TDW after 24 h exposure. This finding suggested that the TDW was inhibitory but not toxic to the mixed community employed during bioprocessing. Although TDW concentration did not adversely affect survival rates of the inoculum in suspension, it affected the culturable growth type. Minimum inhibitory concentrations for antibiotic resistance can be determined by gradient plates (Liu et al. 2011). Similarly, in this study gradient agar plate experiments on the TDW showed that there was a transition in growth mode across such plates. Swarming growth was observed at the low TDW concentration, while growth modes consisted of single colony forming units towards the high TDW concentration side further confirming that the TDW inhibited normal growth of microorganisms.

The mixed community was found to be able to grow in a TDW substrate with glucose and nutrient addition as shown in Figure 4.3. Interestingly, a lag phase in growth was observed under minimal medium and glucose addition. This was probably due to a high proportion of persister cells in the microbial community. Unexpectedly, this was accompanied by a decrease in OD, which was also observed for the glucose-only supplementation mode suggesting that some of the chromophore was initially removed. This could have been due to initial adsorption of compounds onto the inoculum and subsequent precipitation. While there was no observable

growth for the glucose-only mode, the effect of growth in the minimal medium eventually overtook the effect of colour removal from the system. Glucose concentration was found to have no significant impact on the growth curves, suggesting the lowest dose tested of 0.1 % glucose was sufficient to overcome the carbon-limitation of the system.

Although the system was found to be carbon and nutrient limited, the initial COD could not be degraded significantly even under nutrient addition in the bioreactors. However, this clearly demonstrated that biological carbon removal, in the form of easily available glucose, could occur in the reactors, even after 35 days of exposure to the TDW. Established mature bioreactors have been found to be able to withstand long periods without fresh supplies of MWFs. The biofilms typically started to visually thin out during ‘periods of starvation’ and their mechanism of survival in this situation was thought to be by cryptic growth on the trapped necromass. The biofilms were intact after the 43-day exposure to the TDW, returning to previous bioprocessing performances on the MWF.

Since neither aerobic nor anaerobic bioprocessing improved COD removal, this was strong evidence to suggest that the TDW was recalcitrant and non-biodegradable. This is typical of many TDWs (Ghaly et al. 2014; Moore 2004; Robinson et al. 2001) and this is attributed to their biologically inert high molecular constituents (Rajkumar and Kim 2006).

Optimal nitrogen removal was found to occur in anaerobic conditions followed by aerobic bioprocessing with glucose addition. TOC analysis identified that carbon was present in the system, however it was not available to be assimilated for nitrogen removal and the system required activation by glucose addition. This suggested that a partial nitrification

and anaerobic ammonium oxidation process occurred in parallel (Lackner et al. 2014), due to the breakdown of complex nitrogen and reduction of ammonium in the reactors during bioprocessing. A minimal Carbon : Nitrogen ratio is typically required for nitrogen to be sequestered biologically from a system (Ammery 2004), which was restored by the addition of 0.1 % glucose. The system was not thought to be phosphate limited, as nitrogen removal also depends on a Nitrogen : Phosphorus ratio (Ammery 2004) and occurred as soon as carbon was added. Subsequent phosphate additions were not investigated, but may have further improved bioprocessing performance.

The parallel shake flask experiments did not achieve significant COD removal, confirming that biofilms were more effective than planktonic cells at bioprocessing. Indeed, a biofilm was seen to form at the liquid level at the interface between air and TDW after anaerobic incubation, suggesting microaerophilic conditions at the interface might be more favourable for microorganism growth. It was interesting to note that overall aggregate sulphate reduction of $20 \pm 1.9 \text{ mg L}^{-1}$ and total nitrogen removal of $15.8 \pm 3.5 \text{ mg L}^{-1}$ did not affect COD removal significantly. This was unexpected as their sum ($35.8 \pm 2.8 \text{ mg L}^{-1}$) represented approximately 15 % of the initial COD ($223.9 \pm 3.7 \text{ mg L}^{-1}$), so a significant decrease in COD was expected to occur alongside nutrient removal. One possible explanation for this seemingly conflicting result might be that some of the planktonic microorganisms did not survive the TDW and their decay products might have contributed to a parallel increase in COD or there was a possible reduction in oxidation states of some of the TDW contaminants during bioprocessing.

4.10.2 Mechanisms of Action of NZVI Treatment

OD spectra, i.e. decolouration curves, give information on the disappearance of starting molecules, in particular aromatic rings, which have a characteristic absorbance below 300 nm (Wojnárovits and Takács 2008). At wavelengths below 350 nm, several small peaks in the spectrum, presumed to correspond to different chemical compounds, persisted after 1 g L⁻¹ NZVI treatment, as observed in Figure 4.8. This suggested that the initial main mechanism of action during NZVI treatment was adsorption of compounds rather than breakdown of some of these compounds, which was further supported by the flat LC spectrum after NZVI treatment (Figure 4.12).

Interestingly, the continued improvement in OD beyond 1 g L⁻¹ did not occur in parallel with a COD decrease. This suggested that surface-mediated reactions on the NZVI were breaking down the chromophore above 1 g L⁻¹ with breakdown products remaining in solution. The toxicity increase accompanying this step suggested that stable intermediaries were formed, probably in the form of toxic aromatic amines (Bokare et al. 2008).

A decrease in TOC with increasing NZVI doses, suggested that carbon was also being removed. The accompanying reduction in pH was probably due to the removal of some alkaline compounds bringing the overall pH closer to neutrality. Similar to the treatment of MWFs by NZVI in Chapter 3 Section 3.4, no further improvements in treatment performance were observed beyond 10 g L⁻¹. This suggested that the remaining TDW could not be further remediated by NZVI and that the post-10 g L⁻¹- NZVI-treated effluent had no affinity to be either adsorbed or broken down any further on the surface of NZVI.

4.10.3 Mechanisms of Action of E-Beam Treatment

E-beam treatment was found to be effective at colour removal, albeit requiring a very high dose of 1,000 kGy to achieve a 97.6 ± 3.3 % improvement in OD. The initial colour removal during e-beam treatment was found to go in parallel with an initial COD increase by 11.8 ± 7.8 % at 50 kGy decrease up to 35.5 ± 9.1 % of its original value at 1,000 kGy. A similar study reported that colour removal and the COD reduction were directly related to e-beam dose, achieving a COD removal of 32 % and a reduction in OD of 59.6 % at a dose of 9 kGy (Vahdat et al. 2010). In that study, the reduction in COD was probably largely related due to mineralisation. Colour removal also typically depends on the structure of the dye wastewater and its concentration (Bhuiyan et al. 2016) and therefore is difficult to compare quantitatively between studies.

The decrease in absorbance in the visible spectrum observed after e-beam treatment was thought mainly to be caused by the destruction of the extensive conjugation between molecules of the dyeing waste stream, and hence the destruction of the chromophore and accompanying decolourisation, which was observed elsewhere (Wojnárovits and Takács 2008). This was supported by the observed disappearance of several small peaks in the optical spectrum, hypothesised to be radiolytical breakdown of molecules corresponding to these peaks (Figure 4.10). Compared to NZVI treatment, e-beam treatment resulted in a much smoother OD spectral curve below 350 nm, confirming that the main mechanism by which the e-beam was effective was by radiolytical conversion of large organic compounds (Ghaly et al. 2014; Rauf and Ashraf 2009).

Further evidence was the recorded appearance of peaks during liquid chromatography, which were presumed to correspond to the breakdown products (Figure 4.12). The two characteristic LC peaks of untreated TDW were present at a lower intensity and accompanied by several small and indistinct peaks after e-beam treatment. NZVI treatment led to both peaks being removed, even at a dose as low as 0.1 g L^{-1} with no breakdown products visible on the LC at any higher NZVI concentration. In particular, no peaks were observed above 1 g L^{-1} , so at the very least, the stipulated surface-mediated breakdown accompanying the continuation in OD reduction did not result in separable compounds.

In contrast to a similar study (Vahdat et al. 2010), colour removal during e-beam treatment was found to go in parallel with an initial COD increase. This may suggest that some of the radiolytical breakdown products of the TDW generated during e-beam irradiation might have been subsequently reduced, which reflected in an initial increase in COD. This might have implications about the accuracy of using cuvette tests after strong oxidative and reductive effects caused by the e-beam. Alternatively, this could have suggested that some of the components of the TDW were not completely oxidised and were therefore effectively omitted from the COD assessment by cuvette tests. In this scenario, only the free radicals generated during e-beam irradiation would have been able to readily oxidise recalcitrant organic pollutants resulting in a temporary increase in COD. In this scenario, the actual COD of the TDW was likely to have been greater than indicated in Table 4.1.

E-beam treatment was the only treatment showing significant improvement in KPIs. The acute toxicity measurements need to be considered with care, as the OD of the TDW after

bioprocessing was high, so some of the light output of the biosensor might not have been detected. The other three KPIs did not however depend on light outputs and revealed no improvements, so there is a strong case to suggest that e-beam irradiation improved detoxification.

4.10.4 Tracking the Extent of Mineralisation using pH

pH was used to track the extent of mineralisation of the TDW, as the main mechanism of action was expected to be radiolytical breakdown of the chromophore (Vahdat et al. 2012). An initial decrease in pH up to 50 kGy was thought to be due to the conversion and breakdown of aromatic rings and other large molecular weight compounds present in the TDW into small carboxylic acids (Pikaev 2002; Wojnárovits and Takács 2008). The subsequent observed increase in pH suggested that some of these carboxylic acids formed were being mineralised and removed from the system (Ting and Jamaludin 2008). As expected, this was accompanied by an increase in TOC and COD removal efficiency, as carbon is mineralised as CO_2 and removed from the liquid phase of the system.

Hence, it was hypothesised that pH could be used in this study to determine the optimal point at which bioprocessing should be included in a hybrid system and improve COD removal. Though effective, reducing COD by mineralisation using the e-beam is energy intensive and might be unnecessary, especially because bioprocessing has the potential to be effective at this stage. Radiolytically formed small organic compounds, like acids and aldehydes, were predicted to be more susceptible to bioprocessing than untreated TDW containing larger less

KPI	Acute Toxicity	PER	Respiration	PRR	OD	TN _b	SO ₄ ²⁻	COD
Weighting	1	1	1	1	2	1	1	0.5
Change (%)	35.4	80.1	95.4	45.8	99.4	67.7	89.0	54.6

Table 4.4: Strategic selection of KPIs, assigned weighting factors and percentage changes of 10 % TDW after optimal hybrid treatment.

bioavailable compounds and reduce its COD. Contrarily, a very high dose would be required to completely mineralise a TDW stream, which is further aggravated by the fact that carboxylic acids are recalcitrant towards the $\bullet OH$ radical and rely on other radicals and secondary radicals to be fully mineralised (Criquet and Karpel Vel Leitner 2012). However, although the optimal point corresponding to the start of mineralisation was successfully identified by the minimum in pH, subsequent bioprocessing was not found to improve remediation performance in this study.

4.10.5 Remediation Index

The Remediation Index (RI) was established in Chapter 2, Section 2.5.2, Equation 2.7. The reduction of OD was a high priority for the remediation of the TDW, whereas the removal of nitrogen and sulphur were considered to be of medium priority, while a reduction in COD was of low priority. The corresponding KPI weightings are presented in Table 4.4. The tap water control had a unit-RI for this selection of KPIs. The RI of the TDW was 75.2 % after optimal hybrid sequential treatment.

4.10.6 Conclusion

The key issues with the TDW wastewater were its high colour content, toxicity and nutrient content and low COD. It was found that the individual treatments each tackled a different part of the problem. Bioprocessing led to nutrient removal and a slight amelioration in OD, whereas NZVI treatment was best at OD and COD removal and e-beam treatment was best at detoxification as well as OD reduction. To achieve maximum remediation of the TDW, it was therefore found to be essential to combine the individual treatments in a sequential bioprocessing, NZVI and e-beam treatment mode. Practically, biological pre-treatment could correspond to a treatment stage coinciding with storage of the TDW effluent prior to NZVI and e-beam treatment.

The hybrid system exhibited some synergy in that it reduced the NZVI concentration required as the e-beam was effective at breaking down the post-NZVI treatment fragmented chromophore. This was facilitated by the removal of radical scavengers through adsorption onto NZVI. The main mechanisms of action for NZVI and e-beam treatment were postulated to be adsorption and degradation. The remediation of a more concentrated form of the TDW was possible, but required both a 10-fold higher NZVI concentration and e-beam dose. Hybrid treatment of TDW resulted in a remediation index of 75.2 %.

5 Resource Recovery, Disinfection and Footprint

5.1 Outline of Remediation Strategy

After successfully applying the hybrid treatment system to two MWFs in Chapter 3 and a TDW in Chapter 4, the remediation of a distillery wastewater (DWW) was evaluated after individual and hybrid treatment in this Chapter. Due to its high copper content, the scope for resource recovery from the DWW was also explored. Thirdly, the potential for using the e-beam for the disinfection of final effluents in a hybrid system was investigated. Finally, overall process economics and energetics of the hybrid system were evaluated at lab-scale and compared to a hypothetical practical industrial-scale implementation.

5.2 Remediation of a Distillery Wastewater

The main challenge in terms of remediating the DWW was its high toxicity, which was hypothesised to be linked to its high copper content. The stream displayed a greenish appearance from the copper and also smelled rather pleasant. Table 5.1 summarises the physico-chemical parameters of the DWW.

COD (mg L⁻¹)	TOC (mg L⁻¹)	pH	Cu (mg L⁻¹)	SO₄²⁻ (mg L⁻¹)
2,035 (171)	633 (47)	4.5 (0.1)	63.6 (3.8)	109.1 (4.0)

Table 5.1: Distillery wastewater characteristics. Mean of triplicates ($n = 3$) is shown with standard deviations given in parentheses.

Nanoscale zero-valent iron (NZVI) was particularly effective at mitigating toxicity at high concentrations. Figure 5.1 shows that an NZVI concentration of 10 g L^{-1} improved the key performance indicators (KPIs) of the DWW most significantly. This was accompanied by an improvement in COD of $19.5 \pm 4.0 \%$ at 10 g L^{-1} . NZVI concentrations up to 50 g L^{-1} were investigated as well, but did not result in any further significant improvements in either KPIs or COD.

No further significant improvements in toxicity or COD removal were observed after the implementation of bioprocessing, e-beam treatment or sequential hybrid arrangements for the DWW as shown in Figure 5.2. As with the TDW, no culturable fractions were found to be present in the DWW. There was however one more finding, which confirmed that DWW could be detoxified by NZVI treatment. Inoculum from the mature bioreactors was exposed to the DWW for 10 min (vortexed for 10 s) and incubated at serial 10-fold dilutions on nutrient agar plates at 25°C .

Figure 5.3 shows that any hybrid treatment comprising NZVI treatment resulted in recoverable plate counts. Sequential hybrid NZVI treatment at a concentration of 10 g L^{-1} and e-beam treatment at 50 kGy resulted in $41.4 \pm 31.6 \%$ of the inoculum surviving compared to $8.2 \pm 2.2 \%$ of the inoculum surviving after NZVI exposure at 10 g L^{-1} only, which was not statistically significant ($p = 0.067$).

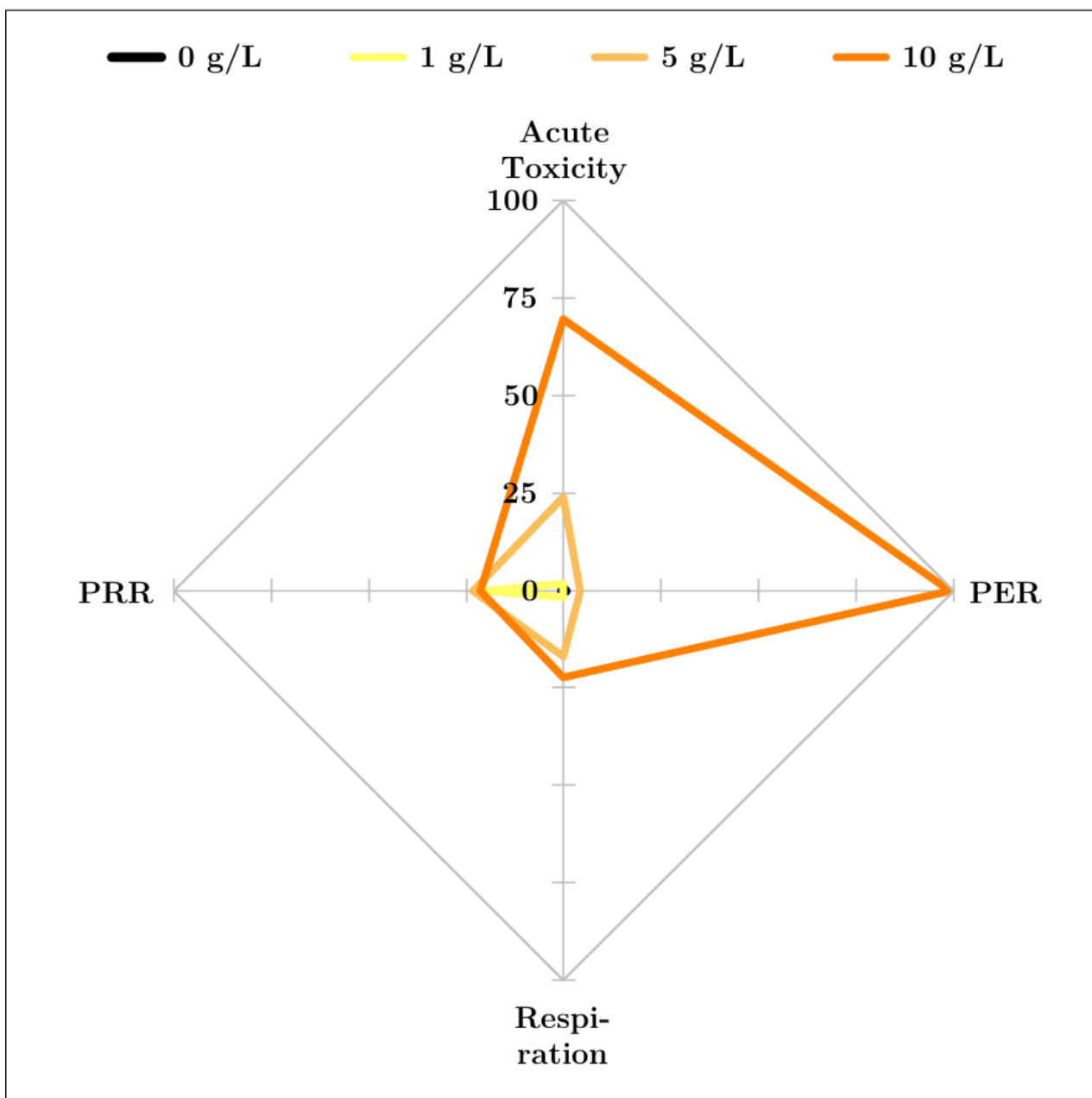


Figure 5.1: Percentage KPI improvements for the DWW after NZVI treatment at concentrations of 0 g L^{-1} , 1 g L^{-1} , 5 g L^{-1} and 10 g L^{-1} .

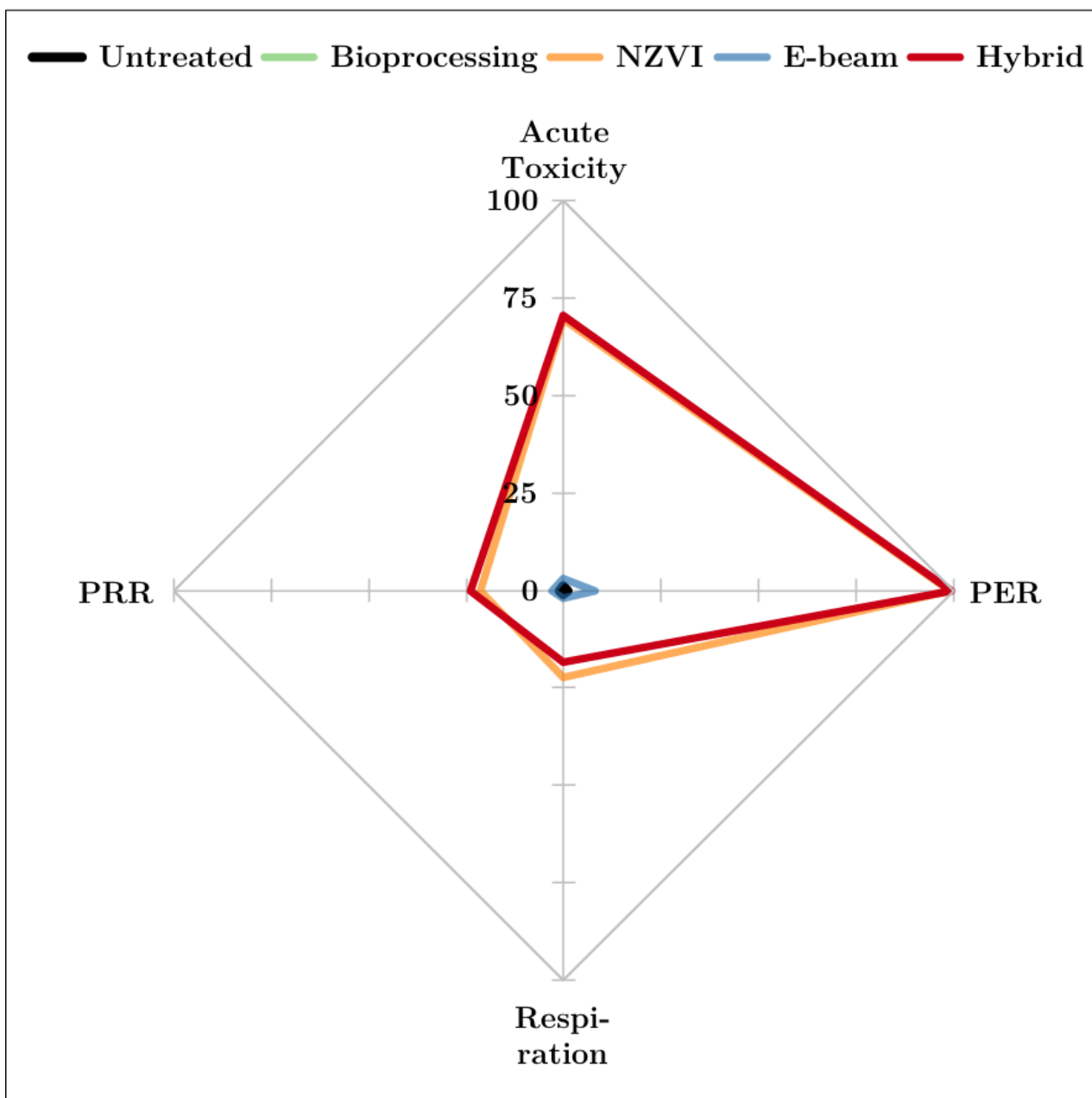


Figure 5.2: Percentage KPI improvements for the DWW after individual bioprocessing, NZVI, e-beam and hybrid treatment.

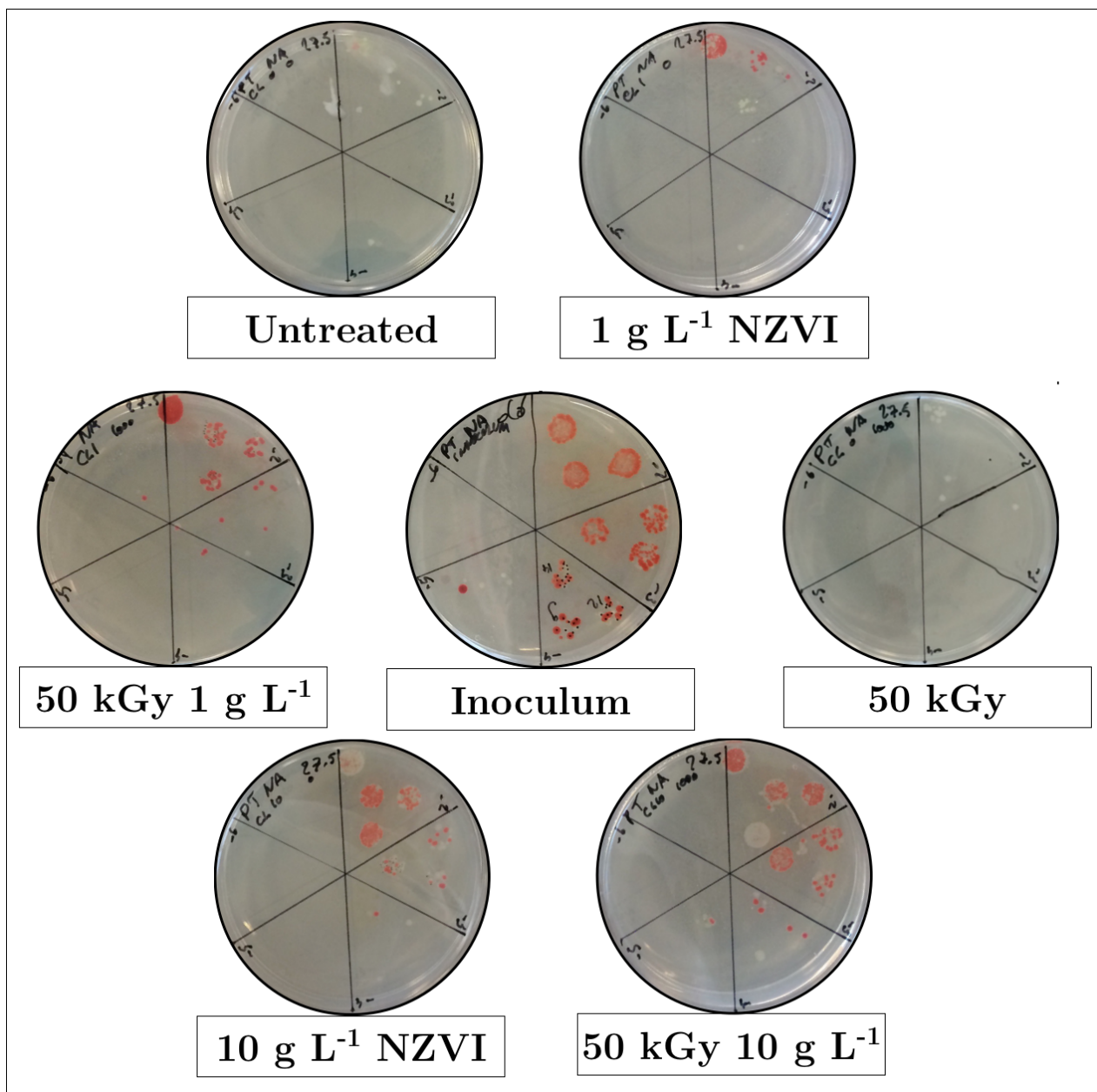


Figure 5.3: Plates showing mature bioreactor inoculum before and after exposure to untreated, NZVI, e-beam and sequential hybrid NZVI and e-beam treated DWW.

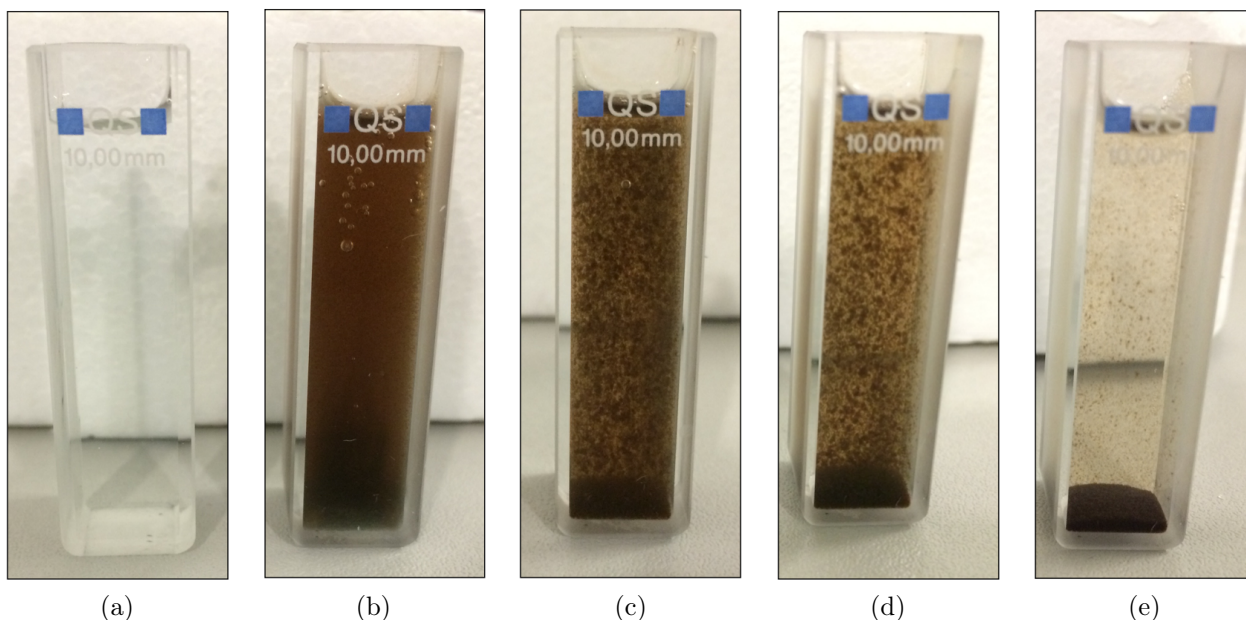


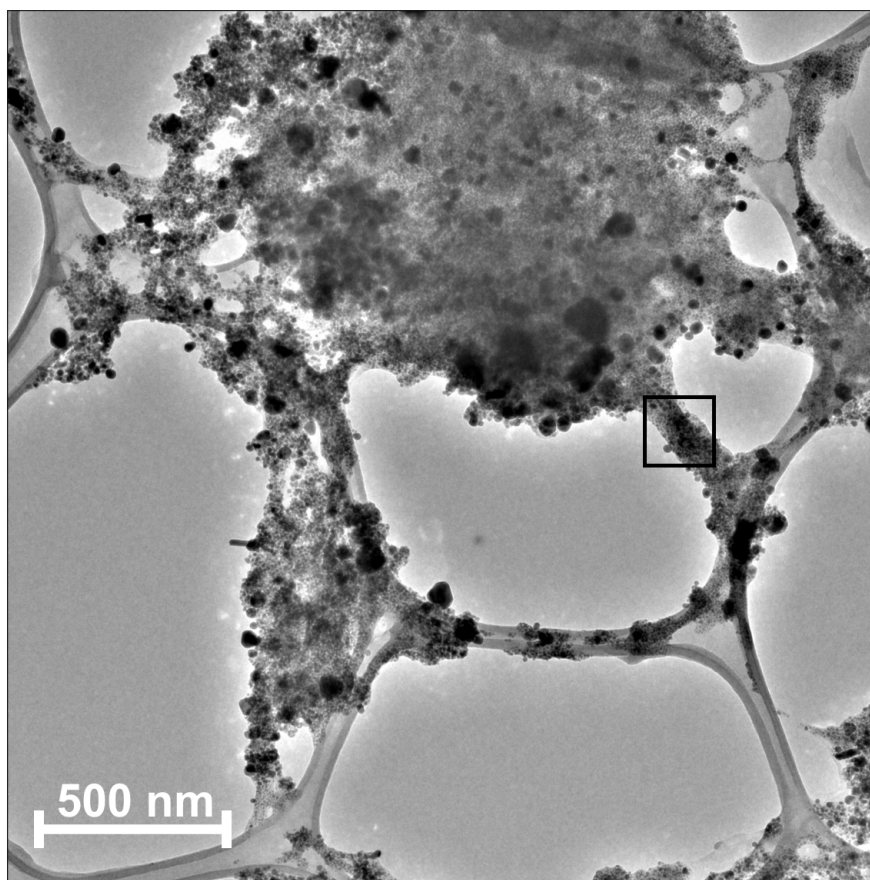
Figure 5.4: DWW sample (a) before irradiation and at (b) 30 s (c) 1 min (d) 2 mins and (e) 10 mins after e-beam irradiation displaying the evolution of precipitation observed after e-beam irradiation.

5.3 Copper Recovery during E-Beam Irradiation

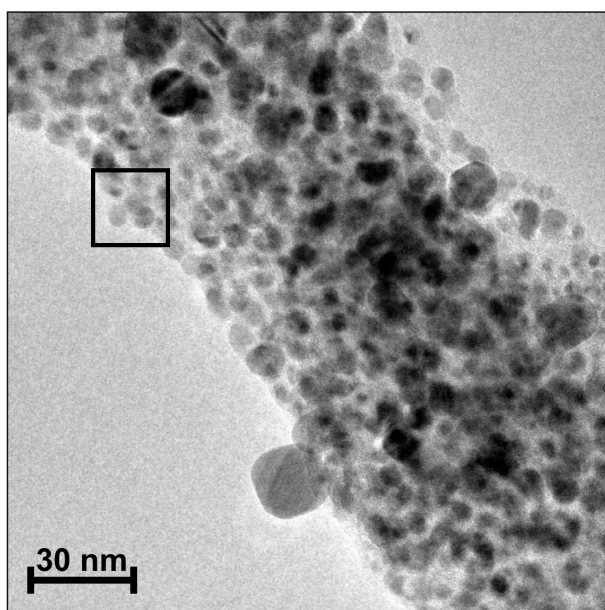
5.3.1 Identification of Precipitate

E-beam irradiation was not found to improve any of the KPIs of the DWW significantly in the previous section. However, precipitation was observed to start occurring shortly after irradiation exposure as shown in Figure 5.4. The precipitate appeared black and settled with gravity.

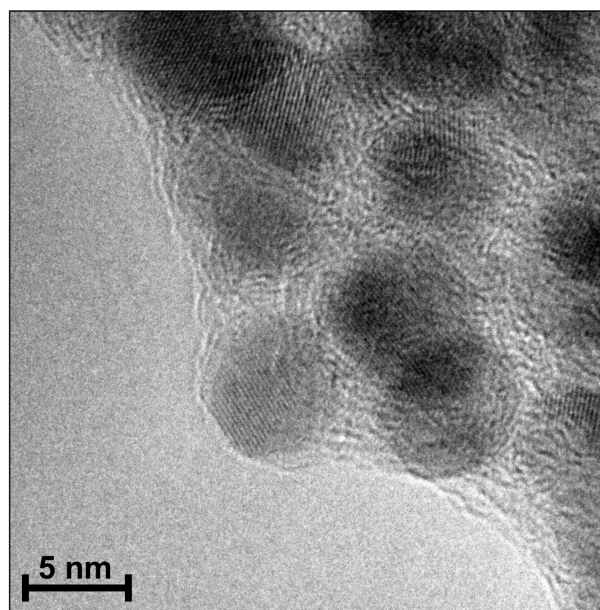
TEM images of the precipitate at different magnifications are shown in Figure 5.5. The structure of the precipitate consisted of nano-crystals, which had formed lattices suggesting a metallic structure.



(a)



(b)



(c)

Figure 5.5: TEM images of precipitate at different magnifications.

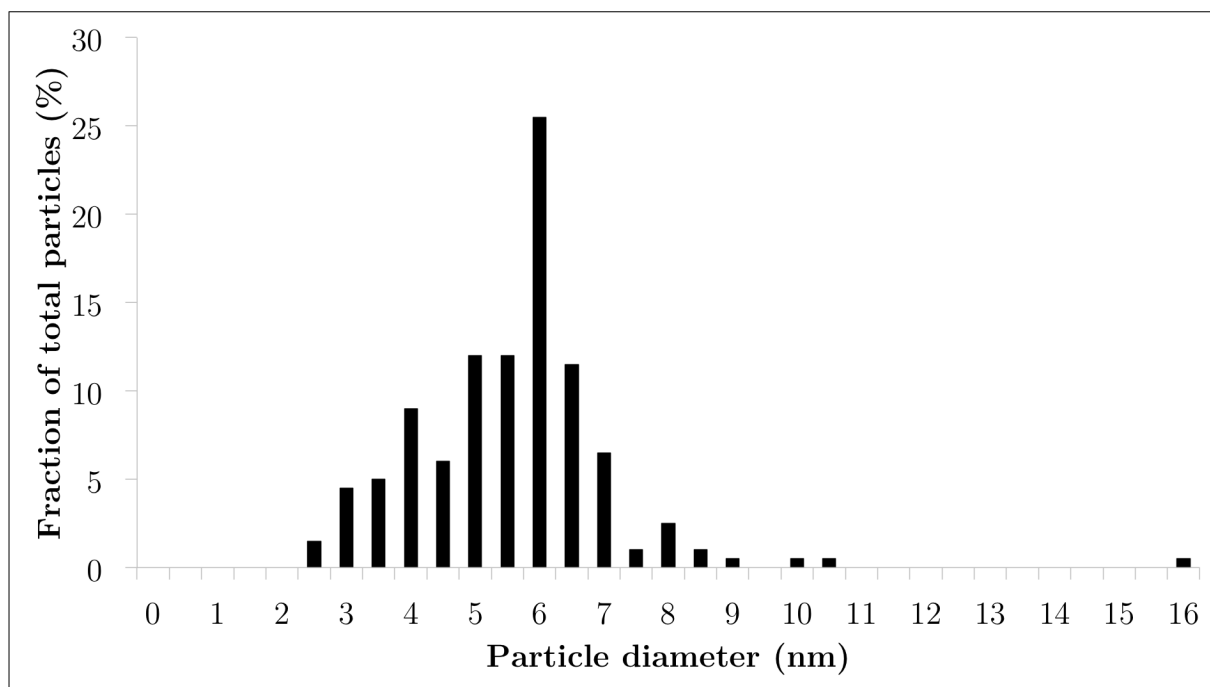


Figure 5.6: Size distribution of precipitate by visual inspection of TEM ($n = 200$).

The size distribution of the precipitate was determined by inspecting 200 particles from the TEM image in Figure 5.5b and the results are shown in Figure 5.6. The observed precipitates were nanoscale particles.

EDX analysis during TEM imaging showed that the sample was mainly composed of copper and sulphur as represented in Figure 5.7. Gold and carbon peaks in the EDX spectrum could be attributed to the substrate and mesh grid onto which the sample was attached during analysis (as described in Chapter 2, Section 2.6), while Indium and Zinc were found to be present in trace concentrations.

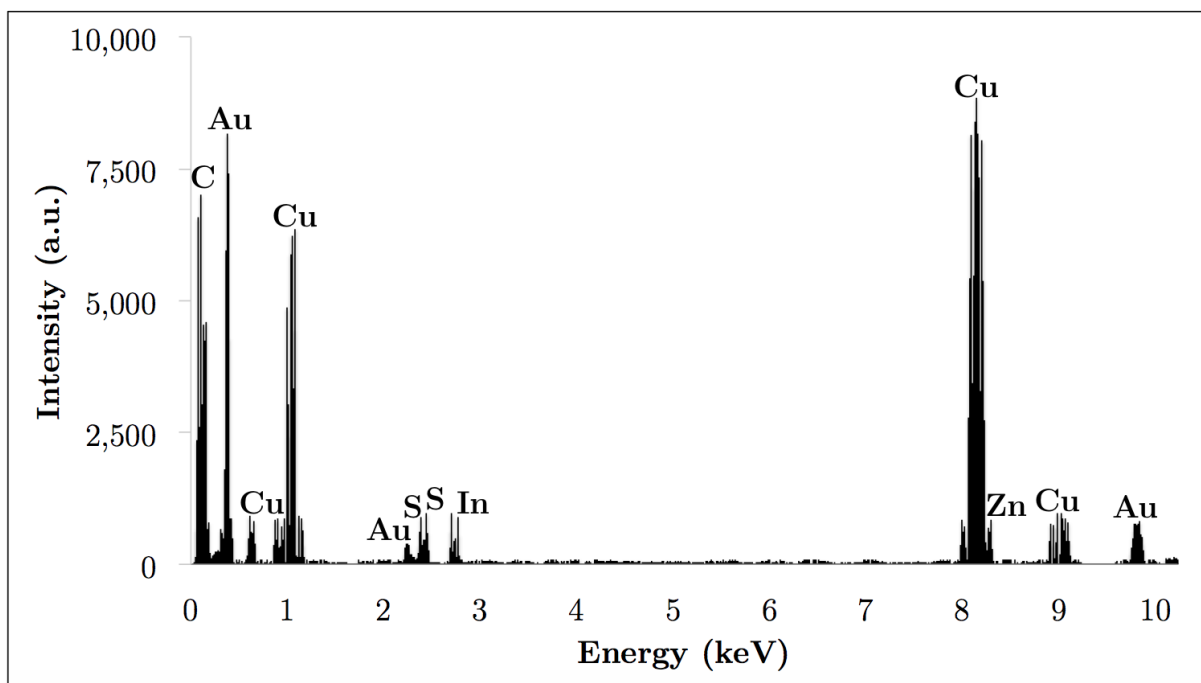


Figure 5.7: EDX Spectrum of TEM in Figure 5.5b.

5.3.2 Mechanism of Removal

The quantitative analysis of the EDX spectra was inconclusive as to whether the copper was copper (I) sulphide (Cu_2S) or copper (II) monosulphide (CuS). The precipitate was found to be insoluble in the treated DWW, DI water and H_2SO_4 , which demonstrated that the precipitate was CuS . CuS is soluble in H_2SO_4 as opposed to Cu_2S , which is insoluble in H_2SO_4 . A common production method for CuS is based on bubbling hydrogen sulphide gas through Cu (II) salt solutions, which results in a black colloidal precipitate of CuS (Greenwood 1997) with the sulphide forming as follows:



The DWW did not contain any significant quantities of sulphide as determined by sulphide cuvette tests, but was found to contain $109.1 \pm 4.0 \text{ mg L}^{-1}$ sulphate. The reduction of sulphate to sulphide was thought to occur as follows:

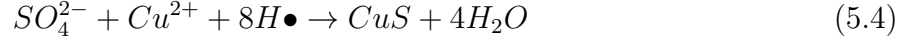


It is well established that sulphate can be reduced to sulphide and variants of this same reaction occur in the biological reduction of sulphate, where sulphate acts as the electron donor during anaerobic respiration (Grein et al. 2013). In lieu of sulphate reducing organisms reducing the sulphate, the radiolysis of water during e-beam irradiation provided the required hydrogen radicals for sulphate reduction in this study. The produced sulphide then combined with some of the copper in solution:



At no stage was a sulphuric smell detected, suggesting the sulphide was short-lived and did not result in any detectable hydrogen sulphide formation. The copper was present as soluble copper in the raw DWW, as dissociated copper salt, as simply increasing the pH of the solution also led to copper removal, by the formation of a blue precipitate, which is a characteristic colour for copper salt precipitates. Combining the above equations enabled

copper removal and sulphate removal to be linked:



As the stoichiometric coefficients (n_i in kmol) of sulphate and copper are both equal to one, their corresponding stoichiometric mass ratio can be determined from their molar masses (M_i in kg kmol⁻¹) and linked to their masses (m_i in kg) and concentrations (c_i measured in mg L⁻¹ in this study) as volumes are constant:

$$n_{Cu^{2+}} = n_{SO_4^{2-}} = \frac{m_{SO_4^{2-}}}{M_{SO_4^{2-}}} = \frac{m_{Cu^{2+}}}{M_{Cu^{2+}}} \quad (5.5)$$

$$\frac{m_{SO_4^{2-}}}{m_{Cu^{2+}}} = \frac{c_{SO_4^{2-}}}{c_{Cu^{2+}}} = \frac{M_{SO_4^{2-}}}{M_{Cu^{2+}}} = \frac{96 \text{ kg kmol}^{-1}}{63.5 \text{ kg kmol}^{-1}} = 1.5 \quad (5.6)$$

In other words, if the above equations represent the main mechanism of CuS formation, the mass of sulphate removed should be proportional to the mass of copper removed by a proportionality constant of 1.5, i.e. their concentrations expressed in mg L⁻¹ should be proportional.

5.3.3 Removal Efficiency

Copper sulphide removal was quantified by evaluating copper removal using AAS and sulphate removal using cuvette tests. Copper removal efficiency increased at higher doses, achieving a 54.6 ± 3.0 % removal at 50 kGy reducing the copper concentration from $63.6 \pm$

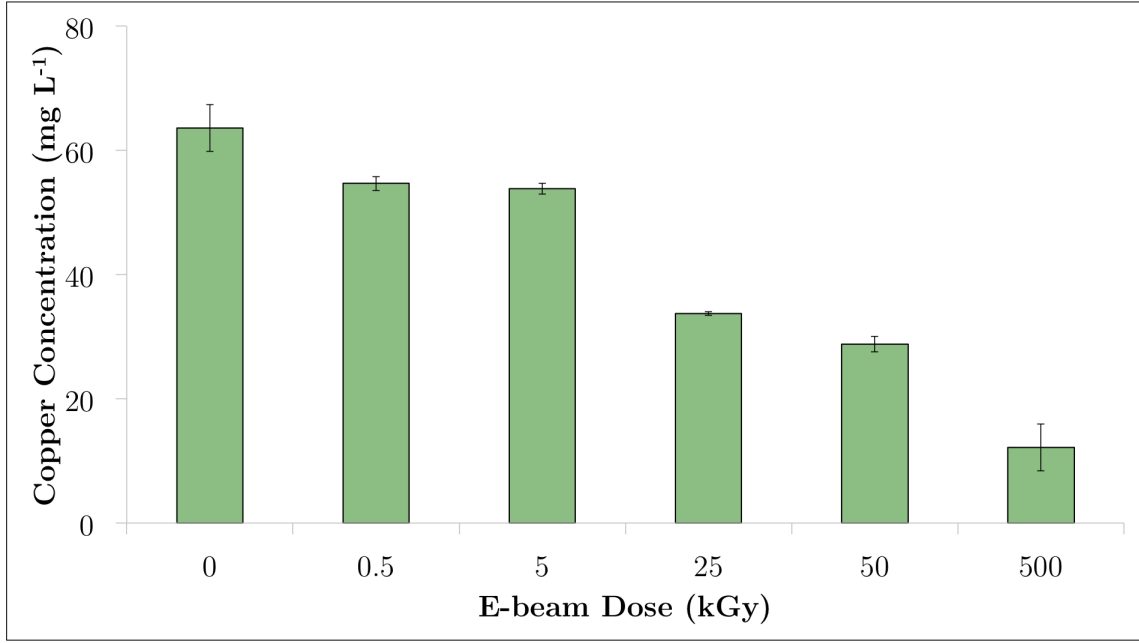


Figure 5.8: Copper concentrations after increasing e-beam dose. Mean is shown and error bars represent standard deviation of triplicate measurements ($n = 3$).

3.8 mg L⁻¹ to 28.8 ± 1.2 mg L⁻¹ as shown in Figure 5.8.

At 50 kGy, sulphate removal was equal to 40.9 ± 4.2 % reducing sulphate concentration from 109.1 ± 4.0 mg L⁻¹ to 64.4 ± 3.8 mg L⁻¹. At 50 kGy, the proportionality constant between sulphate and copper was thus equal to:

$$\frac{\Delta c_{SO_4^{2-}}}{\Delta c_{Cu^{2+}}} = \frac{109.1 - 64.4}{63.6 - 28.8} = 1.3 \quad (5.7)$$

Figure 5.9 shows the sulphate and copper removal values for all doses tested. The regression line equation is given below with R^2 representing the coefficient of determination:

$$c_{SO_4^{2-}} = 1.6c_{Cu^{2+}} + 8.3 \quad R^2 = 0.95 \quad (5.8)$$

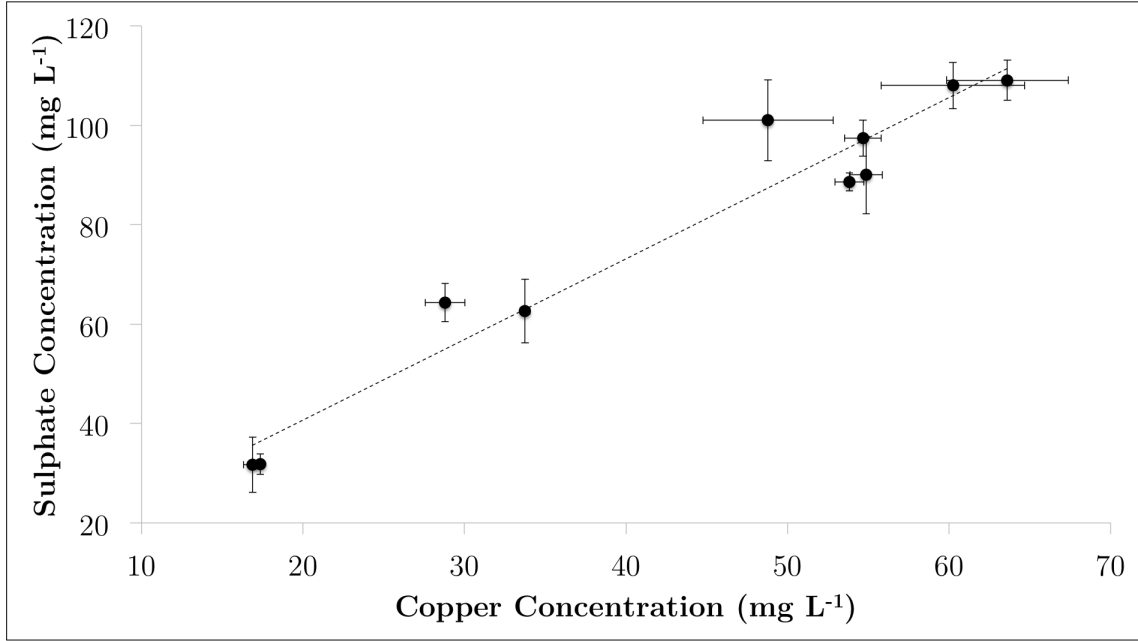


Figure 5.9: Sulphate concentration as a function of copper concentration. Each data point represents a single dose tested. Mean is shown and error bars represent standard deviation of triplicate measurements ($n = 3$). The dashed line represents the linear regression line for this data set and is given by equation 5.8.

The linear regression analysis yielded a constant of proportionality of 1.6, which would mean that to reduce the copper concentration by 1 mg L^{-1} , 1.6 mg L^{-1} of sulphate was required. However, although the relation between copper and sulphate removal was found to be linear, the effect of increasing e-beam dose on their removal was found to be an exponential relationship as shown in Figure 5.10. The equations below for both regression lines with their corresponding R^2 values can be used to predict the sulphate and copper concentrations expected at a given e-beam irradiation dose D (in kGy), valid for $D \geq 0.1 \text{ kGy}$:

$$c_{Cu^{2+}} = -5.2 \ln(D) + 51 \quad R^2 = 0.86 \quad (5.9)$$

$$c_{SO_4^{2-}} = -9.3 \ln(D) + 92 \quad R^2 = 0.94 \quad (5.10)$$

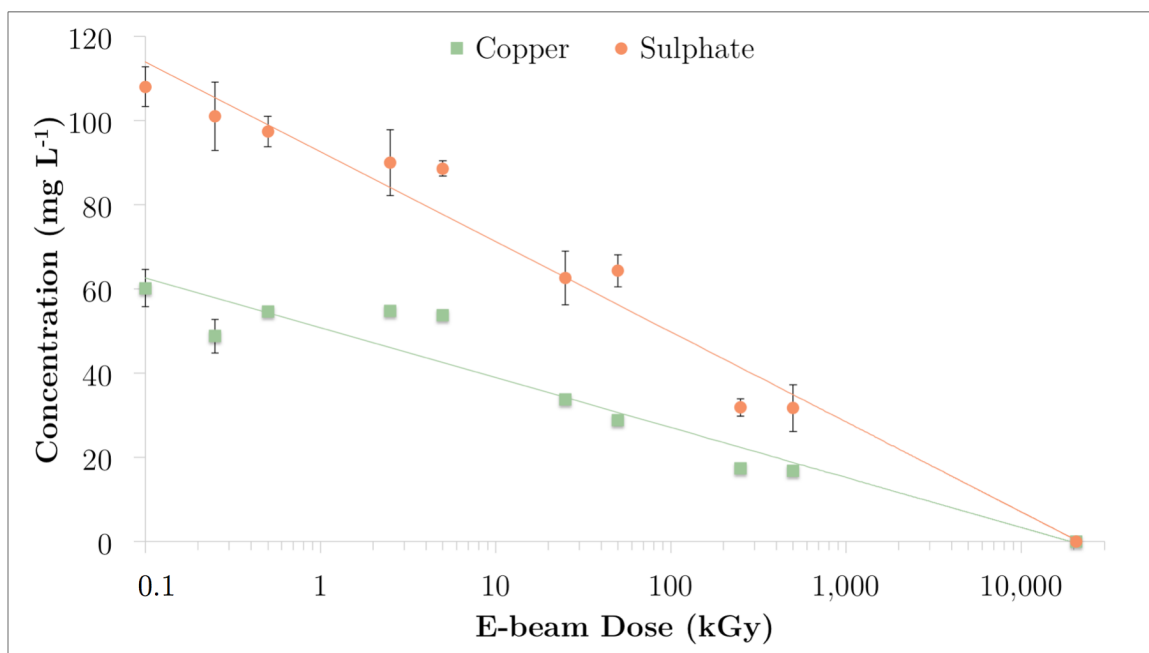


Figure 5.10: Copper and sulphate concentrations at different e-beam doses (on a log-scale). Mean is shown and error bars represent standard deviation of triplicate measurements ($n = 3$). The linear regression lines for the copper and sulphate data sets are given by equations 5.9 and 5.10 respectively.

Both these formulae predict, that the dose required to remove all the copper and sulphate from the system using the e-beam would be approximately e^{10} kGy, which is clearly unsustainably large. They also indicate that both copper and sulphate were present in the untreated DWW at nearly ideal stoichiometric ratios. The total mass of the precipitate at 50 kGy, obtained per 3 mL sample (corresponding to the cuvette volume subjected to the e-beam), was calculated from the reduction in copper concentration and equal to 157 ± 18 μg . This corresponded to a CuS removal of 52.3 ± 6.0 g m^{-3} . However, copper monosulphide precipitation from the system did not translate into any toxicity improvements, suggesting the main source of toxicity of this effluent was not the copper as initially hypothesised.

KPI	Acute Toxicity	PER	Respiration	PRR	Copper	COD
Weighting Factor	1	1	1	1	2	1
Change (%)	69.6	99.0	22.4	21.4	54.6	19.5

Table 5.2: Strategic selection of KPIs, assigned weighting factors and percentage changes of DWW after optimal hybrid treatment.

5.3.4 Remediation Index

The Remediation Index (RI) was established in Chapter 2, Section 2.5.2, Equation 2.7. The top treatment priorities for the remediation of the DWW were to reduce its toxicity, recover its copper and to reduce its COD. The KPIs include a copper-KPI, corresponding to the 54.6 % removal efficiency reported at an e-beam dose of 50 kGy in the previous section. As sulphate removal was related to copper removal, it was not considered as a separate KPI, but the copper-KPI was simply given a weighting factor of two. The corresponding KPI weightings are presented in Table 5.2. The tap water control had a RI of 83.3 % for this selection of KPIs. The RI of the DWW was 58.7 % after sequential e-beam and NZVI treatment.

5.4 Disinfection Potential of the E-Beam

In addition to resource recovery, the e-beam has the potential to achieve other aims typically required in industrial wastewater treatment such as the disinfection of the final effluent after bioprocessing. E-beam treatment is being widely employed as a sterilising method in food production and sterilisation of medical equipment but has seen limited use in industrial

wastewater disinfection applications (Parejo Calvo et al. 2012). The aim of this section was to study the effect of irradiating a single species, the *E.coli* HB101 (pUCD607) biosensor, and a mixed community inoculum taken from the mature reactors. The effectiveness of irradiation for the purposes of disinfection was evaluated using plate count studies and using flow cytometry.

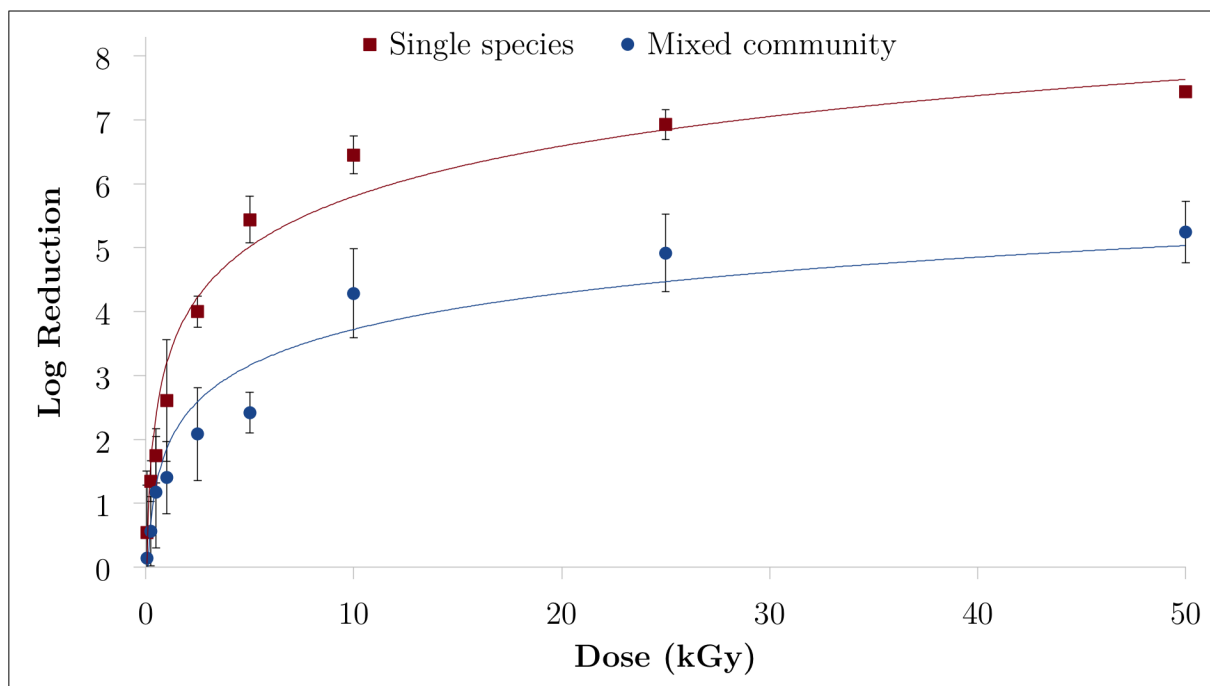
Figure 5.11a shows the log reductions obtained for the single species and the mixed community after e-beam irradiation at increasing dose as evaluated by plate count studies. A selection of plates is shown in Figure 5.11. A dose of 50 kGy achieved a 7-log reduction for the single species, which was found to be statistically higher than the 5-log reduction observed for the mixed community ($p = 0.0003$).

The equations for both regression lines with their corresponding R^2 values are given below and can be used to predict the expected log reduction for both inocula at a given e-beam irradiation dose D (in kGy), valid for $D \geq 0.1$ kGy:

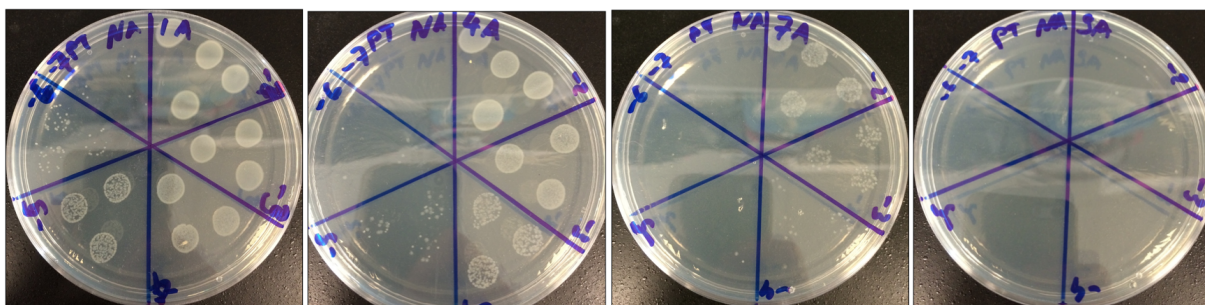
$$\text{Log Reduction}_{\text{Single Species}} = 1.1 \ln(D) + 3.2 \quad R^2 = 0.96 \quad (5.11)$$

$$\text{Log Reduction}_{\text{Mixed Community}} = 0.8 \ln(D) + 1.8 \quad R^2 = 0.93 \quad (5.12)$$

The irradiated samples were also analysed using the flow cytometer. Figure 5.12 shows the live / moribund / dead bacterial cell fractions at increasing doses for both single species and mixed community inocula. The flow cytometry results indicated that the cell viability after e-beam irradiation was higher than compared to what plate count studies suggested. For



(a)



(b)

(c)

(d)

(e)

Figure 5.11: (a) Log reduction in culturable cell counts (measured in CFUs per mL) for disinfection studies on single species and mixed community as a function of e-beam irradiation dose during plate count studies. Mean is shown and error bars represent the standard deviations of triplicate measurements ($n = 3$). The regression lines for the single species and mixed community data sets are given by equations 5.11 and 5.12 respectively. Images of the plates corresponding to irradiation doses of (b) 0 kGy (c) 0.5 kGy (d) 2.5 kGy and (e) 50 kGy for the single species.

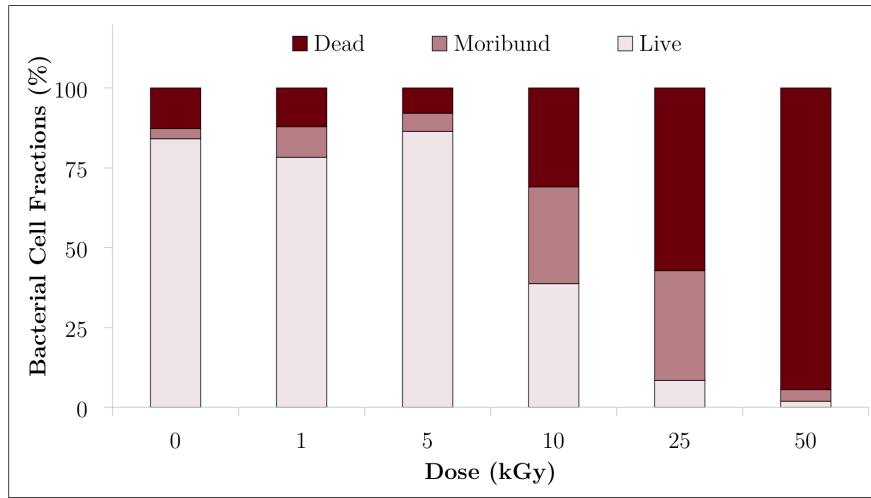
example, at 10 kGy, the mixed inoculum was found to be reduced by 50 % as measured by the flow cytometer compared to the 4.3 ± 0.5 order reduction in plate counts at the same dose. At 50 kGy, 3.7 % of the mixed community inoculum was determined to be culturable, whereas the Miles and Misra plate culture method suggested a reduction of the order 5.2 ± 0.3 , i.e. a 3-order difference in results. Similar results were observed for the single species inoculum.

5.5 Lab-Scale and Industrial-Scale Hybrid System Economics

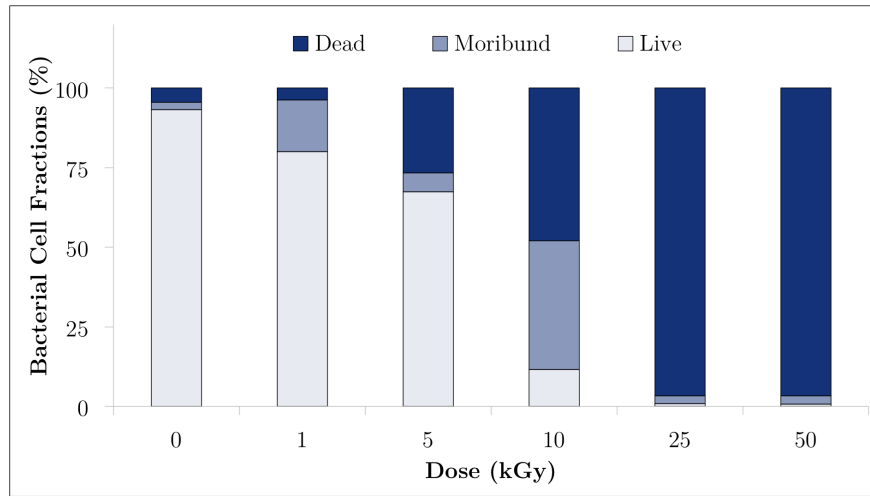
In this section, the objective was to examine the scalability of the laboratory set-up and specifically to investigate its potential to be exploited at industrial-scale. This included a comparison between the lab-scale and a large-scale hypothetical implementation of the hybrid bioprocessing, NZVI and e-beam system, which was then compared to current hybrid treatment options involving an e-beam to determine under which conditions this process would be economical.

5.5.1 Utility Cost Estimates

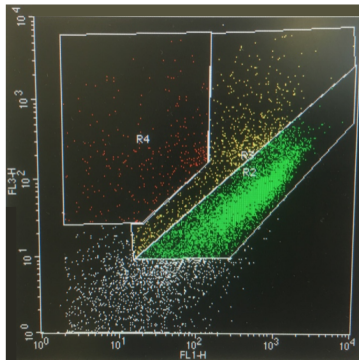
The cost estimations were done with a target volume of one cubic meter to be treated for a standardised hybrid treatment of bioprocessing including 1-day of aerobic biological treatment, NZVI treatment at a concentration of 1 g L^{-1} and e-beam treatment at a dose of 50 kGy. The estimations were undertaken for a simple linear scale-up of the experimental



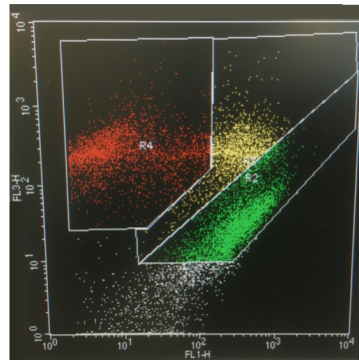
(a)



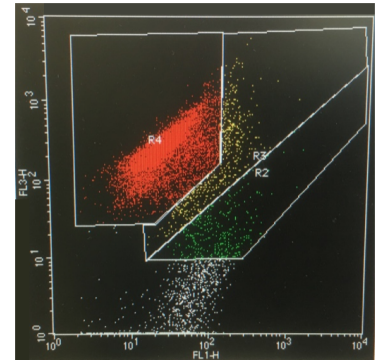
(b)



(c)



(d)



(e)

Figure 5.12: Percentage live / moribund / dead bacterial cell fractions after increasing doses of irradiation for the (a) single species and (b) mixed community inocula. The gated regions used for flow cytometry for the mixed community inoculum are shown at doses of (c) 0 kGy (d) 10 kGy and (e) 50 kGy.

	Electricity (£ kWh^{-1})	Water (£ m^{-3})	DI Water (£ m^{-3})
Cost	0.05	1.09	1.12

Table 5.3: Industrial utility cost estimates.

system used throughout this study as described in Chapter 2, not taking into account any potential economies of scale. The utility costs, as shown in Table 5.3, were taken from current market data (Ofgem 2015; Thames Water 2016) and the DI water cost determined from the power requirements of the Milli-Q® water purification system.

5.5.2 Costs of Bioprocessing

Bio-Blok® matrix cost in the region of £250 per cubic meter capacity and a plastic water storage tank of one cubic meter capacity, serving as a reactor, could be obtained for £85⁵. The air requirement for an aerobic bioreactor would be 400 L min⁻¹ requiring approximately 400 W pumping power (linear scaling of air pump described in Chapter 2 Section 2.8). Aerobic 1-day biotreatment per cubic meter of wastewater would thus cost £0.48 in electricity and £0.98 for the 900 L water typically required for ten-fold dilution of the wastewater amounting to a total cost of £1.46 per cubic meter treated. Any costs associated with anaerobic treatment were considered to be negligible. On an industrial scale, bioprocessing would likely benefit from economies of scale as larger reactors and pumps could be used. However, industrial-scale reactors might also require heating elements to keep the reactors situated outside at optimum temperature for bioprocessing. Insulation, extensive piping, pumping and associated operation and maintenance costs would also need to be considered

⁵<https://www.alibaba.com> (accessed on 7 March 2017)

	Vacuum Pump	Sonicator Probe	Magnetic Stirrer
Power Consumption (W)	100	80	8.5
NZVI Mass (g)	50	1	1
Time (h)	48	0.083	1
Specific Energy (kWh kg ⁻¹)	96	6.7	8.5

Table 5.4: Lab-scale electrical cost breakdown estimation.

and are taken into account in Section 5.5.4 of this chapter.

5.5.3 Costs of Nanoscale Zero-Valent Iron Treatment

The main cost for NZVI production derives from the electrical costs associated with activating and dispersing the NZVI in the industrial effluents (Table 5.4) and the chemicals used during the synthesis of the NZVI (Table 5.5). The costs associated with the recovery of the NZVI using magnetic separation were assumed to be negligible.

The peristaltic vacuum pump employed in this study had a power rating of 100 W and dried 50 g NZVI each 48 h period. A mass of 1 g of NZVI was sonicated each 5 min and subsequently left to react under magnetic stirring for 1 h under the experimental conditions of this study. Total energy consumed was therefore estimated at 111.2 kWh per kg NZVI synthesised. This corresponded to a unit cost of £5.56 per kg NZVI employed.

As shown in Table 5.5^{6 7}, chemical costs amounted to £435.42 per kg for NZVI synthesised in the lab. On a lab-scale, the estimated total cost to synthesise and employ one kg of NZVI amounted to a staggering £440.98. From the above analysis, it became clear that on a lab-

⁶<https://www.alibaba.com> (accessed on 7 March 2017)

⁷<https://www.fishersci.co.uk/gb/en/home.html> (accessed on 7 March 2017)

	FeSO₄	NaBH₄	HNO₃	Ethanol	Acetone	NaOH	H₂O
Required (kg or L)	2.5	1	1	12	12	0.2	36
Lab Cost (£ unit ⁻¹)	35.8	232	16	7.5	3.16	23.6	1.12
Lab Total (£)	89.5	232	16	90	3.16	4.72	0.04
Bulk Cost (£ unit ⁻¹)	0.07	0.83	0.17	0.09	0.05	0.38	1.12
Bulk Total (£)	0.18	0.83	0.17	1.08	0.60	0.08	0.04

Table 5.5: Individual chemicals cost per kg NZVI synthesised in the lab compared to bulk scale cost.

scale 98.8 % of the total cost associated with the NZVI produced in the lab could be attributed to the chemicals required during synthesis. Purchasing chemicals in bulk would bring this cost down significantly to £2.98 per kg NZVI synthesised. It was also hypothesised that using larger pumps, sonicators and stirrers would cut the total utility cost required during NZVI employment in half due to economies of scale resulting in a new unit cost of £2.28 per kg. On an industrial scale, the estimated total cost to synthesise and employ one kg of NZVI amounted to £5.76. Any attempts at further reducing this cost would focus on reusing the NZVI, attempting to use alternative, cheaper synthesis methods as well as investigating novel ways of employing the NZVI.

5.5.4 Start-Up Costs of E-Beam

The two main cost elements of an e-beam are its installation and purchasing cost as well as its operating costs. Section 5.5.4 contains an estimation of what typical start-up costs for an e-beam system might be. Section 5.5.5 contains an estimation of the operating costs associated with e-beam treatment at the dose found to be typically effective in this study (50 kGy) so as to determine a total cost for e-beam treatment.

	Han (2012)	Sampa (2013)	Gehring (1998)	Kurucz (1995)
E-beam Cost (£m)	1.84	0.94	1.21	1.5
Overall Cost (£m)	3.68	2.05	2.23	3.03
E-beam Power (kW)	400	37.5	75	75
E-beam Dose (kGy)	1	6	2.7	4
Capacity (m ³ year ⁻¹)	3m	25k	220k	210k
Cost Factor	245.3	235.0	152.8	133.9

Table 5.6: Price comparisons between different e-beams employed in industrial effluent treatment.

Table 5.6 shows the breakdown of current operational industrial-scale e-beam treatment systems, with costs adjusted by an average yearly inflation of 2% to reflect 2017 prices in GBP using an exchange rate of 1.2 GBP to the USD (US 2017).

The cost factor in Table 5.6 was calculated based on the assumption that optimal treatment delivers the highest dose to the highest volume of effluent treatment (capacity) at the lowest cost and lowest power. According to these criteria, an average cost factor of a custom-made industrial-scale e-beam can thus be determined:

$$Cost\ Factor = \frac{E - beam\ Cost \cdot Power}{Dose \cdot Capacity} \quad (5.13)$$

The average cost factor for the e-beam systems presented in Table 5.6 was calculated to be 191.8. The e-beam cost to deliver a dose of 50 kGy at a flow rate of 50,000 m³ year⁻¹ for a 300 kW rated e-beam (identical power rating as e-beam employed in this study) would amount to £1.60m by this calculation. The choice of flow rate was arbitrary and belonged to the lower end of the spectrum of e-beams typically employed (Pikaev 2000).

Overall cost of the system would include water-handling equipment, cooling, pumping and

	% DEC	Cost (£m)
Direct Costs		
Purchase and delivery of e-beam	100	1.60
Installation of e-beam including shielding and safety features	40	0.64
Instrumentation and controls (installed)	30	0.48
Pumping system, piping and insulation (installed)	60	0.96
Electrical including safety features (installed)	10	0.16
Land, building and service facilities (purchased and installed)	50	0.80
Total Direct Plant Costs	290	4.64
Indirect Costs		
Engineering and supervision	33	0.53
Construction Expenses	30	0.48
Total Indirect Plant Costs	63	1.01
Total Direct and Indirect Plant Costs (TDIC)	353	5.65
Contractor's Fee (5 % of TDIC)	17.5	0.28
Contingency (10 % of TDIC)	35	0.56
Fixed Capital Investment (FCI)	405.5	6.49
Working Capital (15 % of FCI)	61	0.97
Total Capital Investment	466.5	7.46

Table 5.7: Estimation of total capital investment for hypothetical industrial-scale e-beam plant.

safety equipment as well as more general facilities, which would need to be taken into account.

In Table 5.7, the estimated fixed capital investment cost of an industrial-scale implementation of this e-beam was determined. The fixed capital investment consisted of direct costs, indirect costs a contractor's fee, a contingency and working capital as identified in Table 5.7. The cost analysis was performed using the delivered equipment cost method (Peters et al. 2003) with individual percentages estimated for this particular application.

This estimated total cost corresponding to the total capital investment required was estimated at £7.46m. This was approximately twice as high as the second most expensive e-beam, notably due to the high e-beam dose requirement. Cost estimates in previous studies

were potentially underestimates as overall cost was determined to typically be approximately double the cost of the initial e-beam purchase and delivery costs. The total fixed capital investment cost determined in Table 5.7 also has to be considered with caution as costs vary widely depending on the sources of equipment, their price fluctuations, government policies, the exchange rate, local regulations and the percentage DEC assumptions made.

5.5.5 E-beam Treatment Operating Costs

To estimate power consumption during e-beam treatment, the inner functioning of the e-beam, as described in Section 2.10.2 was revisited, and the key power-consuming components were identified as the modulator and the magnetron. The aim was to determine an overall efficiency, which relates the total electrical energy input to the process (estimated in kWh) to effective radiant energy delivered to a cubic meter of sample (determined by Fricke Dosimetry as described in Section 2.10.1).

The e-beam generated 6 MeV electrons delivered at an average current of 50 mA corresponding to an average power of:

$$6 \text{ MeV} \cdot 50 \text{ mA} = 300 \text{ kW} \quad (5.14)$$

Power was delivered to the samples in 4 μ s pulses, corresponding to a total energy delivered per pulse of:

$$300 \text{ kW} \cdot 4 \mu\text{s} = 1.2 \text{ J} \quad (5.15)$$

Using Fricke Dosimetry, each pulse was found to deliver 50 Gy to each 3 mL sample. Assuming

a density equal to water of 1 kg L^{-1} for a hypothetical industrial effluent, 50 Gy was delivered to each 3 g of sample. As $1 \text{ Gy} = 1 \text{ J kg}^{-1}$, 50 Gy per 3 g corresponds to an energy delivered to each sample per pulse of:

$$50 \text{ Gy} \cdot 0.003 \text{ kg} = 0.15 \text{ J} \quad (5.16)$$

This resulted in an irradiation efficiency of:

$$\eta_{\text{Irradiation}} = \frac{0.15}{1.2} = 12.5 \% \quad (5.17)$$

Efficiencies for the modulator and the magnetron were estimated to be 96 % (Nam et al. 1993) and 85 % (Wang and Adolphsen 2013) respectively. Cavity efficiency was estimated from Figure 5.13 as 88 %, which was equal to the difference between forward and reflected power. The overall efficiency estimate was evaluated as the product of the main component efficiencies:

$$\eta_{\text{Overall}} = \eta_{\text{Modulator}} \cdot \eta_{\text{Magnetron}} \cdot \eta_{\text{Cavity}} \cdot \eta_{\text{Irradiation}} = 0.96 \cdot 0.85 \cdot 0.88 \cdot 0.125 = 9\% \quad (5.18)$$

The total energy required to irradiate a cubic meter (i.e. 1,000 kg) wastewater at 50 kGy would amount to:

$$\text{Energy per cubic meter} = \frac{50 \text{ kGy}}{0.09} \cdot 1000 \text{ kg} = 556 \text{ MJ} = 154 \text{ kWh} \quad (5.19)$$

Using the wholesale electricity cost given in Table 5.3, the operating cost per cubic meter

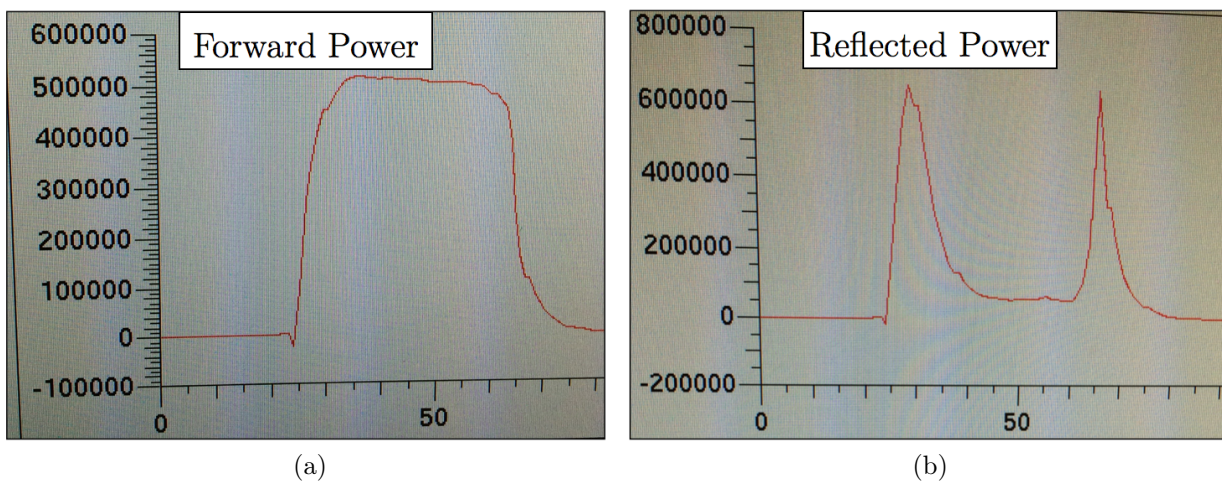


Figure 5.13: (a) Forward, i.e. transmitted and (b) reflected e-beam power in the cavity.

industrial effluent treated at an e-beam dose of 50 kGy in this study was thus equal to £7.72.

5.5.6 Continuous Flow Set-Up of E-Beam

Industrial-scale e-beam arrangements for environmental remediation applications typically consist of lower energy, scanning e-beams with the effluents being run over a weir in a continuous flow set-up (Han et al. 2012; Kurucz et al. 1995; Sampa et al. 1998). This study did not have access to a scanning e-beam but did attempt to investigate the effect of a continuous flow set-up with recirculation as shown in Figure 5.14. The continuous flow set-up was tested for all four industrial effluents investigated in this study and allowed for the treatment of approximately five times larger volumes at equivalent doses to achieve comparable improvements in KPIs. The efficiency was therefore found to increase approximately five-fold in this set-up bringing down the cost of £7.72 calculated in the previous section to £1.55 per cubic meter treated at an e-beam dose of 50 kGy in a continuous flow set-up.

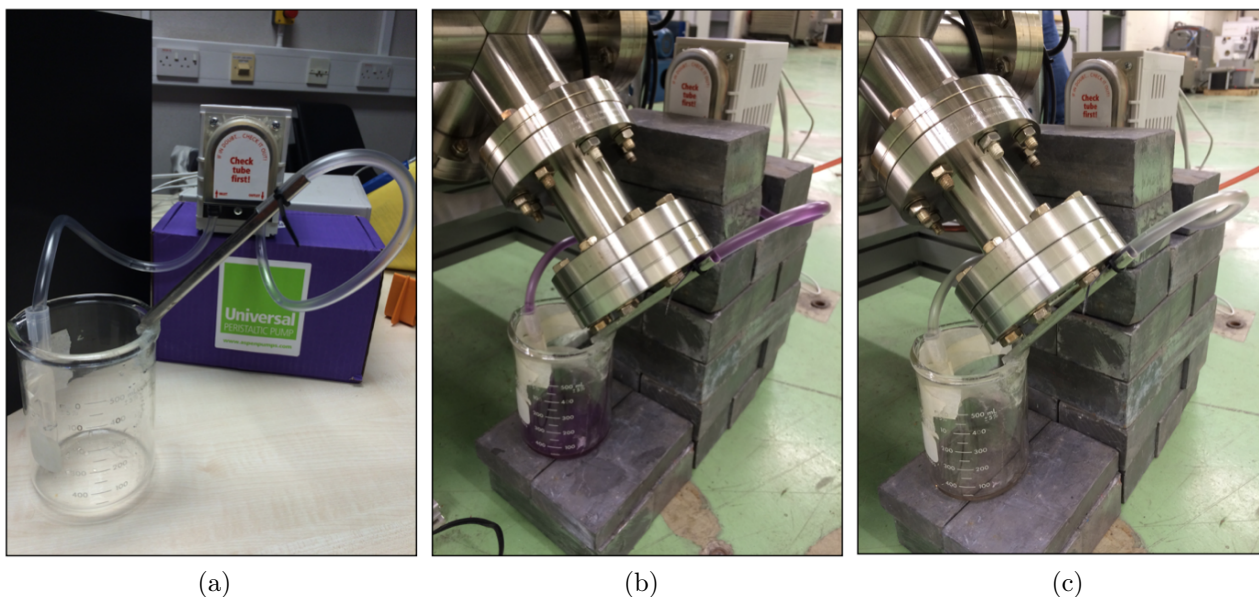


Figure 5.14: (a) Continuous flow set-up showing TDW (b) before and (c) after e-beam irradiation.

For industrial large-scale applications, the e-beam would also be custom-built for the specific treatment task rather than consist of a repurposed medical diagnostic e-beam. Not only would this ensure that a much larger fraction of the high-energy electrons generated would be delivered to the sample but it would further increase e-beam efficiency. The e-beam set-up itself would also be optimisable, which could lead to higher volumes being able to be treated.

5.5.7 Overall Profitability of Hypothetical Industrial-Scale Hybrid System

To determine an overall price per cubic meter at which a hybrid system would be profitable for the design specifications given in Section 5.5.4, a discounted cash flow analysis was undertaken. It was assumed that the fixed capital investments required for bioprocessing and NZVI treatment were negligible compared to those associated with the e-beam. The operating cost

per cubic meter of the hybrid treatment including 1-day aerobic bioprocessing (£1.46), a NZVI concentration of 1 g L⁻¹ (£5.76) and an e-beam dose of 50 kGy (£1.55) amounted to £8.77.

For the discounted cash flow analysis, the construction and operation periods were assumed to be 2 and 30 years respectively, with 30 % of the capital invested in year 1 and 70 % in year 2 and the working capital purchased at the end of year 2. A sum-of-the-years-digit depreciation method was used for 10 years, starting in year 3, assuming a salvage value of 10 % of fixed capital investment, taking into account the decommissioning costs. It was also assumed that the working capital would be recovered at the end of year 32. The tax rate was assumed to be 15 %, and inflation at 2 % applied to all streams in the discounted flow calculation. The results of the discounted cash flow analysis are shown in Figure 5.15 and relate the price per cubic meter to the internal rate of return of the project as follows:

$$\text{Price per cubic meter} = 0.03 \cdot IRR^2 + 1.04 \cdot IRR + 11.08 \quad R^2 = 0.999 \quad (5.20)$$

The internal rate of return (IRR) or discounted cash flow rate represents the discount rate that would make the net present value zero. For an IRR of 10 %, this would result in a price per cubic meter effluent treated of £24.90.

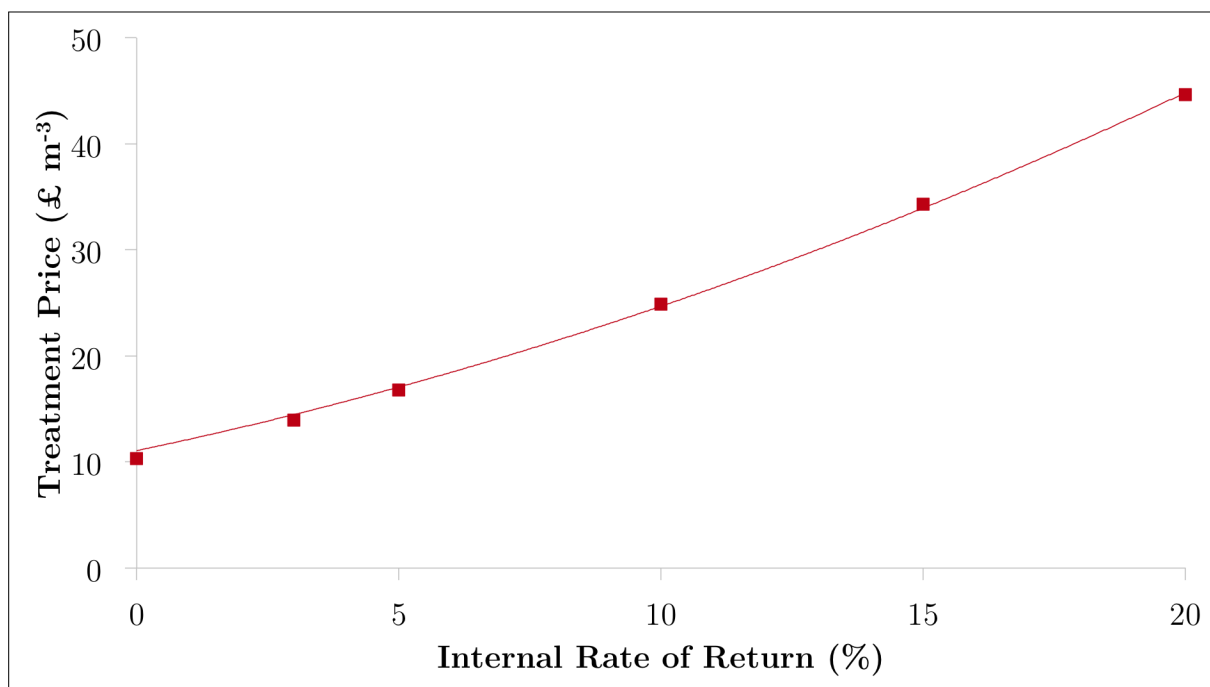


Figure 5.15: Total treatment price per cubic meter as a function of internal rate of return of industrial plant. Quadratic line of best fit is shown and is given by equation 5.20.

5.6 Assessment of Remediation

Two key results were obtained for the DWW experiments. Firstly, NZVI treatment effectively detoxified some of the DWW. It was hypothesised that this was due to NZVI's ability to remediate some of the DWW's toxic and inhibiting contaminants. Although sequential E-beam and NZVI treatment did not show a synergistic effect in terms of plate count numbers, it was found that NZVI treatment after e-beam treatment resulted in the detoxification of the sample. This further strengthened the result obtained in Chapter 3, Section 3.6, where NZVI treatment resulted in both red eukaryotic cells and white prokaryotic cells persisting after exposure compared to only prokaryotic cells surviving after being exposed to untreated or e-beam pre-treated effluent.

Secondly, resource recovery using the e-beam was achieved in the form of nanoscale copper (II) sulphide precipitates. This was the first study reporting this mechanism for copper recovery from an industrial effluent. Mechanisms for this novel process were postulated and the copper recovery was quantified and related to sulphate removal. Increasing the pH of the solution also resulted in copper precipitation, but in a different form, postulated to be copper sulphate. The degree of alkalinity was shown to affect the mechanism of copper removal. Although not evaluated in this study, the buffering potential of the DWW could have been investigated. Buffers are solutions that are able to resist changes in pH (Hem 1959). When there are too many H^+ radicals, a buffer will absorb some of them resisting any reduction in pH (Good et al. 1966). Similarly for an excess of OH^- , buffers will donate some H^+ to reduce the pH. On top of that pH can be a factor in controlling metal toxicity. However, changes down to pH=1 are often considered acceptable in toxicity studies, which may result in considerable biases (de Schamphelaere et al. 2004).

The best way to quantify copper sulphide removal consisted in evaluating copper and sulphate removal efficiencies using AAS and cuvette tests. Another option could have been to measure the weight of the dried precipitate to get a mass of CuS and then infer stoichiometric Cu^{2+} and SO_4^{2-} concentrations. However, this was considered less practical due to the small mass of precipitate generated during e-beam irradiation, which was subsequently quantified as $157 \pm 18 \mu g$ per 3 mL sample at 50 kGy. This would have required precise scales for an accurate measurement.

Experimentally, the constant of proportionality between sulphate and copper was found to

be 1.6, which was in good agreement with the theoretical constant of proportionality of 1.5. The difference could stem partly from the fact that some sulphate could have been removed during irradiation by some other removal mechanism, e.g. mineralisation or precipitation with zinc or indium in the form of metal sulphides, as zinc and indium were both present at trace concentrations in the precipitate.

The e-beam has been shown to be a viable and competitive option compared to UV or ozonation systems for disinfection purposes for municipal effluents (K.-M. Lee et al. 2012). In previous studies on e-beam irradiation on municipal effluents, bacterial viability has been determined by plate count studies, reporting 3- to 5-log reductions in total coliform plate counts at doses varying between 3 and 5 kGy (Borrely et al. 1998; Farooq et al. 1993). However, plate count studies only indicate culturable cell types rather than allowing for the assessment of cell viability. Flow cytometry has been shown to be a valid approach for the determination of cell viability in environmental samples (Pianetti et al. 2008). Flow cytometry has never before been reported to evaluate the disinfection potential of e-beam irradiation but it has been applied to evaluate the inactivation efficiency of *E.coli* during ozonation of a municipal wastewater effluent (Y. Lee et al. 2016).

This study compared both methods for the disinfection potential of the e-beam. The e-beam demonstrated significant potential for the disinfection of industrial effluents, achieving a 5-log and 7-log plate count reduction at 50 kGy for a mixed community and single species inoculum respectively. The flow cytometry assay confirmed that e-beam irradiation significantly reduced live cell counts, but suggested a lower 2-log reduction in culturable cells at the same

dose for both inocula. The significantly greater culturability observed during flow cytometry suggested that plate count studies might underestimate the viability of cells after irradiation and that a proportion of the cells were either sub-lethally injured or were persistent cells in a dormant state. Although plate count studies suggested that a mixed community was more vulnerable to irradiation than a single species inoculum, this was not confirmed by flow cytometry studies. The observed differences in viability between both methods raised doubts concerning the reliability of Miles and Misra plate count studies as the sole indicator for assessing the disinfection potential of e-beam irradiation.

The actual energy and cost requirements for the hybrid system employed were analysed and contrasted with a hypothetical industrial-scale implementation of this hybrid process. The cost was found to be above current cost per cubic meter industrial effluent treated, which is typically an order of magnitude less for a hybrid treatment system.

6 Discussion

The aim of this chapter is to discuss the main findings of this study and relate them to the field of industrial effluent treatment. This chapter also considers the scope for improvements in the hybrid treatment system in the context of future work and concludes with recommendations for prospective implementations of novel hybrid processes in a sustainable fashion.

6.1 A Novel Hybrid Treatment System for the Remediation of Industrial Effluents

In this section, the key challenges for industrial effluent treatment are briefly reviewed. This section proceeds to show how the study objectives have been met by discussing the main findings of this study while highlighting the contribution of this research in the wider context of sustainable remediation technologies for dealing with recalcitrant waste streams.

6.1.1 Current Industrial Effluent Treatment Challenges

A growing population and industry will in the future add to increasing pressures on existing water treatment systems (UN 2014). Many industrial processes will continue to produce large quantities of toxic industrial effluents making their effective treatment directly relevant to ecological health and economic stability. Current treatment technologies such as reverse osmosis and ultrafiltration are effective, but have undesirable process economics and environmental footprints (Petrinic et al. 2015). Even though the current legislative framework

regarding industrial effluents is getting more stringent, e.g. many biocides are being phased out under the EU biocides directive (EU 2012), and industry is being forced to rethink their waste handling systems under the REACH regulation (EU 2006), significant challenges remain. Some industries are rethinking their processes and are designing “greener” biodegradable industrial fluids to minimise their environmental impact post-employment (Massawe and Geiser 2012; Rhee 2002). However, even a swift hypothetical reversal of current practices would still leave large quantities of untreated industrial wastewaters to be processed. Thus, innovative wastewater treatment technologies will be required for the foreseeable future.

6.1.2 Restatement of Research Aims and Objectives

The studies described in this thesis set out to assess the impact and effectiveness of a novel hybrid treatment system on the remediation of three classes of chemically complex and recalcitrant industrial effluents. To adequately assess the hybrid system’s effectiveness, complementary key performance indicators were amalgamated into a remediation index. Various sequential and simultaneous combinations of bioprocessing, nanoscale zero-valent iron (NZVI) and e-beam treatment were investigated for each of the three industrial effluents. The different technologies composing the hybrid system were found to be complementary to varying degrees in terms of their remediation effectiveness, showing great promise and potential to be employed for a wide range of environmental remediation applications.

6.1.3 Metalworking Fluid Results

The studies reported in Chapter 3 demonstrated that bioprocessing, NZVI and e-beam treatment could be successfully applied as a novel hybrid treatment process, achieving synergistic 92.8 ± 1.4 % COD and 92.2 ± 2.8 % OD reductions for a pristine MWF and 68.4 ± 1.6 % COD and 76.3 ± 3.0 % OD reductions for an exhausted MWF. The improvements in COD removal were mainly attributable to bioprocessing, which after optimisation achieved 85.9 ± 3.4 % and 63.0 ± 7.1 % COD removals respectively of the pristine and exhausted MWFs. Even though the COD of the exhausted MWF was not significantly improved during hybrid treatment when compared to bioprocessing, the standard deviation had decreased from 7.1 % to 1.6 % indicating a hybrid system yielded a more reliable remediation.

NZVI treatment in isolation was effective at both OD and COD reduction. However, its most notable effect was that NZVI reduced the toxicity of the MWF more so than bioprocessing. A similar reduction in toxicity has previously been reported for a hybrid NZVI-bioprocessing study for the remediation of a recalcitrant MWF employing a different biosensor (Jagadevan et al. 2012). In contrast, e-beam treatment did not improve COD or OD removal, but was effective at further reducing overall toxicity. E-beam treatment achieved a 92.7 ± 3.1 % reduction in acute toxicity of the pristine MWF at 50 kGy compared to an acute toxicity reduction of 38.1 ± 1.0 % for bioprocessing and 66.4 ± 2.4 % for NZVI treatment at 10 g L^{-1} . This was particularly striking, as the reduction in acute toxicity during e-beam treatment occurred in two stages, with an unexpected calcium carbonate precipitation accompanying the second stage.

6.1.4 Textile Dyeing Wastewater Results

Work reported in Chapter 4 revealed that the hybrid treatment process designed in Chapter 3 could be applied to a different class of industrial effluents: a textile dyeing wastewater (TDW), which was chemically distinct to the MWF. Although virtually all the known physicochemical and biological techniques have been explored for the remediation of dye wastewaters (Hai et al. 2007), this was the first study reporting that hybrid sequential bioprocessing, NZVI and e-beam treatment improved the COD, OD, sulphate and nitrogen contents of a TDW. The optimal bioprocessing treatment mode identified was 1-day of aerobic incubation, followed by 28-day anaerobic and 1-day aerobic treatment with 0.1 % glucose addition.

Individually, bioprocessing was effective at reducing the concentration of sulphate and total nitrogen, whilst NZVI improved COD removal and e-beam treatment reduced overall toxicity. Together, all three techniques improved OD to different degrees. However, they were required to be employed in combination to improve all of the KPIs simultaneously. Additionally, sequential hybrid treatment synergistically reduced the NZVI concentration required to 1 g L⁻¹ from 10 g L⁻¹.

The order of most effective treatment sequence was difficult to predict and waste stream specific. While e-beam and NZVI treatment were initially thought to make the recalcitrant fraction more available for the MWF, it was found that e-beam pre-treatment was actually the most effective option. For the TDW, which was not readily biodegradable, the best sequence position for bioprocessing was as a first step, as that improved nitrogen, sulphate removal and initiated a reduction in optical density.

6.1.5 Distillery Wastewater Results

In Chapter 5, bioprocessing was found to be ineffective at treating a distillery wastewater (DWW) even in a hybrid sequential set-up, despite the fact that biological treatment has previously been proven to be effective for the treatment of some distillery wastewaters (Pant and Adholeya 2007). NZVI treatment on the other hand reduced COD removal by 19.5 ± 4.0 % at a concentration of 10 g L^{-1} and mitigated toxicity most effectively. Similar to the results obtained from the treatment MWF and TDW treatment, e-beam treatment did not improve COD removal for this wastewater at a dose of 50 kGy.

6.1.6 Resource Recovery

Chapter 5 also revealed that e-beam treatment accomplished resource recovery in the form of copper (II) sulphide from a distillery wastewater, achieving 54.6 ± 3.0 % copper removal at a dose of 50 kGy, corresponding to a $52.3 \pm 6.0 \text{ g CuS}$ removal from each cubic meter of DWW. This is of particular importance as the focus in wastewater treatment has shifted from simple treatment of effluents to the regeneration of valuable fractions from waste (Wang et al. 2015). In a framework of resource depletion around the globe, wastewaters are being treated as reusable resources for a wide range of purposes and any effective resource recovery obtained during novel effluent treatment processes is desirable and improves their footprint and sustainability (European Commission 2012).

6.1.7 Disinfection Potential

Chapter 5 also demonstrated significant potential for the disinfection of industrial effluents using e-beam irradiation after bioprocessing, achieving a 5-log plate count reduction for a mixed community and a 2-log reduction in culturable cells as assayed by a flow cytometer at 50 kGy. This level of kill was significant as it is a legal requirement for sewer discharge consent and so may be widely applicable (HSE 2002). Viability, as determined by plate count studies, was comparable to 3- to 5-log reductions reported in previous studies using e-beam irradiation for disinfection, albeit at lower doses between 3 and 5 kGy (Borrely et al. 1998; Farooq et al. 1993). The differing results from the flow cytometry study suggested that widely-accepted plate count studies may underestimate the viability of mixed consortia after disinfection and not be as reliable an indicator as previously thought.

6.1.8 Overall Process Economics

Finally, the cost of a large-scale industrial implementation was investigated in Chapter 5. To achieve an IRR of 10 %, the cost per cubic meter would amount to £24.90 for a financially viable hybrid bioprocessing, NZVI and e-beam treatment system. This is above the current cost per cubic meter industrial effluent treated, which is typically an order of magnitude less for existing hybrid treatment systems containing an e-beam (Han et al. 2012; Kurucz et al. 1995; Sampa et al. 2003).

6.1.9 Contribution

The results describe for the first time a hybrid treatment process, combining bioprocessing, nanoscale zero-valent and e-beam treatment, which is synergistic and effective for the remediation of three distinct, chemically complex recalcitrant industrial effluents. Previous studies on hybrid treatment studies have combined bioprocessing with either NZVI (Kim et al. 2011), e-beam (Sun et al. 2016) or other AOPs such as Fenton's reagent (Zimbron and Reardon 2011) in isolation. Moreover, they typically only investigated the effect of new hybrid systems on a particular class of wastewaters rather than considering their effectiveness across a range of different streams. Metalworking fluids were chosen as they have been proven to be susceptible to biodegradation by the particular consortium employed (van der Gast et al. 2004) and were shown to benefit from being integrated with hybrid treatment techniques (Jagadevan et al. 2011). Effluents from the textile dyeing and distillery industries are some of the most challenging wastewaters to remediate and served as benchmark waste streams for the novel hybrid process designed in this study (Hai et al. 2007; Nataraj et al. 2006).

The main findings of these studies were that hybrid sequential e-beam, bioprocessing, NZVI, bioprocessing led to the synergistic detoxification of two metalworking fluid streams, whereas for optimal remediation of a textile dyeing wastewater, sequential bioprocessing, NZVI and e-beam treatment was required. In addition, the toxicity of a distillery wastewater could be reduced by NZVI treatment, while e-beam treatment of the DWW resulted in the precipitation of copper (II) sulphide, which was a novel way to recover copper from an industrial effluent. E-beam treatment was also found to be effective at disinfecting final effluents.

6.2 Innovative Remediation Strategies

This section reviews the merits of the various strategies employed to adequately assess remediation effectiveness and optimise the hybrid system successfully.

6.2.1 E-beam and Nanoscale Zero-Valent Iron for Detoxification

It was interesting to note that there was no single treatment that was solely effective at detoxification. As previously reported, NZVI treatment consistently reduced CODs (Jagadevan et al. 2012), while e-beam treatment, which had not before been applied to a MWF, did not affect the CODs of any effluents tested. NZVI was particularly effective at detoxifying both the MWF and DWW, whereas the e-beam was found to be best for acute toxicity reduction of the MWF and all toxicity KPIs in the TDW. For the DWW, e-beam treatment did not improve toxicity at all, generating no growth on plates after e-beam treatment. However, growth could be restored after treating the sample with NZVI, suggesting that the mechanisms of action were potentially different for both methods. The main mechanisms of action were therefore hypothesised to be primarily adsorption for the NZVI treatment and radiolytical transformations during e-beam treatment, which was consistent with what has been reported in literature (Pikaev 2000, Scott et al. 2011).

LC analysis comparing the effect of NZVI and e-beam treatment on two components in the TDW supported this hypothesis. Whereas changes in NZVI concentration led to broad-spectrum reduction of the two peaks, e-beam irradiation appeared to break the peaks down

into smaller molecules, corresponding to a range of small peaks in its spectrum (Figure 4.12). This was further supported by the optical density spectra of the TDW after irradiation. Both e-beam treatment and NZVI reduced the main peak corresponding to the chromophore of the TDW (Figures 4.8 and 4.10). However, inspection of the lower end of the spectrum showed subtle differences. While e-beam treatment broke down some of the molecules displaying peaks in the UV spectrum, NZVI treatment did not change the overall shape of the OD curve, but simply reduced its intensity or amplitude, indicating that a broad-spectrum adsorbance phenomenon was observed. Considering the increased reduction in optical density between 1 g L^{-1} and 10 g L^{-1} without accompanying COD reductions however suggested that some of the chromophore was broken down by the NZVI.

Some reports in literature indicate that NZVI has many mechanisms of action, e.g. adsorption, precipitation, co-precipitation and different types of chemical reduction that are generally surface mediated (Crane and Scott 2012; Noubactep 2008). The findings of this study suggested that the main mechanisms of NZVI treatment were initial dominant adsorption accompanied by surface-mediated reactions, which remained active at concentrations up to 10 g L^{-1} . This cut-off concentration, beyond which NZVI treatment was ineffective, was comparable to a concentration of 5 g L^{-1} reported elsewhere (Jagadevan et al. 2012). Repeated removal and re-addition of fresh NZVI did not further improve performance and a NZVI-saturated steady state appeared to have been reached. This suggested that the remaining pollutants showed no susceptibility to further adsorption or surface reductions.

6.2.2 E-beam Pre-Treatment Strategy

A TDW has been previously reported to benefit from e-beam pre-treatment, as some of the larger organics were radiolytically converted to smaller more bioavailable organics during e-beam irradiation, improving subsequent bioprocessing (Han et al. 2012). One of the most surprising and useful findings was that the e-beam had its role in pre-treatment of a different class of effluents: MWFs. Bioprocessing was improved three-fold after e-beam pre-treatment: greater COD removal was achieved at a faster reaction time (Cheng et al. 2005; Connolly et al. 2006; van der Gast et al. 2004) and bioprocessing could occur at high COD, thus reducing dilution requirements. Even though the higher concentration treatment was possible, it came however at the expense of COD removal and a slower reaction time. This was the first study which demonstrated that e-beam pre-treatment of MWFs allowed the operator to freely balance these three factors (COD, dilution requirement, residence time) during bioprocessing and choose whichever would be adequate and most cost effective, with the option to be reconfigured rapidly if required.

6.2.3 Hybrid System Benefits

The results also highlighted that high e-beam irradiation doses combined with high concentrations of NZVI were effective for treating a concentrated form of the TDW, once again reducing dilution requirements. However, this was considered to be less practical for this waste stream and required order increases of both NZVI concentration and e-beam dose.

A two order increase in energy input would be hard to justify for a one order decrease in dilution requirements.

The required NZVI concentration was synergistically reduced in a sequential e-beam, NZVI and bioprocessing hybrid system. Thus, the NZVI concentration, e-beam dose and dilution requirements can be configured according to the treatment aims. E-beam irradiation was also found to disperse NZVI particles enabling them to remain in suspension much longer, as reported before (Zhang et al. 2013). Although not investigated, the improved suspension could potentially lower the NZVI concentrations during hybrid treatment.

6.2.4 Review of Remediation Index Design

Few, if any, studies have considered employing a hybrid system on a range of industrial effluents while comparing its relative treatment effectiveness for different classes of wastewaters, each presenting a different challenge in terms of remediation. In order to successfully incorporate a biological phase into a hybrid treatment system, it is essential to demonstrate that the effluent's toxicity has been mitigated and its biologically inimical chemicals have been remediated and reduced to an appropriate level for end-of-pipe discharge into the environment or onto a subsequent tertiary treatment system (HSE 2002).

Strategies to achieve an effective assessment of remediation in this study consisted of designing a novel multi-faceted remediation index to allow for a holistic quantitative evaluation of the level of decontamination achieved. There is a built-in flexibility into this way of assessing

remediation even though it may not give a definite identification of the mechanisms that drive toxicity.

A variety of water quality indices have been previously designed, such as the wastewater polishing index in the context of reclaimed wastewater in Italy, combining COD, BOD, nitrogen, suspended solids, phosphate and *E.coli* plate counts (Verlicchi et al. 2011). Moreover, the comprehensive pollution index, which combines eleven physicochemical parameters, is being used for severely polluted river waters in India (Mishra et al. 2016). In the field of wastewater treatment, indices rarely integrate chemical methods with biological methods. In soil remediation on the contrary, multiparametric soil quality indices, which combine various chemical and biological parameters, are commonly employed (Andrews et al. 2002; Bastida et al. 2008; Dawson et al. 2007). Similarly, this study's remediation index further developed hybrid bio-chemical indices and aimed to amalgamate suitable chemical and biological indicators into a proprietary remediation index to assess treatment effectiveness.

The remediation index (RI) designed in this study incorporated a range of key performance indicators (KPIs). The KPIs chosen were a selection of well-established chemical analytical methods (such as COD, TOC, pH, nitrogen and sulphate concentrations and measurement of optical density) and a combination of four biological assays that gave insight into the overall toxicity changes of an industrial effluent during treatment taking into account targeted resource recovery.

Acute toxicity was evaluated using a metabolic *E.coli* HB101 (pUCD607) biosensor and post-exposure cell count recovery, whilst the general toxicity was evaluated using respiration

Industrial Effluent	Pristine MWF	Exhausted MWF	TDW	DWW
Remediation Index	71.9 %	56.0 %	75.2 %	58.7 %

Table 6.1: Remediation indices for industrial effluents investigated in this study.

and post-respiration cell count recovery (PRR). Respiration and PRR have previously been combined to measure metabolic inhibition of biofilms and assess the toxicity of individual organics and metals (Adapa et al. 2016). Table 6.1 shows the RIs for the industrial effluents investigated in this study. According to the RI designed in this study, the TDW was most effectively remediated with an RI of 75.2 % followed by the pristine MWF, which had an RI of 71.9 %.

The RI captured the basic dimensions of the remediation achieved using the hybrid treatment system proposed and acted as a proxy for decontamination effectiveness. It was not a comprehensive assessment tool, but could be used during initial screening studies to identify the scope for any novel or existing treatment and potentially become a benchmark indicator for assessing environmental remediation in the area of industrial effluent treatment. In that sense it is not as narrow as existing measures, such as the Mogden formula (OFWAT 2017), which only considers suspended solids and COD as KPIs. The RI designed in this study is a much broader measure informing about the wider aspects of detoxification and decontamination.

However, the RI has limited scope and was not intended to replace widely employed formulas such as the Mogden formula. Rather, it should be viewed as a complement to existing, useful indicators and integrated into current trade effluent regulations to limit ecological and

environmental consequences of the release of treated industrial effluents. For example, on top of any existing requirements, a remediation index of at least 60 % (arbitrary) could be enforced to deem industrial effluents sufficiently decontaminated. This would imply that the exhausted MWF and DWW streams having RIs of 56 % and 58.7 % respectively would require further treatment before discharge.

Moreover, in its current set-up, standard deviations of the individual KPIs were not taken into account during the calculation of the RI. Alternatively, the standard deviations of the KPIs could be incorporated into the calculation of the RI. This would result in the RI being given with lower and upper bounds resulting in a remediation range.

Another limitation of the way that the RI was employed in this study was that the toxicity-KPIs were normalised with respect to the individual effluents (centre points on radar graphs as first described in Figure 2.3). To allow for better cross-effluent comparisons in KPIs, a common standard toxic reference stream could have been included on all radar graphs. In addition, any index always runs the risk of being subject to arbitrary selections of indicators to skew its meaning. In this study, the choice was made transparent and justified with respect to the different remediation aims of the different streams.

6.2.5 Plate Counts as a Qualitative Remediation Indicator

It was revealed that if there were no significant changes in toxicity KPIs, simple and widely-accepted Miles and Misra plate count studies (Miles et al. 1938) could further help assess the degree of detoxification. For the operationally exhausted MWF, a colony count study

demonstrated that e-beam pre-treatment resulted in a higher number of colony-forming units (CFUs) forming than in the absence of pre-treatment. It also revealed that e-beam treatment selectively killed a eukaryotic yeast population and resulted in only prokaryotic cells surviving; an interesting result, which has not previously been reported. Even though NZVI treatment led to lower total plate counts, it allowed for both yeast and bacterial cells to survive. It further confirmed the ability of NZVI treatment to improve the conditions such that both eukaryotic and prokaryotic cells were able to survive, which was found for both the exhausted MWF and the DWW.

During the disinfection experiments, plate counts were also found to be potentially misleading, as the flow cytometer indicated that more cells were viable than the plate counts suggested. They may not have been culturable, indicating that the 5-log respective 7-log reductions predicted by the plate count experiments ought to be considered cautiously.

6.3 Critical Review

6.3.1 Critical Review on Experimental Techniques Used to Measure Toxicity

Acute toxicity was measured using a biosensor in this study. Traditionally, toxicity is measured by exposing organisms from higher trophic levels, e.g. crustaceans daphnia magna or animal bioassays using fish or mice (Guilhermino et al. 2000). Biosensors-based technologies avoid the ethical issues arising from exposing higher trophic level organisms to test for toxicity and there is a drive to move away from animal-based bioassays. Another alterna-

tive would have been to evaluate toxicity by using liquid chromatography coupled with mass spectroscopy (LC-MS), which has been optimised for many toxin groups in the past (Vilarino et al. 2009). A whole-cell biosensor was chosen over traditional analytical techniques such as LC-MS, due to advantages in cost, number of samples processed and higher relevance for complex mixed industrial effluents, in which toxicity related to specific groups of toxins might have been difficult to evaluate. The toxicity key performance indicators post-exposure recovery (PER) and post-respiration recovery (PRR) were evaluated using Miles and Misra plate count studies (Miles et al. 1938). Plate count studies were also contrasted using flow cytometry, which is able to differentiate between culturable and viable fractions of bacterial cells and those results were contrasted. The main alternatives to traditional plate count studies and flow cytometry are molecular based assays, which often display improved sensitivity (FDA 2006) vis-à-vis plate counts. However, they may also run the risk of a higher rate of false positives. Data obtained from biological experiments is inherently variable and plate counts studies remain one of the best interpretations of the number of viable cells present in environmental samples and were justified to be ubiquitously employed throughout this study (Sutton 2011).

6.3.2 Experimental Design and Statistics

Throughout this study, it was assumed, but not verified, that data collected as part of this work followed a normal distribution, which allowed parametric student's t-tests to be applied. Results from biological experiments typically fit the normal distribution well due

to the central limit theorem, which states that the means of a large number of random data points are approximately normally distributed (Glass et al. 1972). A more rigorous approach would have been to validate the assumption of normality by computing appropriate goodness of fit for each data set. For example, Shapiro-Wilks or Anderson-Darling tests could have been employed (Stephens 1974).

Moreover, due to the particular hybrid treatment system chosen, only the bioprocessing experiments yielded independent replicate sets of data. The NZVI batch manufacturing process as well as the fact that the same e-beam was used throughout this study for each set of experiments meant that the results from the NZVI and e-beam experiments consisted of pseudo-replicates. On the radar graphs, the control was tap water. Alternatives for this negative control would have been to use either deionised or bottled water to get more consistent results as it is rigorously controlled. However, due to its industrial relevance tap water was preferred. Moreover there is no consistent positive control (i.e. very toxic, lethal) on these graphs, as the industrial effluents were assumed to be toxic enough to serve as their own positive controls and improvements in key performance indicators with respect to their original effluent were chosen as benchmarks.

6.3.3 Study Limitations and their Effect on the Validity of the Findings

There were several limitations to this study. Work focussed on three industrial effluents, which were diverse in terms of chemical composition. The hybrid system was found to be effective to varying degrees indicating that it may not work on other effluents and on oc-

casion require further processing. The chosen KPIs that were combined into a proprietary remediation index captured a large variety of toxicity aspects and were informative to undertake cross-effluent comparisons for this study. A full toxicity analysis would require further method development, for example the use of biosensors from several trophic levels.

Moreover, the cost estimation was largely hypothetical, which might have resulted in somewhat arbitrary projections. However, as all the relevant assumptions were given, this could readily be adjusted and adopted by an industrial partner intending to commercialise this idea. Another limitation of this study was that it was not possible to analyse the full extent of the chemical reactions. This was not considered in detail as industrial effluents are mixtures of various compounds and blends of proprietary products and a thorough understanding of the chemistry would have been resource intensive to obtain for potentially limited additional insight. For example, the original parent compounds in the exhausted MWF would have degraded with use leading to undefined compositions, rendering chemical analysis ineffective.

The use of a residual gas analyser coupled with liquid chromatography and mass spectrometry would have enabled a more comprehensive analysis of mechanisms occurring during e-beam irradiation and identify recoverable resources, recalcitrant fractions and radical scavenging molecules. The identification of these constraints may have elucidated where improvements could be made.

6.3.4 Lessons to be learned from Dynamic Data Sets

One of the key challenges of industrial effluent treatment is the ever changing nature of the industrial effluents generated, their complex nature and the most effective ways of remediating them. The experimental design in this thesis consisted of an initial choice of three industrial effluents, three proven effluent treatment technologies and a novel remediation assessment tool: the remediation index. Strategies to yield the best hybrid treatment performance were subsequently developed by balancing the classic treatment requirements with a novel remediation process assessment. The dynamic nature of the type of data thus obtained can also be found in many other disciplines. For example, the automotive industry continues to face dynamic challenges, such as globalisation, volatility, shifting marketing conditions and an ever-changing technological landscape in the automotive industry, especially with a recent drive towards electric and smart cars (Albulescu et al. 2015). Not only the automotive industry has to constantly re-adjust its approaches to continue meeting consumer demands, but plenty of other disciplines also require the constant rethinking and redesigning of experimental set-ups to effectively tackle the rapidly changing systems and pertinent issues to be solved.

A field that is generating a lot of interest recently due to its potentially very serious implications for the future health of humans on this planet is that of antimicrobial resistance. Similar to the ever-changing nature of industrial effluents, rapidly changing and evolving antimicrobial resistance due to mutations of bacteria requires clever research strategies and approaches to be overcome. Whereas in this thesis, a strategic selection of key performance indicators

served as proxies for the underlying, often unspecified and poorly understood causes of industrial effluent toxicity; the underlying causes of antimicrobial resistance are not yet fully understood with the overuse of biocides and increasing presence of heavy metals in the environment thought to be contributing to increased antimicrobial resistance (Singer 2017). Recent advances in this field have also come from lateral thinking and novel approaches. For example, as approximately 99% of all species are currently unculturable, several new and innovative methods of cultivation have led to the discovery of the new antibiotic teixobactin (Ling et al. 2015).

Another field, that display ever-changing parameters and moving targets is the field of vaccine development. Flu vaccines are updated and modified every year due to the quick mutations of the influenza virus as well as the human body's declined immune response from vaccination over time⁸. New strategies are needed to effectively tackle this issue of moving targets (Eisenstein 2011). Most vaccines therefore simply look at stimulated response rather than into the detailed mechanisms of action and experimental protocols and approaches have to be adapted constantly (Oyston and Robinson 2012).

Finally, the recent Ebola outbreak in West Africa shows that novel treatment strategies, approaches and multidisciplinary responses are quintessential to effective management of new threats to humanity. For the Ebola outbreak, accelerated vaccine trials have been set up at unprecedented speeds and the response from many experts across fields of science, legislation and public support meant that the outbreak was officially declared over by the

⁸<https://www.cdc.gov/flu/protect/keyfacts.htm> (accessed on 7 July 2017)

World Health Organization in March 2016⁹ (Moll et al. 2016).

Research in the field of vaccine development typically requires experts from a wide array of fields of science, which is becoming increasingly the case for other disciplines (Rappuoli and Medaglini 2014). Even though big data and machine learning is enabling the harnessing of enormous scales of data, it remains paramount to develop innovative strategies to tackle current global challenges.

This thesis has established that the strategic combination of established techniques and novel technologies is a powerful tool for identifying and forecasting system performance, which can give unique insight into intricate problems and this approach may therefore be widely applicable. Nowadays, increasingly complex problems facing humankind will require increasingly complex, innovative and multidisciplinary approaches to be solved effectively.

6.4 Future Outlook

This section contains a discussion on how to improve the scope for each of the three treatment technologies employed in this study. It also identifies the treatment of emerging contaminants as a potential area of application for novel hybrid treatment systems and features a discussion about the importance of social responsibility and sustainability for the design of future industrial fluids and their treatment.

⁹<http://www.who.int/mediacentre/news/statements/2016/ihr-emergency-committee-ebola/en/> (accessed on 7 July 2017)

6.4.1 Scope for Improvement of Bioprocessing

Bioprocessing, employing this study's specific set-up, was faster than previous biological treatment attempts on MWFs using the same consortium, as reported in Section 3.3. It was further improved after e-beam pre-treatment, resulting in shorter residence times and reduced dilution requirements. In the current set-up, carbon and nutrients, in the form of nitrogen and sulphur, were removed from the system either by assimilation and sequestration into biomass or by mineralisation, mainly into CO₂.

Organic nitrogen (e.g. primary, secondary or tertiary amine compounds), found in industrial effluents, can be transformed by microorganisms in the fixed-film bioreactors. Microbes are able to mineralise some of the organic nitrogen (complex nitrogen) into inorganic ammonium, which is readily available to be either taken up by microorganisms and bound as organic nitrogen in their biomass or it can be converted aerobically to nitrate (nitrification), which in turn is denitrified anaerobically into nitrogen gas, thus completing the nitrogen cycle (Moir 2011).

Different types and species of microorganisms have different C:N:P ratios under which they operate optimally, with some having access to larger C:N:P ratios than others (Robertson and Groffman 2015). Microbial C:N:P stoichiometry ratios do affect organic carbon, nitrogen and phosphorus mineralisation (Heuck et al. 2015) and it is therefore directly relevant to proper bioreactor operation.

Microbes are able to convert organic phosphorus from the industrial effluents to orthophos-

phates (H_2PO_4^- , HPO_4^{2-} and PO_4^{3-}), which the microbes can then take up (Sathasivan 2009). Biological phosphorus removal from industrial effluents is typically achieved by phosphorus accumulating organisms, which are able to accumulate it within their cells as polyphosphate (Seviour et al. 2013). In this study, phosphorus concentrations were below detection limits and the breakdown of phosphorus was therefore not further investigated. However, as shown for the TDW, bioreactors were able to process nitrogen once the C:N ratio had been restored, suggesting the N:P ratio remained intact throughout.

However, the scope for diverting some of the carbon and nutrients into more valuable biochemicals remains to be investigated. A study has shown that different conditions in bioreactors could lead to the production of useful bio-precursors to bio-plastics such as polyhydroxyalkanoates (Sakai et al. 2015). Similarly, there is a whole range of other potentially useful products that microorganisms in bioreactors are able to produce, including biofuels (Zhu 2015) and methane, typically formed under anaerobic conditions (Cheng et al. 2016). Producing high value chemicals or chemical precursors in a bioprocess would improve the process economics, providing it was possible to extract these from the reactors.

Moreover, it was observed that the TDW required carbon addition, as it did not contain sufficient bioavailable carbon for bio-treatment. An obvious solution might have been to blend different waste streams prior to treatment to further improve bioprocessing. For example, industrial effluents from dairy plants, paper and pulp industries as well as municipal wastewaters can have high nutrient and carbon contents (Kumar and Pal 2015). However, the current political and legal environment does not yet allow for this, but in a different

future framework, more work should be directed at exploiting this approach.

During this study, biological treatment was performed using a specifically assembled consortium to treat MWFs. Even though this consortium was adaptable to a certain degree to the other two industrial effluents, using more indigenous, acclimatised microorganisms could have improved process performance. It may also be possible to exploit other microbiological species in addition to bacteria, e.g. green algae, which have been shown to be effective for the treatment of a wood-based and paper industry wastewater (Tarlan et al. 2002).

6.4.2 Scope for Improvement of Nanoscale Zero-Valent Iron Treatment

For this study, NZVI was synthesised employing a borohydride reduction method, as this method generated the most reactive NZVI particles and served as a benchmark for a novel hybrid lab-scale study. This method yielded highly reactive NZVI particles of approximately 50-75 nm in diameter and a specific surface area of $17.63 \text{ m}^2 \text{ g}^{-1}$, which was comparable to the 20-100 nm diameter NZVI particles with a specific surface area of $14.8 \text{ m}^2 \text{ g}^{-1}$ reported in a study employing the same synthesis method (Crane et al. 2011).

The limitations of this method are that it is costly in chemical reagents and generates hazardous by-products such as hydrogen gas during NZVI synthesis as shown in Equation 2.8. Any industrial process ought to investigate the scope for alternative greener ways of synthesising NZVI, such as those employing tea polyphenols (Hoag et al. 2009) or clay-supported NZVI (Abbassi et al. 2013). These methods have proven to work to various degrees for both adsorption and removal of different organics, as well as heavy metal ion immobilisation

(Ahmed et al. 2013). Moreover, new ways of synthesising NZVI are being reported as the research area matures (Fazlzadeh et al. 2017; Jamei et al. 2014) and the cost of NZVI is decreasing and is expected to continue to do so (Crane and Scott 2012).

Although in this study it was argued that NZVI was the best type of iron, due to its high reactivity and performance, this assumption could be challenged in terms of balancing cost and performance. Iron is abundant on this planet and if cheap bulk iron, waste iron haematite or other sources of iron became readily available, a comparative optimisation study would inform as to whether NZVI iron could be substituted for a cheaper iron, or even a different nanomaterial altogether, e.g. titanium or mixed alloys, which have shown promise in a variety of applications (El Haggouji et al. 2008; Kharisov et al. 2012).

There is also a question mark over the present method of employment of NZVI, i.e. addition in suspension and magnetic separation and recovery. Novel ways to employ NZVI are constantly being developed. For example, a variety of supports such as carbon nanotubes, activated carbon and silica have been developed to immobilise NZVI (Sheng et al. 2016). Any process, which is designed to reuse, reactivate, regenerate or recycle its NZVI, will improve overall efficiency.

6.4.3 Scope for Improvement of E-beam Treatment

The e-beams employed in this study were not designed with environmental remediation applications in mind and therefore were suboptimal. By employing them in a continuous flow

mode, efficiency could instantly be improved. Hence, at a large-scale purpose-built set-up, their efficiency may be much higher.

Moreover, as the e-beam irradiation process was accompanied by a temperature rise of the sample, post-e-beam effluent could be integrated with a waste heat recovery system to improve efficiency. Waste heat recovery systems have been shown to be effective on hot industrial effluents from the TDW industry (Kandilli and Koclu 2011). An obvious place for synergy would be to use some of that heat to keep the bioreactors at a constant temperature, thereby lowering any bioprocessing heating requirements.

The cost analysis in Chapter 5 has demonstrated that a hybrid system can be established at a realistically applicable cost, especially with further advances in the field, with mobile small-scale e-beams starting to be employed more widely in environmental applications. For example, a mobile e-beam, mounted on the back of a lorry, has recently been introduced for flue gas remediation (Han et al. 2016).

The key considerations for any hybrid system are how they scale up (or down) and whether they should be implemented in a centralised or decentralised set-up. As seen by the differences in set-up required for optimal treatment of three different industrial effluents in this work, it is recommended that any future implementations of this hybrid system should be part of engineered green infrastructures, providing tailor-made solutions for *in situ* treatment.

The results of this study suggested that the e-beam could be used to recover resources from industrial wastewaters. In this work, nanoscale copper (II) sulphide was recovered. In the

cost analysis, the potential sale of the recovered resources was not considered. Copper (II) sulphide, as a well-known p-type semiconductor has a wide range of potential applications in photovoltaic cells and optoelectronic applications (Estrada et al. 2016; Zhao et al. 2009) and new streams of revenue might be generated as part of resource recovery in other industrial effluents. A future investigation into the purity and employability of resources recovered during industrial wastewater treatment would shed light on whether these resources are ready to be used by new industries or whether they require further refining.

6.4.4 Novel Hybrid Systems for the Treatment of Emerging Contaminants

The main subject of this study was the treatment of industrial effluents using a novel hybrid treatment process. This is not the only field that could potentially benefit from a hybrid system such as the one designed in this study. A large variety of emerging contaminants, presenting previously unknown challenges for their remediation, are currently causing concern.

Unprecedented quantities of antibiotics (Llor and Bjerrum 2014), a wide range of pharmaceuticals (Sim et al. 2011) and personal care products (J. Wang and Wang 2016), hormones and endocrine disruptors (Nasirabadi et al. 2016), pesticides (Kock-Schulmeyer et al. 2013), new nano-materials (Ong 2015) and plastics (EPA 2016) are entering the global water systems and exerting disruptive effects across multi trophic levels in the environment at trace-level concentrations (Wilkinson et al. 2015). Another area of concern is that of infectious diseases. Water-borne pathogens, as well as prions are able to transmit infections and often bypass

existing treatment systems (Hinckley et al. 2008; Sozzi et al. 2015), however little is known on how to effectively destroy them.

Conventional treatment processes are not designed to remove emerging contaminants, often resulting in their untreated discharge into the environment (Petrie et al. 2015). Innovative solutions to effluent treatment, which are adaptable to a wide range of contamination scenarios, will be required for the foreseeable future. There is a continuing need to develop novel, effective and robust treatment technologies, which have built-in degrees of flexibility and capacity to be adapted to a myriad of contamination scenarios.

This study further reinforces the recommendation for the introduction of hybrid treatment processes that are tailor-made solutions to specific problems of recalcitrant contaminants. The three methods, albeit not having a unique ideal set-up, could be combined in various sequential combinations to achieve optimal remediation of different industrial effluents.

This multi-sequence complementation offers further flexibility, as small initial screening studies of different industrial effluents would quickly reveal optimal sequential or simultaneous treatment modes, or reveal that this system is not ideal for a particular stream. A threshold in the remediation index, combined with conventional treatment criteria, could for example be used during such a screening.

The built-in flexibility and adaptability, which the hybrid system demonstrates, would also be beneficial in case the waste stream, legal requirements or treatment priorities were to change. This would then solely become an infrastructural, process design consideration. Appropriate designs would allow switching between set-ups quickly and effectively.

6.4.5 Social Responsibility and Sustainability

Every producer of industrial effluents has a social responsibility and duty to ensure adequate treatment. If there is a collective need for mankind to produce certain compounds, which are toxic and cannot or will not be phased out, then there is a collective responsibility to have, or at the very least acquire, the expertise to be able to treat them. If something cannot be treated using conventional (or novel) methods then should we be producing it in the first place?

Policy approaches such as the “Extended Producer Responsibility” mean that increasingly the producer, i.e. polluter, pays for the environmental costs associated with its products (OECD 2001), a cost which is passed on to the consumer. Any increases in cost associated with extensive treatment or penalties will cost the producer more and drive down demand for this particular product and would be a strong incentive to find better alternatives to this pollutant.

The future of sustainable industrial operations will include cradle-to-cradle self-contained water recycling. The design of self-contained, closed-cycle industrial streams, which include *in situ* treatment that satisfy process input requirements could prove to be ground-breaking. Although potentially not possible for each industry, adequate treatment could result in self-contained loops. For example, a textile dyeing wastewater was able to be recycled for a dyeing process after its post-treatment effluent satisfied the process input water quality requirements (Lu et al. 2010).

To take it further, industrial wastewaters could be separated from municipal wastewaters and also from the earth's water cycle, which would contain contamination more effectively and minimise the impact of both types of effluents onto the environment and reduce overall fresh water requirements.

It is already possible today to create a near fully sustainable water cycle as demonstrated by the water recovery system on the International Space Station (Carter 2009) and this technology will be further optimised and taken to Mars (Jones and Kliss 2007) and so this should be easily achievable for the future on Earth.

7 References

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