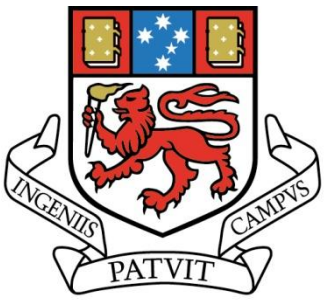
Optimisation of the Norske Skog activated sludge wastewater treatment plant at Boyer: The role of trace metals and vitamins



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Declaration

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Abstract

In January 2008 Norske Skog, Boyer (NSB) , Tasmania, commissioned an activated sludge (AS) wastewater treatment plant to lower the chemical oxygen demand (COD) and suspended solids (SS) in the treated effluent discharged from the mill. In October 2009 the company also changed the feed stock of the mill from a mixed *Pinus radiata* and *Eucalypt* to solely *P. radiata*, altering the pulping process. The cold caustic soda (CCS) plant was decommissioned and replaced with an additional thermo-mechanical pulping (TMP) plant. Trace metals in the mill wastewater samples were analysed before and after this transition to detect any differences due to the changed feedstock and operating conditions, and to determine if metal levels were sufficient for optimum operation of the AS wastewater treatment plant. These analyses indicated deficiencies of cobalt, copper, iron and molybdenum required for optimal theoretical biological growth.

In this thesis the effects of micro-nutrient additions on a number of important parameters were considered by employing porous pots with a capacity of 4.5 L to mimic the operation of the Boyer AS plant. The selected parameters included COD removal, SS level and residual humic fractions. Also determined were the concentrations and potential effects of the trace metals in the effluent and sludge on the abundance of protozoa and filamentous bacteria. This detailed research on the outcomes of additions of trace metals and water-soluble vitamins to wastewater treatment plants had not previously been reported.

Addition of trace metals including calcium, cobalt, copper, iron and magnesium, to wastewater treatment plants were found to significantly increase COD removal by 4 to 5%. At NSB the incorporation of magnesium oxide to the wastewater treatment process significantly increased the COD removal by 6%, this result indicated that the porous pot results were transferable to an industrial plant. The addition of the water-soluble B group vitamins did not have such a significant effect on the COD removal as the trace metals, with the addition of 1.0 mg/L niacin having a detrimental effect on the AS.

The dissolved residual humic substances in the effluent were fractionated to determine the characteristics of the recalcitrant compounds contributing to the COD. They were separated into three distinct acid fractions (hydrophobic (HPhoA), transphilic (TPhiA) and hydrophilic (HPhiA)) through the use of two non-ionic resins, DAX 8 and XAD 4. The term oxygen demanding matter (ODM) was proposed to describe the residual mass of COD in each of the separate humic fractions. The total ODM (mg) in the influent samples comprised of 20 – 30%, 15% and 25 – 35% of the HPhoA, TPhiA and HPhiA fractions, respectively, comparable to previous research. The HPhoA fraction was

the most significant single contributor to the residual ODM in the Boyer effluent. This fraction is most likely composed of hydrophobic lignin derived compounds. Minimal residual COD was detected in the TPhiA and HPhiA fractions separated from the porous pot effluent.

Due to the potential effects of the trace metal additions on the environment through discharge to the Derwent River it was necessary to determine the ultimate fate of the metal additions. It was found that only the addition of 1.0 mg/L copper potentially exceeded the Australian and New Zealand guidelines for discharge to fresh and marine water. In terms of metals in sludge, the main metals of interest were copper and cobalt and both of these were found to be well below the guidelines for sludge application with the additions investigated.

A complete mass balance of the added metals calcium, cobalt, copper, iron and magnesium found that the highest metal recovery was for the addition of copper and cobalt individually (98 – 147%). The recovery of copper decreased to 62 – 108% when calcium, iron or magnesium was added simultaneously. The majority of each metal added to the porous pots was found in the effluent, where divalent cations form cation bridges with biopolymers, giving a possible mechanism for the increased COD removal. Simultaneous metal additions were also found to inhibit the uptake of copper in the sludge.

The addition of 1.0 mg/L copper, 0.5 mg/L zinc, 0.1 mg/L cobalt or 0.05 mg/L molybdenum to the porous pots did not significantly change the abundance of ciliates and rotifers compared to the control pots. The addition of 0.1 to 1.0 mg/L copper was found to affect the abundance of filamentous bacteria but it did not have a detrimental effect on the COD removal or the SS concentration in the effluent. The inhibition of filamentous bacteria through the addition of copper appeared to be neutralized in the presence of excess calcium and magnesium.

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Publications

1. Including results and discussion from Chapters 2, 3 & 4.
   Barnett, J. Richardson, D. Stack, K. and Lewis, T. *Addition of trace metals and vitamins for the optimization of pulp and paper mill activated sludge wastewater treatment plant*, APPITA Journal, 65(3), p. 237-243, 2012.

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Glossary

AAS	Atomic Absorption Spectroscopy
AS	Activated Sludge
ASR	Activated Sludge Reactor
BFR	Biofilm Reactor
BOD	Biological Oxygen Demand
bv	Bed Volume
CCS	Cold Caustic Soda (bleaching)
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOM	Dissolved Organic Matter
F/M	Food to Mass
HMW	High Molecular Weight
HPhiA	Hydrophilic Acid
HPhoA	Hydrophobic Acid
HS	Humic Substances
LMW	Low Molecular Weight
MLSS	Mixed Liquor Suspended Solids
NMR	Nuclear Magnetic Resonance
NSB	Norske Skog Boyer
ODM	Oxygen Demanding Matter
SEC	Size Exclusion Chromatography
SETP	Secondary Effluent Treatment Plant
SVI	Sludge Volume Index
TPhiA	Transphilic Acid
WWTP	Wastewater Treatment Plan

Chapter 1 Introduction

1.1 Activated Sludge Wastewater Treatment Plants

1.1.1 Norske Skog Boyer Activated Sludge Treatment Plant

The Norske Skog paper mill at Boyer is located north of Hobart on the Derwent River in Tasmania's Derwent Valley. Norske Skog Boyer (NSB) produces approximately 40% of Australia's newsprint, about 290,000 tonnes per annum, through thermomechanical pulping (TMP) of plantation *Pinus Radiata.* This operation generates approximately 20 ML of process water daily containing an average chemical oxygen demand (COD) of 1800 mg/L and suspended solids (SS) of 1200 mg/L.

The wastewater from the NSB is currently treated using a recently constructed activated sludge (AS) treatment plant before the effluent is discharged into the Derwent River. The operation of the plant is similar to a municipal sewage treatment plant, although the influent has high cellulose and wood fibre content, with low nutrients.

AS wastewater treatment plants (WWTPs) are frequently used to treat industrial effluents and in Scandinavian pulp and paper plants the treatment of wastewater through activated sludge is common [1]. However, there is significant variability between, and even within, paper mills depending on the operating conditions and feed stock of the plant, making direct comparisons difficult. For example, one of the operational characteristics of the paper mill at NSB is the production of alternating paper grades that changes the chemical composition of the influent to the WWTP as the paper grades are changed. The variability in the wastewater from operational pulp and paper mills affects the efficiency of the biological treatment of the effluent, which decreases the removal of both COD and biochemical oxygen demand (BOD).

While COD is a measure of all substances able to be oxidised by a strong chemical oxidant under reflux in strongly acidic conditions [2, 3], BOD is a direct measure of the amount of oxygen consumed by microbes to degrade organic matter in a water sample over a given time, usually five days [4]. While the latter is possibly more representative of processes that occur in the environment, it does not measure degradation resistant materials that may be present especially in industrial effluents. At high levels oxygen demanding materials may be toxic to aquatic life due to a reduction of the dissolved oxygen (DO) in the water column.

In an AS treatment plant COD and BOD levels are reduced by microbiological breakdown of organic matter, largely suspended solids, for either incorporation into their bodies or as a source of energy.

For optimum COD removal a controlled environment is needed to maximise biological growth rates. The controlling factors include pH, temperature, macro- and micro-nutrient and trace metal levels, dissolved oxygen (DO) concentration and good mixing [5, 6]. However, research has shown that aerobic activated sludge in a pulp and paper effluent treatment plant is not affected by changes in influent pH ranging between 5 to 9.5 and temperatures between 30 to 40°C [7]. This could suggest that other factors, such as nutrient levels, DO or organic loading, have a greater influence on the stability of an AS treatment plant.

The removal rates of BOD and COD in most industrial wastewaters can be expected to be between 95 to 99% BOD and 80 to 90% COD [5, 8, 9]. The removal of COD for the NSB plant between commissioning of the AS treatment plant (January 2008) and July 2009 was between 60 and 90%, with a mean of 74%. Environmental agencies throughout the world are lowering the amount of COD permitted for release into receiving water bodies and Norske Skog had set an internal target of 90% removal of COD by November 2011.

1.1.2 Humic Substances

Humic substances (HS) form a major component of the total dissolved organic matter (DOM) which occurs naturally in the terrestrial and aquatic environments [10]. In the natural environment, HS are dissolved organic humic substances not including insoluble humic substances. They are formed through the partial degradation of plant and animal matter, and are one of the richest sources of organic material available in the environment [11, 12]. Humic substances are a complex mixture of compounds, and their structure is neither known nor can be represented simply. They generally consist of recalcitrant compounds, cellulose, tannin and lignin and more biodegradable proteins, lipids and sugars [13]. Humic substances are important environmental complexing molecules [11], however, due to their complex nature they are some of the least understood groups of molecules in the natural environment [14]. The compounds categorised as humic substances are high molecular weight macromolecules with molecular masses that range from 1 kDa to much greater than 1000 kDa [3, 4, 12, 15, 16]. These HMW HS are biologically inactive [17-19], consequently the major mechanism for their removal from an industrial effluent is the secondary clarification process [20].

1.1.3 Humic Substances in Pulp and Paper Effluent

Untreated effluent from a pulp and paper mill consists of a mixture of phenolic compounds, sugars, acids and inorganic substances originating from the wood and chemical additives used in the pulping and papermaking process. Degradation products of the carbohydrates, lignin derivatives, surfactants

and the wood extractives such as the resin acids can be discharged in the effluent without secondary wastewater treatment and are classified as significant pollutants [21].

The term lignin describes a group of compounds of poorly defined chemical composition, though they are known to contain numerous carboxylic and phenolic functional groups and short aliphatic chains [22]. A general structure for lignin is proposed in Figure 1.1. Lignins are generally derived from the breakdown of wood fibre, and are resistant to degradation due to their hydrophobic nature. The decomposition of lignin and other humic substances in the environment can take up to 20 years [2]. During the decomposition of lignin the abundance of phenolic carbonyl groups increases [15]. The pulping of wood is similar to the natural humification of lignin where recalcitrant lignin derived compounds remain in water ecosystems [22]. The best removal rates of lignin in pulp and paper mill wastewater have been found to occur by adjusting the sludge age to 20 days [23]. However, such a relatively extended sludge age then increases the likelihood of other operational issues which have to be managed, such as bulking and foaming through the excess growth of filamentous bacteria.

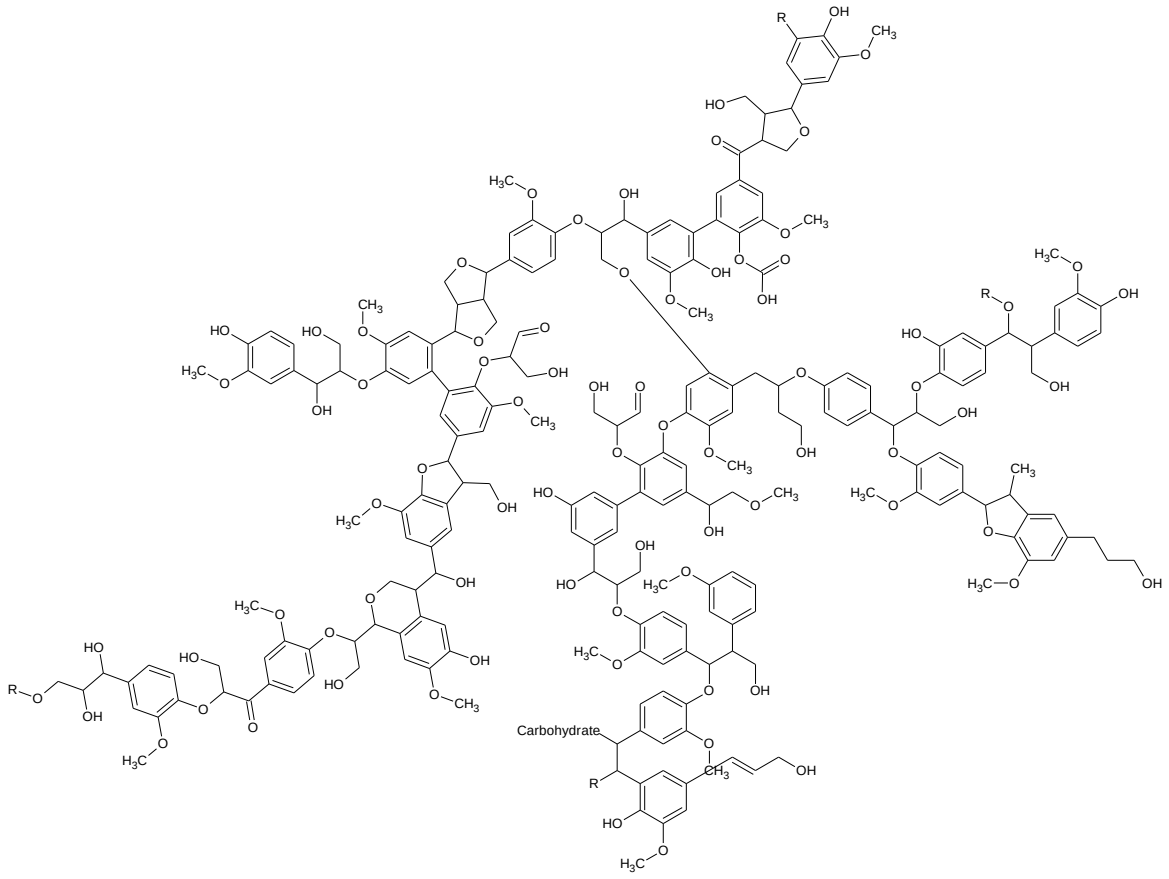


Figure 1.1 Proposed structure of softwood lignin, adapted from Shevchenko and Bailey (1996)

Lignin and its derivatives have been found to be the most significant contributors to the high molecular weight (HMW) compounds detected in pulp and paper mill wastewater [24]. Subsequently lignin and lignin by-products have been found to significantly contribute to the residual COD in pulp and paper mill treated effluent [18, 24-26].

Simple low molecular weight (LMW) resin acids, fatty acids and sterols have also been identified among the hydrophobic acids in treated pulp mill effluent at concentrations < 0.2 mg/L [27].

The resin acids, which include pimaric, sandaracopimaric, isoprimaric, levopimaric, dehydrobietic, abietic and neoabietic acids [20], and the fatty acids, both saturated and unsaturated, have carboxylic acid functional groups. Greater than 95% of resin acids are expected to be removed from TMP pulp and paper wastewater in the SETP[28-30]. Sterols, also isolated from pulp and paper mill treated effluents, contain a polar hydroxide group and non polar aliphatic group and are also characteristic of degraded lignin [27].

1.1.4 Separation and Isolation of Humic Fractions

Due to the complex nature of the humic substances found in pulp and paper waste waters, there has been considerable research undertaken to characterise the separate fulvic and humic acid fractions [20, 31, 32]. The methods for separating hydrophobic and hydrophilic fractions have been developed through fractionation of humic material from natural waters by Leenheer and Aiken *et al.* [33, 34]. The separation of soluble humic substances into hydrophobic, hydrophilic and transphilic fractions has been successfully applied to DOM using two non-ionic resins, Supelite DAX-8 and Amberlite XAD-4 [13, 21, 35-37].

Although each HS fraction (hydrophobic, hydrophilic and transphilic) can be further separated into acid, base and neutral fractions, the acid fractions have been found to be the most chemically significant fractions in water [13]. The focus during this research was on three acid fractions: hydrophobic acids (HPhoA), transphilic acids (TPhiA) and hydrophilic acids (HPhiA). The two major organic acid fractions in natural waters are the HPhoA and HPhiA fractions [34]. Aquatic fulvic acid is the base-soluble fraction which consists of the lower molecular weight (LMW) compounds (≤ 1 kDa). This fraction is a significant source of the hydrophobic acids [13, 33]. The HPhoA fraction includes aliphatic and aromatic carboxylic acids, phenols and highly degraded compounds of lignin and lignocelluloses breakdown products [20, 33, 35, 38]. The HPhiA fraction includes polyfunctional organic acids and aliphatic acids [33]. One assumption is that compounds comprising of the HPhiA fraction are those of a lignin and lignocelluloses origin and simply more degraded than the HPhoA fraction [38]. Transphilic acids are primarily polysaccharides [35].

1.1.5 Adsorption of Humic Substances in Activated Sludge

Activated sludge flocs act as an absorbent for lignin and other humic substance compounds [23]. Biosorption of humic substances occurs in the AS where the organic HS compounds attach to microbial surfaces through physical and chemical bonding, removing the compounds from solution [17]. HS can chemically bond to the surfaces of solids, like metal oxides and clay particles, though retaining similar chemical characteristics to the free HS [2]. At a neutral pH the negative surface charge of HS caused by the deprotonation of the acidic functional groups allows some normally insoluble hydrophobic HS compounds to be soluble. These also maintain the original properties of the solid surface [13].

Biopolymers, comprising mainly proteins and polysaccharides produced by the AS biomass, also contribute to the HS in the SETP effluent [39]. Park *et al.* found that the effluent biopolymer concentration and the effluent COD level increased or decreased in proportion to the concentration of aluminium and iron in the activated sludge floc. As the aluminium and iron increased, the biopolymer and COD concentration decreased [40]. The concentration of divalent cations, especially Ca^{2+} and Mg^{2+} in the sludge liquor has also been found to have a direct effect on the concentration of COD and turbidity in SETP effluent [39].

1.2 Microorganisms in Activated Sludge

One reason an AS process may be unable to cope with the changes in influent may be low levels of the micronutrients required to support optimal microbial growth [1, 8, 41, 42]. For an optimal diversity of microorganisms in the activated sludge an extensive range of micronutrients are essential. In a system with limiting concentrations of nutrients the population shifts to the species that is best adapted for limited nutrients [43]. Of the factors that influence the operation of the AS treatment plant, micronutrients present the best prospects for process enhancement [44]. Most industrial effluents do not contain sufficient nutrients in the form of trace metals and water-soluble B vitamins for the sustainable microbial growth needed for activated sludge plants [8, 45, 46]. For optimal reduction of organic carbon as a component of BOD or COD, it is important to make carbon the limiting nutrient in a AS treatment plant [6]. Of the organic matter removed in the AS treatment plant approximately 60% is synthesised into biological cells, this requires especially nitrogen, phosphorus and trace metals [4]. It is, therefore, logical to propose a decrease in residual COD through the optimisation of the SETP.

1.2.1 Role of Microorganisms in Wastewater Treatment

Bacteria are the main agents for the removal of dissolved and particulate forms of BOD and COD found in many wastewaters. Microorganisms convert the organic matter into simple end products such as CO_2 and additional biomass [5]. The biomass has a greater density than water and is easily separated by gravity in a settling stage.

For sustainable growth and reproduction the bacteria need an energy source, carbon and inorganic macronutrients (nitrogen, phosphorus, sulphur, potassium, calcium, magnesium) [5, 8, 47], and micronutrients or trace metals (zinc, manganese, molybdenum, selenium, cobalt, copper and nickel), often at concentrations below 1 mg/L [5, 6, 8, 47]. Trace metals are generally required by bacteria as constituents for the production of enzymes [48]. Calcium is required for cell transport and osmotic balance, and it also interacts with other metals to aid in their availability [43]. The trace metals have to be in a form which is bioavailable for bacteria to synthesize into biomass, but many of the trace metals form insoluble salts making them unavailable for bacterial uptake [6]. The main roles of "essential" heavy metals have been summarised by Gikas as: (i) catalysis of biochemical reactions, (ii) stabilization of proteins, (iii) regulation of gene expression and (iv) control of osmotic pressure gradients across various microbial membranes [49].

Organic nutrients such as vitamins may also be required for cellular synthesis [5]. In some cases of low nutrient concentrations, the nutrients can become the limiting factor for optimal growth rather than carbon or energy sources [5]. The organic nutrients (growth factors) that are essential in an activated sludge treatment plant are compounds required by microorganisms which cannot be synthesized from other carbon sources [5]. There are three distinct classes of growth factor: amino acids, nitrogen bases and vitamins. The requirements for these varies among different species of microorganism [5, 47].

Without the correct balance of micronutrients for microbial growth, sludge handling and COD removal problems can arise [8]. In an industrial AS treatment plant inorganic and organic nutrients could be either absent, at limiting concentrations, or biologically unavailable due to chemical reactions and precipitation [6]. When adding micronutrients to an AS process there are many variables to consider such as microbial biodiversity, sludge age and the order in which the micronutrients are added. These variables make the reaction between micronutrients unpredictable, and these unpredictable interactions lead to some of the contradictory results that have been reported in the past for the effectiveness of micronutrient additions [8, 49, 50].

1.2.2 Filamentous bacteria, Bulking and Foaming

Filamentous bacteria do not detach following cell division, forming long thin chains of cells [51]. They also play an important role in the activated sludge through forming the backbone of the AS flocs making them an important aspect of any AS treatment plant. Without filaments present pin flocs form that settle rapidly in the secondary clarifier. However, due to low shear strength of the floc, detached bacterial cells contribute to increases in effluent turbidity [52-54]. On the other hand, excessive growth of filaments also causes poor settling of flocs, called bulking.

The character of the influent wastewater can affect the development of a bulking sludge through the absence or presence of certain components such as micronutrients [5]. Bulking can be caused by nutrient deficiencies which increase the prevalence of filamentous bacteria, therefore, decreasing the ability of the sludge to settle [43, 55, 56].

Bulking and foaming affect the efficient removal of COD and SS in the AS treatment plant. Foaming is not as well understood as bulking. There are several mechanisms or processes thought to contribute to foaming in AS. One is through the production of biopolymers by bacteria or the presence of long chain surfactants in the wastewater [54], also a retention time of > 9 days is reported to encourage the growth of bacteria associated with foaming [54], such as *M. parvicella, Gordona amarae* and *Skermania pinensis* [57]. Another cause of foaming is through the production of N_2, largely by denitrifying filamentous bacteria as a by-product of respiring nitrate when the level of DO is too low [58]. Production of N_2 gas is also problematic as the N_2 bubbles become trapped in the floc causing the sludge to float to the surface.

The occurrence of bulking and foaming in a WWTP are symptoms that there may also be other control issues such as low dissolved oxygen (DO) or high organic loading. Some filamentous bacteria are suited to low DO conditions and often an increase in the organic load will lead to a decrease in the DO levels [51, 52]. The filaments commonly associated with low DO are *Haliscomenobacter hydrossis,* Eikelboom Type 1701*, Sphaerotilus natans* and *Microthrix parvicella* [51, 52]. There are more than 30 different filamentous species, mostly bacteria, found in AS with ten being common among most treatment plants in Europe [51]. Of the most common filamentous bacteria found in all AS treatment plants in the USA four of the top six were found in pulp and paper mill effluent treatment plants: *H. hydrossis*, *Nocardia spp*., Type 0092 and Type 1701 [56]. The structure of filamentous communities in Australian municipal AS plants was not the same as those found in other parts of the world [59]. In Australian AS plants *H. hydrossis*, Type 0092, Type 0041/0675 and *M. parvicella* were the most common.

Filamentous bacteria can be used to indicate process problems in a AS treatment plant, the species *Sphaerotilus natans, M. parvicella* and *Nocardia* spp. being most commonly associated with sludge bulking and foaming [6]. The filamentous bacteria Type 1701, Type 021N and Type 1863 with *H. Hydrossis* and *S. natans*, are associated with low dissolved oxygen [60, 61], while *M. Parvicella*, Type 0092, Type 0041 and Type 0961 are associated with low organic loading [61].

Due to the differences between the influents in industrial AS treatment plants the morphology of flocs needs to be investigated on an individual basis to determine what 'normal' floc quality is. The criteria for determining floc quality can include: shape, structure, strength and size [51]. The floc data collected over time can be utilised as a reference to the operation of an individual WWTP plant. Understanding the normal operating conditions will aid in the control of bulking and foaming through adjusting the DO, nutrient level, sludge age and organic loading [62].

1.2.3 Protozoa and Metazoa

Protozoa are single celled organisms including ciliates, flagellates, amoeba and heliozoans [51]. There have been 230 species of protozoa isolated from WWTPs, though of these only a limited number are commonly found throughout the world [63]. Metazoa are a higher order multi-celled organisms, including rotifers, nematodes, worms and tardigrades [51]. Protozoan and metazoan species play an essential role in the clarification of wastewater treated by an AS plant. Protozoa are relatively large and are predators of bacteria which are free in the effluent or attached to the edges of flocs, they also consume whole sludge flocs which decreases sludge production [51]. Bacteria floating free in the effluent do not settle in the secondary clarifier, therefore, predation by the protozoan population is important for COD removal and clarification [51, 64]. The most studied protozoa related to AS are the ciliates.

The structure of protozoan species in activated sludge can be an effective indicator to the operational health of WWTPs [51, 64]. Ciliated protozoa can indicate high-quality effluent due to the predation of bacteria which act as a clarifying process [65, 66]. Protozoan bioindicators *Chilodonella uncinata* and *Trochilia minuta* are crawling ciliates normally found in activated sludge and connected with nitrifying conditions [64]. These two species are highly sensitive to heavy metals and their absence or presence could be an indicator to the concentration of heavy metals [64]. The *Opercularia spp* have been reported to become more abundant in nutrient deficient activated sludge [64].

The effects of metals on bacteria have been widely studied in activated sludge, however, the effects of heavy metals on the inhibition of the protozoan community are less well known. Protozoa have different tolerances for heavy metals compared to bacteria, therefore, bacteria may be inhibited by metal concentrations that may stimulate protozoan species. Madoni *et al.* found that ciliated protozoa are able to survive in higher than normal concentrations of heavy metals in activated sludge [64].

1.3 Vitamins and Trace Metals

1.3.1 The Role of Vitamins and their Biological Requirements in Activated Sludge

Mixed bacterial cultures occur naturally in AS treatment plants and are essential for the reduction of COD and any toxins produced by the metabolic breakdown of the organic constituents [8, 45]. A healthy mixed culture in the activated sludge can provide most of the required vitamins, though industrial sludges have a lower diversity and generally benefit from vitamin addition [43]. Experimentation in industrial AS treatment plants has shown that micronutrient supplementation significantly alters the microbial diversity [42].

Most vitamins function as components of coenzymes and are the most commonly required growth factors for bacteria [47]. Water-soluble vitamins from the vitamin B group are required by bacteria in AS treatment plants for basic cell function, and the concentration and dependence of each vitamin varies. From 300 species of bacteria isolated from a municipal WWTP, 37% had an essential requirement for water-soluble vitamin B and, for a further 20%, growth was stimulated by the addition of vitamins [67]. An industrial AS plant would be expected to have a significantly lower level of micronutrients available for cell growth. Of bacteria isolated from pulp and paper wastewater treatment plants 80-99% have been found to require vitamin B addition for optimal growth [43].

Thiamine is involved in cell growth and carbohydrate metabolism, and microorganisms range from total dependency on thiamine availability in solution to organisms which synthesise their own [6, 43]. Riboflavin (B_2) is required for a wide variety of cellular processes, and plays a key role in energy metabolism [43]. Niacin is needed for growth and dosing stimulates a mixed bacterial culture [43]. Pantothenic acid (B_5) is a growth factor in the stimulation of initial cell growth, propagation and respiration [6, 43]. It is most active when other B vitamins are present such as biotin (B_7) and pyridoxine (B_6). A deficiency results on reduced nitrogen and phosphorus removal efficiencies [43].

Biotin is an essential vitamin for the growth of lactic acid bacteria, however, as most species produce biotin, there is no need for its addition to activated sludge [6, 43]. This is fortunate, as the cost of biotin would make it prohibitive for large scale use (USD $1100 kg in 2010). It has also been reported

that folic acid (B_9) is not required for growth in bacteria [43]. Cobalamin (B_{12}), which is produced during normal growth of some microbes, requires the biochemically rare element cobalt and is essential for growth [43]. The addition of 1 ppm cobalt to AS has been reported to increase the production of cobalamin by 50%, however, no increase in effluent quality was recorded [68].

Research has indicated that external sources of vitamins such as thiamine (B_1), niacin (B_3) and biotin (B_7) are essential for the growth of some bacteria in aquatic environments [69], and a correlation between the concentration of some water-soluble vitamins (thiamine, biotin and cobalamin) and the population of aerobic heterotrophic bacteria has been shown [70]. Madigan *et al.* report that the vitamins which are most commonly required by microorganisms in general are thiamine, pyridoxine, biotin and cobalamin [47].

Table 1.1: Estimated requirements for water-soluble B-vitamins for optimal cell function in wastewater treatment plants.

Nutrient B-Vitamins	**Estimated Micronutrient Requirements (mg/L)[8, 9, 43, 71]**
Thiamine (B_1)	0.3 – 1.2
Riboflavin (B_2)	0.5 – 2.0
Niacin (B_3)	0 – 10.0
Pantothenic acid (B_5)	0.01 – 2.0
Pyridoxine (B_6)	0.1 – 10.0
Biotin (B_7)	0.1 – 1.0
Folic acid (B_9)	N/A
Cobalamin (B_{12})	0.005

There are limited reports on the effects of micronutrient addition to industrial AS treatment plants especially for the pulp and paper industry. From seven industrial and municipal samples the bacteria from pulp and paper sludge samples indicated the highest vitamin requirements [72, 73]. Lemmer *et al.* found that ≥ 99% of the bacteria isolated from an activated sludge sample taken from a pulp and paper industry AS treatment plant required thiamine for growth, and that thiamine, niacin and biotin were essential for growth [72, 73]. Estimated requirements for water-soluble B-vitamins in wastewater treatment plants are given in Table 1.1. For the best enhancement of bacterial cell enzyme activity a multiple vitamin addition has a greater affect than single vitamin addition [43].

In municipal and industrial sludge samples thiamine and riboflavin were found in ranges of 1 to 29 ppm and 18 to 43 ppm, respectively, and the concentration of folic acid in industrial samples was found to be 2 ppm [72]. These concentrations are reported to be sufficient for bacterial growth from isolates tested (See Table 1.1) [8]. The estimated micronutrient requirements of water-soluble vitamins in Table 1.1 have been determined from filtered liquor samples only [8, 9, 43, 71].

No bacteria isolated by Lemmer *et al.* required riboflavin, pyridoxine or folic acid [73]. However, the addition of folic acid to the AS treatment plants of two paper recycling operations led to a 50-70% decrease in the water turbidity of the effluent and increased the control over the settling rates of the sludge [74]. The concentration of folic acid was not given, though it was stated that a higher concentration did cause rapid settling and a loss of the protozoa population.

While the addition of niacin to an industrial AS plant has been reported to increase the COD removal [41], studies undertaken in a textile AS treatment plant showed the addition of 1.0 ppm niacin increased both the level and the speed of COD removal by 2.18 times [42, 45]. In a separate study COD removal in a textile plant was increased by more than 20% with the addition of 1.0 ppm niacin [75].

1.3.2 The Role of Trace Metals and their Biological Requirements in Activated Sludge

A number of trace metals have been identified as being required at low levels for cell function (Table 1.2).

Table 1.2: Estimated trace metal requirements for optimal cell function in wastewater treatment plants.

Nutrient Trace Metals	Estimated Trace Metal Requirements (mg/L)[8, 71, 76]
Calcium (Ca)	3 – 5
Cobalt (Co)	0.02 – 0.05
Copper (Cu)	0.1 – 1.0
Iron (Fe)	1 – 4
Magnesium (Mg)	3 – 10
Molybdenum (Mo)	0.02 – 0.05
Zinc (Zn)	0.01 – 1.0

Laboratory experiments on synthetic wastewater have aided in understanding the effects of trace metal addition on the reduction of COD. Copper is an enzyme activator for bacteria [43, 77], Addition of 0.5 mg/L copper to laboratory scale experiments, led to increased bacteria growth rates and biomass yield [78]. The same researchers reported that addition of 1.0 mg/L copper increased growth rates but there was a decrease in biomass yield [78], indicating some inhibition. Although copper is required for growth, it can inhibit some filamentous bacteria at relatively low levels. The addition of approximately 0.05 mg/L copper to activated sludge has been reported to inhibit the growth of three filamentous bacteria, *Thiothrix sp.*, type 021N and type 1701, which can contribute to SETP control problems [79].

Stover *et al.* reported an AS treatment plant commissioned in New York state by a brewery had increased mixed liquor suspended solids (MLSS) from 6,000 to 17,000 ppm over the first seven years of operation. After a Cu deficiency was found in the wastewater, addition of 14 ppb copper to the aeration basin in the form of $CuSO_4$ reduced the MLSS to initial concentrations and it remained steady for the following six months [44]. Soluble Cu was increased from below detection limits of < 1.0 ppb to between 2 to 5 ppb in the effluent with this addition [44], still under the estimated trace metal requirements for bacteria (Table 1.2).

In a study of the toxic effects of copper and zinc on synthetic wastewater concentrations of 1.5 mg/L and 9 mg/L, respectively, were found to be toxic to activated sludge [50]. In a sewage treatment plant the separate addition of copper and zinc, both at 1.0 mg/L, had a slight stimulatory effect, however, when combined and added at 5 ppm, growth was inhibited [48]. This observed inhibition of activated sludge bacteria was supported by Shuttleworth and Unz who reported that addition of 5 mg/L zinc inhibited the growth of the filamentous bacteria type 1701 [79]. This is not surprising as the estimated biological requirement is between 0.01 mg/L to 1.0 mg/L (Table 1.2).

Zinc is an enzyme activator and stimulates cell growth, but is reported to be toxic at ≥ 1.0 ppm in activated sludge, especially to protozoa [43]. Zinc has been reported to be 100% inhibitory to nitrifying bacteria at 3 ppm, while concentrations of 0.6 – 1.2 ppm have had no effect on the protozoan community [80]. A concentration of 2.1 ppm total zinc was found to give an 80% inhibition of microbial species in activated sludge [80].

The trace element cobalt is also an enzyme activator. It is involved with the synthesis of cobalamin. The addition of < 1.0 ppm cobalt to municipal AS plants has been reported as beneficial [43, 68]. The reported optimum for the concentration of cobalt in municipal activated sludge has been estimated at 0.02 – 0.05 ppm for activated sludge [49]. Increasing concentrations of cobalt to between 0.01

and 0.1 ppm in a sewage treatment plant has increased synthesis of cobalamin by a factor of 1.5 times [6, 68]. There are contradictory reports however of cobalt inhibition and stimulation, with 1 – 20 ppm being reported to reduce respiration while additions of up to 19 ppm have been reported to stimulate growth [49].

The addition of iron(III) to activated sludge to optimise biological growth has not been common. However, it is a growth factor required for enzyme reactions and electron transfer [6, 43, 81]. Magnesium is also required by bacteria for the production and transport of energy [76], as is calcium which is vital for cell transport and osmotic balance [79, 82]. Both magnesium and calcium are reported to increase COD removal in industrial wastewater treatment plants [9, 42], however, they are also reported to reduce COD through bridging between humic material and the floc surface, increasing floc strength and density [17, 39].

Molybdenum is frequently a limiting nutrient in pulp and paper mill wastewater [55]. In a textile AS plant the addition of 2.0 ppm molybdenum increased COD removal rates 1.4 – fold [42]. Separate additions of molybdenum and calcium have also been reported to increase the COD removal efficiency in an industrial AS plant [41].

The level at which microbial growth is stimulated or inhibited by the addition of trace metals is dependent on the concentration that is bioavailable in the treatment system [83]. An important consideration is to treat the wastewater with the optimum concentration of essential trace metals to stimulate maximum growth. Additions of trace metals copper, zinc and cobalt to AS treatment plants at trace quantities have been reported to stimulate microbial growth [49], but excess levels can also be detrimental.

Barth *et al.* found total concentration of 8.9 ppm of four metals (copper, chromium, nickel and zinc) had no significant effect on the overall efficiency of a pilot-scale AS treatment plant treating wastewater from a metal plating factory [84]. Additions of ≥ 3.0 ppm copper to industrial AS treatment plants over time decreased COD removal and increased copper in the sludge [43]. A combination of approximately 5.3 ppm cadmium and 1.2 ppm nickel resulted in a stimulation of microbial growth [83], though the affect on COD reduction was not reported.

1.3.3 Metals and Humic Substances

One of the more significant characteristics of humic substances is their ability to form complexes with metal ions [2-4, 85] and they are believed to play an important role in metal transport and

bioavailability in water [13, 19]. Dissolved humic substances react with metals through ionic or covalent bonds, where alkaline earth metals (Ca^{2+} and Mg^{2+}) form complexes through weak ionic bonds and transition metals (Cu^{2+} and Zn^{2+}) and trivalent cations (Fe^{3+} and Al^{3+}) form strong stable complexes or bidentate chelates through covalent bonds [2, 4, 86].

In natural aqueous environments HS are considered problematic due to the complexing of heavy metals [17], which can stabilise the metals in solution. For example, up to 75 to 87% of the total copper and zinc detected in wastewater effluent has been found to be bound to HS [87]. The mechanism of heavy metal removal in AS is not well understood, however there is a consensus that in the presence of HS the total metal concentration is not representative of the total bioavailable metal in solution [87, 88]. The complexation capacity of HS to bind heavy metals is dependent on the pH, ionic strength, the metal and the concentration of humic substances [17, 86].

1.3.4 The Effects of Mixed Vitamin and Trace Metal Addition

It is probable that greater microbial stimulation would be achieved through a mixed vitamin and trace element addition. In theory the addition of micronutrients and vitamins to nutrient deficient pulp mill AS wastewater treatment plants could decrease retention time and increase COD removal rates [45]. Burgess *et al.* found that the addition of 1.0 ppm niacin had the greatest effect on an industrial AS treatment plant compared to the other water-soluble B-vitamins, however, the stimulatory effect was enhanced with the addition of calcium [8]. The addition of 20 mg/L calcium has also been found to reduce the inhibitory effects of the heavy metals copper and zinc on the filamentous bacteria *Thiothrix sp*. [79]. However, there have also been contradictions in the reports on the effects of mixed micronutrient additions [6, 8, 42, 44, 50, 72]. The contradictions are almost certainly due to the different interactions of metallic ions in different industrial treatment plants.

In a pilot plant experiment on industrial wastewater, the addition of niacin alone increased COD removal. The combined additions of phosphorous, calcium and niacin or phosphorous, calcium and manganese increased the rate of COD removal [71]. Effluent from a chemical plant which was phosphorus limited showed improved COD removal with additions of vitamins riboflavin, niacin and pyridoxine and trace metals calcium, manganese, aluminium, zinc, molybdenum and cobalt [9]. In a textile WWTP the combined addition of niacin, zinc and thiamine had a greater effect on COD removal compared to the individual additions [42].

From all of the literature reviewed the best results reported from the addition of micronutrients to AS treatment plants have been through the addition of vitamins niacin, pantothenic acid, biotin and

pyridoxine with trace metals copper, calcium, cobalt and molybdenum. As previous studies indicate that there are different requirements for individual AS treatment plants, the literature can be a guide only to the implementation of micronutrient additions for the optimisation of a particular wastewater treatment plant.

1.4 Aims

The overall aim of the project was to determine the effectiveness of applying metal or vitamin additions to optimise the performance of a pulp and paper activated sludge wastewater treatment plant.

The specific aims were therefore to determine:

- the differences in the Boyer wastewater trace metal levels over the change from a mixed feed stock of *Eucalyptus sp.* and *Pinus radiata* to solely *P. radiata*,
- the effects of trace metal and water-soluble vitamin additions on the COD removal and SS levels using porous pots to model the waste water treatment plant at Boyer,
- the effect of trace metal and water-soluble vitamin additions on the activated sludge biota,
- the fate of trace metals added to porous pots,
- the composition of humic fractions in the in the porous pot influent and effluent.

Chapter 2 Determination of Metals in Boyer Wastewater and Preliminary SETP Data Analysis.

2.1 Introduction

Four standard types of paper using two paper machines (PM 2 and PM 3) are produced at the Norske Skog, Boyer pulp and paper mill, resulting in different levels of organic loading in wastewater. PM 3 produces solely NorNews which is a standard grade paper used in newsprint, while PM 2 is used to produce higher grade magazine quality paper products including: NorBright, NorStar and NorStar Super and occasionally NorNews. As such, it is PM 2 that contributes to the major difference in the wastewater quality through the different paper grades produced on that machine. A production run on PM 2 can vary from a period of hours up to days, with the possibility of multiple grades in one day. For data treatment the paper grade being produced for the longest time during a particular day was selected as the main contributor to the wastewater quality for that day.

Norske Skog Boyer (NSB) commissioned an activated sludge wastewater treatment plant or secondary effluent treatment plant (SETP) at Boyer in January 2008 (Figure 2.1). The wastewater treatment plant (WWTP) consists of primary and secondary clarifiers and a secondary effluent treatment plant (SETP) comprising a biofilm reactor (BFR) containing small disks to increase surface area for bacterial growth and an activated sludge reactor (ASR). In October 2009 the cold caustic soda (CCS) plant used for the pulping of eucalypt hardwood was decommissioned to allow the integration of a new thermo-mechanical pulping (TMP) plant (TMP 3). Prior to decommissioning the CCS the feed stock to the mill was a combination of eucalypt and *Pinus radiata* (radiata pine). With the decommissioning of the CCS and its replacement with TMP3, the mill converted to solely plantation *P. radiata* feed stock.

Figure 2.1: The NSB SETP. The inner holding tank is the biofilm reactor (BFR) and the outer tank is the activated sludge reactor (ASR).

A schematic of the WWTP showing the main inputs to the SETP from the paper machines (PM2 and PM3), TMP 1 and 2, and CCS/TMP3 and also the screen room and the bleach plant is shown in Figure 2.2. A portion of the settled sludge in the secondary clarifier is returned to the ASR and is known as the return activated sludge (RAS). A portion of the settled sludge in the secondary clarifier, called the waste activated sludge (WAS), is removed from the wastewater treatment process. The sludge age is controlled through the wasting process, the sludge age being the mean cell residence time in the SETP. The food to mass ratio (F/M) determines availability of food for the biomass (mixed liquor suspended solids) in the SETP. Settling can be controlled by optimising the operating conditions (such as the F/M and sludge age), therefore, decreasing SS in the treated effluent [51, 52].

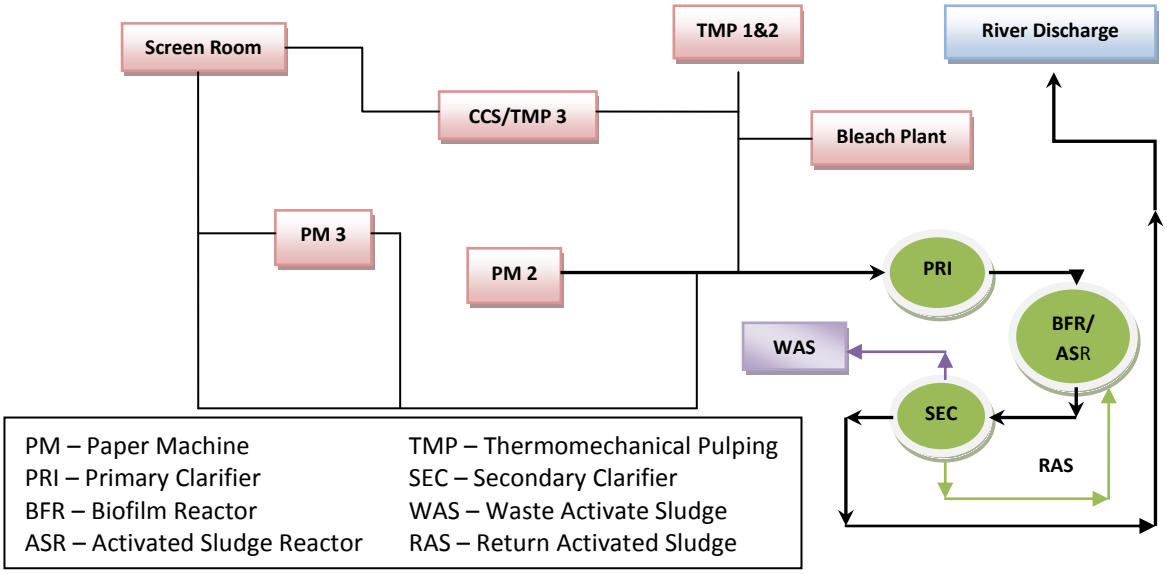


Figure 2.2: Flow chart of the NSB wastewater treatment plant (WWTP) showing input streams from the Pulp and Paper mill.

Wood is composed of three major constituents, cellulose, hemicelluloses and lignin [89]. The sugar based carbohydrate polymers (cellulose and hemicelluloses) comprise between 68 – 75% of the dry weight of wood, with lignin approximately 18 – 35% [89]. Though the differences in the chemistries of hardwood and softwood are well known [89-91], including resin acids, lignin, cellulose and polysaccharides, the effects of these differences on the operation of a WWTP and the effluent quality have not been thoroughly tested. Among the most significant differences between hardwood and softwood species is the abundance of resin acids, where hardwoods contain little or no resin acids and softwoods contain up to 10% resin acid dry weight [90, 91]. However the total extractives, which include fatty acids, resin acids, waxes and tannins in *P. radiata*, has been identified as 1.5% of dry wood weight [92]. Of the resin acids in softwood species the most common are tricyclic acids, predominantly levipimaric and palustric acid [91]. The alkaline pulping of *Eucalypt* species releases significant levels of tannins into the untreated wastewater.

There are also differences in the structure and abundance of celluloses and lignin in hardwood and softwood species [89, 91]. The lignin content in hardwood can be expected to be between 16 – 23% and in softwood it can be between 23 – 33% [90], for example the lignin content determined in *Eucalyptus globulus* and *P. radiata* has been reported to be 22% and 27%, respectively [91]. There is

also an increase in abundance of cellulose and polysaccharides in the *E. globulus* species in comparison to the *P. radiata* of approximately 14% and 11%, respectively [91].

The effects of the feed stock and the mill pulping process changes to the effluent quality were unknown. There was an expectation that there would have been changes in the chemical composition of the wastewater through removal of the CCS inputs from the alkali bleaching of the *Eucalypt* pulp. These changes in chemical composition from an increase in the abundance of lignin derived compounds could be significant in the current work. It is expected that an increase in soluble humic compounds could affect the treated wastewater effluent quality through higher levels of recalcitrant compounds.

While it is a generalisation to call all the wood derived dissolved organic matter "humic substances", they are derived from the degradation of cellulose, hemicelluloses and lignin. The aim of this thesis was not to define individual compounds which contribute to the residual humic material in the wastewater influent or effluent and the humic substances in the influent and effluent are fractionated to better understand their general composition in each fraction.

Trace metals are vital for the health of trees and can be found within the cellular structure of all trees, with trace metals concentrations varying between species [90]. The most important trace metals are calcium, potassium and magnesium [90], which comprise approximately 80% of the wood ash content, equivalent to ≤ 0.5% of dry weight [89]. These metals are generally detected in the dry weight of wood at concentrations of 0.1 – 1.0 ppth [93]. There are fluctuations in the concentration of the metals in softwood and hardwood species. For example, the concentrations of iron and zinc in North American hardwood species was found to be up to 100 ppm and 38 ppm, respectively, while in softwood species the concentration of these metals was approximately 10 ppm [93]. Although there are no reports of the trace metal concentrations in *P. radiata* and *Eucalypt* sp., there was an expectation that by changing the feed stock there could bring about changes in the concentrations of trace metals detected in the Boyer wastewater.

The decommissioning of the CCS and subsequent change in feed stock was then an opportunity to determine any differences in the trace metals in the process water entering the WWTP. This is particularly significant in relation to the microbiological processes in the WWTP, as discussed in Chapter 1. and their effects on the COD and SS removal. Correlations between COD and SS removal and trace metal concentrations and other operating conditions within the plant were considered. Through determining trace metal concentrations and correlations with the COD, SS and operating

conditions, a suite of metals was then selected for further testing using porous pots as a bench scale WWTP.

2.2 Materials and Methods

2.2.1 Materials

Reagents

AR grade Merck HNO_3 was used for effluent stabilization and sludge digestion. Atomic adsorption standards (1000 mg/L) of Co^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Mo^{2+} and Zn^{2+} were obtained from BDH and Ca^{2+} (1000 mg/L) was from AccuTrace. A solution of $Mg(NO_3)_2.6H_2O$ (BDH) (0.3% wt/wt) was used as a chemical modifier for the graphite furnace analysis of Co^{2+}. Solutions of K or La (2000 mg/L) ionization suppressors were used for the flame AAS analysis of Ca^{2+} and Mg^{2+}, respectively. The solutions where made in ultra pure water using BDH SpectrosoL La (10% w/v) or BDH AR grade KCl.

Equipment

The samples were analysed by a GBC XplorAA for flame analysis, with a GBC GF 3000 attachment for graphite furnace analysis, using a GBC PAL 3000 auto sampler. All glassware was acid washed for 24 h in a 10% HNO_3 solution and rinsed three times with ultra pure water. Filtration was undertaken using acid washed glass filters supplied by Advantec GA – 55.

2.2.3 Methods

AAS Procedure

AAS working standards were made by serial dilution from 1000 mg/L standards. Cobalt, copper and molybdenum were analysed using graphite furnace. The graphite furnace temperature ramp tables are given in Appendix A. Iron, magnesium, zinc and copper were analysed by flame AAS using an air/acetylene flame while a nitrous oxide/acetylene flame was used for the analysis of calcium.

Activated Sludge Digestion

A return activated sludge (RAS) sample (50.00 mL) was decanted into a pre weighed 100 mL beaker and dried at 110°C overnight. The dried sample was cooled in a desiccator and re-weighed. An aliquot (50 mL) of 50% HNO_3 was added to the beaker and the solution was digested for 90 min. Following the digestion the solution was allowed to cool and filtered through a glass fibre filter. After

rinsing the glassware the solution was made up to 50.00 mL with ultra pure water. Reagent, sample and procedural blanks were used for the sludge digestion and metals analysis of the digest samples.

Sampling

Boyer staff collected the standard suite of samples from 16 sample sites within the mill on a daily basis. Samples were 24 h composite samples, with the exception of the daily RAS grab sample and a weekend composite sample collected each Monday. Two sampling periods, 28st Sep 2009 to 9th Oct 2009 and 11th Jan 2010 to 22nd Jan 2010, were chosen to represent the pre-decommissioning and post-decommissioning phases of the CCS process, respectively.

The samples were filtered on site and aliquots (70 mL) were decanted into sample containers for transport to Launceston. Reagent blanks and sample blanks were also prepared for the filtered samples. All samples were refrigerated until analysis. Each sample was analysed for seven trace elements: calcium, cobalt, copper, iron, magnesium, molybdenum and zinc.

The wastewater samples collected from the primary and secondary clarifier and the SETP at the Boyer mill were analysed for COD and SS by staff on site. The COD and SS removal in the SETP was determined by using the results from the primary clarifier influent and the secondary clarifier effluent.

Statistical Analysis

Data was analysed using MiniTab 14 statistical software.

2.3 Results and Discussion

2.3.1 Mean COD and SS Removal in Boyer SETP

There had been a long held perception by Boyer staff, based on day to day observations, that the different paper grades contributed to fluctuations in the COD removal in the SETP. No statistical analysis had been undertaken to prove or disprove the hypothesis that the paper grades affected the COD removal. Before calculating the mean COD and SS removal rate, a statistical analysis of the impact of producing different paper grades and of the commissioning of the new TMP plant was required. To this end the data was analysed using a one way ANOVA to compare differences in the COD and SS removal rates and the paper grades, before (Jan 2008 – Oct 2009) and after (Oct 2009 – Feb 2010) TMP 3 commissioning. The COD and SS removal rates from paper machine shutdowns was not included in the analysis as it was not representative of the normal operating conditions in the

plant. The COD and SS samples were 24 h composites, when multiple paper grades were run in a 24 h period the paper grade which was run for the longest time were selected to represent that sample.

There was no significant statistical difference found in the COD removal in the SETP between the paper grades, either before or after the TMP3 commissioning. The COD removal before the TMP 3 plant was 76% for all four paper grades (P – 0.285; df – 416) and between 75% to 77% (P – 0.684; df – 95) after its commissioning (Figure 2.3). This was contrary to expectations, as there are different chemical inputs to the wastewater from the four paper grades.

There was also no significant statistical difference between the SS removal rates and the paper grades in both the before and after commissioning data sets, (P – 0.382; df – 398) and (P – 0.866; df – 93), respectively. The range of the SS removal was 91% to 93% before TMP 3 commissioning and 89% to 93% after commissioning (Figure 2.4). The changes in paper grades did not have a significant effect on the COD or SS removal rates, allowing the before TMP3 commissioning and the after TMP3 commissioning data to be combined for additional analysis (Appendix B).

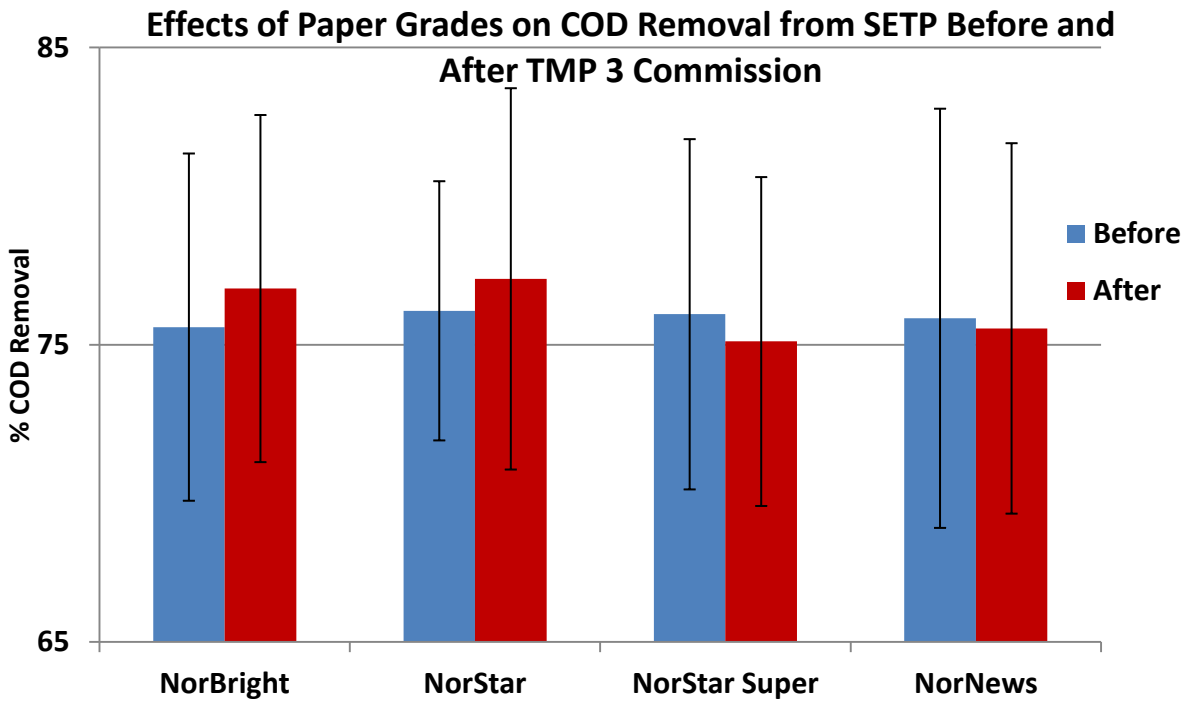


Figure 2.3: The mean percentage COD removal from the SETP for each paper grade produced on paper machine 2, before and after TMP 3 commissioning. The data was collected from Jan 2008 to Feb 2010.

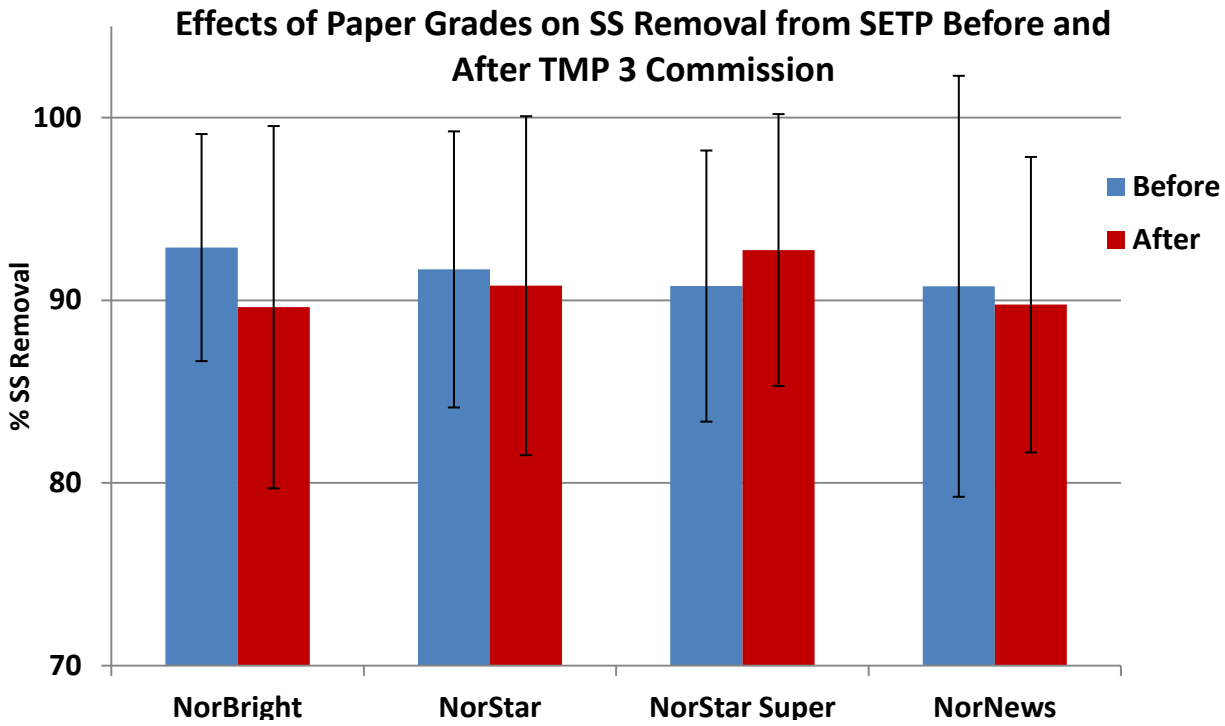


Figure 2.4: The mean percentage of SS removal from the SETP for each paper grade produced on paper machine 2, before and after TMP 3 commissioning. The data was collected from Jan 2008 to Feb 2010.

Through combining all paper grade data the before TMP 3 commissioning and after TMP 3 commissioning mean COD and SS removal rates were compared. Using an ANOVA comparison of means for the data collected there was no significant statistical difference found in the mean COD removal before and after the commissioning of TMP 3 at Boyer (P – 0.867; df - 495). The mean COD removal rate before and after the commissioning of TMP 3 were 76 ± 7% and 76 ± 6%, respectively (Figure 2.5). Softwood species have been reported to contain greater amounts of lignin compared to hardwood species [90, 93]. Lignin derived compounds, being large and complex, are resistant to biodegradation and have been found to be a significant source of residual COD in pulp and paper wastewater in both TMP and KRAFT paper mills [18, 23, 25, 26]. Sjöström reported that the lignin content of *P. radiata* and *Eucalyptus globulus* as a percentage of the dry wood weight was 27% and 22%, respectively [91]. Sjöström also reported that there are differences in the abundance of cellulose and polysaccharides between the two species [91]. Despite these reported differences the process and feed stock changes did not contribute to changes in the wastewater quality.

There was also no significant statistical difference in the SS removal over the same periods (P – 0.559; df – 492). The SS removal rates were 92 ± 8% and 91 ± 9% before and after commissioning,

respectively (Figure 2.5). Due to the absence of a significant statistical difference, all COD and SS data were combined. The combined COD and SS removal rates were 76 ± 6% and 92 ± 8%, respectively, for the period between Jan 2008 and Feb 2010.

One area where there was an appreciable change with the decommissioning of the CCS plant was in the concentration of sodium ions in the WWTP influent. A side effect of the CCS plant was that the sodium hydroxide used as the pulping agent had a significant impact on the pH in the SETP. By removing the CCS bleaching, the pH in the wastewater then required amendments to keep a stable pH of 7 in the SETP. Although sodium hydroxide was used as the amendment the volume required was significantly lower than that used in the CCS. There was an expectation that the decrease in the concentration of the sodium cations would have a positive effect on the COD and SS removal, as research has shown that monovalent cations have an adverse effect on the bond strength of AS flocs, which decreases effluent quality [39, 40]. Again, this was not apparent in the results discussed above.

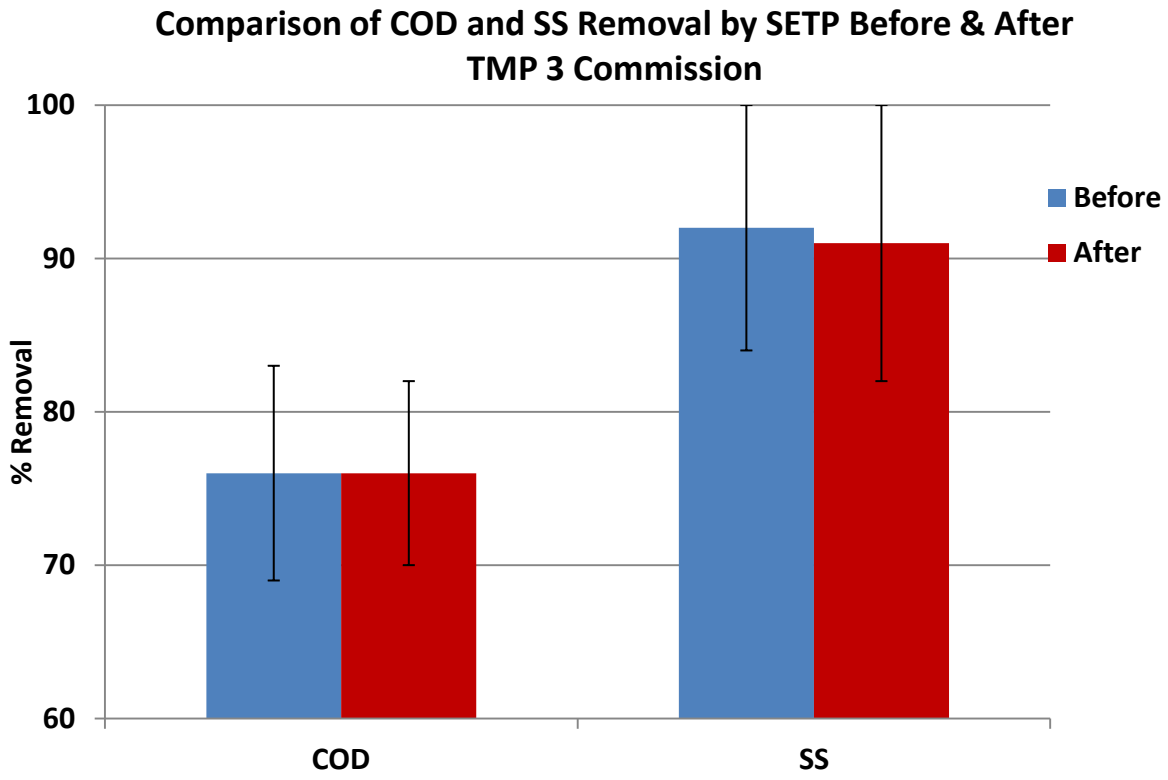


Figure 2.5: The comparison of the COD and SS removal rates before and after the TMP 3 commission.

2.3.2 COD and SS Correlations with Boyer Operating Conditions

Having found no significant difference in the COD and SS data before and after the commissioning of TMP 3, the combined data sets were analysed for correlations with data produced from the SETP controlled parameters. Three of the most important parameters for aiding in the control of activated sludge wastewater treatment are the dissolved oxygen (DO), the sludge age and the F/M ratio. The DO controls the available oxygen for the respiration of the aerobic bacteria in the activated sludge, and keeping the DO concentration stable and above 2.0 mg/L helps control bulking and foaming [51]. Control of the sludge age can also be important in optimising the effective COD removal in activated sludge treatment. It has been reported that the optimal sludge age for the removal of COD is 20 days [23]. The mean sludge age at the Boyer SETP was 8 days. There were, however, no significant correlations detected in the data between both COD and SS in relation to the DO concentration in the ASR and the BFR, or the sludge age (Appendix B).

Analysis of data did show a significant positive relationship (P – 0.000, df 517) between COD removed (mg/L) and primary clarifier influent COD concentration (mg/L) (Figure 2.7). This indicated that the saturation point for the removal of COD in the SETP had not been reached, even at an influent COD of 3,500 mg/L. An indication of saturation would be COD removal plateauing at high COD levels when the SETP is unable to cope with high load. The mean concentration of the Boyer primary clarifier influent was 2005 ± 623 mg/L COD, with a mean removal of 76% equating to removal of 1563 ± 485 mg/L COD. As there was a total removal of 97% BOD, it was assumed that the residual 24% of COD, approximately 440 mg/L, could be attributed to dissolved humic substances that resist degradation in the SETP. To increase the COD removal in the SETP it is necessary to remove these recalcitrant humic substances by microbiological breakdown in the treatment plant. Humic fractions in the porous pot effluent were studied during the porous pot experiments and the results are reported in Chapter 6.

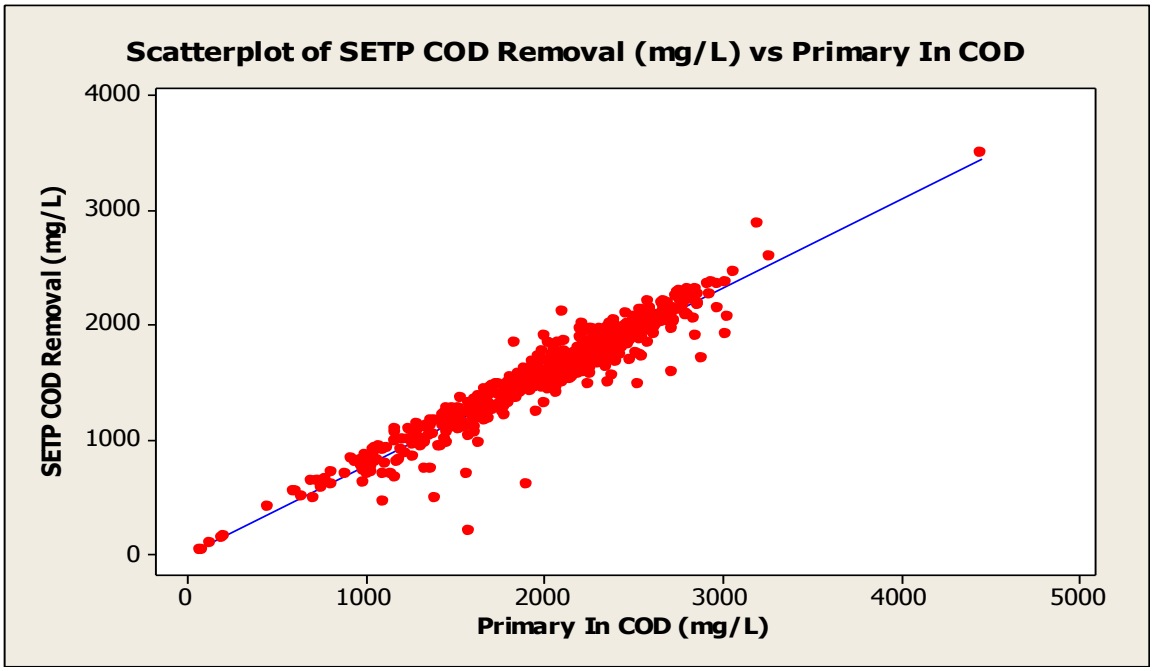


Figure 2.7: Primary clarifier influent COD concentration (mg/L) against the concentration of COD removed (mg/L) in the SETP. The regression line has a P-value of 0.000.

2.3.3 Preliminary Boyer Metals Analysis

An initial grab sample from the primary clarifier influent was collected on the 17th May 2009 and analysed for trace metals by an ELEMENT 1 High Resolution Inductively Coupled Plasma Mass Spectrometer in the University of Tasmania Central Science Laboratory, Hobart. The initial metals analysis was undertaken to determine the levels of a broad suite of trace metals for comparison with the estimated trace metal requirements for optimal biological growth reported in the literature (Table 2.1). The concentration of metals in the initial grab sample can be found in Appendix 2.

The concentrations of molybdenum and cobalt in the grab sample were an order of magnitude below the estimated trace metal requirements (Table 2.1). The concentration of copper was also below the estimated requirement, with iron and magnesium within the estimated required range for optimal biological growth. Calcium and zinc were detected at significantly higher concentrations than the reported biological requirements (Table 2.1). The measured concentration of zinc (1.35 mg/L) was so high that it would be toxic to AS biota if it remained at this level for extended periods [50, 94]. During the analysis reagent blanks were prepared, however, there were no sample blanks prepared at the time the sample was collected. Therefore, contamination cannot be ruled out as a source of the higher than expected zinc concentration detected in the grab sample.

Due to the complex nature of the wastewater the concentrations detected in the grab sample from the Boyer wastewater samples may not be a true representation of the concentration that is bioavailable. Complexation, adsorption and precipitation of metals in activated sludge would decrease the bioavailable portion of some metals especially iron [3]. This will be discussed in full in Chapter 5.

Table 2.1: Experimental results obtained for a range of metals from a Primary Clarifier Influent grab sample collected from the Boyer plant on 17th May 2009. The preliminary analysis of the Boyer sample was determined using ICP MS.

Trace Metals	Ca	Cu	Co	Fe	Mg	Mo	Zn
Primary Clarifier Concentration (mg/L)	11.1	0.08	0.004	1.0	5.9	0.006	1.35
Estimated Trace Element Requirements (mg/L)[8, 71, 76]	3 – 5	0.1 – 1.0	0.02 – 0.50	1 – 4	3 – 10	0.02 – 0.05	0.01 – 1.0

Based on a comparison between their measured concentrations and microbiological requirements calcium, iron, magnesium, copper, zinc, molybdenum and cobalt were selected for further investigation. Calcium, iron and magnesium were included even though they are within or above the required concentrations in the primary clarifier sample as they are major components for biological cell activity, as indicated by their use as nutrient additions for the standard BOD_5 analysis. As the concentration of zinc was above the maximum estimated requirement for biological growth it was also included in further work.

Two 24 hr composite samples, one from the primary clarifier influent and a secondary clarifier effluent were collected as a quality control measure. The samples were filtered through glass fibre filters and refrigerated by laboratory staff at Norske Skog Boyer on the 17th Nov 2009. The samples were transported to Hobart on the same day, acidified with HNO_3 and divided for separate analysis in Launceston by AAS and Hobart by ICP-MS on the following day (See Table 2.2).

The results from the two inter-laboratory survey samples were comparable (See Table 2.2). From Table 2.2, there were significant changes in the concentrations of metals in the quality control samples compared to the initial grab sample. The quality control samples were collected following the commissioning of the new TMP 3 plant. At the time, operational trials at the plant were using lime as an amendment for pH control as there was no input from the CCS wastewater. Though the

use of lime was not implemented as a standard procedure, the calcium concentration in the 24 hour composite samples was four times higher than in the initial grab sample of 17th May. The zinc concentration in the quality control samples was 0.22 mg/L and 0.20 mg/L (Table 2.2) in the primary clarifier influent and the secondary clarifier effluent respectively. This was considerably lower than the 1.35 mg/L detected in the initial grab sample (Table 2.1). Due to the variations in the concentration of zinc detected in the Boyer wastewater and the inability to discount contamination there were no conclusions made in relation to the normal zinc concentration in the SETP.

Table 2.2: Comparative results from selected metal analysis in Launceston and Hobart using AAS and ICP MS, respectively.

	Primary Clarifier Influent (mg/L)		Secondary Clarifier Effluent (mg/L)	
Element	AAS	CSL - ICP MS Check Sample	AAS	CSL - ICP MS Check Sample
Ca	42.18	37.12	47.05	40.42
Co	0.01	< 0.00	0.01	< 0.00
Cu	0.02	0.01	0.02	0.01
Fe	0.37	0.16	0.54	0.24
Mg	8.75	7.21	7.78	6.73
Mo	< 0.00	0.01	< 0.00	0.01
Zn	0.22	0.20	0.20	0.19

It was apparent from the inter-laboratory survey samples that the concentration of iron and copper were significantly lower than the initial grab sample at ≤ 0.5 mg/L and ≤ 0.02 mg/L, respectively, justifying their inclusion as metals of interest. There was no apparent change in the concentrations of cobalt, magnesium and molybdenum between the two sampling events.

From the concentrations of metals detected in the initial grab sample and the quality control samples the selected metals of interest (calcium, magnesium, iron, copper, cobalt, molybdenum and zinc) were retained for further investigation.

2.3.4 Boyer SETP Trace Metals

As the Boyer mill treats water from the Derwent River, known as “clearwater”, for use in the paper making process, metal levels in the Derwent represented baseline trace metal concentrations. Clearwater influent samples were collected daily by the Boyer staff, and refrigerated before

transport to Launceston for AAS analysis. As the changes in the operation of the mill did not affect the quality of the clearwater influent samples the mean trace metal concentrations of both sampling periods were simply combined and averaged (Table 2.3). The trace metals were close to the limit of detection for flame AAS analysis, excepting calcium and magnesium where the clearwater contributed approximately 30% of the total concentration detected in the SETP.

Table 2.3: Concentration of selected trace metals in the Boyer pulp and paper mill treated clearwater influent averaged over 21st Sep 2009 to 9th Oct 2009 and 11th Jan 2010 to 5th Feb 2010.

	Ca (mg/L)	Co (mg/L)	Cu (mg/L)	Fe (mg/L)	Mg (mg/L)	Mo (mg/L)	Zn (mg/L)
Inlet Clearwater	5.5 ± 1.2	0.002 ± 0.001	0.008 ± 0.006	0.02 ± 0.035	1.6 ± 0.475	< 0.001 ± 0.0008	0.016 ± 0.01

Two sample periods were then selected for further testing to determine metal levels in the mill wastewater and the effects of the plant changes brought about by the commissioning of TMP 3. The samples to be collected included the primary and secondary clarifier samples and the SETP samples. The two sampling periods were selected to include periods before and after the plant changes, from 21st Sep 2009 to 9th Oct 2009 and 11th Jan 2010 to 5th Feb 2010 (Appendix A).

In total there are 16 wastewater sampling sites at the NSB mill, to allow any pollutant to be traced back to a particular source. For this study the sample sites within the WWTP are the most important, especially those in the SETP. The SETP comprises of four sample sites: activated sludge reactor (ASR), biofilm reactor (BFR), return activated sludge (RAS) and waste activated sludge (WAS). The samples were collected by NSB staff and refrigerated until analysis by flame AAS. After metal analysis a one-way ANOVA statistical analysis comparing the mean metal concentrations at each of these four sample sites before and after the TMP 3 commissioning was undertaken.

The mean metal concentrations from the before and after the TMP3 commissioning and the respective P-values for the statistical analysis from the WWTP samples are given in Table 2.4 (a summary table of all sample sites is given in Appendix C). There were significant differences between before and after the TMP3 commissioning in ten instances, two for copper, three for iron, three for magnesium and two for zinc . Increases in the iron and zinc concentrations in hardwood species have been reported compared to softwood species [93]. The mean concentrations of iron and zinc were higher in the SETP samples before the TMP3 commissioning where hardwood was a portion of the

feed stock. However, the differences in the iron and zinc mean concentrations in the primary and secondary clarifier were higher in the post TMP3 commission (Table 2.4). In all the sample sites the mean magnesium concentration was higher in the post-TMP3 commission sample. Of those, the increase in mean concentration in three sample sites was statistically significant. Magnesium is an important trace metal for all trees and has been found to be at concentration of between 0.1 ppth and 1.8 ppth dry weight in both soft and hard woods of North America [89, 93].

Table 2.4: Mean concentrations (mg/L) of metals detected in the WWTP from the samples collected before and after the TMP 3 commissioning. P-values for the analysis of mean trace metal concentration of the before and after TMP 3 samples (one-way ANOVA; 95% confidence interval). P-values in red indicate a significant difference.

	(mg/L)	Pri In	Pri Eff	Sec In	Sec Eff	BFR	RAS	WAS	ASR
Ca	Before	21.4	22.1	19.9	20.7	20.2	21.7	24.4	20.1
	After	19.1	20.1	18.3	18.5	18.2	18.4	18.9	18.7
	P-Value	0.49	0.54	0.57	0.44	0.46	0.23	0.06	0.59
Co	Before	0.004	0.004	0.004	0.004	0.003	0.004	0.004	0.004
	After	0.003	0.003	0.003	0.004	0.004	0.003	0.003	0.004
	P-Value	0.12	0.19	0.16	0.40	0.69	0.34	0.13	0.60
Cu	Before	0.05	0.03	0.03	0.03	0.02	0.03	0.03	0.04
	After	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03
	P-Value	**0.01**	0.22	**0.03**	0.44	0.87	0.62	0.88	0.21
Fe	Before	0.27	0.31	0.22	0.28	0.27	0.54	0.87	0.27
	After	0.36	0.27	0.16	0.20	0.25	0.26	0.53	0.18
	P-Value	**0.01**	0.35	0.22	0.11	0.58	**0.00**	**0.00**	0.08
Mg	Before	5.0	4.9	4.7	4.7	4.3	4.8	5.4	4.5
	After	5.5	5.5	5.7	5.9	5.1	5.4	5.7	5.3
	P-Value	0.32	0.16	**0.05**	**0.01**	0.06	0.17	0.56	**0.05**
Mo	Before	0.004	0.003	0.002	0.002	0.002	0.002	0.001	0.002
	After	0.003	0.003	0.002	0.002	0.002	0.002	0.001	0.002
	P-Value	0.20	0.99	0.55	0.90	0.98	0.84	0.72	0.84
Zn	Before	0.16	0.17	0.21	0.11	0.20	0.33	0.26	0.22
	After	0.19	0.15	0.24	0.19	0.24	0.23	0.25	0.25
	P-Value	0.11	0.11	0.07	**0.00**	0.18	**0.00**	0.68	0.20

Calcium is another important trace metal that is required by trees for growth [89, 93]. There was no significant difference in the mean concentrations of calcium in the before and after the change over from mixed feedstock to solely *Pinus radiata*. Similarly with the mean cobalt and molybdenum detected there was no significant difference between the two means from before and after the mill feed stock change. The mean concentration of cobalt and molybdenum in the WWTP sample sites ranged between 0.003 – 0.004 mg/L and 0.001 – 0.004 mg/L respectively.

As the concentrations of metals in the initial grab sample and the inter-laboratory samples were different (Table 2.1 and Table 2.2), it was not unexpected to have some differences between the samples collected prior to and following commissioning of TMP 3. However, if there were major changes in the trace metal concentrations from the change of feed stock, then it would be expected that these would be seen in all the WWTP samples, which was not the case. The individual mean metal concentration at each sample site can be found in Appendix C.

As there were only four statistically significant differences found between the mean metal concentrations detected within the SETP (that is, from the BFR, RAS, WAS and ASR sample points) from the samples collected before the TMP 3 commissioning and those following the TMP 3 commissioning (Table 2.4), the data from each SETP sample sites were compared. From this comparison there were no significant differences in the mean concentrations of metals from the SETP sample points and the four SETP sites were combined to determine the long term mean trace metal concentrations (Table 2.5).

The long term mean metal concentrations were seen to differ from the levels detected in the initial grab sample (Table 2.1), notably for calcium, copper, iron and zinc. The mean calcium concentration was found to be higher than the grab sample while the mean concentrations of copper, iron and zinc were all at most 50% of the concentration in the initial grab sample.

As can be seen in Table 2.5 the concentrations of calcium, magnesium and zinc were found to be either above or within the estimated microbial trace metal requirements. The analyses of zinc indicated that the grab sample result was not typical and the concentration was generally within the estimated requirements with the mean zinc concentration of 0.25 mg/L. The concentration of zinc in the initial primary clarifier grab sample was 1.35 mg/L, significantly higher than the accepted requirement. It could not be determined if the high zinc level in the sample was from sample contamination or process inputs. However, if the concentration of zinc in the SETP was prolonged over consecutive days above 1.0 mg/L it most likely would have had a toxic effect on the SETP biota [50, 94].

The mean concentrations of cobalt, copper, iron and molybdenum were below the accepted microbial requirements. The reported cobalt concentration for optimal microbial growth in municipal activated sludge has been between 0.02 – 0.05 mg/L [49], while the maximum estimated nutrient requirements from Table 2.5 has been calculated to be up to 0.5 mg/L. The mean concentration of cobalt in the SETP was found to be approximately 0.003 mg/L, significantly lower than the estimated requirements given in the literature (see Table 2.5).

Table 2.5: Mean concentration of selected trace metals (mg/L) in SETP from Boyer.

Metal (mg/L)	**Ca**	**Co**	**Cu**	**Fe**	**Mg**	**Mo**	**Zn**
Mean Concentration of Combined SETP Samples	19.9 ± 7.8	0.003 ± 0.001	0.03 ± 0.03	0.38 ± 0.29	5.1 ± 1.3	0.002 ± 0.002	0.25 ± 0.09
P-Value Between SETP Sample Sites	0.994	0.794	0.717	0.232	0.741	0.105	0.801
Accepted Microbial Trace Element Requirements[8, 71, 76]	3 - 5	0.02 – 0.50	0.1 – 1.0	1 - 4	3 - 10	0.02 – 0.05	0.01 – 1.0
SETP Metal removal*	4%	3%	35%	33%	0%	24%	20%
Concentration of Metals in Sludge (mg/kg)	1.35×10^{-4}	7.89×10^{-7}	2.74×10^{-5}	6.18×10^{-4}	3.52×10^{-3}	1.21×10^{-5}	2.48×10^{-5}
Sludge digest St Dev	5.99×10^{-4}	1.99×10^{-7}	1.52×10^{-6}	1.03×10^{-4}	3.88×10^{-4}	1.02×10^{-5}	1.60×10^{-5}

*The removal of metals calculated from the primary clarifier effluent and the secondary clarifier influent.

The removal of metals in the SETP was determined by subtracting the secondary clarifier influent metal concentration from the primary clarifier effluent concentration. Estimating the concentration of individual metals removed in the SETP was important in understanding how they react with the sludge and in estimating the bioavailable concentration for each metal. As expected, due to the solubility of calcium and magnesium there were only limited amounts removed in the SETP process. Additionally only minimal amounts of cobalt were removed in the SETP, something observed in previous studies [49]. On the other hand between 20 and 35% of the copper, iron, molybdenum and zinc in the primary clarifier were removed in the SETP. The fate of metals in activated sludge will be discussed in Chapter 5.

There was only a single sludge sample collection period between the 21st Sep and 9th Oct 2009 where samples were collected daily prior to the commissioning of the TMP 3. The concentration of metals in the sludge was low compared to the guidelines for the land application of biosolids [95], ranging between 7.9×10^{-7} mg/kg Co to 3.5×10^{-3} mg/kg Mg (Table 2.5). The acceptable metal levels given in the Tasmanian Biosolids Reuse Guidelines are copper and zinc, 100 mg/kg and 200 mg/kg, respectively [95] while the acceptable levels of cobalt and molybdenum have been reported to be 40 mg/kg and 5 mg/kg, respectively in the Land Application of Municipal Sewage Sludge Guidelines [96].

Metals with the higher concentration in solution generally had a greater concentration in the sludge. Although, the concentration of iron was 6.18 x 10^{-4} mg/kg and the concentration of calcium was 1.35 x 10^{-4} mg/kg where the concentration of iron in the aqueous phase was significantly lower than that of calcium, at 0.38 mg/L and 19.9 mg/L, respectively. The greater level of iron in the sludge compared to calcium reflects the solubility of the two metals at pH 7. The removal of iron, copper and zinc from the SETP was also an indication of the affinity of those metal ions to bind to solid surfaces in the sludge or complex with the organic ligands and precipitate out of solution [97, 98]. Chapter 5 gives detailed discussion on the fate of metals in activated sludge.

A single spike of 1.20 mg/L molybdenum was also detected in the PM2 effluent, on Monday 28th Sep 2009. This decreased over 3 days to the base line concentration of 0.002 mg/L. In two separate studies Burgess *et al.* have found the addition of 0.5 mg/L molybdenum to be beneficial to activated sludge in one experiment [8] and in another they reported that molybdenum had a toxic effect [9]. The spike in the PM2 effluent had a limited effect on the molybdenum concentration in the primary clarifier influent increasing from a mean 0.002mg/L to 0.007 mg/L on the day.

2.3.5 Boyer Trace Metal Correlations with COD Removal

To investigate possible correlations between the concentration of metal ions and the efficiency of COD removal a regression analysis for the analysis of variance was undertaken with a confidence interval of 95%. For the regression analysis two outliers were observed: the copper and molybdenum outliers detected on 22nd Jan 2012 and 28th Sep 2009, respectively. The concentration of the copper and molybdenum in the outliers were one and three orders of magnitude greater than their respective means. The outliers were removed from the data set due to the potential of their inclusion giving a false positive correlation between the concentration of the metals and the COD and SS removal.

There were four individual sample sites found to have correlations between an individual metal ion and the COD removal rate with P-value below 0.05 (Table 2.6), however, in all cases the R^2 value was < 0.2.

Table 2.6: MiniTab 14 Regression analysis of COD removal and trace metal concentration for each SETP sample site. A P-value of ≤0.05 indicates a significant correlation between the increased COD removal and increased metal concentration.

	Ca	Co	Cu	Fe	Mg	Mo	Zn
Primary Influent	0.79	0.36	0.10	**0.05**	0.69	0.53	0.82
Primary Effluent	0.85	0.33	**0.03**	0.26	0.92	0.44	0.36
ASR	0.60	0.44	**0.01**	0.38	0.84	0.29	0.81
BFR	0.89	0.88	0.12	0.17	0.66	0.75	0.91
RAS	0.63	0.49	0.36	0.43	0.91	0.32	0.72
Secondary Influent	0.76	0.94	**0.03**	0.47	0.80	0.38	0.75
Secondary Effluent	0.54	0.08	0.41	0.29	0.53	0.47	0.51

There was a correlation detected in the primary clarifier influent between the COD removal and the concentration of iron (P – 0.05) (Table 2.6). As there was only one correlation detected in the WWTP sample sites with iron the finding was not conclusive. The correlation indicated that there was a 10% increase in COD removal from approximately 70% to approximately 80% associated with an increase in iron concentration from 0.1 mg/L to 0.6 mg/L.

Correlations between the COD removal and copper concentration in the primary effluent and in the secondary influent were found, both (P – 0.03) (Table 2.6). It was assumed that if the correlation between the metals and COD removal was found in the primary clarifier, then that correlation would be seen throughout the SETP and the secondary clarifier. It is of some consequence that there were a total of three samples where a correlation was detected, as they were detected in the SETP influent and effluent and from a sample within the SETP. With correlations in multiple samples there is a greater likelihood that the concentration of copper within the WWTP could be affecting the COD removal.

There was also a 10% increase from approximately 70% COD removal to 80% as the concentration of copper increased from 0.005 mg/L to 0.1 mg/L, this occurred in the three sample sites where there was a significant correlation between the copper concentration and the COD removal. The addition of up to 1.0 mg/L copper has been found to improve COD removal in an unspecified industrial wastewater treatment plant [43]. The concentration of copper detected in the SETP was 0.03 mg/L, clearly lower than the minimum required concentration for optimal microbial cell function [8, 71]. Therefore, the addition of up to 1.0 mg/L copper was expected to increase the rate of COD removal from the Boyer SETP.

As there were correlations between COD removal and the concentration of both copper and iron, there were two methods of COD removal proposed: firstly through increased microbial growth brought about by having the optimum concentration of copper (between 0.1 – 1.0 mg/L Cu), and secondly through the metal ions complexing dissolved humic material and improving settling in the secondary clarifier.

2.3.6 Boyer Trace Metal Correlations with SS Removal

Another suite of regression analyses were performed to determine whether correlations existed between the metal concentrations in the WWTP and the rate of SS removal (see Table 2.8). There were correlations detected with P-values < 0.05 between the concentrations of calcium, magnesium, zinc and molybdenum and SS removal, however, the R^2 was < 0.2 Regardless, a correlation exists between SS removal and the concentration of calcium at all seven sample points indicating that increasing calcium concentration decreased SS removal. There was approximately a 4% decrease in the rate of SS removal when the concentration of calcium increased from 5 mg/L to 45 mg/L. This was unexpected as the concentration of divalent cations, especially magnesium and calcium have been reported to increase the rate of SS removal in the WWTP effluent [39, 40, 99]. The fact that the correlation was found at all seven sample points gives greater confidence that the concentration of calcium has a real effect on the SS concentration in the effluent, whereas a correlation with one sample could be an anomaly.

Table 2.8: MiniTab 14 Regression analysis of SS removal and trace metal concentration in each SETP sample site. A P-value of ≤0.05 indicates a significant correlation between the rate of SS removal and individual metal concentrations.

	Ca	Co	Cu	Fe	Mg	Mo	Zn
Primary Influent	**0.03**	0.29	0.90	0.78	0.12	**0.03**	0.20
Primary Effluent	**0.01**	0.60	0.81	0.17	**0.02**	**0.03**	0.39
ASR	**0.01**	0.68	0.71	0.55	0.08	0.27	0.17
BFR	**0.03**	0.22	0.76	0.77	0.10	**0.02**	**0.04**
RAS	**0.00**	0.82	0.77	0.80	0.06	0.36	**0.02**
Secondary Influent	**0.00**	0.79	0.69	0.45	**0.04**	0.09	**0.01**
Secondary Effluent	**0.00**	0.71	0.99	0.30	0.07	0.13	0.93

Similarly the correlations between the SS removal and concentration of magnesium were negative, though this was seen only in the Primary Effluent and Secondary Influent samples. There was an indication that as the concentration of magnesium increased from 3 mg/L to 8 mg/L that the SS removal decreased from approximately 98% to 94%. This is despite the expectation that the divalent cations would complex the suspended matter and reduce the SS in the SETP. As previously stated, there would also be an expectation that if there was a correlation, it would be seen throughout the entire SETP. As this was the case only with calcium, it is expected that there are other variables interfering.

There were also negative correlations detected in the BFR, RAS and secondary clarifier influent samples between the SS and zinc concentrations. The decrease in SS was between 4 to 6% when the zinc concentration increased from 0.05 to 0.5 mg/L. The negative correlations were unexpected as calcium, magnesium have the potential to bind, through either complexation or chelation, to the suspended matter in the wastewater, therefore, decreasing the residual SS in activated sludge effluent [39, 99].

On the other hand, increasing molybdenum concentration in the primary influent and effluent and the BFR was associated with increased SS removal. The SS increased by 4% from approximately 95% to 99% when the concentration fluctuated between 0.00 mg/L to 0.01 mg/L. The mean concentration of molybdenum in the SETP was approximately 0.002 mg/L, and although there were three correlations detected between the concentration of molybdenum and the removal rate of SS it would be highly unlikely that the fluctuations in the molybdenum concentration would have such a large effect on the removal of SS. The concentration of molybdenum in the SETP would be unlikely to have an effect on the binding or settling of suspended matter, making it hard to explain how such a low concentration could have a significant effect without assuming that there could be an unknown variable effecting the SS removal.

2.4 Conclusions

The Norske Skog, Boyer paper mill produces four grades of paper, which generate wastewater containing different chemical inputs. The changes in the paper grades throughout the normal daily operations of the plant did not significantly affect the rates of COD and SS removal from the SETP. The mean COD and SS removal from January 2008 to Feb 2010 was 76 ± 6% and 91 ± 8%, respectively, which were below internal targets set by Norske Skog.

In October 2009 there was a significant operational change at the Boyer mill. The CCS plant was decommissioned and a new TMP plant was commissioned to replace it, leading to changes in the chemical inputs to the SETP. The feedstock also changed at this time from a mixed *Pinus radiata* and *Eucalypt* feed to solely *P. radiata*. Statistical analysis of the data collected across the changeover showed that the changes in effluent quality anticipated by Boyer Staff based on the changes in process and feed stock did not eventuate. The mean COD before and after the TMP commission were 76 ± 7% and 76 ± 6%, respectively (P – 0.867; df – 495), while the mean SS removal was 92 ± 8% and 91 ± 9% before and after commissioning, respectively (P – 0.559; df – 492).

There were no significant changes in the trace metal concentrations in the wastewater samples at Boyer during the changeover in plant and feedstock. There were also no statistical differences in the metal concentrations from the four sample sites within the SETP. From published trace metal requirements for optimal microbial activity the combined mean concentrations in the SETP of the trace metals cobalt (0.003 ± 0.001 mg/L), copper (0.03 ± 0.03 mg/L), iron (0.38 ± 0.29mg/L) and molybdenum (0.002 ± 0.002 mg/L) were below the reported microbial requirements. These deficiencies could be a contributing factor for the lower than expected COD removal rates from the treatment plant. The trace metals calcium (19.9 ± 7.8 mg/L), magnesium (5.1 ± 1.3 mg/L) and zinc (0.25 ± 0.09 mg/L) were either at or above the accepted microbial requirements.

There were also no significant statistical correlations between COD or SS removal, as measured between the primary clarifier influent and the secondary clarifier effluent, and the concentration of the trace metals analysed in the SETP, further indication that the changes in process inputs and feed stock did not affect the final effluent quality. Adding the essential trace metals which were found to be deficient within the Boyer wastewater could increase biological activity in the activated sludge reactor, therefore, potentially increasing the COD removal rates.

There was also a significant relationship found between the concentration of COD in the primary clarifier and the concentration of COD removed from the SETP (P – 0.000; df – 517), indicating that the saturation point, the point where the influent COD was too high to treat in the SETP, had not been reached, therefore, the COD removal rates were not limited by the capacity of the treatment plant.

Chapter 3 Trace metal addition to porous pots

3.1 Introduction

3.1.1 Addition of Trace Metals to Activated Sludge

Initial analysis of wastewater samples at Norske Skog, Boyer indicated that seven (7) trace metals could be beneficial as amendments to the mill's secondary effluent treatment plant (SETP) (see Table 2.5)(P 34). Cobalt, copper, iron and molybdenum were found to be below the accepted microbial trace element requirements in an activated sludge plant. The objective of adding trace metals and water-soluble vitamins to the porous pots was to stimulate bacterial growth in a micro-nutrient deficient wastewater, and thereby, increase the rate of COD and SS removal. Even though they were within or above the accepted microbial requirements, calcium, magnesium and zinc were included in the experiments due to negative correlations between the SS removal rate and the concentration of the individual metals in the SETP at Boyer.

3.1.2 Porous Pots

Porous pots were selected as a means of running batch experiments on a laboratory scale to investigate the use of trace metals and water-soluble vitamins to improve chemical oxygen demand (COD) and suspended solids (SS) removal in the Norske Skog, Boyer SETP. Porous pots have a solid outer vessel with a porous high density polyethylene liner (see Figures 3.1 and 3.2) and are recommended by the USEPA as a standard test for the simulation of activated sludge treatment plants

There were a number of operational requirements for the porous pot experiments including the F/M ratio, the sludge age and the dose rates of macro-nutrients. The F/M ratio calculation aims to balance the COD entering the system and the mass of activated sludge in the system to consume that COD. At the time of these experiments the Boyer SETP was employing an F/M ratio of 0.28. Sludge age, a measure of the retention time of the activated sludge in the porous pots, incorporates the wasting rate of activated sludge to encourage renewed bacterial growth and control the concentration of MLSS in the porous pots. The aim was to have a sludge age between 5 and 7 days. The ratios of nitrogen and phosphorus added to the Boyer SETP were COD:N 45:1 and COD:P 1000:1, set so that nitrogen and phosphorus were not limiting. The dissolved oxygen levels and temperature were initially set at 2.0 ppm and 35^{o}C, respectively.

Figure 3.1: Porous pot experimental set up.

From the left: 55 L storage container fitted with a mechanical stirrer; peristaltic pumps to transfer feedstock to the porous pots (top), additive reservoirs (centre) and peristaltic pumps to transfer additives to the porous pots (bottom); six porous pots fitted with waste taps, air tubes and heaters.

3.2 Materials and Methods

3.2.1 Materials

Reagents

The macronutrients N and P were added in the form of LR grade $CO(NH_2)_2$ and H_3PO_4, respectively, both supplied by BDH. The trace elements for the micronutrient additions were added as solutions of $CaCl_2$, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, $NaMoO_4.2H_2O$ and $ZnSO_4.7H_2O$ (BDH), $CuSO_4.5H_2O$ (Ajax) and $CoSO_4.7H_2O$ (M & B Laboratory Chemicals). To inhibit the formation of iron hydroxide precipitates in the bulk solution, its pH was adjusted to 2 with H_2SO_4. The B-group vitamins were supplied by Sigma Chemicals.

$K_2Cr_2O_7$ (Ajax), $HgSO_4$ and AR grade H_2SO_4 (Merck), Ag_2SO_4 and $C_8H_5KO_4$ (BDH) were used for COD digestion. The phosphate buffer reagents for BOD_5 $NaHPO_4$ (anhydrous), KH_2PO_4 and K_2HPO_4 were obtained from BDH and NH_4Cl from Ajax chemicals. The BOD_5 nutrient solutions were prepared using $MgSO_4.7H_2O$, $CaCl_2$ and $FeCl_3.6H_2O$ supplied by BDH.

Potassium antimonyl tartrate (AR) and ammonium molybdate (AR) for the total PO_4^{3-} analysis were obtained from M & B Laboratory Chemicals and AJAX Chemicals, respectively. Ascorbic acid (AR) was supplied by Sigma Chemicals.

Concentrated hypochlorite solution (12.5% OCl^-) was obtained from Hunters Products, Tasmania.

Equipment

A 1000 L container was used to transport bulk samples from Boyer to Launceston, where the sample was decanted into 25 L containers and stored at 4°C. The method for reproducing the conditions in an activated sludge reactor was adapted from the USEPA guidelines for a simulation test using porous pots (OPPTS 835.3220, Porous Pot Test) [100, 101]. The method uses a porous high density polyethylene liner (70 µm, Scientific Commodities Inc, Arizona, USA) as an inner vessel to overcome problems with circulating sludge and avoid the loss of sludge in the effluent [102] (Figure 3.2) The pots were manufactured by the Central Science Laboratory, Hobart.

An Elite 802 aquarium air pump with a 5 mm open tubing and a commercial fish tank aeration stone for aeration and mixing was fitted to each porous pot with 306 marine grade stainless steel nuts used as weights to keep the aeration stones in the bottom of the pot. Standard 200 W Fluval Tronic aquarium heaters were used in each pot to maintain a temperature of 35^oC.

Minipuls and Alitea peristaltic pumps were used to supply feed and nutrient solutions with flow rated tygon pump tubing (TACS Australia). The pH was measured using an Orion 420A pH meter, and temperature and DO were measured with an OxyGuard DO probe. A Pharmacia Novaspec II UV-Vis spectrophotometer was used to measure absorbance for the COD, PO_4^{3-} and NH_3 analyse, while a Ratek dry block heater was used for COD digestions. Advantec GA – 55 glass fibre filters were used to filter suspended solids (SS) and mixed liquor suspended solids (MLSS) samples. The turbidity was analysed using a Hach 2100P turbidimeter and the BOD incubation bottles and stoppers were supplied by DKSH Australia.

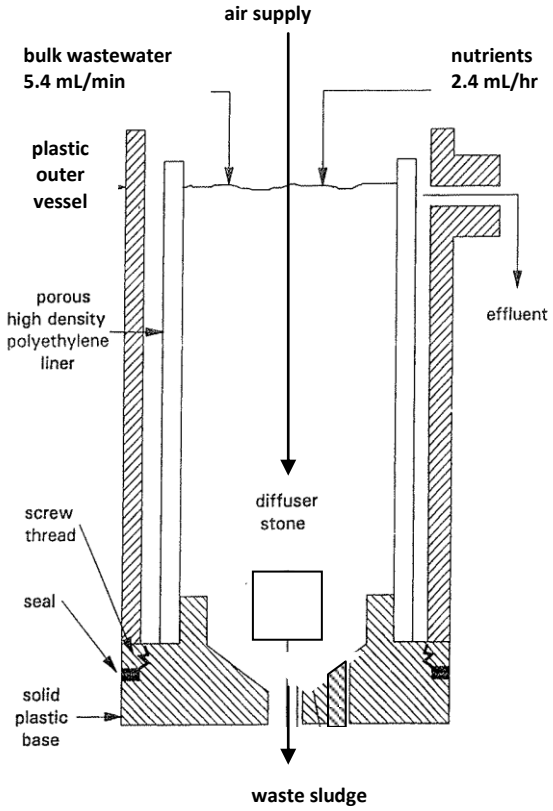


Figure 3.2: The schematic of a porous pot. Adapted from the USEPA [100]

3.2.3 Porous Pot Methods

Procedure

Runs were completed using eight 1000 L primary clarifier effluent samples collected from the Boyer SETP on 10th Aug 2010, 4th Oct 2010, 12th Nov 2010, 5th Jan 2011, 7th Mar 2011, 8th Aug 2011, 8th Nov 2011 and 30th Jan 2012. The pots were seeded in Launceston on the day of collection with 2 L of return activated sludge no more than 3 h old. A 55 L container was used to supply the primary treated sample to the pots. The bulk solution was stirred using a Eurostar digital stirrer at 300 rpm and delivered to each pot at 5.4 mL/min. The flow rate for the macro and micronutrients was 2.4 mL/h. Nitrogen and phosphorus were supplied at a ratio of COD:N of 33:1 and COD:P of 1000:1, the levels used in the Boyer SETP at that time. The target residual nitrogen was 1.0-2.0 mg/L and phosphorus was 0.5-1.0 mg/L. An initial stabilisation period of 3-4 days was allowed during which adjustments to flow rates and air supply levels were made before the trace element additions started.

The run duration times were between 21 and 26 days following the stabilisation period. Only data collected after day 15 was processed to evaluate the results. The temperature was maintained at 35^{o}C, and the DO level at ≥ 2.0 mg/L. The full-scale SETP plant operation was replicated by setting the influent flow rate at 5.3 mL/min to give a retention time of 14 h, with a target sludge age and food to mass (F/M) ratio of 5-7 days and 0.28, respectively.

Analysis

The temperature, pH, DO, and peristaltic pump flow rates were recorded daily, with a waste activated sludge (WAS) sample discarded daily so that a given F/M ratio could be maintained. The tests for COD, Sludge Volume/MLSS, NH_3, PO_4^{3-} and SS were undertaken 2 to 3 times per week. In the last two weeks of a run the COD and SS were determined daily. The BOD_5 analysis were performed weekly.

As SS is measured daily at the Boyer effluent treatment plant, it was important to measure it on a similar basis during the experimental runs to have comparable results between the two. The APHA Standard Method for Total Suspended Solids Dried at 105°C [103] was initially used to determine SS. This required that the contents of a porous pot be decanted into a 5 L beaker and allowed to settle for 30 min without aeration or heating. A 100 mL aliquot of clear liquor was pipetted from the top before the remainder was poured back into the porous pot. The 100 mL aliquot was then filtered through pre-weighed glass fibre filters, dried in an air oven and weighed to calculate SS. To eliminate the risk that the 30 minute settling period with no aeration or heating would affect the porous pot run, turbidity was introduced as a fast and simple method for suspended solids at the beginning of Run 3. The turbidity analysis required 15 mL of clear supernatant sample which was collected after a 200 mL WAS sample was settled for 30 min.

The MLSS method was adapted from the USEPA Porous Pot Test [100]. A 50 mL aliquot was collected from each pot and placed in a 50 mL measuring cylinder. After a settling period of 30 min the SV was recorded in mL/L by multiplying the sludge height by 20. The samples were then filtered through glass fibre filter papers, dried and weighed to calculate the MLSS (mg/L). The sludge volume index (SVI) was calculated using Equation 3.1

3.1

The sludge age was determined through Equation 3.2.

3.2

Trace Metal and Vitamin Addition

The trace elements calcium, iron(III) and magnesium were added to the porous pots by peristaltic pumps from 500 mg/L bulk solutions to achieve an increase in concentration of 4.0 mg/L in the pots. Copper and zinc were added from 60 mg/L solutions and cobalt and molybdenum from 6 mg/L solutions to increase the concentrations by 0.5 mg/L and 0.05 mg/L, respectively. The water-soluble vitamins were added to the pots from bulk solutions of 120 mg/L to give an increased concentration of 1 mg/L.

Run 7 of the porous pot experiments revisited the additions of copper and cobalt from previous experiments at various concentrations. Three Cu trial pots were employed with increased concentrations of 1.0, 0.25 and 0.1 mg/L in the pots obtained from bulk copper solutions of 180 mg/L, 45 mg/L and 18 mg/L, respectively. The target increase concentrations in the two cobalt pots were 0.1 mg/L from a 6.5 mg/L bulk solution and 0.025 mg/L from a 1.6 mg/L solution.

Run 8 of the porous pot experiments used multiple metal additions to individual pots. The run was performed using a control pot (pot 1) plus 5 trial pots. Copper was added to each trial pot, to give an increased concentration of 0.5 mg/L. Iron(III) (pots 2 and 3), magnesium (pots 5 and 6) and calcium (pot 4) were added to give an increased concentration of 4.0 mg/L of the metal in the respective pots. The metals were delivered from separate bulk solutions of 68 mg/L copper and 540 mg/L iron, magnesium and calcium. The ratio of COD:N was 45:1 and COD:P was 1000:1. The standard dose rate of nitrogen (as urea) and phosphorus (as H_3PO_4) was 22 mg/L and 1.0 mg/L, respectively.

Cleaning

While the contents of the pots were decanted into 5 L beakers, the empty pots were soaked for 2 h in a 5:1 water:hypochlorite 20 L bath once per week. These were then rinsed thoroughly with running water for half an hour. Aeration, macro- and micronutrient additions and heating were continued in the 5 L beakers during the cleaning procedure.

3.2.2 Analytical Methods

Prior to analysis the samples were filtered through Advantec GA – 55 glass fiber filters.

BOD

Analysis of BOD_5 was by the APHA standard method (507 Oxygen Demand Biochemical) [103], where the porous pot effluent was diluted 1:5 and influent was diluted 1:500 with aerated ultra pure water.

COD Method

The method for COD analysis was adapted from the APHA standard method (508 C Oxygen Demand Chemical, Closed Reflux, Colorimetric Method) [103].

Reagent Preparation

Potassium dichromate (12.5 g) was dried in an oven at 105°C ± 5°C for 2 h and allowed to cool in a desiccator. An aliquot of sulphuric acid (1 L) was added to a 3 L beaker and stirred with stirrer bar while 10.2145 g of the dried potassium dichromate was slowly added. The solution was stirred for a further 1 h, then allowed to stand for 10 min. The supernatant was decanted into another clean beaker leaving any undissolved potassium dichromate behind. About 30 mL of MilliQ water was added to dissolve the remaining potassium dichromate and this was then carefully added to the sulphuric acid/potassium dichromate solution. The acid solution was allowed to cool before adding 15.0 g of silver sulphate and 20.0 g of mercuric sulphate while continuously stirring. After stirring the solution for a further 2 h, 2.0 mL aliquots were pipetted into clean Hach tubes while continuing to stir. Tubes were then sealed and stored until use.

Standard calibration

The 1000 mg/L COD standard was prepared by drying 1.0 g of potassium hydrogen phthalate at 120°C overnight. 0.8500 g was then dissolved with MilliQ water in a 1 L volumetric flask and made up to the mark with Millipore water. The standards were prepared in 10 mL Hach vials following Table 3.1.

Table 3.1: Preparation of standards for COD analysis

	COD Concentration (mg/L)	Volume of 1000 mg/L Standard (mL)	Volume of MilliQ water (mL)
Blank	0	0.0	3.5
Std 1	250	0.5	3.0
Std 2	500	1.0	2.5
Std 3	1000	2.0	1.5

Sample Preparation and Analysis

All COD samples were duplicated, blank samples were prepared by pipetting 3.5 mL of MilliQ water into a pre-prepared 10 mL Hach vial containing 2 mL reagent. Table 3.2 was used to estimate the dilution factor for influent and effluent samples, and the required volume of MilliQ water was added before the required sample volume. The vial caps were secured and placed onto a Ratek dry block

heater at 150°C for 2 h. The vials were allowed to cool before determining absorbance at 590 nm using the blank to zero the spectrophotometer.

Table 3.2: COD sample preparation

Sample Concentration (mg/L COD)	Volume of Sample (mL)	Volume of MilliQ Water (mL)	Dilution Factor
< 250	3.5	0	0.57
250 – 1000	2.0	1.5	1
1000 – 2000	1.0	2.5	2
2000 – 4000	0.5	3.0	4

Nitrogen Ammonia (Salicylate Method)

Residual NH_3 was determined by the Salicylate Method using pre-prepared Hach tubes according to Hach method 10205 using reagent blanks, sample blanks and check samples [104]. The 1000 mg/L ammonium standard solution was prepared by making 3.819 g of ammonium chloride up to 1 L using Milli Q water. An intermediate 20 mg/L standard was prepared by dilution with Milli Q water and 2.5 mg/L, 5.0, mg/L, 7.5 mg/L and 10.0 mg/L standards were prepared by dilution from the 20 mg/L intermediate standard. The blank was prepared using Milli Q water and was used to zero the spectrophotometer at 690 nm for the calibration.

PO_4^{3-} (Soluble P) Method

Analysis of PO_4^{3-} was undertaken using the ascorbic acid method according to the APHA standard methods (424 F. Ascorbic Acid Method) [103], by pipetting 6.25 mL of filtered sample into a clean 10 mL Hach vial and adding a single drop of phenolphthalein indicator. 1.0 mL of combined reagent was added to the vial and mixed, following which the solution was allowed to stand for 10 min before analysis using a spectrophotometer set at 880 nm. Milli Q water was used as a blank to zero the spectrophotometer.

3.3 Results and Discussion

3.3.1 Preliminary Porous Pot Run

The porous pot tests were commenced on a bulk sample collected from Boyer on the 10th Aug 2010, from wastewater generated through TMP of *P. radiata*, with a preliminary trial run over two weeks with the addition of only the macro-nutrients, nitrogen and phosphorus. If there was any visible evidence of microbial growth in the bulk macro-nutrient solution during any run, such as, suspended matter or a settled green film on the base of the bulk container, the solution was discarded and it was replaced with a fresh solution.

Following an initial stabilisation period of 3 days the COD removal in each of the four pots was analysed using an ANOVA comparison of means. There was no significant statistical difference between the COD removal in the four pots (P - 0.883) over the initial two week period. The COD removal from the four pots was 77 ± 4%, 78 ± 3%, 78 ± 4% and 78 ± 4%. For comparison the removal at Boyer on the 10th Aug 2010 was 81%. Due to the good agreements between pots in the preliminary run, the subsequent porous pot runs were undertaken with one control pot and a number of trial pots. Using one control porous pot has been used by Burgess et al. [41] and was suggested as a minimum by USEPA Porous Pot Test [100].

3.3.2 Bulk Influent Samples

To verify that the refrigerated bulk samples were stable over each porous pot run the COD of the feedstock was determined daily for the duration of the run. The mean of these results over a run was then compared to COD of a 24 h composite sample collected by Boyer staff on the day of collection (Table 3.3). Two important points are clear from this table. First that there is significant statistical difference between the eight bulk samples collected between August 2010 and January 2012 (P – 0.000; df – 117), indicating that, due to the plant operational variability, the COD between runs fluctuated considerably. Second, as indicated by the result for Run 2, the single sampling event represented by the collection of the 1000 L bulk sample can be quite different to the result for the 24 h composite.

Table 3.3: Comparison of Boyer Primary Clarifier Effluent COD (24 h composite sample) and Porous Pot mean COD.

Run	Sample Collection Date	Porous Pot Mean COD (mg/L)	Boyer Primary Clarifier Effluent COD (mg/L)	Difference
Run 1	10 Aug 2010	1390 ± 178	1335	4%
Run 2	04 Oct 2010	1663 ± 159	1257	24%
Run 3	08 Nov 2010	1614 ± 104	1511	6%
Run 4	05 Jan 2011	1325 ± 145	1286	3%
Run 5	07 Mar 2011	1637 ± 141	1291	21%
Run 6	08 Aug 2011	2213 ± 143	1897	14%
Run 7	08 Nov 2011	1030 ± 97	1170	12%
Run 8	30 Jan 2012	1048 ± 125	1204	12%

Daily variations in the quality of the wastewater produced at Boyer are due to the changes in paper grades leading to changes in organic loading. The decision to opt for the variability of "real" wastewater samples was to ensure that the results would be representative of the operation of the Boyer SETP, that is the micro-nutrients would be tested on wastewater samples truly representative of the Boyer mill. Theoretically as a synthetic wastewater would have no variability in chemical properties, the micro-nutrient addition experiments could have been more reproducible. On the other hand, by using Boyer samples there would be a greater understanding of the micro-nutrient interactions within the activated sludge and wastewater matrix.

3.3.3 Initial Trace Metal Porous Pot Runs (Runs 1 and 2)

Due to the time required for each run, an experimental design was implemented which allowed for an initial seven trace metals and six water-soluble vitamins to be tested over four separate porous pot runs. Each of Runs 1 and 2 consisted of one control pot and a single pot for each trace metal or water soluble vitamin addition to determine which micro-nutrients had a positive effect on COD removal. The results from the water-soluble vitamin additions will be discussed in Chapter 4. The micro-nutrients which had a positive effect on the COD removal were then duplicated or triplicated in later runs to gain a statistically sound data set.

Run 1 directly followed the preliminary porous pot run (Section 3.3.1) with the trace element addition commencing on the 25th Aug 2010 and continuing for a further 22 days. The run was performed using a control pot plus three (3) trial pots: trace elements were added individually from 500 mg/L bulk solutions to the three test pots to increase the concentration by 4.0 mg/L iron(III),

magnesium or calcium. The total mass of trace elements added in each trial pot for the duration of the experiment was approximately 630 mg iron(III), magnesium or calcium over the 22 days.

The mean F/M ratio during the test period was within the range of 0.24 – 0.26 for all pots. The average sludge age calculated in all pots was between 6 – 13 days for the duration of the recording period of the COD removal.

The mean COD removal in the control pot in Run 1 was 76 ± 1%, the mean removal for pots with additions of iron(III), calcium or magnesium was 83 ± 4%, 83 ± 4% and 83 ± 5%, respectively. Using an ANOVA comparison of means with a confidence interval of 95% a significant statistical difference was found between the control and all metal additions: iron(III) (P – 0.003), calcium (P – 0.001) and magnesium (P – 0.008).

The initial concentration of iron(III) in the SETP was 0.38 mg/L, well below the theoretical requirements for optimum bacterial growth (1 to 4 mg/L) [76]. Though the concentration of metals in the effluent were not determined during this run, iron was added to give an increase in concentration of 4.0 mg/L, expected to be within the optimum iron range. The fact that the optimum iron level coincided with improved COD removal could imply that microbiological activity had been increased by the iron addition, leading directly to an improvement in COD removal.

Due to its ability to act as a flocculent, iron could also improve COD removal by a number of mechanisms, including complexation or precipitation of humic substances [76]. Humic substances adsorbing to the iron(III) hydroxide precipitates could aid in the settling of the humic materials to the extent that they could even precipitate soluble humic matter. The large surface area of the iron(III) flocs increases the capacity to adsorb organic compounds [105], where the iron(III) floc can react with negatively charged humic substances to form organo-metallic complexes and precipitates, removing them from solution [105, 106].

During Run 1 the sludge in the iron(III) pot became markedly darker from the second day, indicating the formation of iron oxy-hydroxide precipitates, or iron sulphide, formed when ferrous iron reacts with hydrogen sulphide [4, 47]. The presence of hydrogen sulphide would most likely indicate anaerobic conditions in the porous pots. However, the DO was ≥ 2 mg/L and there was no difference in the odour of this pot compared to the others, indicating the absence of significant levels of H_2S. The most probable explanation for the darker sludge in the iron(III) amended pot was due to the formation of iron phosphate precipitates which are stable and insoluble. Determining the levels of iron phosphate precipitation in the activated sludge from an iron(III) amended porous pot will be discussed in Section 3.3.4.

Calcium and magnesium are soluble at pH 7, therefore, they will react differently in the wastewater compared to iron(III). The addition of 1.0 mg/L calcium has been reported to enhance COD removal from a chemical manufacturing plant [9] while the addition of 5.0 mg/L magnesium to a textile wastewater has been reported to have doubled COD removal through the stimulation of biological growth [42]. Calcium is reported to act as a bridge between negatively charged humic substances and activated sludge surfaces, increasing the floc size, density and strength [17, 39] this then contributes to an increase in the efficient removal of COD in wastewater treatment [60, 107].The addition of calcium and magnesium has also been reported to aid in controlling bulking and settlability [107] by cation bridging and biosorption [39], a physical – chemical mechanism of removal of compounds by complexation to microbial surfaces [17].

The target residual PO_4^{3-} in the porous pot effluent was between 0.5 and 1.0 mg/L, though this was reached in only the pot amended with iron(III) (Table 3.4). Though the macro-nutrient phosphorus can be biologically limiting in activated sludge, the variations in the residual PO_4^{3-} concentrations did not affect the COD removal from the trace metal amended porous pots. Iron(III) phosphate is insoluble at pH 7 and as the effluent samples were filtered before PO_4^{3-} analysis, one would expect that any insoluble iron(III) phosphate would be retained by the filter. Iron is a common method employed to remove excess phosphate from water, which makes it difficult to explain the residual PO_4^{3-} in the iron(III) amended pot being higher than the remaining pots. There was only a single PO_4^{3-} analysis in Run 1 which may not have been representative of the whole run. The relatively high residual PO_4^{3-} did not occur in subsequent porous pot runs where iron(III) was added.

Table 3.4: Run 1 mean residual PO_4^{3-} and BOD removal

Treatment	Control	Fe(III) Pot	Ca Pot	Mg Pot
Residual PO_4^{3-} (mg/L)	0.076	0.760	0.221	0.085
% BOD Removal	95 ± 2	97 ± 1	96 ± 2	96 ± 0

A bulk 1000 L grab sample was collected from Boyer for Run 2 on the 4th Oct 2010. There was a settling in period of 3 days before the trace element addition commenced on the 7th Oct 2010 with the additions continuing for a total of 22 days. The run was performed using a control pot plus four trial pots: trace elements were added individually to the four test pots to give a target increase in concentration of 0.50 mg/L copper or zinc, or 0.05 mg/L cobalt or molybdenum. The total mass of trace elements added in each trial pot for the duration of the experiment was approximately 75 mg copper and zinc, with 8 mg cobalt and molybdenum over 22 days.

The mean F/M ratio during the sampling period was within the range of 0.27 – 0.32 for all pots. The average sludge age calculated in all pots was 6 to 9 days for the duration of the recording period of the COD removal.

There was a significant statistical difference between the control and the copper and cobalt addition pots (P – 0.022, df – 7) and (P – 0.044, df – 7), respectively. The increase in COD removal for the copper and cobalt pots was 12% and 9%, respectively (see Figure 3.3). In the case of copper addition this result is as expected as the addition of between 0.014 and 1.0 mg/L copper to industrial wastewater treatment plants has been reported to increase both COD removal and effluent quality [44, 78]. However, there are conflicting reports on the benefits of adding copper to activated sludge treatment plants, where adding 0.4 mg/L copper has been reported to make no difference to the COD removal [84]. Clearly this is an indication of the separate nutrient requirements of individual wastewater treatment plants.

The increase in COD removal brought about by the addition of cobalt was anticipated based on separate work by Burgess *et al*. and Gikas, who reported that additions of between 1.0 and 5.0 mg/L cobalt enhanced COD removal in industrial wastewater treatment plants [49, 71]. It was proposed that improvements in COD removal in cobalt amended activated sludge, where the bacteria were observed to increase both the respiration and the maximum specific growth rate, were through the biosynthesis of cobalamin [49]. The biological synthesis of cobalamin (Vitamin B_{12}), needed for metabolism and cell function, is stimulated through the addition of between 0.01 and 0.1 mg/L cobalt [6, 68]. On the other hand, the concentration at which cobalt has a toxic effect on the activated sludge has been reported to be as low as 1.0 mg/L [43].

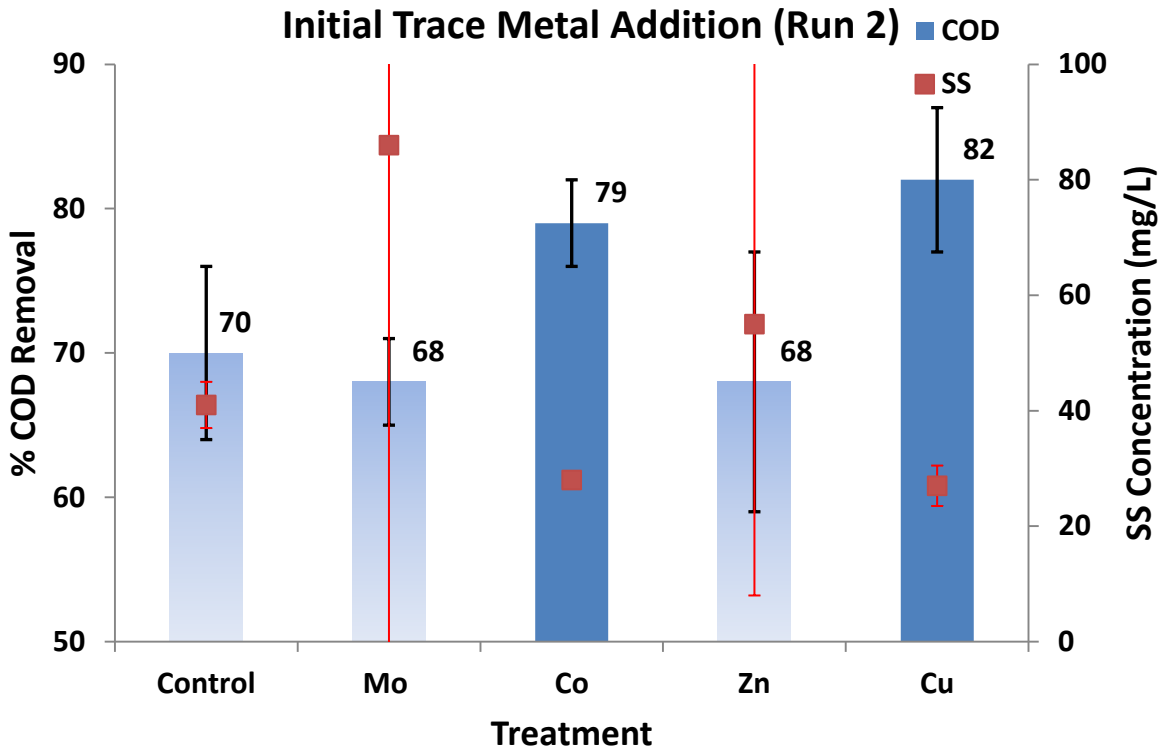


Figure 3.3: Effect of trace metal addition (Mo, Co, Zn and Cu) on %COD removal and SS concentration in porous pots (Run 2).

(Legend: Black error bars are 1 SD for %COD mean; Light blue bar = no significant difference to control; Dark blue bar = statistically significant difference to control; Red square = SS concentration mean; Red Error bars = SD of SS concentration mean)

The mean COD removal for the control pot on this occasion was 70 ± 6%. It was found that there was no significant difference for the molybdenum and zinc amended pots compared to the control pot, with 68 ± 3% and 68 ± 9% COD removal, respectively (see Figure 3.3). There have been conflicting results reported for the addition of molybdenum. Burgess *et al.* reported in two separate studies on industrial wastewater that 0.5 mg/L molybdenum had both beneficial and toxic effects on the effluent quality [8, 9]. The same authors also reported that 2.0 mg/L molybdenum increased COD removal by a factor of 1.4 times [42].

The mean COD removal for the zinc amended pot was 68%, while a maximum of 85% was recorded on day 11 which then decreased to 61% on the final day of the run. This is reflected in the relatively high standard deviation (9%) of the COD results for this pot. It is believed that, though the total zinc concentration in the pot was approximately 0.75 mg/L (0.25 mg/L background, plus 0.50 mg/L addition), well within the 0.1 to 1.0 mg/L target range, there could have been a residual zinc build up

in the sludge. While the addition of zinc has been found to be toxic to activated sludge microorganisms at concentrations > 1.0 mg/L [8, 9], the same addition of zinc has also been reported to increase microorganism growth [48]. Further, in a separate study on wastewater from a textile mill, zinc addition to this level increased COD removal by a factor of 1.3 times [42]. While the zinc concentration in the porous pot liquor could have accumulated to such an extent as to be toxic to the activated sludge biota, the concentration of zinc in the sludge was only 1.0×10^{-3} mg/kg dry weight.

In Run 2 the residual PO_4^{3-} was consistently lower than the target range of 0.5 to 1.0 mg/L, while the residual NH_3 was significantly above the target residual concentration of 1.0 to 2.0 mg/L (Table 3.5). The residual ammonia concentration detected in pulp and paper wastewater treatment effluent has been as high as 10 mg/L, with fluctuations in the ammonia levels a part of the operational variations [108]. However, excess ammonia and limited phosphorus were detected in the porous pot effluent from Runs 1 to 6. The target COD:N:P ratio was the same as used at the Boyer plant at the time and, to reduce variation between runs, it was not altered from this ratio. It should be noted that the ammonia analysis was performed on the final day of Run 2, which unfortunately coincided with overdosing of macro-nutrients to the copper pot for between 1 – 2 h due to pump irregularities. The flow rate of the pumps was checked daily throughout the run and no fluctuations in the flow rate had been previously detected. This is reflected in the ammonia level of 108 mg/L in the copper amended pot based on a one-off reading, while the phosphate level is an average of 8 analyses over 22 days. The higher NH_3 result for the porous pots compared to pulp and paper WWTP could be due to insufficient time for the biological assimilation of the nitrogen before it passed through the porous membrane. In the Norske Skog Boyer SETP there is a secondary clarification process and a separate ASR and BFR that allows this to occur.

Table 3.5: Initial trace metal addition (Run 2) residual macro-nutrient and BOD data

Treatment	Control	Mo Pot	Co Pot	Zn Pot	Cu Pot
Residual NH_3 (mg/L)	24	32	11	40	108
Residual PO_4^{3-} (mg/L)	0.14 ± 0.17	0.28 ± 0.02	0.15 ± 0.14	0.30 ± 0.13	0.30 ± 0.57
% BOD Removal	97 ± 2	96 ± 0	97 ± 2	97 ± 2	98 ± 2

The BOD removal from the porous pots was ≥ 96% indicating that the majority of the readily biologically degradable compounds, that also contribute to the COD, were being removed in the

porous pots. This shows that the low residual PO_4^{3-} was not a limiting factor in the removal of BOD from the porous pots. In Run 6 an increased phosphorus concentration was employed in two porous pots to check the effects of excess phosphorus in the activated sludge on the COD removal, as determined by the target residual PO_4^{3-}.

Turbidity and SS Relationship

During Run 3 a comparison analysis of SS and turbidity was undertaken, to determine a suitable surrogate to replace the time consuming SS method. Although the SS and turbidity results are discussed in this chapter, Run 3 was a vitamin addition run and the complete suite of results will be discussed in Chapter 4.

The analysis of suspended solids required the sludge to be settled (without aeration) for 30 – 45 minutes, followed by collection of 100 mL of supernatant. As there was a high risk that the suspended solids analysis itself may disturb the balance of the biological populations in the porous pots, the suspended solids analysis was only determined weekly, during the porous pot cleaning regime, in Runs 1 and 2. The limited data obtained from such infrequent analysis reduced the ability to compare the COD removal and suspended solids levels. To allow the determination of a parameter directly related to SS on a daily basis to give a more complete data set, turbidity was considered an indication of suspended solids. The analysis was undertaken by comparing the SS level (mg/L) with the turbidity (NTU) results for the same samples. The relationship found between turbidity and suspended solids determined in Run 3 was: $SS = 5.089 \times (Turbidity)^{0.5452}$, $R^2 = 0.8154$ (Figure 3.4). After the relationship was determined between the SS and turbidity the equation was applied to the turbidity results throughout the remainder of the Porous Pot runs to measure SS.

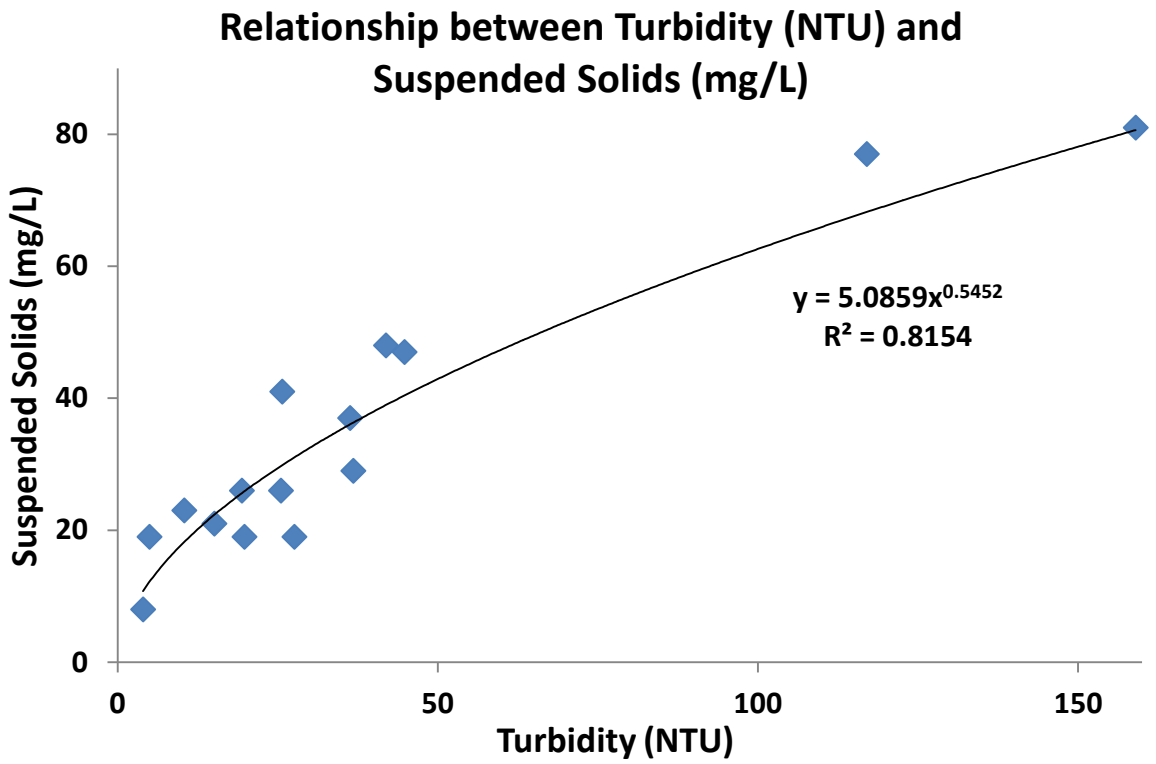


Figure 3.4: Plot of suspended solids (mg/L) and turbidity (NTU), with a power trend line.

3.3.4 Duplicate Trace Metal Porous Pot Runs (Run5)

The purpose of the duplicate trace metals run was to allow statistical verification of the earlier single runs. To this end the separate COD removal and SS data for each pot from the duplicate run were statistically analysed using an ANOVA comparison of means with a 95% confidence level. The trace metals added in the duplicate run were those that had produced significant statistical improvement in the COD removal from the initial trace metals runs.

A bulk 1000 L grab sample was collected from Boyer on the 7th Mar 2011. There was a settling in period of 4 days before the trace element addition commenced on the 11th Mar 2011 with the additions continuing for a total of 25 days. The run was performed using a control pot plus five trial pots. Trace elements were added individually to the five test pots to give additional concentrations of 0.50 mg/L copper, 0.05 mg/L cobalt or 4.0 mg/L iron(III), magnesium or calcium. The total mass of trace elements added in each trial pot for the duration of the experiment was 97 mg copper, 9.7 mg cobalt or 778 mg iron(III), magnesium or calcium over 25 days.

The mean F/M ratio during the sampling period was within the range of 0.30 – 0.36 for all pots. The average sludge age calculated in all pots was between 5 – 6 days for the duration of the recording period of the COD removal.

On day 21 of the run the power for the air pumps failed, resulting in the pots being without O_2 for approximately 12 to 14 h. One hour after the resumption of aeration the DO in the porous pots was at the minimum concentration of 2 mg/L. An ANOVA statistical analysis was employed to compare the mean COD removal before and after the event. As the power failure occurred towards the end of the run, the pots were sampled on four occasions after the event. This gave eight data points for a statistical analysis, (four before and four after the power failure) to compare the mean COD removal before and after the power failure. Though the statistical validity of the results could be questioned, the results are of interest.

All the pots recovered well from the O_2 failure, with exception of the magnesium amended pot (Table 3.6). The apparent increase in COD removal after the O_2 was restored in the cobalt amended pot was found to be statistically significant (P – 0.015, df 6), while the decrease in the COD removal in the magnesium pot was also statistically significant (P – 0.002, df – 7). Although the porous pots were designed to be fully aerated, it is common for activated sludge treatment plants to have anoxic regions [109]. In anaerobic sludge trace metals iron, cobalt and copper are required to convert methanol to methane [110], indicating that the addition of trace metals could be beneficial to both aerobic and anaerobic bacteria in nutrient deficient wastewater. Although the control pot was not affected by the O_2 failure as it was aerated by a pump on a different power switch, the trace metal additions to the experimental pots could have contributed to the activated sludge biota adapting to the anaerobic conditions. It appears that in this case the activated sludge biota was able to adjust to the anoxic conditions and subsequently recover following the restoration of the O_2 supply except in the case of the magnesium treated porous pot.

Table 3.6: Run 5 data for mean % COD removal before and after aeration pump failure

		Control	Cu Pot	Co Pot	Fe Pot	Mg Pot	Ca Pot
%COD Removal	**Before aeration failure**	84 ± 1	87 ± 1	83 ± 3	88 ± 2	81 ± 2	86 ± 1
	After aeration failure	85 ± 2	88 ± 1	88 ± 1	89 ± 2	70 ± 4	87 ± 3
	P - value	0.104	0.311	0.015	0.393	0.002	0.573
SS (mg/L)	**Before aeration failure**	43 ± 46	7 ± 1	14 ± 8	8 ± 3	27 ± 9	13 ± 5
	After aeration failure	9 ± 2	23 ± 23	8 ± 1	9 ± 2	84 ± 23	14 ± 4
	P - value	0.183	0.216	0.175	0.593	0.006	0.722

The difference in the SS level before and after the DO excursion was also analysed. Again though there was a limited data set the results of the ANOVA analysis of means are interesting. The SS in the iron(III) and calcium had the least variance before and after the O_2 failure (P – 0.593,df – 7) and (P – 0.722, df – 7), respectively. Following the aeration failure the SS in both the iron(III) and calcium pots increased by 1 mg/L, to 9 mg/L and 14 mg/L respectively. The only statistically significant difference was in the magnesium amended pot (P - 0.006, df – 6), which was expected due to the reduced COD removal. While there was no significant statistical difference in the SS from the remaining pots, the SS decreased from 43 mg/L to 9 mg/L in the control and from 14 mg/L to 8 in the cobalt amended pots. The SS in the copper amended pot increased following the aeration failure from 7 mg/L to 23 mg/L, although there was no indication of adverse effects in the COD removal results the presence of increased SS in the activated sludge liquor can be an indication of toxicity and poor biota health. Other than the magnesium amended pot it appeared as though activated sludge biota in the remaining porous pots were not adversely affected by the anoxic conditions.

As there was limited significant statistical difference in the COD and SS removal data between the before and after aeration failure in Run 5, the data sets were combined for further statistical analysis.

The COD removal rates from the duplicate trace metals run (Run 5) were relatively high in comparison to the previous runs. The mean % COD removal for the control pot over the final 14 days was 84 ± 2% (Figure 3.7). There was a significant statistical increase in the COD removal in pots amended with copper, which had a COD removal of 87 ± 2% (P – 0.001, df – 15), iron(III) (88 ± 2%, P – 0.000, df – 15) and calcium (86 ± 2%, P – 0.046, df – 15). There was no significant difference between the control and the pot amended with cobalt, which had a COD removal of 86 ± 3% (P – 0.352, df – 14). A significant decrease in COD removal was found in the magnesium amended pot 76 ± 6% (P – 0.003; df – 15). This was probably brought about by low DO, down to 1.0 mg/L, over the last eight days of the run. Though the cause of the low DO was unknown during the run, an unserviceable aeration adjustment knob was identified during the clean up stage as the most probable cause. The initial low DO (0.7 mg/L) recorded the day before the excursion could have affected the ability of the biomass in the magnesium amended porous pots to recover from the major stress event. This could explain the COD removal of 67% in the magnesium amended pot on the day following the re-introduction of O_2 where the DO levels were ≤ 1.4 mg/L. In the remaining pots where there appeared to be a full recovery, the COD removal was between 86 – 89% with DO levels ≥ 2.0 mg/L. The low DO level in the magnesium pot also affected the removal of BOD, analysed weekly for each pot. BOD removal for the control and remaining trace metal amended pots varied

between 98% and 99% (Table 3.7), while the mean BOD removal in the magnesium amended pot was 91%, with a removal of only 79% determined for a sample taken on the final day.

The magnesium trial was a re-run in a subsequent porous pot set, the duplicate water-soluble vitamins run (Run 6). In that subsequent run the bulk influent COD was significantly higher than in the previous runs, causing bulking issues which will be discussed in Chapter 4. Though it will be further discussed in Chapter 4, there was no significant difference in the COD removal between the control and the magnesium amended pots in the re-run (P – 0.714; df – 19) where the mean removal was 85 ± 3% and 86 ± 2%, respectively.

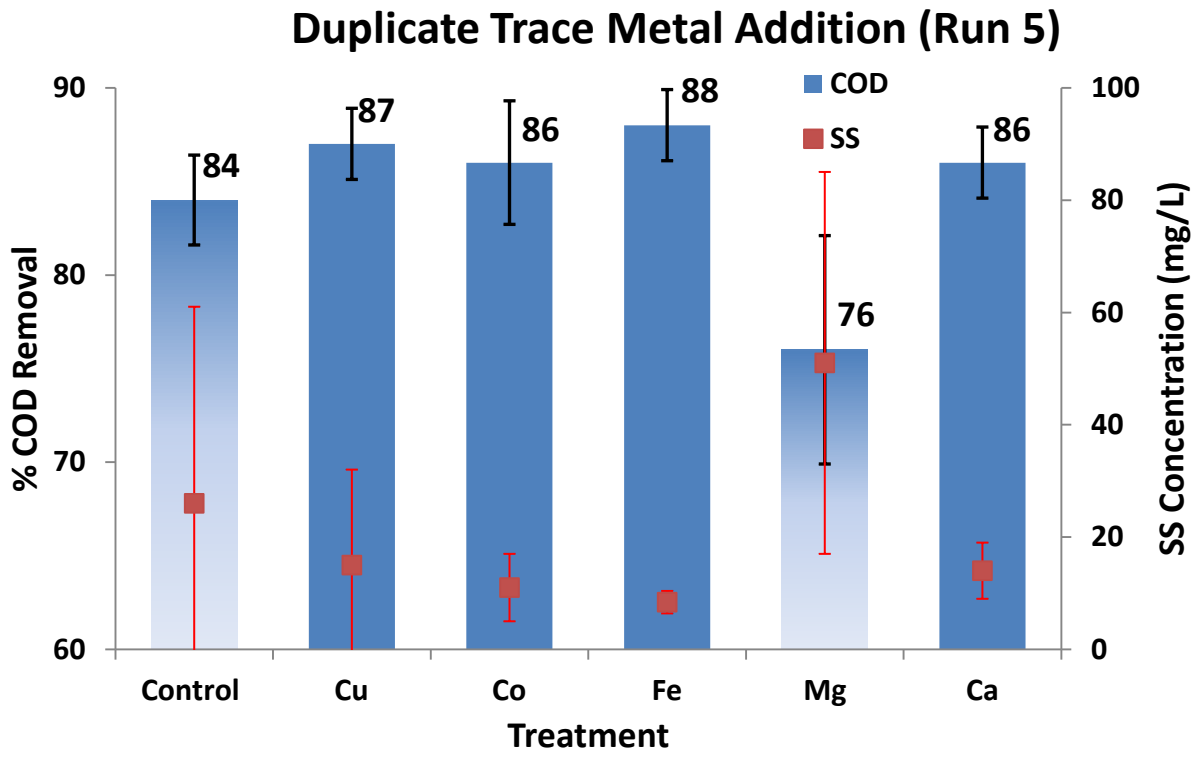


Figure 3.7: Effect of trace metal addition (Cu, Co, Fe, Mg and Cu) on %COD removal and SS concentration in porous pots (Run 5).

(Legend: Black error bars are 1 SD for %COD mean; Light blue bar= no significant difference to control; Dark blue bar = statistically significant difference to control; Red square= SS concentration mean; Red Error bars= SD of SS concentration mean).

The SS data was analysed using an ANOVA comparison of means. Though the suspended solids level in the magnesium amended pot was higher than the control (Figure 3.7), it was a statistically

significant difference (P – 0.185, df – 16). The mean concentration of SS in the remaining pots was below the control mean, though no statistical significant difference was found: copper (P – 0.330, df – 19), cobalt (P – 0.163, df – 19), iron(III) (P – 0.084, df – 17) and calcium (P – 0.195, df – 17). The standard deviation calculated from the SS data in the pots amended with iron(III), cobalt and calcium was relatively low at ± 2 mg/L, ± 6 mg/L and ± 5 mg/L, respectively, while standard deviation for the control was ± 35 mg/L. In previous runs a large standard deviation was an indication of activated sludge inhibition, seen in the magnesium amended pot during Run 5. In the control pot on Day 17 and 18 the SS levels were 100 mg/L and 59 mg/L, respectively. They subsequently dropped to between 6 to 11 mg/L from day 19. This resulted in the standard deviation being considerably larger than the remaining trace metals amended pots.

Due to the high residual NH_3 concentrations in the effluent of the previous runs the COD:N ratio was reduced from 33:1 to 45:1 before the commencement of Run 5. These high residual NH_3 levels remained even though the COD:N ratio had been reduced (Table 3.7). The mean range of the residual concentration of NH_3 was between 11 mg/L and 18 mg/L, at least one order of magnitude higher than the target level. Overall these results were lower than the four initial trace metal and water soluble vitamin porous pot runs that preceded this run and this can be attributed to the reduction in the COD:N ratio.

Table 3.7: The average residual NH_3 and PO_4^{3-} concentration (mg/L) from each pot for Run 5 with the standard deviations shown.

Treatment	**Control**	**Cu**	**Co**	**Fe**	**Mg**	**Ca**
Residual NH_3 (mg/L)	16 ± 6	12 ± 7	14 ± 7	11 ± 9	12 ± 13	18 ± 13
Residual PO_4^{-3} (mg/L)	0.28 ± 0.31	0.13 ± 0.07	0.15 ± 0.05	0.14 ± 0.10	0.16 ± 0.10	0.14 ± 0.10
% BOD Removal	99 ± 0.1	99 ± 0.4	98 ± 1.0	99 ± 0.7	91 ± 10	99 ± 0.5

The mean residual PO_4^{3-} concentration during Run 5 ranged from 0.13 mg/L to 0.28 mg/L (Table 3.7), well below the target residual PO_4^{3-} in the effluent (0.5 – 1.0 mg/L). The low PO_4^{3-} concentrations in the effluent could be due to the formation of insoluble metal phosphates in the sludge. The PO_4^{3-} ligand is known to have a high affinity for iron(III) [111] and calcium can form insoluble calcium phosphate. However, this does not explain why the residual PO_4^{3-} was also low in the remaining trace metal amended pots. The addition of trace metals could have had the intended effect and stimulated microbial growth, therefore, increasing the phosphorus demand.

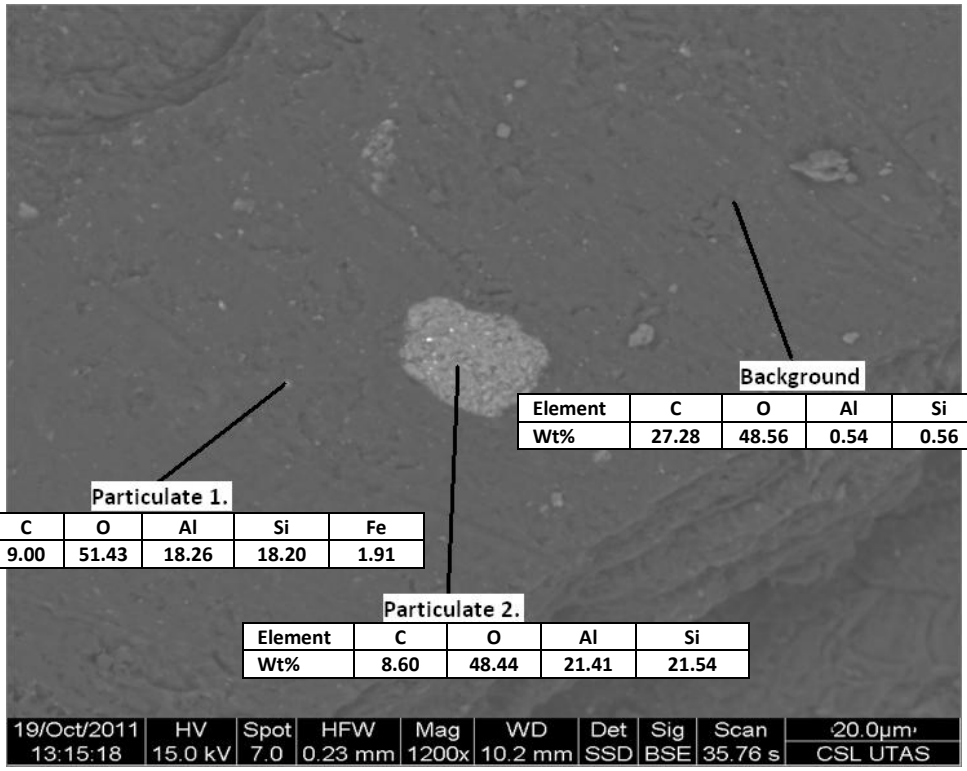
To determine if there was a significant quantity of iron(III) being bound in the sludge as iron phosphate, three sludge samples from the control and iron(III) amended pots were collected. The sludge samples were filtered through glass fiber, dried in an over at 105°C and analysed by FEI Quanta 600MLA environmental scanning electron microscope (ESEM), at the Central Science Laboratory in Hobart, to determine iron and phosphorus levels and give a mechanism for phosphorus removal in the porous pot liquor.

Four micrographs of the control and five of the iron amended samples were obtained. Magnified to 1200 x the majority of the sludge sample appeared to be one consistent texture, this was determined to be the background. There was also some particulate matter approximately 1 – 20 µm in diameter observed at 1200 x, both particulate matter and the background sludge were analysed by the microprobe technique on each occasion.

From the four micrographs of the control pot sludge, the background was found to contain mainly carbon and oxygen at a ratio of 2:1, representing approximately 93 ± 4% of the total elements in the background. There were a number of minor peaks representing other elements present at levels ≤ 1.0% of the total abundance, but no iron was detected (Figure 3.8). However, the mean abundance of iron in the background of the sludge from the iron(III) amended pot was found to be 0.91 ± 0.62% (Figure 3.9). The mean abundance of phosphorus in the control and iron(III) amended sludge was 0.56 ± 0.36% and 1.01 ± 0.54%, respectively. As only nine samples were collected for SEM analysis, there was no significant statistical difference found between the mean abundance of phosphorus in the control and iron(III) amended sludge (P – 0.198; df – 8).

The SEM analysis indicated the presence of iron precipitates in the sludge from the pot amended with iron(III). However, as there was no significant increase in the abundance of phosphorus in the sludge it was unlikely that it was in the form of an iron phosphate. Due to the limited data collected further SEM analysis would be needed before any firm conclusions could be drawn concerning the formation of iron phosphates in the sludge.

In one of the control samples (see Figure 3.8), the light particle in the centre of the SEM image contained oxygen, aluminium and silicon, at a ratio of 4:1:1. This could be a strong indicator of the presence of kaolinite clay particles or colloids in the sludge. Kaolinite is used in paper making and has a chemical composition of $Al_2Si_2O_5(OH)_4$ [4]. The high cation exchange capacity of the kaolinite clay could be involved in the binding of the metals that will be discussed in greater detail in Chapter 5.

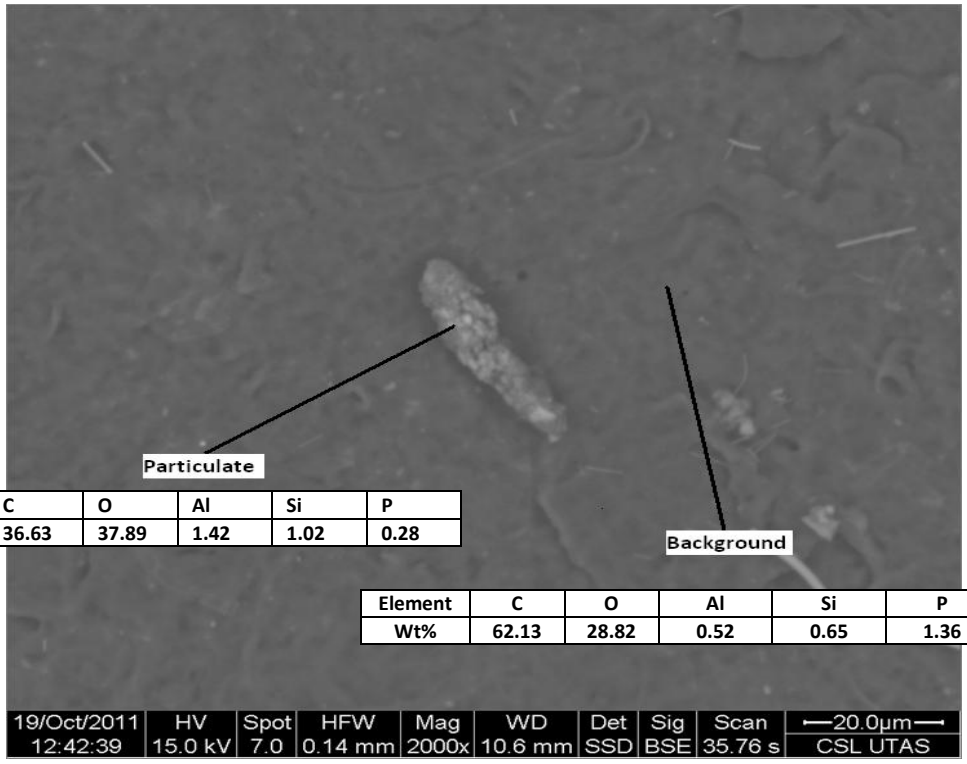


Element	C	O	Al	Si	P	Ca
Wt%	27.28	48.56	0.54	0.56	0.37	1.99

Element	C	O	Al	Si	Fe
Wt%	9.00	51.43	18.26	18.20	1.91

Element	C	O	Al	Si
Wt%	8.60	48.44	21.41	21.54

Figure 3.8: SEM image and data from the control porous pot sludge collected during the duplicate trace metals run.



Element	C	O	Al	Si	P
Wt%	36.63	37.89	1.42	1.02	0.28

Element	C	O	Al	Si	P	Fe
Wt%	62.13	28.82	0.52	0.65	1.36	3.05

Figure 3.9: SEM image and data from the iron(III) amended porous pot sludge collected during the duplicate trace metals run.

Combined Trace Metal Data

The COD removal results from initial trace metal runs (Run 1 and Run 2) were added to the duplicate trace metal results (Run 5). A paired, one way, single tail t-test with a 95% confidence level was used to compare the mean difference in COD removal between the control and the trace metal additions. In all cases, the trace metals selected for duplication showed an overall statistically significant increase of 4-5% in COD removal compared to the control (Figure 3.10).

For calcium, magnesium and iron additions, this improvement in COD removal could be explained by a combination of at least two mechanisms: improved biological activity and flocculation (both bioflocculation and particle flocculation). The addition of divalent cations, especially calcium and magnesium, to activated sludge has been found to increase biofloculation, lowering biopolymer concentration and, hence, the soluble COD [39]. Though this effect has not been directly reported for copper nor iron additions, it is expected that iron (III) would have an even greater impact by adsorbing onto biopolymers directly and also by acting as a flocculating agent for "suspended" particles and colloids [112]. This latter mechanism would assist in the removal of colloidal and small humic material that would otherwise pass through the porous pot liner (pore size 70 μm) and contribute to the soluble COD in the effluent. The addition of iron to nutrient deficient wastewater has been reported to increase the COD removal rates through chemical reactions, not biological metabolism, where the most significant differences in the activated sludge were in the size and structure of the flocs [113]. Iron may not be limiting for biological metabolism in the porous pots, however, the addition of iron did improve the removal of COD.

An improvement in COD removal of 5 ± 5% (P-0.002) was found for copper (see Figure 3.10). This result is as anticipated as the addition of between 0.014 – 1.0 mg/L copper to industrial wastewater treatment plants has been reported to increase both COD removal and effluent quality [44, 78]. This occurs as copper is an enzyme activator required for cell synthesis, and the addition of 0.04 mg/L of copper to wastewater has been reported to increase COD removal through biological stimulation [113].

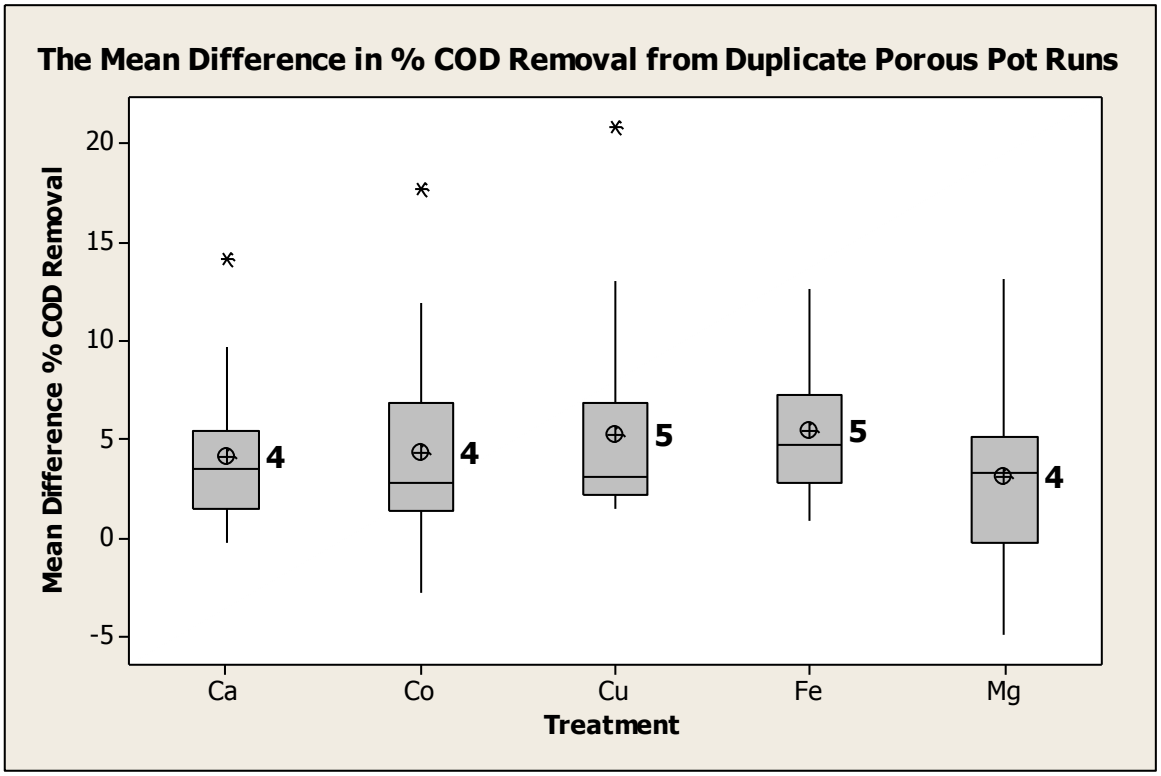


Figure 3.10: Effect of duplicated trace metal addition (Ca, Co, Cu, Fe and Mg) on %COD removal in porous pots (Run 1, 2 and 5).

(Legend: The outliers are shown as asterisk; The mean symbols are represented by a circle).

The mean increase in COD removal for cobalt was 4% (P-0.012). There is limited research available on the effects of these trace metal additions to activated sludge pulp and paper wastewater treatment plants. However, there have been increases in the removal of COD reported through the separate additions of 1.0 mg/L calcium and cobalt to a chemical plant which was phosphorus limited [9]. In this case the improvement in COD removal was reported to be through biological stimulation [9].

While the addition of 4.0 mg/L magnesium to the porous pot had a significant statistical effect on the COD removal of 4% (P-0.000), Wei *et al* reported that the addition of 5.0 mg/L magnesium to textile wastewater doubled COD removal, measured in kg COD/kg MLSS day, compared to the control [42]. However, in the current study magnesium was already present in excess for biological stimulation [76, 113], so increasing the concentration by a further 4 mg/L gave only a marginal improvement. On the 27th April 2010, before the first porous pot run, Norske Skog introduced MgO at Boyer as a replacement for sodium hydroxide for pH control. Following the introduction of MgO, the mean concentration of magnesium in the effluent increased from 5.1 mg/L to 41 mg/L. The data

collected by the Boyer staff over the 12 month period before and after MgO introduction was statistically analysed using an ANOVA comparison of means to compare the SETP COD removal rates before and after the change in the pH chemical amendment. The COD and SS data collected during multiple paper machine shut downs were removed as they were deemed to not represent the standard operating conditions of the SETP. There were significant statistical differences found in the COD removal (P – 0.000; df – 457). The mean COD removal before and after the MgO addition was 76 ± 6% and 82 ± 5%, respectively (Figure 3.11).

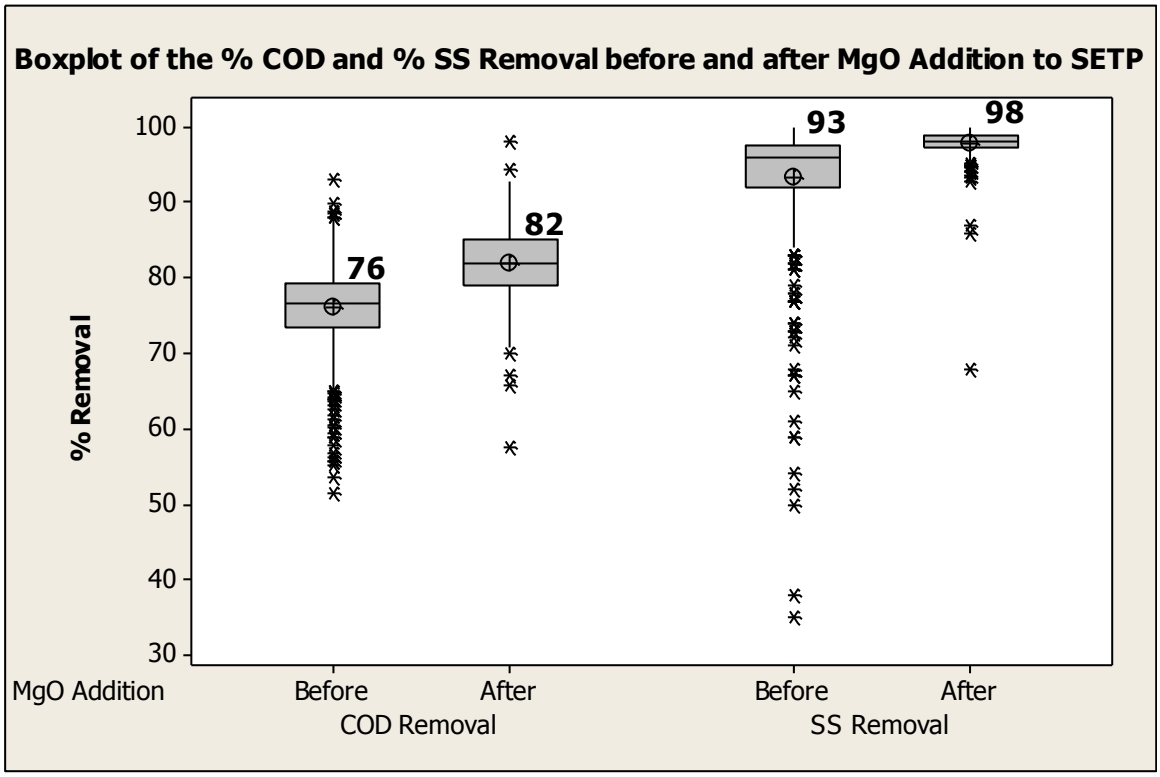


Figure 3.11: Effect of MgO addition on the %COD removal and SS concentration in the Boyer SETP. (Legend: The outliers are shown as asterisks; The mean symbols are represented by a circle).

The increase in COD removal brought about by the addition of MgO was not unexpected as magnesium addition to wastewater treatment plants has been shown to improve effluent quality [114]. At least in part this is brought about by the formation of cation bridges between negatively charged biopolymers and the divalent cations calcium or magnesium [40]. Normally biopolymer slime produced by the bioflocs adds to the COD in the effluent, however, with higher levels of magnesium present in the biopolymeric network, the biopolymers in solution could bind to magnesium, increasing the effluent quality. This effect is enhanced because the monovalent cation

sodium, replaced by magnesium in the Boyer changeover, has been shown to decrease floc settling properties in secondary clarifiers [39, 40, 115]. A statistically significant difference in the removal rate of SS in the SETP was also found between pre- and post- MgO addition (P – 0.000, df – 773). The mean before the MgO addition was 93% ± 8, and the mean SS removal after was 98% ± 3 (Figure 3.11).

Effluent COD and SS Relationship

A significant relationship was found between the COD of the effluent and the SS concentration for data collected from Run 2 to Run 5, inclusive, with an R^2 value of 0.751 and a Y intercept at 132 mg/L COD (Figure 3.12). From this relationship the expected COD would be approximately 132 mg/L even if 100% of the SS was removed. This equates to approximately 89 – 90% COD removal, with the remaining 10% being in solution, probably consisting of dissolved or colloidal humic material. The maximum COD removal expected in an industrial wastewater treatment plant is approximately 90% [9]. The average COD removal for copper and iron(III) additions in Run 5 was 87% and 88%, respectively, suggesting that the COD removal is at the upper limit under the biological and physical conditions of the porous pots. The removal of the BOD component in the wastewater during Run 5 was ≥ 98%, indicating that the residual 10% COD in the effluent was dissolved or colloidal humic substances, likely lignin derivatives which are resistant to biodegradation. The humic fractions will be discussed further in Chapter 6.

The increases in COD removal in later runs may have been modest compared to the increases observed in Run 1 and Run 2, but the COD removal from the trace metal amended pots may have already reached the maximum level before the need to invoke different mechanisms to remove the dissolved degradation resistant humic substances.

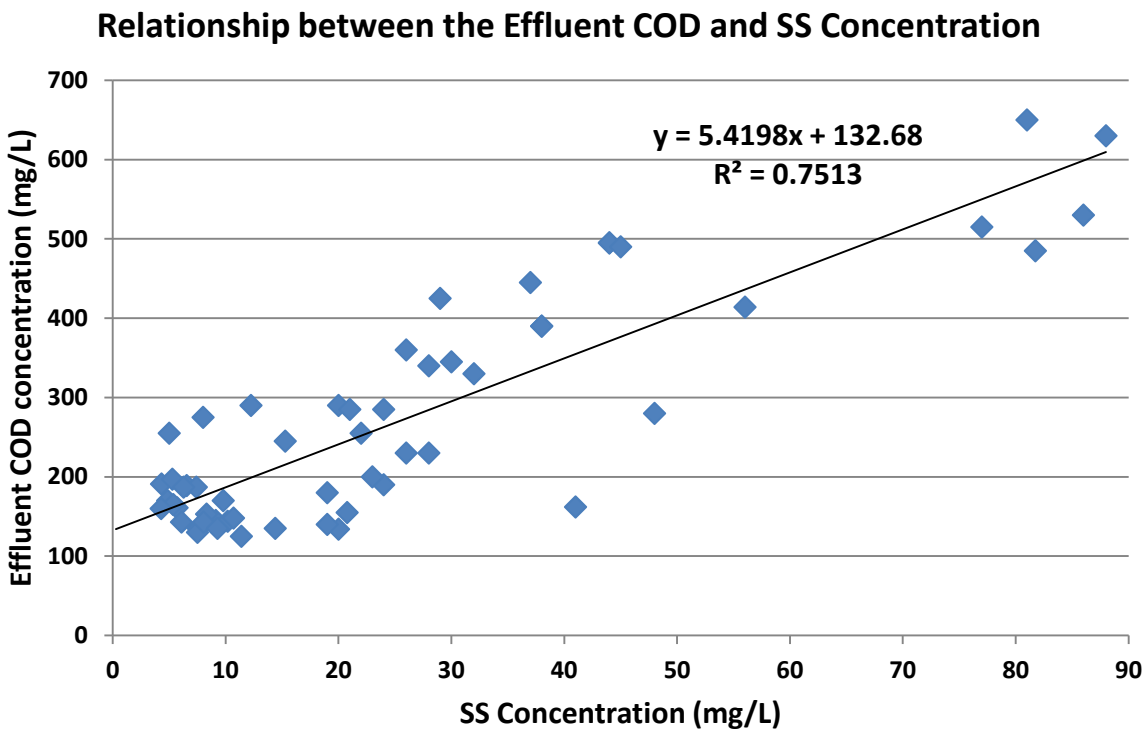


Figure 3.12: The relationship between the COD concentration in the porous pot effluent and the SS concentration.

3.3.5 Additions of Various Concentrations of Copper and Cobalt (Run 7)

The purpose of Run 7 was to determine the effects of a range of concentrations of copper and cobalt, to determine the fate of the metals and the effects of copper addition on the filamentous bacteria. These latter two points will be discussed in Chapters 5 and 7, respectively. The bulk sample for Run 7 was collected on the 8th Nov 2011 during a PM3 shut down and surge basin cleaning. During this operation the surge basin effluent was pumped back into the primary clarifier, which contributed to the high level of suspended matter in the bulk sample. The mean COD concentration of the bulk sample was 1030 mg/L, significantly lower than the 1359 – 1687 mg/L from previous Runs 1 to 5. The run was performed using a control pot (pot 1) plus 5 trial pots. There were three Cu pots with the target additions in the pots of 1.0 mg/L, 0.25 mg/L and 0.1 mg/L in pots 2 to 4 and two cobalt pots of 0.1 mg/L and 0.025 mg/L in pots 5 and 6.

The average F/M ratio from 8th Nov – 5th Dec 2011 was within the range of 0.21 – 0.24 for all pots. The average sludge age calculated in all pots was between 15 –37 days for the duration of the recording period of the COD removal (day 15 – 24). The high sludge age was due to keeping the influent flow rate to the porous pots at 5.4 mL/min, the same for all runs. Due to the low COD of the

feedstock, the F/M ratio was kept within target values. This reduced the biomass and the need for wasting which allowed the sludge age to increase.

An ANOVA comparison of means with a 95% confidence level was used to statistically analyse the difference in the data from each pot compared to the control. The mean COD removal in the control pot was 84 ± 3% (Figure 3.13). The only COD result that was significantly different to this was from the 0.1 mg/L copper amended pot with a COD removal of 87 ± 2% (P – 0.038; df 19).

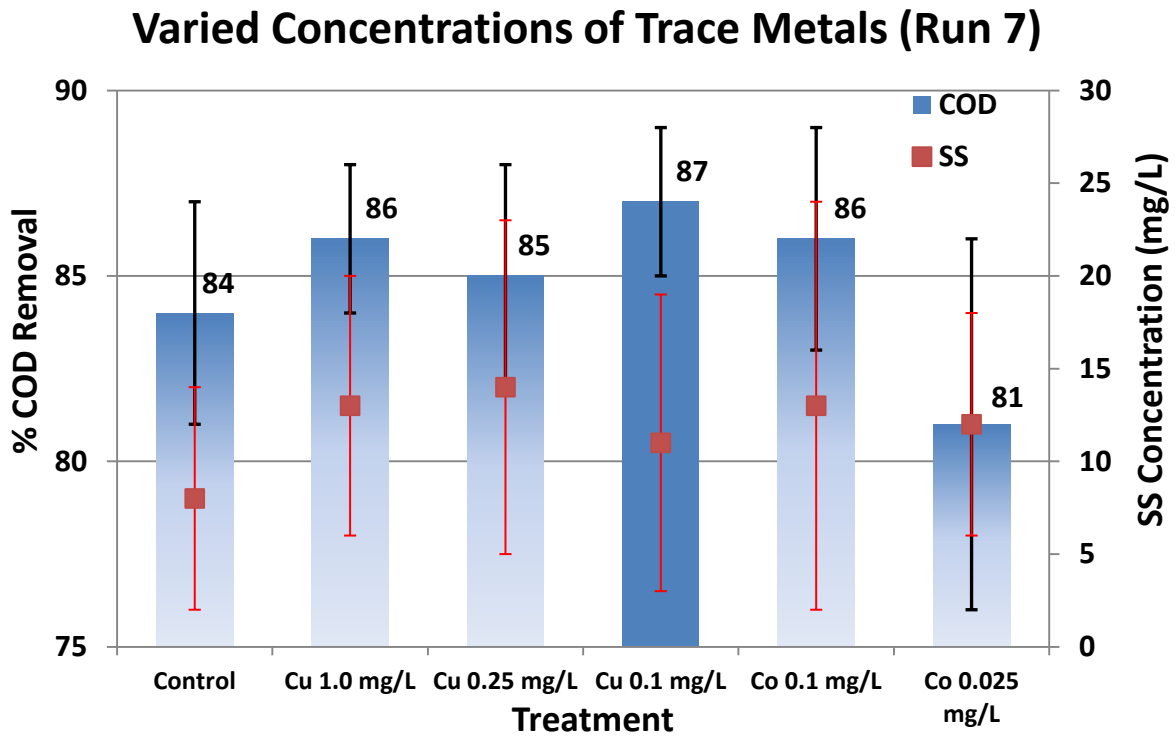


Figure 3.13: Effect of trace metal addition (Cu and Co) on %COD removal and SS concentration in porous pots (Run 7).

(Legend: Black error bars are 1 SD for %COD mean; Light blue bar= no significant difference to control; Dark blue bar = statistically significant difference to control; Red square= SS concentration mean; Red Error bars= SD of SS concentration mean).

There was no statistical difference found between the remaining pots and the control: with the 1.0 mg/L copper amended pot having a COD removal of 86 ± 2% (P – 0.363; df – 19), the 0.25 mg/L copper amended pot having 85 ± 3% (P – 0.827; df – 19) removal, the 0.1 mg/L cobalt pot having 86 ± 3% (P – 0.159; df – 19) removal and the 0.025 mg/L cobalt pot having 81 ± 5% (P – 0.083; df – 19)

removal. These results contradicted the previous COD removal rates calculated from the initial and duplicate trace metals runs, where the mean combined difference in COD removal for a 0.5 mg/L copper addition and 0.05 mg/L cobalt addition was 5% and 4%, respectively.

The failure of the trace metal amendments of 1.0 mg/L copper or 0.025 mg/L cobalt to improve COD removal are, in part, understandable as a concentration of 1.0 mg/L copper in a SETP has the potential to be toxic [5, 50] and the addition of 0.025 mg/L cobalt may have been insufficient to stimulate growth. Copper added to SETPs to increase the concentration in the MLSS to between 0.5 mg/L and 1.0 mg/L has been shown to stimulate growth in activated sludge biomass [48, 49, 78]. However, an increase of 1.0 mg/L copper over a prolonged period could have an inhibitive effect on the diversity of the activated sludge biomass. The addition of cobalt at levels between 0.01 mg/L and 0.1 mg/L has been reported to stimulate Vitamin B_{12} synthesis [6, 68]. The method of COD removal in the porous pots amended with cobalt at concentrations of 0.1 mg/L and 0.05 mg/L could have either been through direct biological stimulation or through high molecular weight compounds binding to activated sludge biomass through attraction divalent cations [39, 40].

The mean SS level across the six pots in Run 7 was between 8 – 14 mg/L, much lower than previous porous pot runs, with no significant difference between any of the porous pots (P – 0.415; df – 64) (Figure 3.13). Although the COD removal in the 0.025 mg/L cobalt amended porous pot was 3% lower than the control pot, there was no statistical difference in the mean suspended solids level in this pot compared to the control. Previously when COD removal rates in the experimental pots were lower than the control pot, the SS concentration in the test pot increased, indicating adverse effects of the trace metal additions to the activated sludge biota. In the case of the 0.025 mg/L cobalt amended pot the SS level was comparable to the control pot, supporting the statistical analysis of the COD results indicating that the biomass was not adversely affected.

An important observation from this Run was the reduction in the abundance of filamentous bacteria in the copper amended pots. This will be discussed in detail in Chapter 7.

BOD_5 tests were not undertaken in Run 7 and Run 8 as the high removal of up to 98 – 99% BOD in the previous runs indicated that the major contributing factor to the effluent quality was the residual soluble COD fraction, not BOD. There were also time limitations with the increased metals analysis undertaken to calculate mass balances. The residual humic fractions in the effluent will be discussed in Chapter 6.

Throughout the porous pot runs the concentration of the added phosphorus was kept constant to reduce variables in the experimental regime. For the same reason the concentration of nitrogen

added was kept constant until Run 5 where the COD:N ratio was increased from 33:1 to 45:1 and kept at this level for the remainder of the porous pot runs. The residual NH_3 concentration found in runs prior to Run 7 was generally significantly greater than its target residual concentration of 1.0 to 2.0 mg/L NH_3, while PO_4^{3-} was significantly lower than its target of 0.5 to 1.0 mg/L PO_4^{3-}. In Run 7, the mean residual NH_3 and PO_4^{3-} concentrations were close to the target values in one pot, the 0.025 mg/L cobalt amended pot, the pot with the lowest COD removal in the run (Table 3.8). In previous runs there was limited difference in the mean residual concentrations of NH_3 and PO_4^{3-} between the pots, however, in Run 7 there was significant variation in the residual NH_3 and PO_4^{3-} concentration in the pots.

Table 3.8: The average residual NH_3 and PO_4^{3-} concentration (mg/L) from each pot over the duration of Run 7 with the standard deviation shown.

Run 7	**Pot 1 Control**	**Pot 2 Cu 1.0 mg/L**	**Pot 3 Cu 0.25 mg/L**	**Pot 4 Cu 0.1 mg/L**	**Pot 5 Co 0.1 mg/L**	**Pot 6 Co 0.025 mg/L**
Residual NH_3 (mg/L)	8.4 ± 5.8	0.6 ± 0.4	7.5 ± 7.7	0.4 ± 0.1	0.3 ± 0.3	2.0 ± 2.6
Residual PO_4^{3-} (mg/L)	0.42 ± 0.3	0.31 ± 0.4	0.11 ± 0.06	0.07 ± 0.04	0.13 ± 0.16	1.2 ± 0.7

There appeared to be no explanation for the changes in the residual NH_3 and PO_4^{3-}, other than the observation that the greater the mean COD removal, the lower the residual PO_4^{3-} in the effluent, seen in the mean PO_4^{3-} variation between Pot 4 and Pot 6 (Table 3.8). This could be an indication of increased microbial activity, however, there are no correlations with the residual NH_3 in the effluent to support increased biological activity. As discussed there were no changes in the operating conditions of the porous pots other than the bulk wastewater sample, and this is possibly the single most significant contribution to the porous pot variability. What is not understood is the effects the different samples have on the chemistry in the porous pots and how the differences affected the capacity of the activated sludge biota to assimilate the compounds contributing to the COD.

3.3.6 Multiple Metals Addition (Run 8)

From Runs 2, 5 and 7 it was apparent that the addition of copper had improved both settlability and COD removal. Though, with somewhat less certainty, it could also be seen that additions of calcium, iron and magnesium also improved COD removal. This led to the question “Can multiple trace metal additions magnify the improvements in the COD removal?” The purpose of Run 8 then was to determine the effects of multiple metal additions on the COD removal, humic fractions and bacterial

populations in the porous pots, and the fate of the added metals. The bulk sample for Run 8 was collected on the 31st Jan 2012, the mean COD was 1048 ± 125 mg/L, again significantly lower than the mean COD concentration in the bulk wastewater samples collected for Runs 1 - 5. On this occasion the paper mill was in normal operation. The run consisted of a Control Pot (Pot 1) and five test pots each with addition of two metals: Pot 2 and Pot 3 with 0.5 mg/L copper and 4.0 mg/L iron, Pot 4 with 0.5 mg/L copper and 4.0 mg/L magnesium, and Pot 5 and Pot 6 with 0.5 mg/L copper and 4.0 mg/L calcium. The mean range of F/M ratios were calculated to be 0.22 – 0.27 and the average sludge age calculated in pots 1 – 5 was between 11 –19 days for the duration of the run, while the sludge age in the pot 6 was 26 days. The performance of pot 6 will be discussed in greater detail later in this section.

An ANOVA comparison of means with a 95% confidence level was used to statistically evaluate the difference in the data from each pot compared to the control. The increase in the COD removal from the trial pots was not as significant as expected from previous work (Figure 3.14). The mean COD removal in the control pot was 85 ± 2%, and again there was minimal increase in the mean COD removal of the test pots compared to the control (see Figure 3.14).

The mean combined COD removal from the duplicate iron/copper pots was 87 ± 2%, a statistically significant increase of 2% (P – 0.005; df – 29) over the control pot. Although the mean COD removal in the magnesium/copper amended pot was also 87 ± 2%, the analysis of the data in this case determined that it was not statistically significant (P – 0.077; df – 19). One of the calcium/copper amended pots (Pot 5) gave a significant increase in COD removal (87 ± 2%) compared to the control (P – 0.036; df – 19). In the duplicate calcium/copper pot (Pot 6), there was no statistical difference between the COD removal (85 ± 2%) and the control pot (P – 0.766; df – 19). While the mean COD removal for the duplicate calcium/copper pots was 86 ± 2%, further analysis showed that this was not a statistically significant increase (P – 0.181; df – 29). The difference in the COD removal between the two pots amended with calcium and copper could have been due to a considerable blockage in the pot 6 influent that led to the concentration of copper increasing in the sludge and effluent, this will be discussed in Chapter 5.

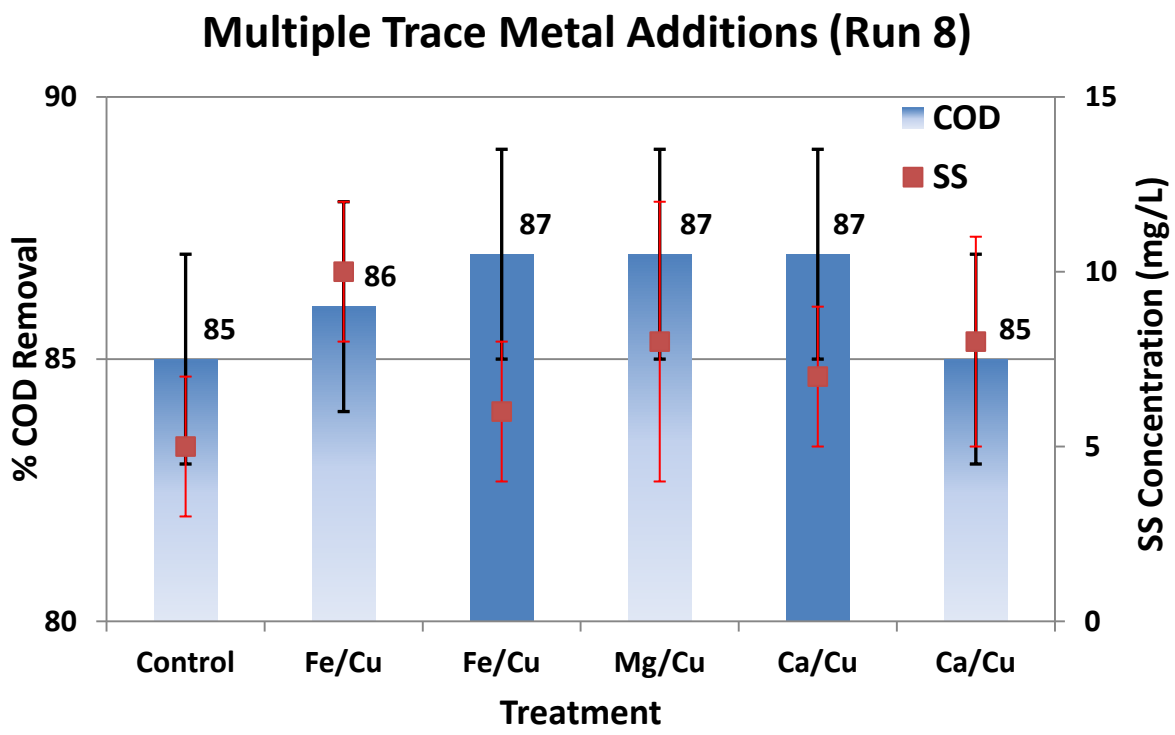


Figure 3.14: Effect of multiple trace metal additions (Fe/Cu, Mg/Cu and Ca/Cu) on %COD removal and SS concentration in porous pots (Run 8).

(Legend: Black error bars are 1 SD for %COD mean; Light blue bar= no significant difference to control; Dark blue bar = statistically significant difference to control; Red square= SS concentration mean; Red Error bars= SD of SS concentration mean)

The removal rates of COD and the residual SS concentration and their corresponding standard deviations were similar in both Run 7 and Run 8. The mean COD removal of 87% during Run 8 was again at the upper level of the COD removal where one would expect to see a 100% removal of SS according to Figure 3.12. The residual COD in the porous pot effluent is comprised of dissolved humic substances which will be discussed in Chapter 6, while the effects of the copper addition on activated sludge biota observed during Run 8 will be discussed in Chapter 7.

There was a statistically significant difference in the mean effluent SS concentration between the porous pots in Run 8 (P – 0.003; df – 54), Figure 3.14. The mean SS in one of the pots amended with iron and copper (Pot 2) was the highest recorded during Run 8 (10 mg/L). However, the COD removal from that pot was also higher than the control at 86%. The general trend observed in all other porous pot runs has been that an increase in SS would be associated with a decrease in COD

removal. As the SS was measured by turbidity of unfiltered WAS samples, there was an expectation that the concentration of SS in the iron amended pots would be lower than the control due to iron acting as a flocculant and aiding in the settling of SS.

As mentioned above the concentrations of the added nitrogen and phosphorus were kept constant to reduce variables in the experimental regime. The residual NH_3 and PO_4^{3-} concentration found in previous runs was generally significantly greater and lower, respectively, than the target residual concentrations. The mean residual NH_3 and PO_4^{3-} concentrations in Run 8 were within the target range in the control, Pot 1 (Table 3.9). The mean residual concentration of PO_4^{3-} was within the target range excepting for Pot 6, which could be an indication of reduced microbial activity. Comparing the residual PO_4^{3-} concentration in the effluent from all the pots there was no significant difference found (P – 0.605; df – 70). From previous runs the PO_4^{3-} residual concentration has generally been well below the target range. While the mean residual NH_3 concentration across all pots except for Pot 6 was significantly lower than previous runs. One explanation for the changes in the residual NH_3 and PO_4^{3-} could be that due to a more diverse biological community in the activated sludge. There were differences observed in the abundance of filamentous bacteria which is discussed in Chapter 7.

Table 3.9: The average residual NH_3 and PO_4^{3-} concentration (mg/L) from each pot over the duration of Run 8 with the standard deviation shown.

Run 8	**Control**	**Fe and Cu**		**Mg and Cu**	**Ca and Cu**	
	Pot 1	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
NH_3 (mg/L)	2.0 ± 1.9	0.7 ± 0.8	2.1 ± 1.9	4.0 ± 6.0	2.3 ± 4.6	6.5 ± 7.7
PO_4^{3-} (mg/L)	0.76 ± 1.1	0.62 ± 0.8	0.64 ± 0.94	0.78 ± 1.0	0.69 ± 0.69	1.3 ± 1.3

There was an expectation that the COD removal would be affected by deviations in the target residual concentration of the macro-nutrients nitrogen and phosphorus. However, there was no difference in the mean COD removal in control pots from Runs 5, 7 and 8, which remained between 84% and 85%. In these runs the mean residual nitrogen was in excess, up to 16 mg/L in Run 5, and the mean PO_4^{3-} was deemed to be limiting at 0.28 mg/L without affecting the removal of COD. The mean residual NH_3 and PO_4^{3-} in Run 8 was within the target residual concentration, 2.0 mg/L and 0.76 mg/L, respectively, without altering the COD removal. The divergence from the target residual concentration of NH_3 and PO_4^{3-} in the effluent may have operational consequences not measured during the porous pot runs.

3.4 Conclusions

As expected the wastewater samples collected from the surge basin at the Boyer Mill were variable in the COD and SS concentrations, and this variability affected the rate of COD removal in the control pots over the 5 runs discussed in this chapter. The mean COD in the bulk influent samples ranged from 1030 mg/L to 2213 COD mg/L and the removal in the 5 control pots ranged from 70 to 85%.

The COD removal in the initial trace metal runs (Run 1 and Run 2), showed a 7% increase from the separate addition of 4 mg/L iron, calcium or magnesium, a 12% increase from a 0.5 mg/L addition of copper and a 9% increase from 0.05 mg/L cobalt addition. There was no significant difference in the COD removal from pots amended with 0.05 mg/L molybdenum or 0.5 mg/L zinc. In these pots the SS concentration and the SS standard deviation increased compared to the control pot, which can be an indication of biological inhibition.

By combining the data from the initial trace metal additions and the metal duplicate run (Run 5) a significant statistical increase of COD removal of 5% was observed for iron and copper additions and a 4% increase for calcium, cobalt and magnesium additions.

The mean residual PO_4^{3-} concentration in all trace metal amended pots during the duplicate trace metals run was lower than the control. This could be due to increased biological activity brought about by trace metal addition or by the removal of phosphate as a metal phosphate precipitate. However, there was no conclusive evidence suggesting that there were iron phosphates precipitating in the porous pots that would explain the lower residual PO_4^{3-} in the iron amended pots, though there was evidence for the presence of kaolinite clay colloids in the sludge which could adsorb the added trace metals.

During the addition of various concentrations of copper (1.00 mg/L, 0.25 mg/L and 0.10 mg/L) and cobalt (0.10 mg/L and 0.025 mg/L) (Run 7) only the 0.10 mg/L addition of copper had a statistically significant increase of 3% in COD removal. The addition of multiple metals (Run 8) to the porous pots again showed improvements in the COD removal. From the combined data of two pots amended with 4.0 mg/L iron and 0.5 mg/L copper there was a statistically significant increase in the COD removal of 2%. An individual pot amended with 4.0 mg/L calcium and 0.5 mg/L copper also increased the COD removal by 2%. However, a second pot amended with calcium and copper did not show an improvement, though this could have been due to an overdose of copper inhibiting the activated sludge biomass.

The COD removal in the control pot was also relatively high in Runs 7 and 8 at 84% and 85%, respectively, while the residual SS in these pots was relatively low. In both cases this corresponded

to a relatively low influent COD. The mean concentration of SS during Run 7 and Run 8 was also low relative to the preceding runs, ranging between 8 – 14 mg/L and 5 – 10 mg/L, respectively, indicating the porous pots were operating at a high level of efficiency.

The highest mean removal of COD from all porous pots amended with trace metals was found for the addition of iron (88%, Run 5). The maximum mean COD removal in the control pot was 85% in Run 8. A relationship between the residual COD in the effluent and the SS concentration had an R^2 value of 0.751 and a y intercept at 132 mg/L COD. This relationship indicated that at 100% SS removal the residual COD would be approximately 10 - 11%. As the addition of trace metals may have increased the COD removal to a maximum, it would appear to be necessary to include other removal technologies to further reduce the residual recalcitrant dissolved humic substances in the effluent.

The addition of MgO as a pH amendment to the Boyer SETP has improved the COD and SS removal rates by 6% and 5%, respectively. The mean COD removal from 1 year before and 1 year after the switch from NaOH to MgO increased from 76 ± 6% to 82 ± 5%, and the SS removal increased from 93 ± 8% to 98 ± 3%. The increase in COD removal at Boyer from the addition of MgO supported the overall mean increase associated with magnesium addition during the porous pot runs of 4 ± 5%.

Chapter 4 Water Soluble Vitamin Additions

4.1 Introduction

4.1.1 Adding Vitamins to Activated Sludge

Microbial diversity in activated sludge is an important aspect of effective industrial secondary effluent treatment plant (SETP) operation. Industrial SETPs generally have lower bacterial diversity in the activated sludge compared to municipal treatment plants and should, therefore, benefit from the addition of micro-nutrients, including water-soluble vitamins [42, 43]. Most bacteria isolated from activated sludge have some requirement for water-soluble vitamins to optimise growth especially those found in the activated sludge from pulp and paper mills [43, 67].

The vitamins which have been found to have the greatest effect on bacterial cell function are thiamine (Vitamin B_1), pyridoxine (B_6), biotin (B_7) and cobalamin (B_{12}) [47]. This has been supported by research that has shown biotin, thiamine and niacin to be essential for the growth of some bacteria species isolated from industrial and municipal activated sludge [6, 73].

As mentioned earlier biotin, required for bacterial growth, is produced naturally by most species of bacteria [6, 43]. It is also cost prohibitive for an industrial application. Cobalamin is another vitamin which is produced during normal growth of some microbes, however, bacteria require cobalt to be bioavailable for biosynthesis [43]. Cobalt was added during the trace metal addition runs as an amendment, especially to stimulate the production of cobalamin as the vitamin itself is also cost prohibitive for industrial applications.

Industrial wastewater has been found to contain insufficient niacin and therefore dosing would appear to be required [55]. In industrial SETPs the addition of 1.0 mg/L niacin has been reported to increase COD removal from 20 – 200% [41, 42, 45, 75]. Thiamine has also been reported to be required by bacteria which have been isolated from pulp and paper activated sludge [72, 73].

The water-soluble vitamins riboflavin, pyridoxine and folic acid were generally not required by bacteria isolated from industrial activated sludge [43, 73]. However, the addition of folic acid decreased turbidity and increased the control over the settling rates of the sludge [74]. With the addition of pyridoxine there were contradictory reports concerning the bacterial requirements, as stated it has been reported to either be required [47] or not required [43, 73] by bacteria in activated sludge.

It has also been reported that some additions of micronutrients and vitamins increased the metabolic rate of the sludge in a manufacturing plant while other additions decreased the metabolic

rate. From this it would appear that the application of micronutrient supplements has the potential to have a positive or a negative effect on industrial activated sludge treatment plants [8, 41].

As with the trace metal addition (Chapter 3), there are contradictory reports on the stimulation or inhibition of activated sludge bacteria from the addition of water-soluble vitamins. The different results from the additions of water-soluble vitamins to various industrial treatment plants reinforces the need to run experiments based on Boyer activated sludge. The water-soluble vitamins selected for the initial porous pot runs were: thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6) and folic acid (B_9).

4.2 Materials and Methods

The general materials and methods were outlined in Chapter 3. The B-group vitamins were supplied by Sigma Chemicals and were added to the porous pots from bulk solutions of 120 mg/L to give a final concentration of 1 mg/L of the respective vitamin.

4.3 Results and Discussion

4.3.1 Initial Vitamin Addition (Run 3 and Run 4)

The initial water-soluble vitamin additions were undertaken in Runs 3 and 4. Thiamine (B_1), riboflavin (B_2) and niacin (B_3) were added in Run 3 and pantothenic acid (B_5), pyridoxine (B_6) and folic acid (B_9) were added in Run 4.

The mean COD removal for the control pot in Run 3 was 78 ± 4%, with no significant difference between the COD removal in the control pot and thiamine and riboflavin amended pots (see Figure 4.1). Though the riboflavin result is in agreement with a report by Burgess *et al*. [8] that the addition of 1.0 mg/L riboflavin to industrial wastewater gave no significant improvement in COD removal, the thiamine result is a little surprising. Wei *et al.* report that the addition of 1.0 mg/L thiamine to wastewater from a textile mill increased COD removal by a factor of 1.6 times [42], a result that was clearly not reflected in the current work. Addition of 1.0 mg/L thiamine has also been reported to be required for bacterial growth in pulp and paper mill activated sludge [72, 73], however, these improvements in growth were not necessarily reflected in improvements in COD removal.

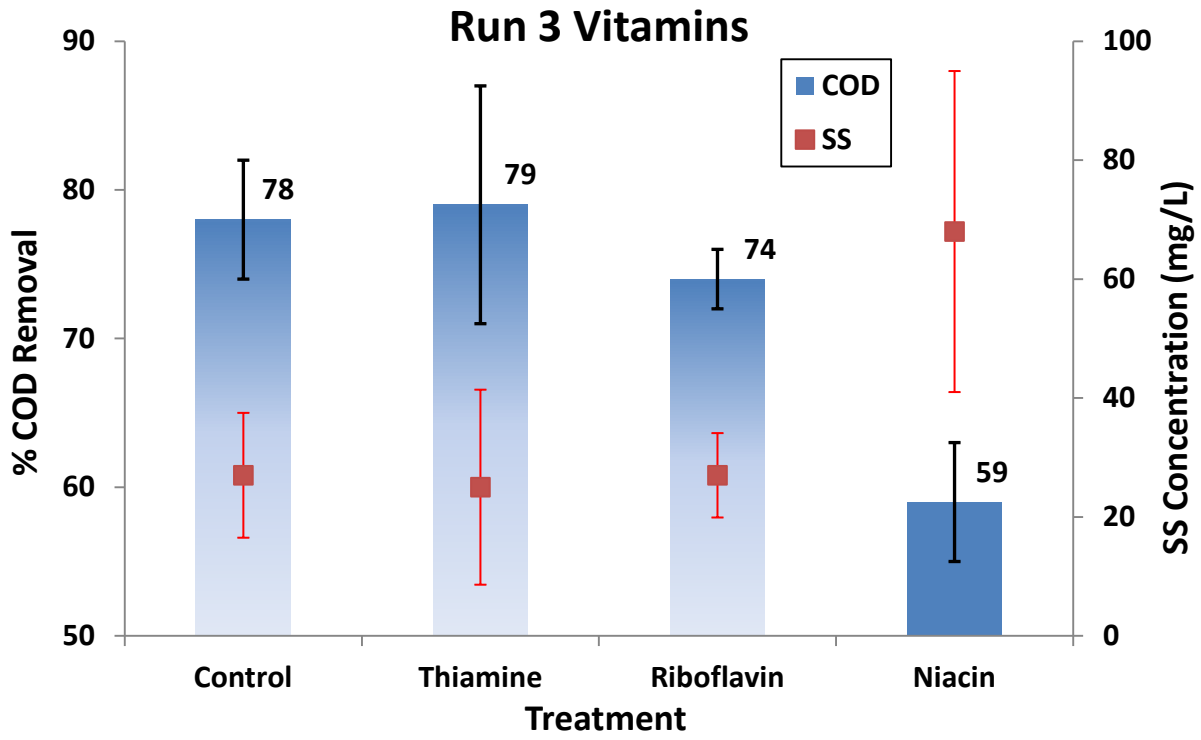


Figure 4.1: The Effect of vitamin addition on % COD removal in Run 3 (black error bars) and SS concentration (red squares and red error bars).

(Error bars are one standard deviation. Light blue bars represent control pot and pots with no significant difference to the control. Blue bar indicates significant difference.)

Thiamine and niacin have been found to be essential for the growth of some isolated bacteria species [6, 73], with 10 mg/L being at the top end of the required micro-nutrient range for niacin [71]. However, niacin addition to the porous pots in the current work gave a significant negative difference with mean COD removal of 59 ± 4%, a decrease of 19% compared to the control. This contradicts a number of other reports where the addition of between 0.1 – 1.0 mg/L niacin to synthetic and industrial wastewater has been observed to increase COD removal in batch experiments by 15 – 200% [8, 9, 42, 43, 45, 71, 75]. There was no operational explanation for the decreased removal associated with the addition of niacin, as DO, temperature, pH and flow rates were maintained within set limits during the run. Lemmer reported that bacteria isolated from pulp and paper AS had a low requirement for niacin [72] and the initial level was not measured before the amendment, as it was assumed that the concentration of niacin was effectively zero. It is unlikely that additional niacin at 1.0 mg/L to activated sludge would be toxic, as this would have been reported in other studies. However, it was difficult to explain the apparent inhibitory effects on the activated sludge biota through the addition of 1.0 mg/L niacin.

There was no significant difference in the SS levels between the Control, the thiamine and the riboflavin amended pots with suspended solids of 27 ± 10 mg/L, 25 ± 16 mg/L and 27 ± 7 mg/L, respectively (Figure 4.1). As there was no difference in the COD removal between the control and the thiamine and riboflavin amended pots it was expected that SS levels in those pots would be similar. There was a significant increase (2.5 times) in the concentration of SS in the niacin amended pot 68 ± 27 mg/L (P – 0.006), which validated the significant decrease in the COD removal compared to the control.

Table 4.1: The mean residual NH_3 and PO_4^{3-} (mg/L) and % BOD removal from porous pots from Run 3.

Porous Pot Amendment	**Control**	**Thiamine**	**Riboflavin**	**Niacin**
Residual NH_3 (mg/L)	13 ± 18	7 ± 7	7 ± 5	6 ± 4
Residual PO_4^{-3} (mg/L)	0.16 ± 0.10	0.20 ± 0.10	0.21 ± 0.12	0.11 ± 0.06
% BOD Removal	96 ± 1	97 ± 1	98 ± 1	96 ± 2

The mean residual PO_4^{3-} concentration ranged between 0.11 mg/L to 0.21 mg/L (Table 4.1), which was significantly lower than the target residual concentration of 0.5 – 1.0 mg/L. There was no significant difference in the residual concentration of dissolved PO_4^{3-} (P – 0.220; df – 47) across the pots. The mean residual NH_3 was again significantly above the target residual concentration of 1.0 – 2.0 mg/L. Excess NH_3 in anoxic conditions can lead to the production of N_2 gas, which causes the sludge to float [47, 52], although the mean residual NH_3 concentration was appreciably higher than the target, bulking was not an issue during Runs 3 or 4.

The trend of the residual PO_4^{3-} and NH_3 being below and above the target concentrations, respectively, was carried over from the initial trace metal runs, Runs 1 and 2. To ensure that the data collected from each run could be compared between runs, the dose rate of nitrogen and phosphorus was not adjusted over the series. The residual concentration of PO_4^{3-} and NH_3 in the porous pots is an indication that the conditions in the pots are not identical to those in the Boyer SETP. The SETP at Boyer comprises of a separate biofilm reactor (BFR), an activated sludge reactor (ASR) and a secondary clarifier, where the porous pots have a single porous liner that contains all the biological activity to a single stage. The available nitrogen may be discharged through the porous pot liner before the AS biota has time to assimilate the macro-nutrient, increasing the residual NH_3 in the effluent.

As expected there was no statistically significant difference in the BOD removal between the control and the vitamin amended pots (Table 4.1). Burgess *et al.* reported that though there was a stimulation of bacterial growth or improvement of COD removal through the addition of micro-nutrients in batch experiments. The BOD removal was not effected [8]. As stated in Chapter 3, the BOD removal was ≥ 96% indicating that the majority of the biologically degradable compounds that contribute to COD were being removed in the porous pots. This would also suggest that the removal of recalcitrant dissolved humic matter contributing to the residual COD in the porous pot effluent was improved. This could also suggest that the low residual PO_4^{3-} was not a limiting factor in the removal of BOD from the porous pots.

The water-soluble vitamins pantothenic acid (B_5), pyridoxine (B_6) and folic acid (B_9) were added in Run 4 to give target concentrations of 1.0 mg/L in the porous pots. The mean COD removal for the control pot was relatively high at 86 ± 3%. The COD removals in pantothenic acid and folic acid pots were both 88 ± 2% (P – 0.089) and (P – 0.104), respectively (see Figure 4.2). Though these were apparently better COD removals than the control, they were not within the set statistical parameters of 95% confidence level to report a significant difference. While the addition of 1.0 mg/L pantothenic acid has been reported to increase COD removal [8], this was not found in the current work. Similarly, while the effects of folic acid addition on COD removal has not been reported directly, it has been reported to improve settling and decrease turbidity by 50 – 70% [74]. Again, this effect was not observed here.

The mean COD removal in the pyridoxine pot was less than the control but not significantly lower due to the comparatively high standard deviation, 78 ± 14%. Reports of the effects of B_6 addition in the literature have varied considerably. Burgess *et al.* has reported that the addition of 1.0 mg/L pyridoxine trebled COD removal in phosphorus limited wastewater treatment compared to the control [41]. It could be argued that based on the residual PO_4^{3-} in the effluent the porous pots were phosphorus limited, making the comparison to Burgess' work relevant. Further, in earlier work Burgess *et al.* reported that the addition of only 0.01 µg/L pyridoxine stimulated bacterial growth, but, did not increase the rate of COD removal [8].

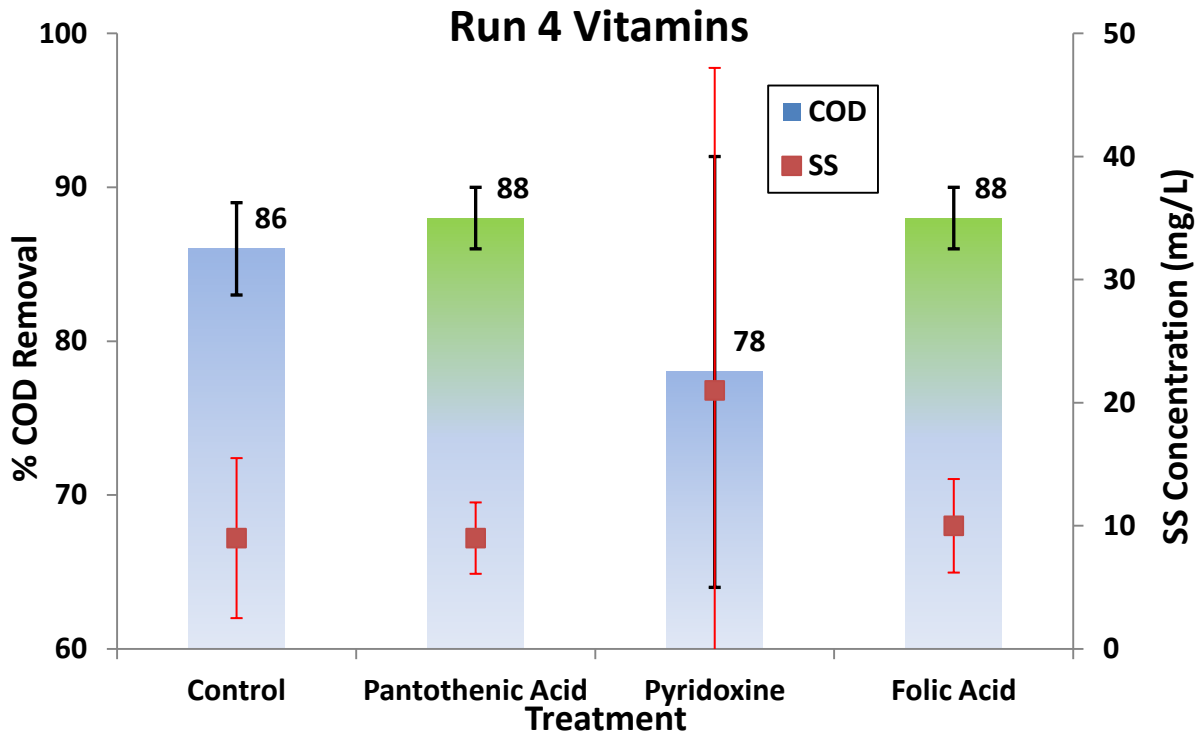


Figure 4.2: The Effect of vitamin addition on % COD removal in Run 4 (black error bars) and SS concentration (red squares and red error bars).

(Error bars are one standard deviation. Light blue bars represent control pot and pots with no significant difference to the control. Light green bar indicates minimal increase).

The SS in the control, the pantothenic acid and the folic acid amended pots ranged between 9 and 10 mg/L, with no statistically significant difference between them (Figure 4.2). However, there was a significantly higher (2 x) mean SS, with an extremely high standard deviation (21 ± 26 mg/L), in the porous pot amended with pyridoxine. There was a noticeable transformation in this pot from day 21 reflected in a decrease in the COD removal and an increase in SS. The mean COD removal prior to day 21 was 89% with a mean SS level of 9 mg/L, both similar to the control and other vitamin amended pots in the run. From day 24 to day 26 there was a linear decrease in the COD removal from 89% to 56% with a corresponding increase in SS from 9 mg/L to 82 mg/L (see Figure 4.3). This was associated with the appearance of flagellates and excessive foaming. Although foaming can indicate a low DO level [51, 52], the DO in the pyridoxine pot remained between 1.8 and 4.8 mg/L throughout the run. It was unlikely that the pyridoxine concentration increased to a toxic level in the activated sludge, as levels as high as 10.0 mg/L are reported to improve COD removal [8] and there are no reports of pyridoxine toxicity. The increase in flagellates and foaming implies that there could

have been other operational issues in the porous pot, even though there were no noticeable changes in the temperature, pH or flow rates.

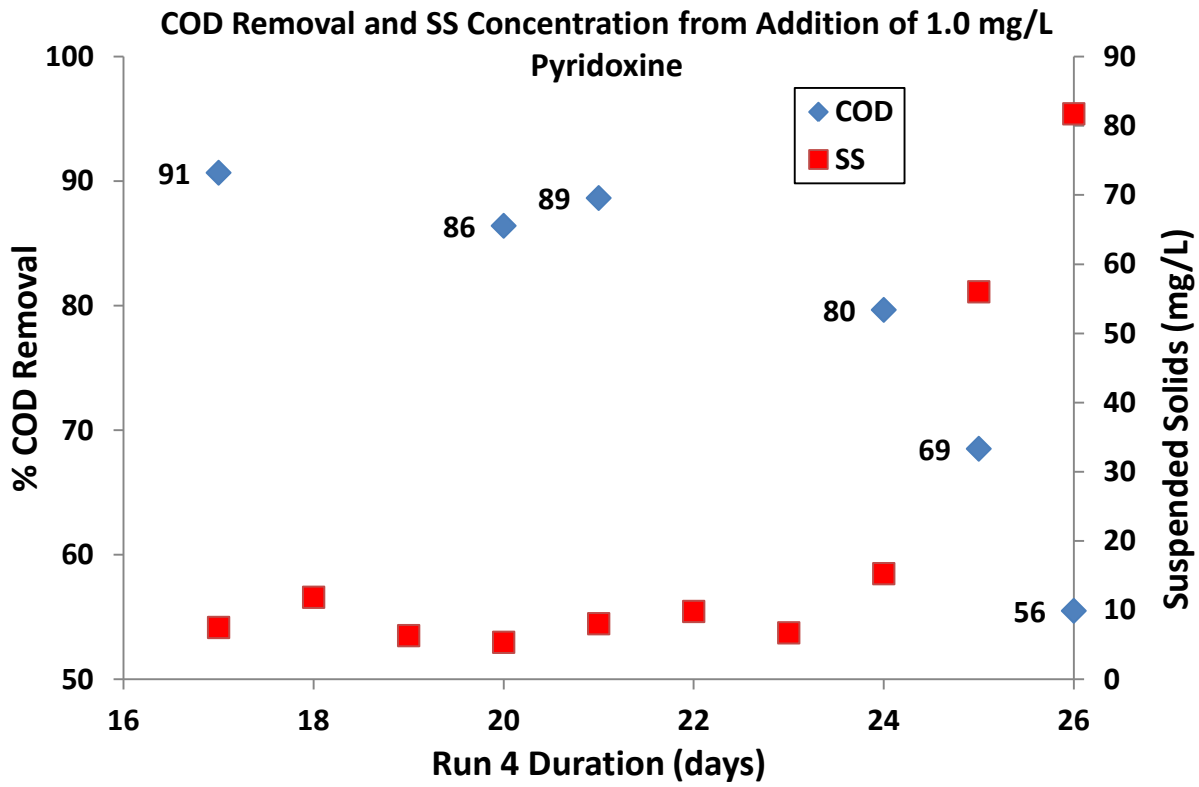


Figure 4.3: Comparison of the COD removal and SS level in the porous pot amended with 1.0 mg/L pyridoxine in Run 4.

The mean range of the residual NH_3 in Run 4 was between 15 and 30 mg/L, at least one order of magnitude higher than the target level. Möbius [108] found that the residual ammonia and phosphate concentrations in a tertiary treated pulp and paper mill wastewater fluctuated independently of each other and was unable to explain the possible chemical reactions taking place. The excess NH_3 in the effluent could be due to an over sight in the experimental design, where the porous pot experiments were designed to mimic the Boyer plant as closely as possible. In turn the design differences of the porous pots and the industrial treatment plant were not considered with the macro-nutrient addition in mind. This does not explain the low residual PO_4^{3-} concentration unless the bacteria have higher requirements for phosphorus or it is precipitated as a metal phosphate or adsorbed onto sludge in the system, which has been found to occur in pulp and paper mill wastewater treatment plants [55].

Table 4.2: The mean residual NH_3 and PO_4^{3-} (mg/L) and % BOD removal from each pot over the duration of Run 4 with the standard deviation shown.

Run 4	Control	Pantothenic Acid	Pyridoxine	Folic Acid
Residual NH_3 (mg/L)	19 ± 15	21 ± 11	24 ± 17	30 ± 24
Residual PO_4^{-3} (mg/L)	0.31 ± 0.4	0.21 ± 0.3	0.22 ± 0.3	0.27 ± 0.3
% BOD Removal	98 ± 2	98 ± 2	98 ± 3	98 ± 2

The mean residual PO_4^{3-} concentration during Run 4 in all the pots ranged between 0.21 to 0.31 mg/L (Table 4.2). The minimum concentration of soluble phosphorus for AS has been reported to be 0.5 mg/L, however, a lower concentration was also found not to inhibit COD removal [55]. Consequently the concentration of phosphorus in the porous pots may not be limiting biological growth and effecting the COD removal. There was no significant difference in the residual concentration of dissolved PO_4^{3-} in the experimental pots compared to the control pot. In Run 4 there was no change to the residual concentration of NH_3 and PO_4^{3-} compared to the previous runs, where the residual NH_3 and PO_4^{3-} were above and below the target concentrations, respectively.

4.3.2 Duplicate Vitamins (Run 6)

The results from the addition of water-soluble vitamins were unexpected in comparison to previous published results. Some of the available literature is contradictory in reports on water-soluble vitamin requirements in AS and on their effects on COD removal. These differences are an indication of the variations between industrial wastewater treatment plants and highlight the need to trial micro-nutrient additions with specific wastewater and AS seed.

The purpose of Run 6 was to gain a separate cohort of data to allow statistical analysis of the COD removal rates associated with vitamin additions in Runs 3 and 4. Though the initial COD removal results for pantothenic acid and folic acid additions indicated that there was no significant difference in the removal rates of COD when using an ANOVA comparison of means with a 95% confidence level, the difference was deemed sufficient to select the vitamins for duplicate studies. There is minimal literature on the effects of folic acid addition and this research was, in part at least, aimed at filling this void. The improvement in COD removal from the additions of thiamine and riboflavin

were deemed to be insufficient to warrant further work. Niacin and pyridoxine where excluded from further runs due to their poor performance in Runs 3 and 4, respectively.

A bulk 1000 L grab sample was collected from Boyer on the 8th August 2011. There was a settling in period of 4 days before the trace element addition commenced on the 12th August 2011 with the additions continuing for a total of 25 days. The run was performed using a control pot plus five trial pots. Water-soluble vitamins, pantothenic acid and folic acid were added individually to two test pots to give added concentrations of 1.0 mg/L. The opportunity to verify that sufficient phosphorus was being added was also taken during this run. As the standard COD:P ratio of 1000:1 gave a significantly lower residual PO_4^{3-} in the effluent than the target level it may have been too low to gain the full advantages from micro-nutrient additions. The phosphorus dose rate was increased to a target COD:P ratio of 440:1 in two porous pots (Pots 5 and 6) amended with "high" phosphorus. Phosphorous was added to all the remaining porous pots at a ratio of 1000:1; COD:P. The remaining pot was amended with 4.0 mg/L magnesium which was discussed in Chapter 3.

The mean COD of the bulk sample was 2210 mg/L, significantly higher than that for previous runs (1359 to 1687 mg/L for Runs 1 to 5). The average F/M ratio for the duration of the run was within the range of 0.29 to 0.35 for all pots. At the time the RAS sample was collected for seeding the porous pots, there was significant foaming in the BFR and at the RAS grab sample collection site. This problem continued throughout Run 6 with foaming, bulking and settleability being ongoing problems. Due to the bulking and settleability issues daily SS samples were unable to be collected during Run 6.

The average sludge age calculated in all pots was between 20 to 23 days for the duration of the recording period (day 15 to 25). The high sludge age was due to attempts to maintain the desired F/M ratio of 0.28 by reducing wasting to retain the required mass in the mixed liquor suspended solids (MLSS). It may have been possible to control the sludge age by decreasing the influent flow rate to decrease the food, allowing for more wasting of the mass (MLSS). It was incorrectly thought at the time that the influent flow rate had to be maintained to keep the retention time comparable to Boyer.

While the COD in the bulk influent sample was significantly higher than in previous runs, the COD removal rates during Run 6 were comparable to the relatively high COD removal rates in Run 5 (76 to 88%, discussed in Chapter 3). The mean % COD removal for the control pot was 85 ± 3% (Figure 4.4). There was a statistically significant increase of 3% in the COD removal in the pot amended with 1.0 mg/L folic acid (88 ± 2%, P – 0.051, df – 19). Folic acid was included as an amendment in the porous

pots due to the limited available literature on its addition to AS, with reports limited to improvements in sludge handling [116]. Although folic acid is a growth factor required by bacteria for cell development [6], it has also been reported that it is not required by AS bacteria [72, 73]. This work indicates that the addition of folic acid does improve COD removal. Surprisingly the results from the porous pot vitamin additions have contradicted previous research where vitamins such as thiamine and niacin have been reported to significantly increase COD removal while folic acid has been regarded as having no particular merit.

The mean COD removal from the pot amended with pantothenic acid in Run 6 was comparable to the previous results for this vitamin in Run 4, where the 2% increase in COD removal was not statistically significant. In Run 6 there was also no significant statistical difference in the COD removal between the pantothenic acid amended pot and the control pot (87 ± 2%, P – 0.279; df – 19). There have been limited reports on the effect on COD removal from the addition of pantothenic acid. However, Burgess *et al*. has reported an increase in the COD removal associated with pantothenic acid addition [8] and Clark and Stephenson have reported that it can stimulate bacterial respiration but that the increase in respiration was not associated with an increase in COD removal [6].

As stated previously, SS was not analysed daily due to bulking and settleability problems, and those results that were collected were deemed to be unreliable due to the limited volume of clear liquor available. However, from the limited data collected, even though folic acid has been reported to improve sludge settling [74], this was not observed in the folic acid amended pot during Run 6.

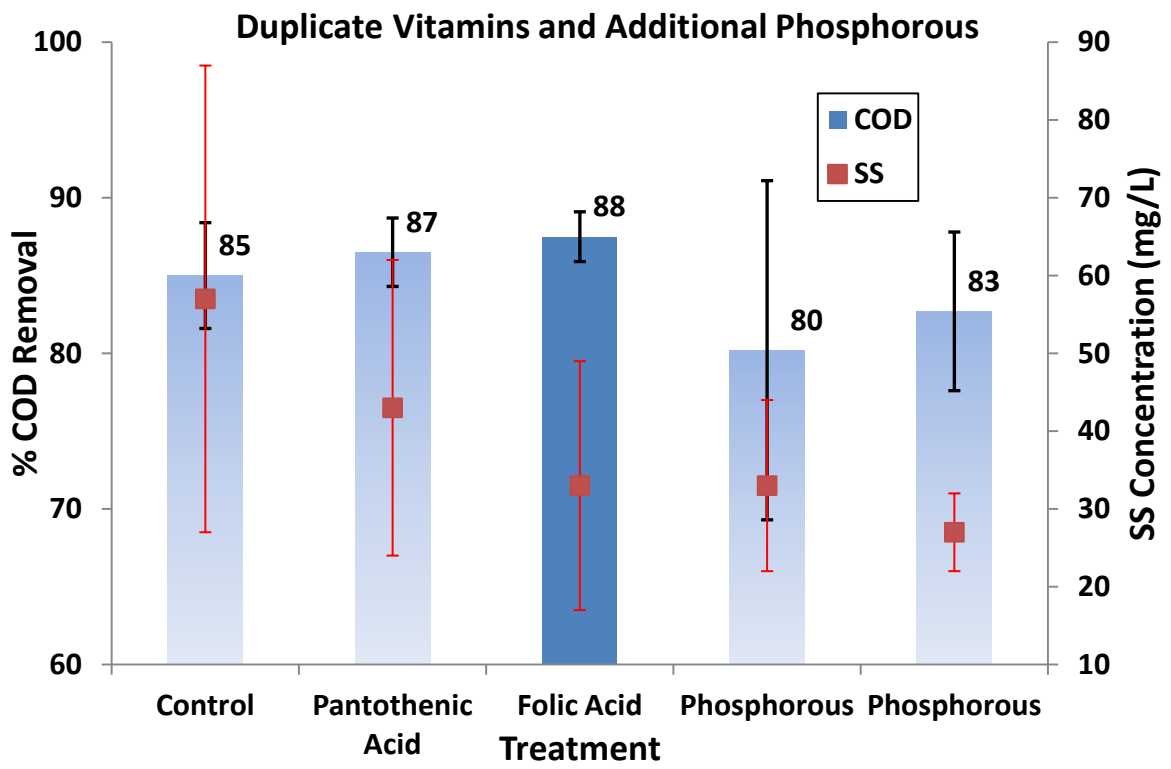


Figure 4.4: The Effect of vitamin addition and increased phosphorus on % COD removal in Run 6 (black error bars) and SS concentration (red squares and red error bars).

(Error bars are one standard deviation. Light blue bars represent control pot and pots with no significant difference to the control. Blue bar indicates significant difference.)

During the trace metal addition porous pot runs, a relationship was established between the residual COD concentration in the porous pot effluent and the level of SS. Through this relationship it was estimated that COD removal at zero SS was approximately 89 – 90%. The mean COD removal rate of 88% in the folic acid amended pot was close to the level of COD removal where complete SS removal was expected. The mean BOD removal in this pot was in the range 98 to 99%, indicating that the residual compounds contributing to the COD were likely to be recalcitrant dissolved humic substances which are resistant to biological decomposition. Therefore, the increase in the COD removal brought about by folic acid addition could be due to increased biological degradation of the recalcitrant dissolved humic substances.

The mean range of the residual NH_3 was between 12 and 34 mg/L, significantly higher than the required residual (Table 4.3). The increased phosphorus concentration in two porous pots (Pots 5 and 6) appeared to decrease the residual NH_3 in the effluent in those pots. As discussed in Chapter 3, the low residual PO_4^{3-} in the previous runs could be due to phosphate precipitation [55]. However, in

Run 6 with a higher influent COD and a relatively high COD removal, there was a higher residual PO_4^{3-} and bulking issues. The noteworthy difference in Run 6 was the high influent COD, which increased the oxygen demand in the pots causing DO deficiencies. During the run there appeared to be gas evolving from the settled WAS, an indication of denitrification, which in turn can be an indication of anoxic conditions [47]. There was also a noticeable increase in the abundance of filamentous bacteria in the sludge during Run 6, this will be discussed in more detail in Chapter 7.

Table 4.3: The average residual NH_3 and PO_4^{3-} (mg/L) and %BOD removal from each pot over the duration of Run 6 with the standard deviation shown.

Porous Pot Amendment	**Control**	**Pantothenic Acid**	**Folic Acid**	**High P**	**High P**
Residual NH_3 (mg/L)	21 ± 14	27 ± 18	34 ± 16	12 ± 10	12 ± 19
Residual PO_4^{3-} (mg/L)	0.6 ± 0.3	0.7 ± 0.5	0.8 ± 0.7	1.6 ± 0.4	1.2 ± 0.7
% BOD Removal	99 ± 2	98 ± 2	98 ± 2	99 ± 2	98 ± 2

The standard target ratio of phosphorus was 1000:1 COD:P which gave a mean residual PO_4^{3-} concentration in the control pot and the two vitamin amended pots in Run 6 of from 0.6 mg/L to 0.8 mg/L. The concentration of residual PO_4^{3-} in the pots amended with high phosphorus (440:1; COD:P), was significantly higher at 1.6 mg/L and 1.2 mg/L (Table 4.3). The increase in the residual PO_4^{3-} could also be due to the denitrifying bacteria having a lower requirement for phosphorus than the standard aerobic bacteria found in the sludge [117].

There was an expectation that the two pots amended with a higher concentration of phosphorus would have had an increased COD removal if the standard amendment of phosphorus in earlier runs had been limiting. However, there was no significant difference in the COD removal rates in the pots amended with phosphorus at a ratio of 440:1; COD:P compared to the control 80 ± 10% (P – 0.201; df – 19) and 83 ± 5% (P – 0.238; df – 19). It is apparent from these results that increasing the phosphorus in the porous pots did not improve the COD removal and the runs amended with phosphorus at a ratio of 1000:1 COD:P were legitimate.

The COD removal results from initial vitamin addition runs were included with the current duplicate results to allow a statistically sound determination of the benefit of vitamin addition. The data could not be statistically analysed using an ANOVA comparison of means, as the mean COD removal from the two separate control pots was different. A paired, one way, single tail t-test with a 95%

confidence level was used to compare the mean difference in COD removal between the control and the vitamin amended pots.

When the results from folic acid amended pots in Runs 4 and 6 were combined the overall increase in COD removal was 2 ± 2% (P – 0.016). While the effect of folic acid addition on COD removal in an industrial waste water treatment plant has not been reported directly, its addition to a recycled paper plant treatment plant has been shown to improve control over settling [74]. The combined statistical analysis of the pantothenic acid results indicated no significant difference (P – 0.078) with an overall improvement in COD removal of 1 ± 3%. Burgess *et al.* found that the addition of 1.0 mg/L pantothenic acid to a WWTP treating effluent from a chemical manufacturing plant stimulated the growth of AS bacteria [8]. It is evident from these porous pot amendments with water-soluble vitamins that stimulation of bacterial growth does not automatically transfer to an increase in COD removal. Even though the bacteria are stimulated, they may not be able to biosynthesise the recalcitrant compounds in the effluent of a pulp and paper mill.

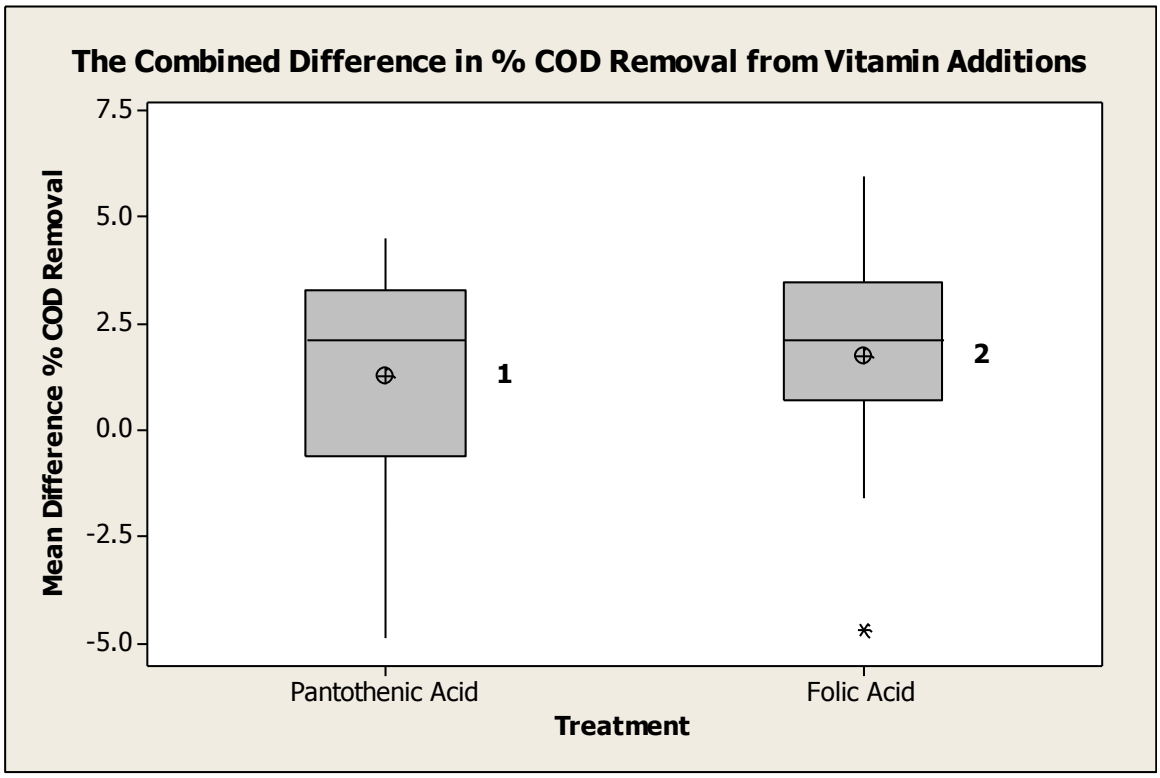


Figure 4.5: Effect of pantothenic acid and folic acid additions on % COD Removal.

(Outliers shown in asterisks, mean symbols represented by circle, combined mean %COD removal shown on right of boxplot).

4.4 Conclusions

Though there were great expectations for the water-soluble vitamin additions, encouraged by literature reports of past success, it is evident that individual treatment plants not only have unique wastewater characteristics but also have separate micro-nutrient requirements.

An initial study indicated that the addition of 1.0 mg/L thiamine or riboflavin did not significantly improve COD removal compared to the porous pot control. Although the COD removal rate of pyridoxine amended pot was not significantly different to the control, the COD removal deteriorated and mean removal was 8% lower than the control. Had the addition of pyridoxine been continued, it was expected that there would have been a significant decrease. Another indication that the pyridoxine amended pot was in decline was the mean SS were twice the control at 68 mg/L compared to 27 mg/L in the control.

In Run 3 the addition of 1.0 mg/L niacin caused a statistically significant decrease in the mean COD removal of 19% compared to the control. In this instance there was also a 2.5 times increase in the mean SS of 21 mg/L compared to 9 mg/L in the control pot. The individual additions of 1.0 mg/L thiamine, riboflavin pantothenic acid and folic acid to porous pots treating pulp and paper wastewater in Runs 3 and 4 did not significantly affect the level of SS in the effluent compared to the control.

Though the initial trials of both pantothenic acid and folic acid addition increased COD removal by 2% when compared to the control pot, these increases were not statistically significant (P – 0.089) and (P – 0.104), respectively. However, these increases were considered sufficient to justify duplicate experiments to test their validity. In the duplicate runs of pantothenic acid and folic acid additions COD removal was found to increase by 2% and 3%, respectively. However, only the COD removal improvement from the addition of folic acid was statistically significant (P – 0.051) and (P – 0.279), respectively. The combined mean increase in COD removal from the initial and duplicate results through the additions of 1.0 mg/L pantothenic acid and folic acid indicated that there was an increase in the COD removal rates of 1% and 2%, respectively. By analysing the combined data using a students t-test the mean increase in COD removal from the addition of 1.0 mg/L folic acid was statistically significant (P – 0.016), however, the overall 1% increase found from the addition of 1.0 mg/L pantothenic acid was not significantly greater than the control (P – 0.078). Folic acid should be considered as an amendment to the NSB SETP at a concentration of 1.0 mg/L.

The amendment of additional phosphorus to two porous pots at a ratio of 440:1; COD:P did not significantly change the rate of COD removal compared to the control (P – 0.201; df – 19) and (P –

0.238; df – 19). The mean COD removal in the two phosphorus amended pots and the control pot was 80 ± 10%, 83 ± 5% and 85 ± 3%, respectively.

To achieve the target residual concentration of NH_3 and PO_4^{3-} in the porous pot effluent 1.0 to 2.0 mg/L and 0.5 to 1.0 mg/L, respectively, future macro-nutrient amendments of nitrogen and phosphorus may have to be reviewed and altered to give direct equivalence to the COD nutrient ratios of an industrial plant.

Chapter 5 Fate of Metals in Effluent and Sludge from Porous Pot Experiments

5.1 Introduction

Knowledge of the fate of added metals is essential as there will be no real benefit to improving COD removal if the concentration of an added metal discharged in the effluent or sludge exceeds environmental guidelines. The aim is to have maximum effect on COD removal either through chemical reactions with metals and organic ligands or through increasing biological activity without exceeding legislated environmental regulations for the discharge of metals. As metals that are added to the waste treatment plant will ultimately reside in either the treated effluent or the waste sludge it is imperative that additional metal load in the wastewater and sludge be considered. The added metals could reside in the treated effluent as dissolved or colloidal material, or in the sludge as part of an inorganic precipitate or organic-bound material. To this end the treated effluent and sludge from the Duplicate Metal Run (Run 5), Varied Metal Concentration Run (Run 7) and Multiple Metals Run (Run 8) were analysed to determine the fate of the added metals. The operating conditions of each experiment are given in Chapter 3.

While there have been a number of studies into the effects of trace metal addition on lowering the chemical oxygen demand (COD) in activated sludge wastewater treatment plants (WWTP), there are very few reports on the fate of added trace metals from the research undertaken to date. In Chapter 3 the effect of metal addition on the COD removal from porous pots was considered and it was determined that the addition of calcium, cobalt, copper, iron(III) and magnesium had a positive effect on the removal of COD. During the initial porous pot runs, determining the concentration and mass of metals in the effluent and sludge, respectively, was not a priority and limited metals analysis was undertaken. In the duplicate experiments of individual metal additions (Runs 5, 7 and 8) this deficiency was corrected and there was a focus on the metals in the effluent and sludge. As the additions of zinc and molybdenum in the initial porous pot runs did not significantly improve effluent quality, these additions were not repeated in later runs and there remains only limited data available on zinc and molybdenum in the effluent and the sludge.

Initially in Run 5 calcium, cobalt, copper, iron(III) and magnesium were analysed weekly in effluent and waste activated sludge (WAS) samples. The target concentration of calcium, iron(III) and magnesium in the porous pots was 4.0 mg/L, while it was 0.5 mg/L for copper and 0.05 mg/L for cobalt. The data obtained was insufficient to determine a mass balance and to detect possible fluctuations in metal concentrations at potentially toxic levels. Consequently in Runs 7 and 8,

effluent and WAS samples were collected daily and analysed to determine metal concentrations and daily fluctuations.

In considering the fate of added metals, understanding metal speciation will help predict how metals will behave in solution, especially in regard to precipitation, sorption, complexation and chelation [118]. Individual metal hydroxides or oxy-hydroxides have different solubility constants (see Table 5.1), and precipitate at different pH values with a diverse range of concentrations at their optimum pH [119] (see Figure 5.1). However, there are variables other than solubility and pH which affect the aqueous metal concentration in activated sludge such as the concentration of complexing compounds and the extracellular polymer concentration [98]. A feature of iron(III) in aqueous solution is that it readily hydrolyzes and/or forms complexes [111]. In ideal conditions iron(III) will precipitate as an iron(III) oxy-hydroxide, forming a flocs at pH 7 [3].

Table 5.1: Solubility of metal hydroxides. Adapted from Aylward and Findlay (1998)

Metal Hydroxide	**$Ca(OH)_2$**	**$Co(OH)_2$**	**$Cu(OH)_2$**	**$Fe_2(OH)_3$**	**$Mg(OH)_2$**	**$Zn(OH)_2$**
K_{sp}	6.4×10^{-6}	1.0×10^{-15}	4.8×10^{-20}	2.0×10^{-39}	7.1×10^{-12}	3.8×10^{-17}

This provides a possible mechanism for the removal of metals from solution by co-precipitation [120]. Co-precipitation occurs when hydroxide groups attached to the precipitate react with soluble metal ions and bind to its surface [4]. Co-precipitation or adsorption of the heavy metals will decrease the metallic ion concentrations to below that predicted through theoretical solubility constants. For example, while the copper ion is soluble at pH 7, it also has an affinity for iron(III) flocs where it will bind to the floc and precipitate at a pH below that indicated by the solubility of the metal hydroxide [97]. On the other hand, magnesium and calcium are highly soluble at a neutral pH, well above the concentrations detected in the wastewater [97], and neither has a particular affinity for iron flocs [3].

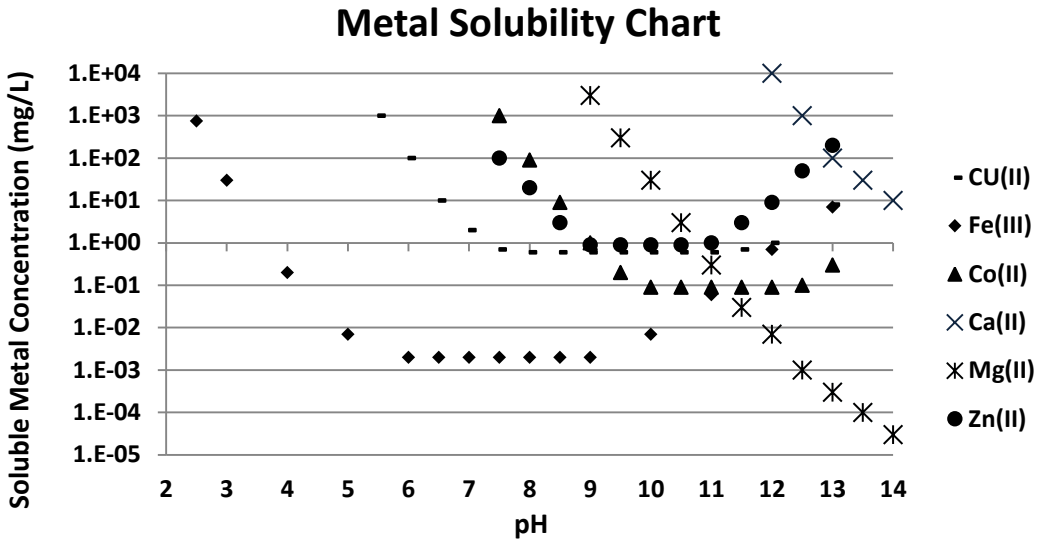


Figure 5.1: Metal solubility plot, indicating the concentrations in mg/L of individual species at a range of pH in pure water.

Note the total concentration axis is a log scale. The plot of the metals shows the amphoteric nature as the pH increases and the solubility decreases. Adapted from: Dyer et al. (1998).

In general, metals have an affinity for many organic compounds [4], especially those containing oxygen or nitrogen. There are a diverse range of organic ligands found in wastewater, with varying functional groups (eg aliphatic and aromatic amino acids and carboxylates) [4]. Pulp and paper mill wastewater consists of a high level of lignin derived compounds, as well as extracellular polymers that are produced in the biological treatment in the SETP. Both of these can contribute to the effluent COD and both are capable of binding metal ions present in solution. Extracellular polymers can be either in capsule form, surrounding the cell, or a slime material that can be free from the cell wall [121, 122]. Metal ions are thought to complex with the capsule surface and by direct ion exchange with cell walls [122]. Some metals such as calcium complex to soluble ligands [99] and discharge in the effluent.

The living biomass in the activated sludge can act as a biosorbent for metal species [17]. Biosorption is the removal of compounds through complexation to microbial surfaces by physical and chemical mechanisms [17, 123]. As the metal is adsorbed on the sorbent surface, a new hydroxide phase is formed allowing further mass transfer of the metal ion to the solid phase [124]. Surface precipitation becomes the principal sorption mechanism following surface saturation [124-126]. Biosorption of humic substances has been found to increase with increasing concentration of calcium [17].

These removal mechanisms, co-precipitation and adsorption are efficient and effective methods for the removal of heavy metals from wastewater. However, the purpose of adding trace metals to the Boyer SETP was to have them bioavailable and not part of a stable precipitate.

5.2 Materials and Methods

5.2.1 Materials

Reagents

The trace elements for the micronutrient additions were added as solutions of: $CaCl_2$, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, $Na_2(MoO_4).2H_2O$ and $ZnSO_4.7H_2O$ (all AR grade supplied by BDH), $CuSO_4.5H_2O$ (AR, Ajax) and $CoSO_4.7H_2O$ (AR, M & B Laboratory Chemicals). AR grade Merck HNO_3 was used for effluent stabilization and sludge digestion. 1000 mg/L AAS standards of cobalt, copper, iron(III), magnesium, molybdenum and zinc were obtained from BDH and 1000 mg/L calcium was from AccuTrace. A 0.3% $Mg(NO_3)_2.6H_2O$ (BDH) modifier solution was used for the graphite furnace analysis of cobalt. 2000 mg/L K or La ionization suppressors were used for the flame AAS analysis of calcium and magnesium, respectively. Both solutions where made in ultra pure water using BDH SpectrosoL 10% w/v La or BDH AR grade KCl.

Equipment

The samples were analysed for iron, calcium and magnesium using a GBC XplorAA for metal analysis. This was fitted with a GBC GF 3000 attachment and a GBC PAL 3000 auto sampler for graphite furnace analysis for copper and cobalt. A Hamilton Microlab 500 series automatic diluter was used to dilute samples. IEC hotplates were used to digest samples with Advantec glass fiber filter papers GA-55, used to filter the digested samples.

5.2.2 Methods

Procedure

Metals were added to the porous pots as described in Chapter 3.

Duplicate Metal Run (Run 5) - One control pot (Pot 1, no metal addition) plus 5 trial pots (calcium, iron(III), magnesium, copper or zinc additions): Calcium, iron(III) and magnesium were added to obtain additional concentrations in the pots of 4.0 mg/L. Copper and zinc were added to give additional concentrations of 0.5 mg/L and cobalt and molybdenum to give 0.05 mg/L. The iron(III)

bulk solution was acidified to 1% HNO_3 to eliminate the precipitation of iron (III) hydroxide in the dosing solution. The total mass of trace elements added in each trial pot for the duration of the experiment was 97 mg copper, 9.7 mg cobalt and 778 mg iron(III), magnesium and calcium over 25 days.

Varied Metal Concentrations Run (Run 7), One control pot (Pot 1, no metal addition) plus 5 trial pots (copper or cobalt additions): There were three copper pots with target additional concentrations in the pots of 1.0 mg/L, 0.25 mg/L or 0.1 mg/L and a total mass delivered for the duration of the experiment 187 mg, 47 mg and 19 mg, respectively. The target additional concentrations in the two cobalt pots were 0.1 mg/L and 0.025 mg/L, the total mass of cobalt delivered was 19 mg and 5 mg, respectively. There was a settling period of three (3) days before metals were added to the porous pots.

Multiple Metals Run (Run 8), One control pot (Pot 1, no metal addition) plus 5 trial pots (copper plus iron(III), magnesium or calcium additions): Run 6 employed multiple metal additions to individual pots. Copper was added to each trial pot, with a final additional concentration of 0.5 mg/L, while iron(III), magnesium or calcium was added to give final additional concentration of 4.0 mg/L in addition to the copper. The iron(III) additions were duplicated in pots 2 and 3, and calcium in pots 5 and 6. Magnesium was added to pot 4. The total mass of metals delivered for the duration of the experiment was 82 mg copper and 655 mg of iron(III), magnesium or calcium. A four (4) day settling period was required to stabilize the porous pots before the metal additions were commenced.

Chemical Analysis

AAS standards were made by serial dilution from 1000 mg/L standards. Cobalt and copper were analysed using graphite furnace, with calcium, iron(III) and magnesium concentrations determined through flame AAS. Graphite furnace temperature ramps for cobalt and copper can be found in Appendix 5: Tables 5.1 and 5.2, respectively. Flame AAS analysis was performed with air/acetylene with the exception of a nitrous oxide/acetylene flame for calcium. The absorbance of calcium, cobalt, copper, iron(III) and magnesium were measured at wavelengths 422.7 nm, 240.7 nm, 324.8 nm, 248.3 nm and 202.6 nm, respectively. A 0.3% $Mg(NO_3)_2$ modifier for cobalt analysis was automatically added through the auto sampler at 5 µL per sample. 2000 mg/L K and La ionization suppressors were used for the flame AAS analysis of calcium and magnesium, respectively, and added when samples were diluted.

Statistical analysis was undertaken using ANOVA comparison of means and regression analysis utilizing the MiniTab statistical package.

Sampling

50 mL aliquots of effluent were collected daily, filtered and acidified with HNO_3 (to 1%) and refrigerated until analysis. 25 mL aliquots of homogeneous WAS were taken directly from each pot daily, the sample was washed into a previously weighed beaker and dried at 110°C overnight. The beaker was placed into a desiccator and weighed after cooling. The dried sludge from the WAS sample was digested by boiling on a hotplate in 50 mL of 50% HNO_3 for 90 min. The digest was filtered through glass fiber filters and the solution rinsed into a 50 mL volumetric flask using ultra pure water. Blanks (50% HNO_3) and digest controls were used as quality control.

5.2.3 Calculating Metal Addition Potential Effects on Derwent River

Combined Effluent

The combined effluent discharged into the Derwent River includes the effluent from the SETP plus the water used only for the heat exchangers. The mean daily flow rate from the SETP and the combined effluent was 22 ML and 60 ML, respectively. In calculating the estimated effects of metal additions to the Derwent River, the metal concentration detected in the porous pot effluent was inserted into Equation 5.1

(5.1)

Derwent River

To calculate the estimated additional concentration in the Derwent River (Median flow 4888 ML) the combined effluent from equation 5.1 was inserted into equation 5.2.

(5.2)

By calculating the potential additional metal concentration in the Derwent River from the porous pot effluent samples the suitability of metals can be determined based on the Australian and New Zealand Guidelines for Fresh and Marine Water Quality [127].

5.2.4 Mass Balance

Metals were added to porous pots through peristaltic pumps at a rate between 2 mL/h to 3.2 mL/h. The total mass of metals delivered was determined through equation 5.3.

Mass of Metal Delivered

(5.3)

A mass balance of metals was calculated using the data from the metals analysis for the daily effluent and sludge samples. The calculation for the mass balance was separated into daily effluent, aqueous phase samples (Equation 5.4) and WAS samples (Equation 5.5).

Mass of Dissolved Metal in Effluent

(5.4)

Mass of metal in WAS

The mass of metal in the WAS is the total metal in the wasted sludge each day which varies daily depending on porous pot operating conditions. This metal is the total metal that is determined in the sludge digest.

(5.5)

Mass of Metal in Porous Pot

At the conclusion of the run there is a residual mass of metal in each porous pot, which has to be accounted for through Equation 5.6.

(5.6)

The equation for the sum of metals detected over the duration of each run adds the daily totals and accounts for the total mass of metal remaining in the pot at the completion of the run (Equation 5.7).

Total metals detected

(5.7)

5.2.5 Calculating the Mass of Metals in dried sludge (mg/kg)

The digest of the WAS includes both free metals in the aqueous phase and the metals bound to the sludge, to determine the mass of metal in the dried sludge, the aqueous phase was subtracted from the total metals in the WAS (Equation 5.8).

(5.8)

5.3 Results and Discussion

5.3.1 Run 5: Duplicate Metal Run and Initial Metal Runs

During the duplicate metal run (Run 5), the metals were analysed from five effluent and sludge samples collected weekly.

The mean concentration of calcium and magnesium in the Control effluent was significantly higher than the 4.0 mg/L of each metal added to the test pots. The remaining trace metal concentrations detected in the Control effluent were significantly lower than the targeted increases added to the pots (see Table 5.2).

The mean concentration of calcium and magnesium in the treated effluent was 14.3 mg/L and 44.6 mg/L, respectively. Due to the relatively low concentration of calcium and magnesium added and the relatively high standard deviations in the analysis, the difference between the control and treated effluent concentrations could be hidden in the analytical variation. For example the mean concentration of magnesium in the effluent was 41.4 ± 6.8 mg/L and the experimental mean was 44.6 ± 4.4 mg/L, that is the standard deviation of both means was greater than the target metal addition of 4.0 mg/L (Table 5.2).

Approximately 50% of the iron(III) added to the porous pot was detected in the effluent. This is much higher than expected at this pH and in the presence of free dissolved oxygen. Under these conditions it would be expected that almost all of the iron present would precipitate as some form of iron(III) oxy-hydroxide [111] and be found in the activated sludge. The unexpected result can be explained by the presence of high levels of humic substances which have been found to decrease the rate of hydrolysis of iron(III) in natural waters with increasing humic substance concentration [112]. However, metal cations have also been found to have a significantly effect on the hydrolysis reactions of organic compounds, though they need to be available in sufficient concentrations [16]. As a result humic substances in the wastewater are expected to decrease the rate of metal hydroxides forming, making the metals available to act as a catalyst for hydrolysis with organic compounds such as humic substances. Thus, these reactions could also be interfering with the natural solubility of iron(III).

Table 5.2: Trace metals detected in the effluent of porous pots from a Control and metal treated pots. Samples collected and analysed weekly

Trace Element	Target Porous Pot Addition (mg/L)	Effluent* (mg/L)			Estimated Increase [Metal] in Derwent River (mg/L)	Australian Freshwater Guidelines (mg/L) [128]
		Control Mean	Treated Pot Mean	Increase in Effluent		
Fe	4.00	0.8 ± 0.17	2.6 ± 0.47	1.83	1.2×10^{-2}	0.3
Ca	4.00	13.8 ± 2.15	14.3 ± 1.74	0.53	6.3×10^{-2}	N/A
Mg	4.00	41.4 ± 6.76	44.6 ± 4.42	3.23	1.7×10^{-1}	N/A
Cu	0.50	0.07 ± 0.03	0.14 ± 0.04	0.066	9.3×10^{-4}	1.4×10^{-3}
Zn**	0.50	0.15 ± 0.07	0.19 ± 0.11	0.039	7.9×10^{-4}	8.0×10^{-3}
Co	0.05	0.002 ± 0.001	0.031 ± 0.013	0.029	1.3×10^{-4}	N/A
Mo**	0.05	0.002 ± 0.000	0.012 ± 0.005	0.010	4.9×10^{-5}	N/A

*Mean from weekly samples; **Mean from two grab samples

High proportions of the added magnesium and cobalt were also detected in the effluent, approximately 80% and 60%, respectively, of the added metals (Table 5.2). As both metals in aqueous form are highly soluble at neutral pH, even in the presence of high levels of humic material, detecting them in the aqueous effluent is not surprising [97].

The rationale for adding trace metals was to increase biological growth and, hence, the biodegradation of the recalcitrant organic compounds contributing to the COD in the effluent. It is clear that as the majority of calcium and cobalt are found in solution, they should be bioavailable.

However, if metals are complexed to humic substances in the wastewater they may be unavailable for biological synthesis. There have been two mechanisms proposed for the removal of metals from solution in the presence of biomass: firstly the biosynthesis through living cells, and secondly through complexation with negatively charged biopolymers and/or the negatively charged external cell wall [129]. However, direct precipitation of some metal hydroxides is also likely at neutral pH.

There have been few reports of either the bioaccumulation of metals or metal concentration in the activated sludge or effluent in the AS process. Rudd *et al.* reports that the removal of metals in batch experiments was due to surface binding and not incorporation to the cell [122]. However, Burgess *et al.* reported that increases in COD removal were due to increased availability of trace metals for metabolism [41].

Whatever the process, there were orders of magnitude increases in the masses of copper and zinc detected in the sludge in comparison to the Control pot (Table 5.3), with only 13% and 8%, respectively, of the added metal in the porous pot effluents (Table 5.2). Rudd *et al.* found that copper hydroxide precipitates were visibly enmeshed within the activated sludge floc structure [98]. In the presence of humic substances, especially biopolymers, copper is known to form strong complexes [98, 118], however, the interaction between zinc and humic substances is much weaker [98]. This is contradicted by the results presented here, implying that, as zinc is weakly adsorbed by humic matter and yet only 8% of the added zinc remains in solution, it is most likely incorporated in the micro-organisms.

Table 5.3: Trace elements detected in the sludge of a control pot and pots treated with trace elements. Samples collected and analysed weekly

Trace Element	Metal Addition (mg/L)	Sludge* (mg/kg)			Land Application Guidelines (mg/kg)
		Control Mean	Treated Pot Mean	Increase in Sludge	
Fe	4.0	$9.23 \times 10^{-4} \pm 3.4 \times 10^{-5}$	$5.52 \times 10^{-3} \pm 2.8 \times 10^{-3}$	4.60×10^{-3}	N/A
Ca	4.0	$2.53 \times 10^{-3} \pm 3.1 \times 10^{-4}$	$2.78 \times 10^{-3} \pm 1.1 \times 10^{-4}$	2.50×10^{-4}	N/A
Mg	4.0	$4.76 \times 10^{-3} \pm 3.3 \times 10^{-3}$	$3.67 \times 10^{-3} \pm 3.9 \times 10^{-4}$	-1.09×10^{-3}	N/A
Cu	0.50	$3.31 \times 10^{-5} \pm 5.3 \times 10^{-6}$	$3.02 \times 10^{-4} \pm 8.6 \times 10^{-5}$	2.69×10^{-4}	100 [95]
Zn**	0.50	8.82×10^{-5}	1.01×10^{-3}	9.22×10^{-4}	200 [95]
Co	0.05	$4.41 \times 10^{-7} \pm 3.8 \times 10^{-7}$	$2.4 \times 10^{-6} \pm 1.7 \times 10^{-6}$	1.96×10^{-6}	40 [96]
Mo**	0.05	1.01×10^{-5}	2.32×10^{-5}	1.31×10^{-5}	5 [96]

*Control Mean from 5 samples; **Analysis from single grab sample

Of the metals trialled in this project the Australian and New Zealand Guidelines for Fresh and Marine Water Quality only recommends guidelines for the concentrations of copper and zinc in freshwater [128]. Based on the median flow of the Derwent River (4888 ML/day from 1985 – 2009) and the mean effluent discharge from the wastewater treatment plant (20 ML/day, diluted to 60 ML/day by the cooling water for the heat transfer unit), the estimated total concentration of each of these metals in the Derwent were well below the freshwater guidelines when copper and zinc when added at 0.50 mg/L (see Table 5.2).

The mechanism for improvements in COD removal brought about by the addition of magnesium and calcium was not clear. It may have been either direct biological stimulation, metal complexation with humic substances followed by binding to solid activated sludge surfaces and precipitation, or breakdown of organic compounds by metal cations acting as a catalyst for their hydrolysis. As indicated in Table 5.2 there was an increase of 3.23 mg/L magnesium in the effluent from the magnesium treated porous pot, and an apparent decrease of 29% magnesium in the sludge from the experimental pot compared to the control, though this difference is within the experimental error associated with the control pot sludge analysis (Table 5.3). However, in the case of calcium, there were increases in the calcium detected in both the effluent (a 4% increase) and the sludge (a 10% increase) compared to the control pot (Table 5.3).

As calcium and magnesium are both normally soluble at pH 7, the majority of both metals would be expected to be found in the effluent [97]. However, calcium and magnesium have the potential to interact with negatively charged resin acids in the porous pot [39]. Metal ions react with resin acids to form metal resinates, and calcium had been found to form octahedral complexes through the carboxylic acid functional group on resin acids, significantly decreasing the solubility of calcium in water [99].

While there was limited difference between the mass of calcium and magnesium detected in the Control Pot sludge and the mass in the sludge from their respective metal treated pots, the mass of the remaining metals increased by approximately an order of magnitude in the sludge from each treated pot. Importantly though, the levels of the heavy metals copper, zinc, cobalt and molybdenum in their respective metal amended pot sludge was significantly below the land application guidelines by at least 5 orders of magnitude (see Table 5.3).

The five weekly samples were not a sufficient data set to observe fluctuations of metals in either the effluent or the sludge. To gain more information on the daily variations and a clear picture of the metal behaviour over time, a daily sampling regime was undertaken. This also allowed the

introduction of a mass balance analysis in following experiments to gain a sounder understanding of the amounts of individual metals in either soluble or insoluble phase. The mass balance results are reported in Section 5.3.4.

5.3.2 Run 7: Varied Metal Concentrations

To produce a more accurate metals data set from a complete porous pot experiment the effluent and sludge samples were collected for analysis daily. Of the metals of environmental concern only copper and cobalt additions were continued as the addition of molybdenum and zinc gave no improvement in COD removal in previous runs. There was one control pot and five experimental pots. The two metals, copper and cobalt were added to the porous pots at various concentrations, copper at 1.0 mg/L, 0.25mg/L and 0.1mg/L and cobalt at 0.1 mg/L and 0.025 mg/L. There was a three day stabilization period before the metal additions started.

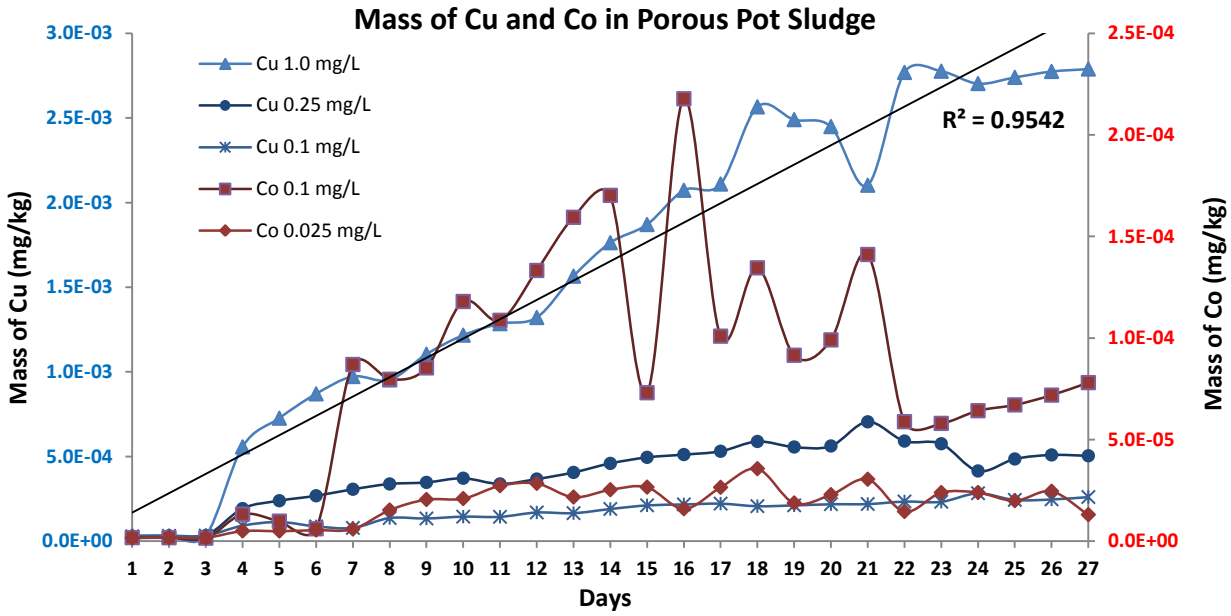


Figure 5.2: Mass of copper and cobalt detected in daily porous pot sludge samples from Run 7. With three pots amended with copper at 1.0 mg/L, 0.25 mg/L and 0.1 mg/L and two pots amended with cobalt at 0.1 mg/L and 0.025 mg/L.

There were two significant operational problems that impacted on the experiment. Firstly, the retention basin was undergoing routine maintenance at the time the bulk sample was collected from

Boyer. This increased the suspended solids in the bulk sample, causing the inlet tubing to occasionally block during the porous pot experiment. Secondly, there was a power failure on day 15, which resulted in the loss of aeration, and influent and nutrient feed. While these operational issues did not cause significant fluctuations in the copper concentration in the sludge of the copper amended pots (Figure 5.2), the same could not be said for cobalt amended pots.

From Figure 5.2 a significant increase in the concentration of metals in the sludge is apparent from the time the metals were added to the porous pots after the three day stabilization. In both the cobalt amended pots there was no clear trend until day 7, four days after the metal amendment started, where there was a significant increase in the amount of cobalt detected in the sludge. At the same time the concentration detected in the effluent was close to the target concentration for each cobalt amended pot (Figure 5.3). As cobalt is expected to be soluble at pH 7, hydroxide precipitation is unlikely to affect the concentrations of the metal in solution. The delay in the uptake of cobalt in the sludge suggests that physical binding is not the primary mechanism and that there is a biological lag in the uptake of the available cobalt. On Day 15 in the 0.1 mg/L cobalt amended pot a lower mass of cobalt detected coincides with the power failure. There could be two explanations for the reduction in the mass of cobalt detected in the sludge following the power failure (Day 15): firstly there was no mixing to promote complexation by available binding sites on the sludge or onto biopolymers in solution, secondly the facultative aerobic bacteria using cobalt to synthesis cobalamin (vitamin B_{12}) were not active in the anoxic conditions. On day 16 the significant increase in cobalt in the sludge coincides with a blocked inlet tubing. As can be seen from Figure 5.2 accumulation of cobalt in the sludge became very erratic following this blockage until Day 22. There was little variation in the amounts of cobalt detected in the effluent or sludge throughout the run in the 0.025 mg/L cobalt amended pot, indicating that at 0.025 mg/L cobalt may not have been sufficient to stimulate biological synthesis or to cause significant complexation in the activated sludge.

It is significant that the metal reduction associated with the power failure in the sludge occurred only in the pot amended with cobalt (at the higher level) and not in any of the pots amended with copper. The mass of copper detected in the sludge of the 1.0 mg/L amended pot appeared to be steadily increasing, while the mass of copper in the sludge in the pots with the lower concentration amendments was relatively stable throughout the run. As the power failure had no noticeable effect on the pots amended with copper it would appear that copper binding in the sludge is not affected by direct biological cell synthesis. Rudd *et al.* found that of the heavy metals cadmium, cobalt, copper and nickel, copper had the strongest affinity for binding to activated sludge [98].

There are conflicting reports on the affect of sludge age on the adsorption of copper to activated sludge. The sludge age refers to the average cell resonance time in the SETP before being wasted, and is controlled through increasing or decreasing the volume of activated sludge wasted. It is known that as the sludge age fluctuates the concentration of biopolymers being produced also changes [39, 40]. However, Rudd *et al.* suggest that sludge age has no affect on the level of copper complexation with biopolymers [98], while Santos *et al.* suggest that the greater the sludge age the greater the concentration of copper in the sludge [130]. To determine which of these claims apply to the porous pot sludge, the percentage of copper in the sludge in Runs 7 and 8 was determined by back calculating the copper in the effluent from Tables 5.3 and 5.5. The mean percentage of copper in the sludge from Runs 7 and 8, with mean sludge ages of 24 days and 16 days, was between 34 – 54% and 7 – 44%, respectively. However, the results from the initial metals run (see Section 5.3.1), indicate that 74% of the copper was in the sludge when the mean sludge age was only 11 days. This would appear to contradict Rudd et al., who reported that the complexation of metals to activated sludge and biopolymers was not affected by the sludge age, where both studies were undertaken using sludge ages between 3 – 12 days [98, 130]. This is an indication of the complicated nature of activated sludge treatment where incorporating the results from plants treating different quality wastewater may not be acceptable.

Due to the ability of copper to complex with activated sludge and dissolved organic matter (DOM), the copper present may not be bioavailable [118]. In Figure 5.2, the trend from the 1.0 mg/L copper amended pot appears to indicate that there is a steady accumulation of copper in the sludge (R^2 – 0.9542). However, the mass of metals detected in the sludge appeared to be stabilizing following day 23, where equilibrium could have been reached between activated sludge wasting and the available complexation sites.

As opposed to the metal level in the sludge, the power failure and tube blockage affected both copper and cobalt levels in the effluent. In the copper 1.0 mg/L and the cobalt 0.1 mg/L amended pots (Figure 5.3), the highest concentrations of copper and cobalt in the effluent also coincide with the power failure on day 15. The remaining spikes in concentration of copper and cobalt detected in Figure 5.3 were due to influent tube blockages. Copper and cobalt appeared in the effluent on the day that metal additions commenced. In the pots amended with cobalt the final concentrations detected on day 27 in the effluent were comparable to the target dose level. The mean residual concentration of copper in the effluent of the pots amended with 1.0 mg/L, 0.25 mg/L and 0.1 mg/L copper was 49%, 46% and 66% of the target concentrations, respectively. The mean copper concentration detected in the effluent from the initial run (Run 2; Section 3.3.3) containing copper

indicated that there was a residual 14% in the effluent. The difference could be due the limited number of initial samples taken in Run 2 not accurately representing the results for the entire run.

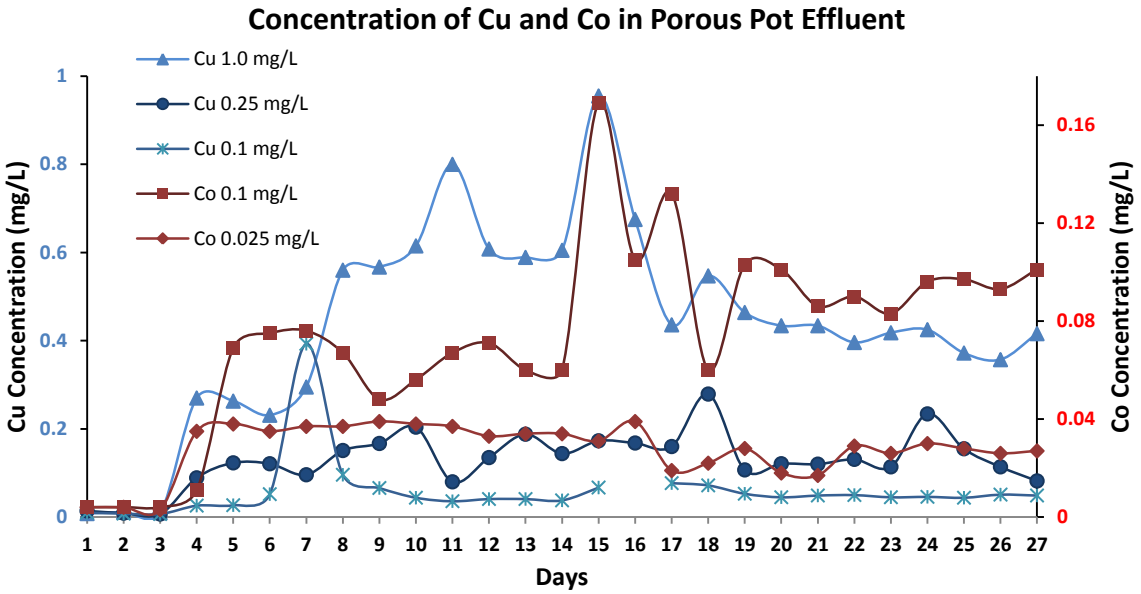


Figure 5.3: The daily concentration of copper and cobalt detected in effluent samples from Run 7. Blue represents the three pots amended with copper at 1.0 mg/L, 0.25 mg/L and 0.1 mg/L and brown represents the two pots amended with cobalt at 0.1 mg/L and 0.025 mg/L. Day 16 copper 0.1 mg/L sample was lost.

The mean concentration of copper in the effluent from the pot dosed to a target concentration of 1.0 mg/L copper was found to be 0.49 ± 0.17 mg/L. The estimated additional concentration of copper in the Derwent River calculated from the mean porous pot effluent was determined using Equations 5.1 and 5.2. Based on the metal levels in the Porous Pot effluent and taking into account the plant discharge volume and Derwent River flow, the estimated copper concentration in the Derwent River from adding 1.0 mg/L copper to the Boyer SETP would increase to approximately 1.9 x 10^{-3} mg/L. Discharging effluent from the SETP following the addition of 1.0 mg/L copper would exceed the Australian and New Zealand Guidelines for Fresh and Marine Water Quality of 1.4 x 10^{-3} mg/L [128] (See Table 5.4). The effluent from the remaining copper amended pots is estimated to be well below the freshwater guidelines for copper.

Cobalt has a lower affinity for adsorption to dissolved organic matter and activated sludge than copper [98], which is verified by the residual concentrations in the effluent. The mean concentration of cobalt in the effluent from the pots with the target increase concentration of 0.1 mg/L and 0.025

mg/L was 0.066 mg/L and 0.031 mg/L, respectively. Clearly, the mean concentration detected in the pot amended with 0.025 mg/L cobalt was higher than the target value. Taking into account the mean baseline concentration from the Boyer treated influent mill water (Clearwater, Table 2.3), the concentration would still exceed the target value, being 0.029 mg/L, though this would bring the result to within experimental error. Regardless, as there was minimal variation in the concentration of cobalt detected in the effluent it was apparent that the blockages that occurred around Day 16 did not have a significant effect on the concentration of cobalt in the porous pot effluent.

Table 5.4: The mean concentrations and mass of copper and cobalt detected in the effluent and sludge during porous pot runs involving the addition of cobalt and copper at various levels.

Pot	Element	Target Porous Pot Increase (mg/L)	Mean M^+ Concentration in Effluent (mg/L)*	Estimated Additional M^+ Concentration in Derwent River (mg/L)	Freshwater Guidelines (mg/L)[128]	Mean M^+ concentration in sludge (mg/kg)	Land Application Guidelines (mg/kg)
Control	Cu		0.005 ± 0.006		1.40×10^{-3}	$2.7 \times 10^{-5} \pm 1.1 \times 10^{-5}$	100 [95]
Control	Co		0.005 ± 0.001		NA	$2.2 \times 10^{-7} \pm 9.1 \times 10^{-7}$	40 [96]
Pot 2	Cu	1.0	0.489 ± 0.17	2.20×10^{-3}	1.40×10^{-3}	$1.9 \times 10^{-3} \pm 7.7 \times 10^{-4}$	100
Pot 3	Cu	0.25	0.114 ± 0.05	5.13×10^{-4}	1.40×10^{-3}	$4.4 \times 10^{-4} \pm 1.3 \times 10^{-4}$	100
Pot 4	Cu	0.10	0.066 ± 0.07	2.97×10^{-4}	1.40×10^{-3}	$1.8 \times 10^{-4} \pm 5.8 \times 10^{-5}$	100
Pot 5	Co	0.10	0.082 ± 0.03	3.69×10^{-4}	NA	$9.2 \times 10^{-5} \pm 5.1 \times 10^{-5}$	40
Pot 6	Co	0.025	0.031 ± 0.01	1.39×10^{-4}	NA	$2.0 \times 10^{-5} \pm 8.4 \times 10^{-6}$	40

*Mean 24 samples

Though there are no Australian and New Zealand guidelines for the concentration of cobalt in freshwater or marine environments, there are guidelines for the concentrations of copper and cobalt in sludge designed for land application. The maximum accepted level of copper and cobalt in sludge recommended for the application to agricultural land is 100 mg/kg copper [95] and 40 mg/kg cobalt [96] dry weight (see Table 5.5). The concentrations of copper and cobalt in the dry weight of sludge was at least five (5) orders of magnitude below the recommended maximum levels.

Although it has been reported that the binding capacity of cobalt to DOM and activated sludge is lower than it is for copper and that the concentration of cobalt detected in the effluent was approximately equal to the target concentration added to the experimental pot, there was an increase in the mass of cobalt detected in the sludge of two orders of magnitude (Table 5.3). However, the total increase of cobalt calculated in the sludge was still minimal (see Section 5.3.4).

The relatively low level of cobalt added to the porous pot could be too low to stimulate biological synthesis of cobalamin, the primary cell requirement for cobalt. Previous work has indicated that there is a significant increase in the biosynthesis of cobalamin from the addition of 1.0 mg/L cobalt [68], however, at 1.0 mg/L cobalt led to a decreased COD removal in activated sludge, potentially being toxic [8]. The estimated requirements for optimal cell growth has been reported between 0.02 – 0.05 mg/L [76], and the theoretical required concentration of cobalamin is 0.005 mg/L [43]. As such, the addition of between 0.025 – 0.1 mg/L cobalt should be significant enough to stimulate the biosynthesis of cobalamin, though this appears not to have occurred in the current work.

5.3.3 Run 8: Multiple Metals Additions

From the work described above, the addition of copper alone had a significant effect on COD removal (see Chapter 3) and also the settleability as the abundance of filamentous bacteria decreased (see Chapter 7). It was also clear from the work above that an addition of 0.5 mg/L copper is optimal. As individual amendments iron(III), calcium and magnesium also had significant effects on the rate of COD removal. This section considers the effects of a 0.5 mg/L copper amendment when combined with these other beneficial metals. The multiple metal additions therefore had a fixed target concentration of 0.5 mg/L copper in all pots with an additional 4.0 mg/L iron(III) to two pots (Pots 2 and 3), a single 4.0 mg/L magnesium (Pot 4) and 4.0 mg/L calcium in a further two pots (Pots 5 and 6). Pot 1 was again used as a Control Pot with no metal addition.

There was a significant statistical relationship found between the mass of iron(III) and copper detected in the sludge (see Figure 5.3a). As the concentration of iron(III) detected in the sludge increases the concentration of copper also increased (P – 0.000; df – 41). There was no relationship found between the calcium and copper (see Figure 5.4b) or the magnesium and copper concentrations detected in the sludge (P – 0.452; df – 41) and (P – 0.095; df – 20), respectively.

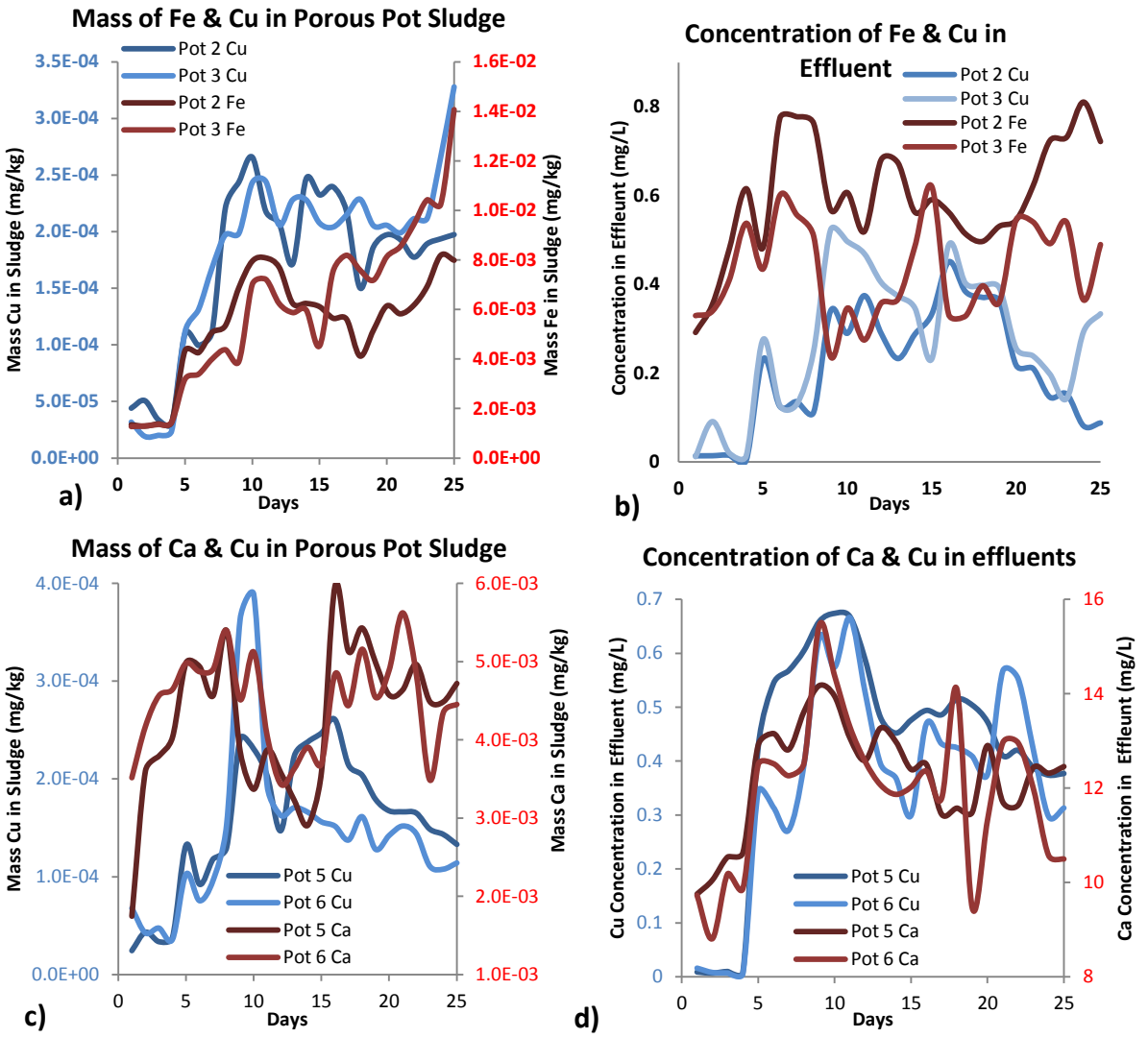


Figure 5.4: Mass of metals in porous pot sludge and concentration of metals in effluent from Run 8. Comparisons of the mass of metals detected in the sludge (top) and the concentration of metals detected in the effluent from the iron(III) and calcium amended pots (bottom). a) Mass iron and copper in sludge, b) Mass calcium and copper in sludge, c) Concentration iron and copper in effluent, d) Concentration calcium and copper in effluent.

While copper, calcium and magnesium are very soluble at the pH of the Porous Pots, iron(III) is effectively insoluble at any pH ≥ 5, forming stable iron(III) oxy-hydroxide flocs [111]. It is also known that though copper is quite soluble at this pH, it has an affinity for the iron(III) oxy-hydroxide floc. Once adsorbed onto the iron floc, copper will settle out of solution with the floc to give concentrations well below its natural solubility level. This floc formation followed by adsorption does not occur with magnesium and calcium cations which are more stable in solution at pH 7 [111], eliminating this mechanism for copper removal with these metals.

There was a statistically significant increase in the mean copper concentration in the effluent of the pot amended with calcium compared to that of the iron(III) amended pot, with 0.47 ± 0.11 mg/L copper in the calcium amended pot and 0.29 ± 0.12 mg/L in the iron(III) amended pot (P – 0.000; df – 83). There was also a significant statistical inverse relationship between the concentration of iron(III) and copper in effluent, as iron(III) concentration decreased the copper concentration increased (P – 0.000; df – 41). As was discussed above, this is expected as copper has an affinity for the iron(III) complexes and hydroxide precipitates. In the presence of iron(III) it would appear that approximately 50% of the copper is bound to the iron floc and not bioavailable for cell synthesis. There was also a significant statistical direct relationship between the concentration of calcium and copper in the effluent (P – 0.000; df – 41). It was found that as the concentration of calcium detected in the effluent increased the concentration of copper also increased.

Though blockages of the influent to the porous pots during the multi metal addition run were uncommon, there was a major blockage in Pot 6 on day 9. This can be seen in the spike in calcium and copper detected in the effluent and the level of copper detected in the sludge on day 9 (see Figure 5.4). However, there was no significant increase in the calcium detected in the sludge even though the concentration of calcium and copper were being increased in the porous pot (as the metal addition continued though the pot feed was blocked). This is in line with the discussion above that the binding mechanisms and the ability of calcium to form complexes with DOM is significantly different to those of iron(III) and does not occur at the same rate.

Following the blockage the COD removal rates in pot 6 decreased to below the control and did not recover until toward the end of the run. As indicated by a decrease in COD removal and an increase in SS, the spike in copper concentration appears to have been toxic to some of the porous pot biota. It is known that the toxicity of copper is dependent on the availability of free copper in solution [118]. The concentration of copper has previously spiked (see Section 5.3.2), with no adverse affects on the porous pot biota, however, during this run Pot 6 had a consistently high concentration of copper ≥ 0.5 mg/L in the effluent for seven (7) days. The maximum concentration of copper estimated for optimal biological growth has been reported to be 1.0 mg/L (see Table 2.5) [5]. The adverse reaction to the copper concentration ≥ 0.5 mg/L, also indicated that prolonged exposure to copper even at levels below 1.0 mg/L was toxic to the activated sludge biota from the Boyer SETP.

Table 5.5: The mean concentrations of metals in the effluent during the Multiple Metals (Run 8) porous pot run.

	Effluent* (mg/L)			Estimated Additional copper Concentration in Derwent River (mg/L)	Freshwater guidelines [128] (mg/L)
	Mean Control M^+ Concentration	Mean M^+ Concentration	Mean copper Concentration		
Cu (Control)			0.009 ± 0.007		
Fe (4 mg/L)	0.294 ± 0.218	0.535 ± 0.145	0.286 ± 0.121	1.2×10^{-3}	1.4×10^{-3}
Mg (4 mg/L)	31.16 ± 3.12	36.08 ± 6.53	0.362 ± 0.156	1.5×10^{-3}	1.4×10^{-3}
Ca (4 mg/L)	9.77 ± 0.77	12.69 ± 0.89	0.467 ± 0.110	1.9×10^{-3}	1.4×10^{-3}

*mean 21 samples

As discussed in relation to Table 5.4, the copper concentration in the Derwent River would increase to approximately 1.2×10^{-3} mg/L copper when copper is added to the Boyer SETP at 0.5 mg/L, below the guidelines [128]. The estimated contribution to the Derwent River from adding iron at 4 mg/L to the Boyer plant would be 2.19×10^{-3} mg/L iron(III), well below the guidelines for total iron(III) in a freshwater aquatic environment of 0.3 mg/L iron(III) [128]. The mean copper concentration detected when adding 0.5 mg/L copper in the presence of 4.0 mg/L iron(III) was 0.29 mg/L and the mean for the initial copper run, when copper was also being added at 0.5 mg/L was 0.14 mg/L. The percentage of copper in the effluent from the copper plus iron(III) amended pots (56%) and the copper amended pots in the varied concentration runs (44% to 66%) was similar.

The amount of individual metals available as soluble metals for cell biosynthesis is unknown. Through the formation of iron hydroxide flocs new adsorption surfaces form to allow metal ion adsorption to a sorbent metal hydroxide such as iron hydroxide [124, 126, 131]. As the metal is adsorbed on the sorbent surface, a new hydroxide phase is formed allowing further mass transfer of the metal ion to the solid phase [124, 131]. However, the presence of DOM can inhibit Fe(III) hydrolysis due to the formation of metal ion complexes with DOM [112]. As the concentration of humic substances in the porous pots was significantly higher than natural waters, it would be expected that the formation of iron(III) hydroxide precipitates would be inhibited. The iron(III) would bind to humic substances, including biopolymers in solution and to the negatively charged organic compounds on the surface of the flocs, directly competing with the copper and not forming hydroxide precipitates that would co-precipitate with copper.

There was an expectation that due to co-precipitation of copper with iron(III) there would be less copper detected in the effluent from the Multiple Metal Addition Runs with iron(III) than the pots amended with only copper. This was not the case. The lack of difference in the copper concentration between metal additions of copper with iron(III) and individual copper additions could be due to co-precipitation of copper with iron(III) to give the greatest removal rate of copper, approximately 50%. Although cobalt does not have a strong affinity to bind with activated sludge or biopolymers and is stable in solution at pH 7, the humic substances in the sludge could be inhibiting co-precipitation with iron(III).

The mean concentration of copper detected in the effluent increased in the presence of magnesium and calcium to 72% and 93% of the target increase, respectively (Table 5.5). As expected due to the solubility constants and consideration of their binding to organic components, there was no significant increase in the levels of magnesium or calcium in the sludge (Table 5.6). The competing organic and inorganic chemical reactions within the activated sludge are complicated and varied. It appears that when copper was added to the porous pots with either magnesium or calcium there was an inhibition of copper complexation and adsorption. It is known that metal ions, especially calcium, iron(III) and aluminium(III), complex with the polar lignin derived acidic humic substances [16]. It is also known that the acidic humic fraction is the most significant residual organic matter in pulp and paper wastewater [13] and that divalent cations such as calcium and magnesium react with negatively charged biopolymers to form cation bridges [40]. Competition from calcium and magnesium for complexing sites with humic substances or solid sludge surfaces could affect the residual copper concentration in the effluent of the porous pots amended with the multiple metal additions. There does appear to be a chemical or physical mechanism that limited the adsorption or complexation of copper when added with calcium and magnesium.

There was a statistically significant difference in the mean copper concentration in the sludge observed in all the metal addition pots in this Run (P – 0.029; df – 104). As expected the concentration of copper in the sludge from the pots amended with iron(III) was significantly higher (P – 0.008; df – 83) than the concentration of copper detected in the calcium amended pots, $2.02 \times 10^{-4} \pm 4.5 \times 10^{-5}$ mg/kg Cu and $1.70 \times 10^{-4} \pm 6.4 \times 10^{-5}$ mg/kg Cu, respectively (Table 5.6).

Table 5.6: Multiple Metals Run (Run 8) sludge data from the constant addition of 0.5mg/L Cu to all pots plus the addition of 4.0 mg/L Fe, Mg and Ca to individual pots.

	Sludge* (mg/kg)			Cu Land Application Guidelines [95] (mg/Kg)
	Mean Control Concentration M^+	Mean Concentration M^+	Mean Concentration Cu	
Cu (Control)			$3.63 \times 10^{-5} \pm 1.9 \times 10^{-5}$	100
Fe (4 mg/L)	$1.86 \times 10^{-3} \pm 2.43 \times 10^{-5}$	$6.60 \times 10^{-3} \pm 2.1 \times 10^{-3}$	$2.02 \times 10^{-4} \pm 4.5 \times 10^{-5}$	100
Mg (4 mg/L)	$3.48 \times 10^{-3} \pm 9.63 \times 10^{-4}$	$4.12 \times 10^{-3} \pm 2.17 \times 10^{-3}$	$1.99 \times 10^{-4} \pm 4.7 \times 10^{-5}$	100
Ca (4 mg/L)	$2.37 \times 10^{-3} \pm 1.12 \times 10^{-3}$	$4.48 \times 10^{-3} \pm 7.2 \times 10^{-4}$	$1.70 \times 10^{-4} \pm 6.4 \times 10^{-5}$	100

*mean 21 samples

From Table 5.6 there is no significant increase in the mass of magnesium and calcium in the sludge, possibly as the concentrations of these metals in control pot solution was relatively high, approximately 31 mg/L and 10 mg/L, respectively. The theoretical requirements for optimal cell function of magnesium and calcium have been reported as 3 mg/L – 10 mg/L and 3 mg/L – 5 mg/L, respectively [8, 71]. As such, biological synthesis was not restricted prior to the addition of either metal as calcium and magnesium are effectively completely soluble at pH < 9 [97]. The addition of divalent cations, calcium and magnesium was included due to reports that they decrease residual COD in WWTP through forming cation bridges with biopolymers [39, 40]. The concentrations of copper detected in the sludge were orders of magnitude below the guidelines (Table 5.6).

5.3.4 Mass Balance

Determining the total mass of metals delivered during porous pot runs was calculated by multiplying the stock metal solution (mg/L) by the volume delivered (L/day) and multiplying the daily mass of metals delivered by the number of days (Equation 5.3). There were three separate calculations taken into consideration before determining the total mass of metal recovered from the porous pots. The calculations for the mass of dissolved metal in the effluent and the mass of metals in the WAS, Equations 5.4 and 5.5 respectively, were determined daily. On the final day the total mass of residual metals in the porous pot was determined through the final sludge digest (Equation 5.6), the total mass of metals recovered was the sum of the results from Equations 5.4, 5.5 and 5.6.

The mass balance calculation for the copper and cobalt in the effluent and sludge from the varied metal concentration run (Section 5.3.2) indicated a recovery of between 98% and 105% for both copper and cobalt. The exception was the copper pot with the target concentration of 0.1 mg/L, in

which the calculated recovery was 147% (Table 5.7). The apparent copper recovery of 147% could have been due to the relatively low concentration of copper added and potentially false high concentrations detected in the effluent samples after digestion. There was limited difference in the percentage of copper detected in the sludge between the varied copper additions in Run 7 (22 – 26%).

Table 5.7: Varied metal concentration addition mass balance from Run 7.

Trace Metal	Mass Delivered (mg)	Mass Recovered (mg)	% Recovery	Mass (mg) in Sludge	% M^+ in Sludge
Cu (1.0 mg/L)	187	184	98%	42	22%
Cu (0.25 mg/L)	47	47	100%	12	25%
Cu (0.1 mg/L)	19	28	147%	5	26%
Co (0.1 mg/L)	19	20	105%	2	10%
Co (0.025 mg/L)	5	5	100%	4.4×10^{-4}	0.008%

Effectively no cobalt was detected in the sludge of the pot with target concentration of 0.025 mg/L cobalt, while the mean concentration detected in the effluent was 0.031 mg/L. However, from the mass balance calculation the recovery was 100% (see Table 5.7). There was only 10% and 0.008% of cobalt detected in the sludge from additions of 0.1 mg/L and 0.025 mg/L respectively (Table 5.7).

From the varied metal concentration additions, the percentage of copper accounted for in the porous pot sludge was between 22% and 26% (see Table 5.7). The percentage of copper detected in the sludge from the multiple metals addition with iron(III) was significantly lower at approximately 10% and 11% (see Table 5.8). Also in the presence of additional calcium and magnesium there was between 6 – 8% of the recovered copper in the sludge, where as stated in the single additions of copper from Run 7 there was between 22 – 26% detected in the sludge.

The percentage recovery of copper from the multi-metal addition run (Section 5.3.3) was between 62% and 108% (Table 5.8). The pot with the lowest copper recovery (62%) coincided with a low recovery of iron(III) (74%). The copper and iron(III) recovery in the other multiple metal addition pot was 83% and 96%, respectively. Without taking into account the biological uptake of iron(III), the two main mechanisms for iron(III) removal from solution are: firstly to form oxy-hydroxide precipitates and secondly to form complexes with organic compounds, either in solution or attached

to the floc surface. There is no explanation for the low recovery of both metals in one pot as copper and iron(III) should easily dissolve in the acid digest and both pots were treated the same.

Table 5.8: Multi-metal addition mass balance data from Run 8 Pot 2 0.5 mg/L Cu + 4.0 mg/L Fe, Pot 3 0.5 mg/L Cu + 4.0 mg/L Fe, Pot 4 0.5 mg/L Cu + 4.0 mg/L Mg, Pot 5 0.5 mg/L Cu + 4.0 mg/L Ca, Pot 6 0.5 mg/L Cu + 4.0 mg/L Ca.

655 mg M^+ Delivered (4.0 mg/L)	**Pot 2 Cu + Fe**	**Pot 3 Cu + Fe**	**Pot 4 Cu + Mg**	**Pot 5 Cu + Ca**	**Pot 6 Cu + Ca**
Total Mass of Cu Recovered (82 mg from 0.5 mg/L addn.)	51 mg	68 mg	67 mg	89 mg	75 mg
% Cu Recovered	62%	83%	81%	108%	91%
Mass Cu (mg) Detected in Sludge	8 mg	9 mg	7 mg	7 mg	5 mg
% Cu Detected in Sludge	10%	11%	8%	8%	6%
Total Mass (mg) M^+ Recovered	483 mg	632 mg	960 mg	491 mg	434 mg
% M^+ Recovered	74%	96%	147%	74%	66%
Mass M^+ Detected in Sludge	168 mg	198 mg	65 mg	29 mg	21 mg
% M^+ Detected in Sludge	26%	30%	9%	4%	3%

As expected there was limited calcium and magnesium detected in the sludge and the recoveries were 66 – 74% and 147%, respectively. An explanation for the reduced calcium recoveries could be due to the formation of calcium phosphate which is insoluble and may not have been extracted in the acid digest. The copper recovery from the calcium amended pots was between 91 – 108%, indicating that the digest was sufficient for the recovery of the copper from the sludge. The base line concentration of magnesium in the control pot effluent was approximately 31 ± 3 mg/L, with the experimental pot 36 ± 7 mg/L.

5.4 Conclusions

There was a limited increase in the mass of calcium and magnesium detected in the sludge following the multiple metals porous pot runs with an extra copper (0.5 mg/L) addition. The mass detected from a mass balance of the porous pot sludge amended with calcium and magnesium at 4.0 mg/L, was 3 – 4% and 9% of the delivered mass respectively. From separate calcium and magnesium additions of 4.0 mg/L without copper, where the mean sludge mass was compared to the control

pots and experimental pots, an increase of 10% calcium and a decrease of 29% magnesium was detected in the sludge.

When iron(III), calcium and magnesium were added to the porous pots with copper there appeared to be an inhibition of copper adsorption to the sludge, either through precipitation, binding to the solid surface of flocs or through direct biosynthesis by living organisms. In porous pots amended solely with copper between 22 – 26% of the added copper accumulated in the sludge from additions ranging from between 0.1 mg/L to 1.0 mg/L. The percentage of copper in the sludge from porous pots amended with copper plus iron(III), calcium or magnesium was between 6 and 9%.

Of the metals considered here, potentially only the addition of 1.0 mg/L copper exceeded The Australian and New Zealand guidelines for freshwater (1.4×10^{-3} mg/L copper), with the mean estimated increase in concentration calculated for the Derwent River of 2.2×10^{-3} mg/L copper. The estimated increase in the Derwent River of copper from porous pots amended with copper at 0.5 mg/L, 0.25 mg/L and 0.1 mg/L was calculated to be 9.3×10^{-4} mg/L, 5.13×10^{-4} mg/L and 2.97×10^{-4} mg/L, respectively, well below the fresh water guidelines.

In the porous pot amended with 1.0 mg/L copper there was a significant positive relationship between time and the mass of copper detected in the sludge with an $R^2 = 0.9542$ (Figure 5.2). It is unknown if the mass of copper would have continued to accumulate in the sludge or if an equilibrium would have been reached over a longer period. There was also a significant statistical relationship (P – 0.000; df – 41), between the mass of iron(III) and copper detected in the sludge from multiple metal additions of 4.0 mg/L and 0.5 mg/L, respectively. The amount of copper detected in the sludge increased proportionally to the amount of iron(III) detected in the sludge.

The guidelines for biological sludge application to agricultural land were not exceeded by any of the metals studied in this work. As iron(III), calcium and magnesium are not considered to be toxic, the guidelines for the application of sludge to agricultural land did not include those metals. With regards to land disposal of the sludge, the metals of interest from this research were copper and cobalt. The highest concentrations detected in the sludge from the addition of 1.0 mg/L copper and 0.1 mg/L cobalt were 1.9×10^{-3} mg/kg and 9.2×10^{-5} mg/kg, respectively. The guidelines for these metals are 100 mg/kg and 40 mg/kg, respectively.

Chapter 6 Residual Humic Substances Affecting Effluent Quality

6.1 Introduction

6.1.1 Pulp and Paper Mill Effluent Characteristics

In natural waters the dissolved organic matter (DOM) contains acidic polar groups including hydrophilic, phenolic and carboxylic functional groups, and hydrophobic aromatic and aliphatic structures [3, 15, 16]. Compounds derived from the degradation of lignin are characterized by oxygen containing functional groups such as carboxy-, phenoxy-, hydroxyl and carbonyl groups [16]. The DOM in untreated pulp and paper wastewater contains compounds with both hydrophobic and hydrophilic functionality which are a heterogeneous mixture with widely differing molecular weights [3, 13, 21]. This lack of distinctive structure contributes to the challenging task of characterizing individual compounds.

Untreated pulp and paper mill effluent can significantly contribute to anthropogenic dissolved humic matter in receiving waters [25]. The levels of BOD_5 can be effectively reduced through secondary treatment, however, recalcitrant organic compounds can contribute to residual COD in pulp and paper mill effluent [21]. This oxygen demanding material is from the degradation of lignin and has both hydrophobic and hydrophilic characteristics [16, 24, 26]. Norske Skog Boyer (NSB) commissioned its secondary effluent treatment plant (SETP) to reduce organic load in the effluent through the biological degradation of both the carbohydrate and residual humic substances.

The effluent from pulp and paper SETPs are generally dominated by a fraction of hydrophobic acid (HPhoA) which is resistant to biodegradation [37, 132]. The HPhoA fraction is comprised of wood extractives including resin acids, fatty acids and sterols [25, 27, 132]. Although hydrophobic in nature, they are at least partially soluble in water due to the hydrophilic functional groups attached to a mainly non-polar structure. The chief characteristic of the hydrophilic acid (HPhiA) fraction is that it is highly polar, with a significant portion comprised of polysaccharides [90].

6.1.2 Fractionation of Humic Substances

Most research on dissolved organic matter fractionation has been focused on natural waters. As most DOM in natural waters is wood and plant derived, it was appropriate to apply these methods to the organic matter in pulp and paper mill effluent. The DOM in water originating from wood

products is divided into three groups: hydrophobic extractives, lignin and polysaccharides [32]. The methods employed for the fractionation of humic substances have been adapted from Aiken *et al.* and Ciputra *et al* [21, 33].

There are a number of fractions of humic substances including acid, base and neutral fractions [20, 31, 32] with the acidic fraction generally the most significant [13]. Two non-ionic resins, Supelite DAX-8 an acrylic ester based polymer with moderate polarity ideal for isolation of fulvic and humic acids to 150 kDa, and Amberlite XAD -4 a styrene-divinylbenzene based copolymer for small hydrophobic compounds, were used sequentially to isolate acidic humic substances (HS). Hydrophobic acid (HPhoA) is the humic fraction which is retained on the DAX-8 resin and eluted with 0.1 M NaOH (Figure 6.3), and has been reported as comparable to the fulvic acid fraction [14, 132, 133]. HPhoA has also been found to contain small aliphatic and aromatic carboxylic acids and 1 and 2 ring phenols which could give rise to ^{1}H NMR resonances in 6.0 ppm – 8.0 ppm regions [132]. The Amberlite XAD-4 resin was used to retain low molecular weight (LMW) (< 20 kDa) hydrophobic compounds. The transphilic acid (TPhiA) fraction, which was first described by Croué *et al.* was not retained on the DAX-8 resin, but subsequently retained on the XAD-4 resin and desorbed with 0.1 M NaOH. The hydrophilic acid (HPhiA) fraction was not retained on either the DAX-8 or XAD-4 resins.

The hydrophobic base fraction, which is eluted from DAX-8 resin with dilute HCl and which contains aromatic amines, was not found in municipal wastewater treatment plant (WWTP) or natural waters [134]. The fractions retained on the DAX-8 and XAD-4 resins following elution with 0.1 M NaOH were hydrophobic neutrals and transphilic neutrals, respectively [35]. The hydrophilic neutral fraction containing carbohydrate compounds which are easily oxidized by treatment plant biota were found in municipal WWTPs and natural waters at low concentrations [134].

6.2 Materials and Methods

6.2.1 Reagents

The non-ionic resins, Supelco Supelite DAX-8 and Amberlite XAD-4 were supplied by Sigma-Aldrich. DAX-8 is an acrylic ester polymer with a pore volume of 0.79 mg/L, a surface area of 160 m^2/g and a mean pore size of 225 Å and XAD-4 is a styrene-divinyl benzene polymeric resin with a pore volume of 0.98 mg/L, a surface area of 725 m^2/g, and a mean pore size of 50 Å. Schatlau HPLC grade methanol and HPLC grade Merck acetonitrile were used in separate soxhlet extractions to prepare the resins. Solutions of Merck AR NaOH (0.1 M) and Ajax AR 32% HCl (0.1 M) were used to elute and

regenerate the resins respectively. LR grade HNO_3 was used for acid washing glassware. Size exclusion chromatography (SEC) standards glucose, lactose and maltose were supplied by BDH Chemicals and the dextran standards were obtained from Sigma-Aldrich.

6.2.2 Equipment

Influent and effluent samples were filtered through GA-55 Advantec glass fiber filters and MicroAnalytix 0.45 µm cellulose filter papers with Millipore filtration glassware. Soxhlet extraction of resins was undertaken using Advantec grade 84 thimble filters and Quickfit soxhlet extraction glassware. A Heildolph VV 2000 rotary evaporator was used to concentrate solutions of dissolved humic substances and samples for NMR analysis were dried in a dessicator under vacuum.

Separation of humic fractions was undertaken using two glass frit columns, 450 mm long with an internal diameter of 20 mm sequentially, with attached Quickfit 250 mL dropping funnels and Teflon stopcocks.

All glassware was cleaned by washing and rinsing in distilled water, soaking in a 10% HNO_3 acid bath for 24 h, and then rinsed 3 times in distilled water and dried overnight in an oven at 60°C.

6.2.3 Resin Preparation and Regeneration

Preparation

The DAX-8 and XAD-4 resins were prepared in a similar manner. The resin was washed thoroughly with Milli Q water before use to ensure no soluble contaminants or fine particulates were present. Resins (100 mL) were cleaned by slurrying in a 0.1 M NaOH solution with stirring for 15 min. The resin was allowed to settle and the fines and solution were decanted off and discarded. The process was repeated twice and the resin then rinsed with ultra pure water. The resin was stored in a Schott bottle (250 mL) in a NaOH solution (0.1 M) for 24 h. It was then separated by filtration using a Millipore filter, and washed with 4 x of distilled water (250 mL). The resin was then extracted in a soxhlet apparatus with methanol (300 mL) for 24 h. This procedure was then repeated with acetonitrile replacing methanol. The clean resin was stored in methanol.

The glass frit columns were packed as a slurry in distilled water and 25 mL resin to a bed height of 60 mm and rinsed with 50 bed volumes (bv) of distilled water. The resin was then washed in 5 bv of 0.1

M NaOH, followed by 5 bv of 0.1 M HCl and rinsed with 50 bv of distilled water until the residual COD was ≤ 1 mg/L in the final 50 mL distilled water.

Regeneration

Superlite DAX-8 and Amberlite XAD-4 resins were regenerated during a porous pot run after each individual separation with two cycles of 50 mL (2 bv) of 0.1 M HCl and 2 bv of 0.1 M NaOH solution and then rinsed with 50 bv of distilled water until the residual COD was 1 mg/L or less in the final 50 mL distilled water. Following a complete porous pot run the resins were cleaned by the soxhlet extraction process and stored in methanol until the next run.

6.2.4 Humic Fractionation Method

Two glass frit columns were used sequentially with the initial column packed with 25 mL of DAX-8 resin and the second packed with 25 mL of XAD-4 resin. The flow rate was set at 3.0 mL/min.

For a complete humic fraction separation of treated or untreated pulp and paper mill effluent a 300 mL sample was filtered through a 0.45 µm cellulose filter paper and acidified to pH 2 with concentrated AR HCl. An aliquot of 50 mL was taken for COD analysis and stored at 4°C.

The remaining 250 mL was passed through the DAX-8 column. The first 2 bvs (50 mL) were discarded and the remaining permeate was collected. A 50 mL aliquot of the DAX-8 permeate was separated and stored at 4°C for future analysis. The remaining 150 mL of DAX-8 permeate was passed through the XAD – 4 column, the first two bv were discarded and the remaining 100 mL XAD- 4 permeate (HPhiA) sample was stored at 4°C (see Figure 6.1).

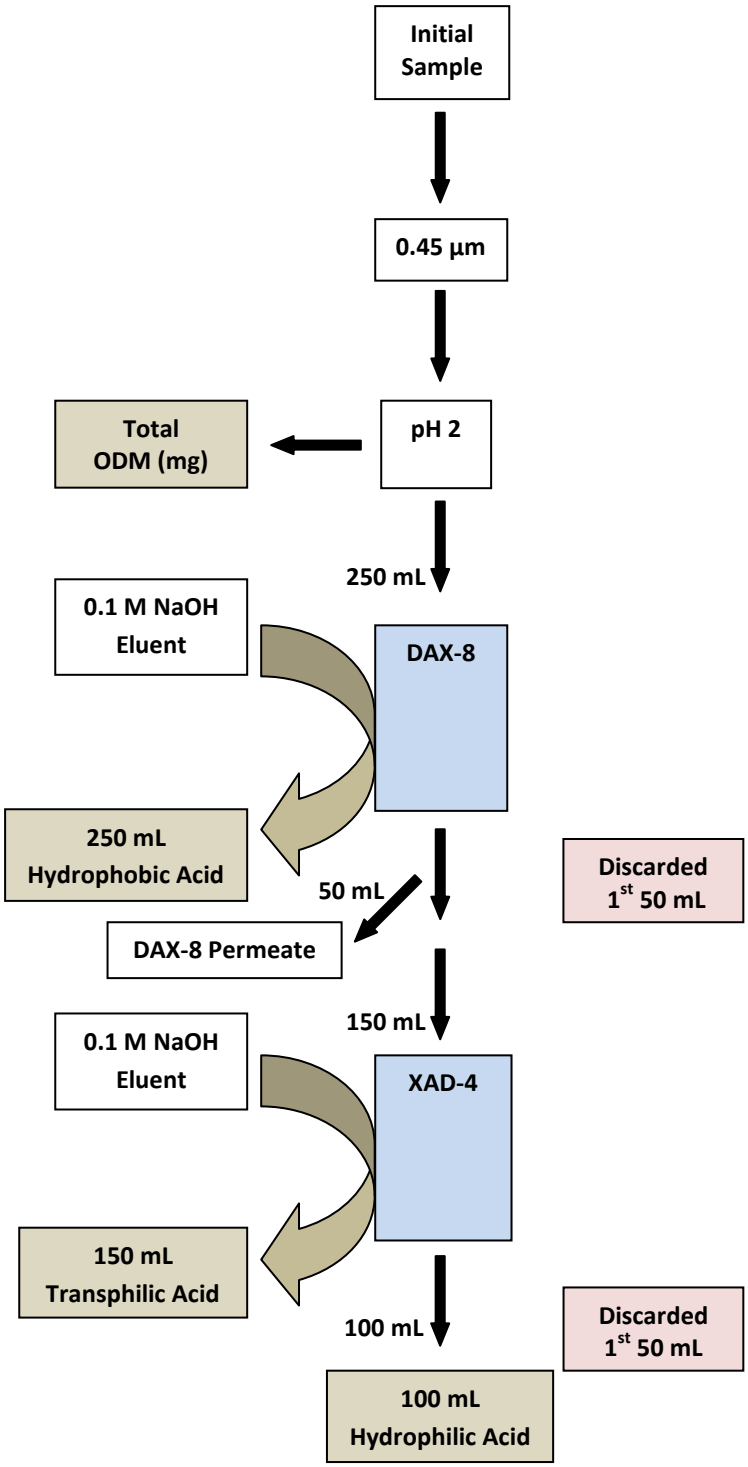


Figure 6.2: Scheme of the isolation of humic fractions in pulp and paper mill SETP effluent.

The retentate from the DAX-8 column (HPhoA) was eluted with 5 bv 0.1 M NaOH (see Figure 6.2) followed by 5 bv distilled water. The XAD-4 retentate (TPhiA) was eluted with 3 bv NaOH followed by 3 bv distilled water. The collected samples were stored at 4°C.

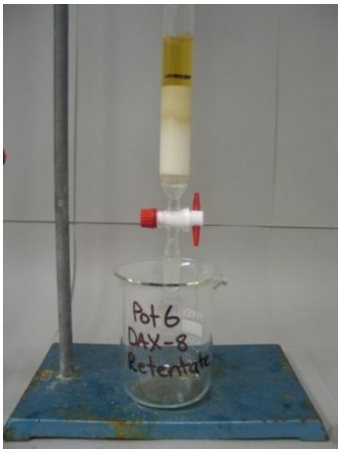


Figure 6.3: DAX – 8 resin column with HPhoA fraction eluted with 0.1 M NaOH.

Following sample elution the resins were regenerated as previously described.

6.2.5 Humic Fraction Sample Analysis

Calculation of Oxygen Demanding Matter (ODM mg)

Standard COD digestions were used to determine the oxygen demanding matter (ODM) in the humic fraction samples, with each sample analysed in duplicate. This thesis used ODM to be able to undertake a mass balance of the residual humic fractions in the porous pot influent and effluent. Samples were fractionated into different volumes'. Therefore, COD would not be a representative measure of the total ODM in each fractionated sample to then accurately determine the mass of ODM in each fraction. As the final 50 mL rinse discarded from each resin had ≤ 1 mg/L COD, the COD in the final rinse waste would not affect the calculation of ODM in the humic fraction samples.

Total Mass of ODM

The total mass of ODM in the sample to be fractionated was calculated from an aliquot of the initial sample following filtration and pH adjustment. The total initial sample volume, 250 mL, was multiplied by the COD in mg/L. (See Equation 6.1)

(6.1)

Hydrophobic Acid Mass

The mass of ODM in the 250 mL DAX-8 retentate (HPhoA) fraction was calculated similarly to the total ODM as the eluted volume is the same as the initial sample volume. (Equation 6.2)

(6.2)

Hydrophilic Acid Mass

The mass of ODM in the 100 mL HPhiA sample was calculated by multiplying the COD of the XAD-4 permeate by the volume and corrected for the fraction of the DAX-8 permeate (total volume 200 mL) that was passed through the XAD-4 column (150 mL). (See Equation 6.3)

(6.3)

Transphilic Acid Mass

The mass of ODM in the 150 mL of eluted TPhiA was calculated similarly using the total TPhiA volume of 150 mL in Equation 6.3. (See Equation 6.4)

(6.4)

Size Exclusion Chromatography

Aliquots (1 mL) of 0.45 µm filtered effluent and humic fraction samples were pipetted into 1.5 mL centrifuge tubes and stored at -17°C prior to SEC analysis. SEC chromatograms were acquired using a Waters 2695 HPLC equipped with a 10 µm Ultrahydrogel Linear 300 x 7.8 mm ID column, Empower 3 software and a Waters refractive index model 410 detector.

The column was calibrated using glucose (180 Da), lactose (360 Da) and maltose (829 Da) supplied by BDH, and a set of dextran standards supplied by Sigma-Aldrich (5 kDa, 12 kDa, 25 kDa, 50 kDa and 2000 kDa) (Figure 6.3). All standards were dissolved in Milli Q water and diluted to 100 mg/L. Water was employed as the eluent at a flow rate of 0.8 mL/min with sample injection volumes of 100 µL. The column temperature was 50°C. The equation for the calibration line was found to be $y = 10^8 + 23\ x^{-19.44}$, with an $R^2 = 0.9955$ (see Figure 6.3). The upper exclusion limit was 2000 kDa and the lower limit was 180 Da.

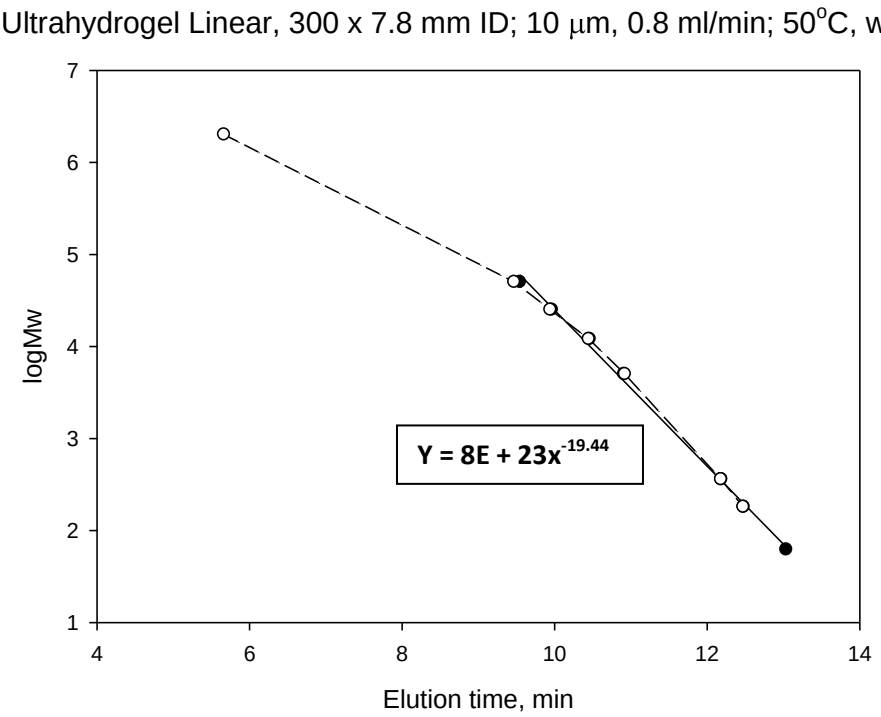


Figure 6.3: Calibration of the SEC Ultrahydrogel Linear column

NMR

The relatively low concentration and available volumes of fractionated samples limited the NMR analysis to ^{1}H NMR and not the better suited solid state C^{13} NMR analysis for the investigation of the humic fraction functional groups. Aliquots (50.00 mL) of humic samples and fractions were rotary evaporated at 80°C and transferred into 1.5 mL centrifuge tubes. The rotary evaporator glassware was rinsed with ultra pure water, which was added to into the centrifuge tube with the concentrated humic samples. The humic samples were then air dried in a dessicator under vacuum. The ^{1}H NMR spectra were acquired on a Varian Unity Inova Wide Bore 400 MHz spectrometer using a 5 mm ID-PFG probe. Samples were dissolved in 600 µL of D_2O. Proton spectra were recorded at 25°C with a presaturation at the residual water frequency. Data were recorded with 128 transients and 32K data points and a spectrum width of 4396 Hz. Spectra were processed with zero-filling to 64K data points and exponential multiplication of the free induction decay (FID) by 1 Hz.

As the humic fractions samples were of a complex nature NMR was employed solely to determine the functional groups in the humic fraction and not to identify individual compounds. C_{13} NMR was attempted on the most concentrated humic fraction samples, however, the concentration was insufficient for spectra to be meaningful. Due to the volume of sample and resin required to

concentrate the humic fractions in preparation for C_{13} NMR it was decided that it would be impractical to employ C_{13} NMR analysis of the porous pot effluent.

UV-VIS Spectroscopy

A Varian – Cary 1E Ultraviolet–Visible spectrophotometer was used to obtain UV-vis spectra from 500 – 200 nm in a 1 cm path length quartz cell. Due to relatively high concentrations of humic matter in the samples they were diluted 1:5 with MIlli Q water prior to scanning.

6.3 Results and Discussion

6.3.1 Oxygen Demanding Matter

Based on work by Imai *et al.*, who reported that the neutral and base humic fractions in the SETP process are either biologically degraded or at insignificant concentrations to contribute to the final effluent quality [134] , from this research it was assumed that the significant portion of the residual ODM was due to the acidic humic fractions . The effluent quality, as measured by COD removal, fluctuated during the porous pot runs. In order to investigate the differences between “optimal” and “sub-optimal” quality effluent samples, as judged by COD removal, where “optimal” indicates > 85% COD removal and “sub-optimal” indicates < 80% removal, and the effects on the humic fractions, samples were collected at times when there were extremes in effluent quality. The two types of effluent quality are referred to as “optimal” and “sub-optimal”, and are not intended to be representative of individual metal additions.

The examples in Figure 6.5 were selected to provide examples of how the humic fractions contribute to the ODM when the porous pots were operating optimally and sub-optimally. The relatively low recovery of ODM, which is approximately 70% of the influent sample’s value, could be attributed to the neutral and base fractions which were not expected to significantly contribute to the effluent quality [13, 35, 134]. Separation of humic acids from the porous pot effluent into the fractions HPhoA, TPhiA and HPhiA showed that the TPhiA and HPhiA fractions were the most readily degradable (Figure 6.5).

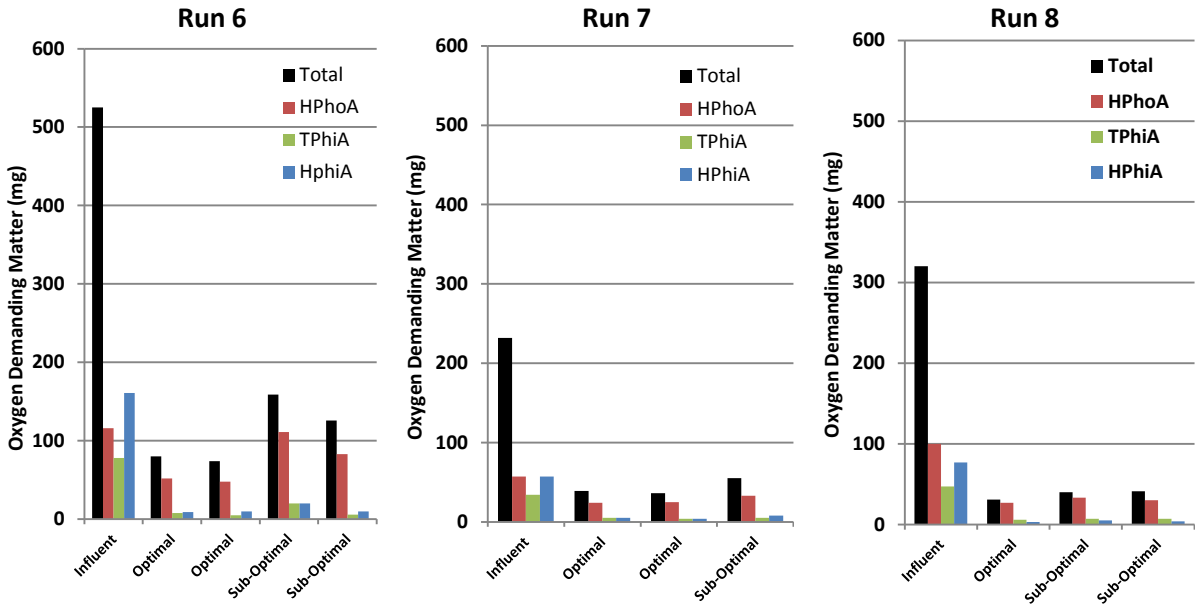


Figure 6.5: A comparison of ODM in the influent and effluent samples when porous pots were operating at optimal and sub-optimal levels during Run 6, Run 7 and Run 8.

The percentage of HPhoA, TPhiA and HPhiA in the influent of municipal WWTP has been reported as 35%, 20% and 45%, respectively [36], and the composition of the pulp and paper influent humic fractions from Run 6 to Run 8 was comparable having compositions of 20 to 30%, 15% and 25 to 30%, respectively (Table 6.1).

Table 6.1: The fractionation of oxygen demanding matter in the humic fraction samples from Runs 6, 7 and 8. "Optimal" and "Sub-Optimal" refer to the performance of a particular pot at that time.

ODM (mg)	Run 6					Run 7				Run 8			
	Influent	Optimal Pots 1 & 2		Sub-Optimal Pots 5 & 6		Influent	Optimal Pots 1 & 2		Sub-Optimal Pot 6	Influent	Optimal Pot 3	Sub-Optimal Pots 1 & 6	
Total	525	80	74	159	126	232	39	36	55	320	31	40	41
HPhoA	116	52	48	111	83	57	24	25	33	100	27	33	30
TPhiA	78	8	5	20	6	34	5	4	5	47	6	7	7
HPhiA	161	9	10	20	10	57	5	4	8	77	3	5	4
% Recovery	68	86	85	95	78	64	89	92	84	70	115	111	99

There was no difference in the relative contribution of the acidic fractions when comparing optimal and sub-optimal effluent quality samples. From Table 6.1 the mean percentage of HPhoA in either sample set was 69%, while the mean in the TPhiA and HPhiA fractions was 12%, indicating that the

composition of ODM in the effluent does not change when the porous pots were operating at optimal or sub-optimal efficiency. Antony *et al*. found three distinct fractions in pulp and paper effluent which correspond with the proportions of the acid fractions separated, where there was 71% HS, 12% each of, building blocks and LMW neutrals [135].

Another interesting observation was that there were better recoveries of ODM after treatment than before treatment. This indicates that there is a significant fraction not detected in the untreated influent samples, and that this was removed in the treatment process. These were likely to be base and neutral hydrophilic and transphilic fractions which are easily degraded and which have been reported to have a minimal contribution to treated wastewater effluent quality [35, 134].

The HPhoA fraction was most resistant to degradation, suggesting that the majority of the ODM in the treated effluent was due to HPhoA, supporting previous research in this area [132, 136]. However, Imai *et al.* found that the hydrophilic fraction in treated municipal wastewater effluents was more important than the hydrophobic fraction [134]. The residual mass of HPhoA in the later runs (Runs 7 and 8) was between 24 mg and 33 mg, and under the current operating conditions this could be the lowest residual HPhoA mass achievable.

Carvalho *et al.* found the HPhoA fraction was the major constituent of a Kraft pulp mill effluent, representing 73% of DOM [136]. Though DOM is not necessarily ODM, it would appear to represent the considerable majority of the overall ODM in paper mill effluents. The residual HPhoA fraction in the porous pot effluents was between 60 – 87% of the total residual ODM, with a mean of 69% (Table 6.1), very close to the 73% reported by Carvalho *et al.* Low molecular weight (LMW) HPhiA has been found to dominate municipal wastewater effluent [137]. However, municipal wastewater does not contain the high degree of high molecular weight (HMW) compounds, derived from the hydrolysis of lignin, that are present in paper mill effluent. The HPhiA fraction in the influent was greater than or equal to the HPhoA fraction (Figure 6.6), but the residual HPhiA in the porous pot effluent was dramatically reduced.

Before the Boyer SETP was commissioned, Viney found the residual concentrations of the HPhoA and HPhiA fractions remaining in the effluent were 46% and 56%, respectively [20]. The residual fraction was the percentage of each individual initial humic fraction detected in the effluent. In general, the residual HPhoA fractions in the optimal samples were comparable to Viney's results (see Table 6.2). However, there was a dramatic decrease in the residual HPhiA fraction. When the porous pots were running at an optimal level the residual HPhiA fraction in the effluent was reduced to 4 – 9% of the initial HPhiA from the influent samples (Table 6.2).

Table 6.2: Percent residual ODM of initial influent humic fractions detected in the porous pot effluent samples.

% Residual ODM	Optimal					Sub-Optimal				
	Run 6*		Run 7**		Run 8	Run 6		Run 7	Run 8***	
HPhoA	45	41	42	44	27	96	72	58	33	30
TPhiA	10	6	15	12	12	26	8	15	15	15
HPhiA	6	6	9	7	4	15	6	14	7	7

Influent ODM: *525 mg ** 232 mg ***320 mg

The TPhiA fraction contains proteins and amino acids which are highly biodegradable, and subsequently, this fraction was not expected to contribute significantly to the residual ODM in the porous pot effluent [35]. Viney found that TPhiA accounted for 11 – 17% of the total DOM in the Boyer effluent before the commissioning of the SETP [134]. During the current study the residual TPhiA fraction was found to be comparable at 10 – 20%, suggesting that the SETP has had the greatest effect on the reduction of the HPhiA fraction.

6.3.2 Molecular Weight Distribution and NMR Spectroscopic Studies of the Influent Humic Substances

The untreated porous pot influents for Runs 6, 7 and 8 were separated into the three acidic fractions for size exclusion chromatographic (SEC) analysis to shed further light on the removal efficiencies of the various components. Although widely employed as a detector for SEC, ultraviolet absorbance is limited to qualitative analysis of molecular weight fractions and is not suitable for a quantitative analysis [138]. The functionality of dissolved organic matter causes inaccuracies in UV detection in SEC due to unequal molar absorptivities, as the molecular weight increases the molar absorptivity also increases which makes HMW compounds appear more abundant [138]. The samples prepared for SEC were not intended for quantitative analysis, as the peak height on the SEC chromatograms is not an accurate representation of abundance. This research incorporated SEC to indicate the presence or absence of particular molecular weight fractions in comparative influent and effluent samples.

There are distinct differences in the SEC chromatograms between the influents of Run 6 and Runs 7 and 8. In Run 6 there are two distinct peaks corresponding to approximately 45 kDa and ≤ 180 Da, respectively, while in Runs 7 and 8 there was a significant single peak indicating compounds with apparent molecular weight between 800 – 3000 Da (Figure 6.6). Though there was also a difference

in the COD in the bulk sample collected for Run 6, which was approximately double (at 2210 mg/L) that of Runs 7 and 8, it was not clear what operational events caused the differences. A higher paper grade may have contributed to the increase in COD in the bulk sample, but this would not have precluded compounds with a similar molecular weight distribution to Runs 7 and 8.

It should be noted that samples for NMR analysis for Run 6 and Runs 7 and 8 were prepared differently and the intensity of the peaks cannot be directly compared. The NMR samples from Runs 7 and 8 were prepared by concentrating 50.00 mL aliquots of each fraction and the Run 6 samples were concentrated from aliquots of between 50 to 150 mL. ^{1}H NMR of complex samples can be employed to indicate the type of functional groups associated with protons and, therefore, broad classes of compounds. The NMR spectrum of humic fractions would be expected to show two distinct types of peaks, a sharp peak indicating smaller molecules and a series of broad peaks indicating complex mixtures or larger compounds with more efficient relaxation [14]. In natural DOM from rivers and lakes the peaks are generally broad and ill-defined indicating complex mixtures with overlapping resonances. In the porous pot influent samples some defined peaks were evident, indicative of particular functional groups and structures [14]. The structure of DOM in natural water samples differs from that in pulp and paper effluent, although there are some similarities. In natural water samples there are generally peaks associated with aliphatic protons in the region of 0.9 ppm to 1.8 ppm up-field, and compounds with methoxyl structures in the region of 3.1 ppm to 3.5 ppm [139, 140]. The initial ^{1}H NMR spectra from the un-fractionated influent samples (Figure 6.6), showed no evidence of significant amounts of simple aromatic and aliphatic compounds in the expected chemical shift region between 6 ppm – 8 ppm and 0.9 ppm – 1.5 ppm, respectively.

An initial influent sample for Run 6 was not obtained, which was unfortunate given the significant difference in the SEC chromatogram compared to Runs 7 and 8. The main difference observed in the influent ^{1}H NMR samples of Runs 7 and 8 was found in the region of 0.0 ppm to 3.3 ppm (Figure 6.6). Generally peaks between 0.9 ppm and 1.5 ppm in ^{1}H NMR spectra are assigned to protons in aliphatic side chains [141], and mirror resonances from protons associated with aliphatic compounds were detected upfield at < 1.6 ppm. In Run 8 a greater diversity and intensity of peaks was observed in the aliphatic region compared to Run 7.

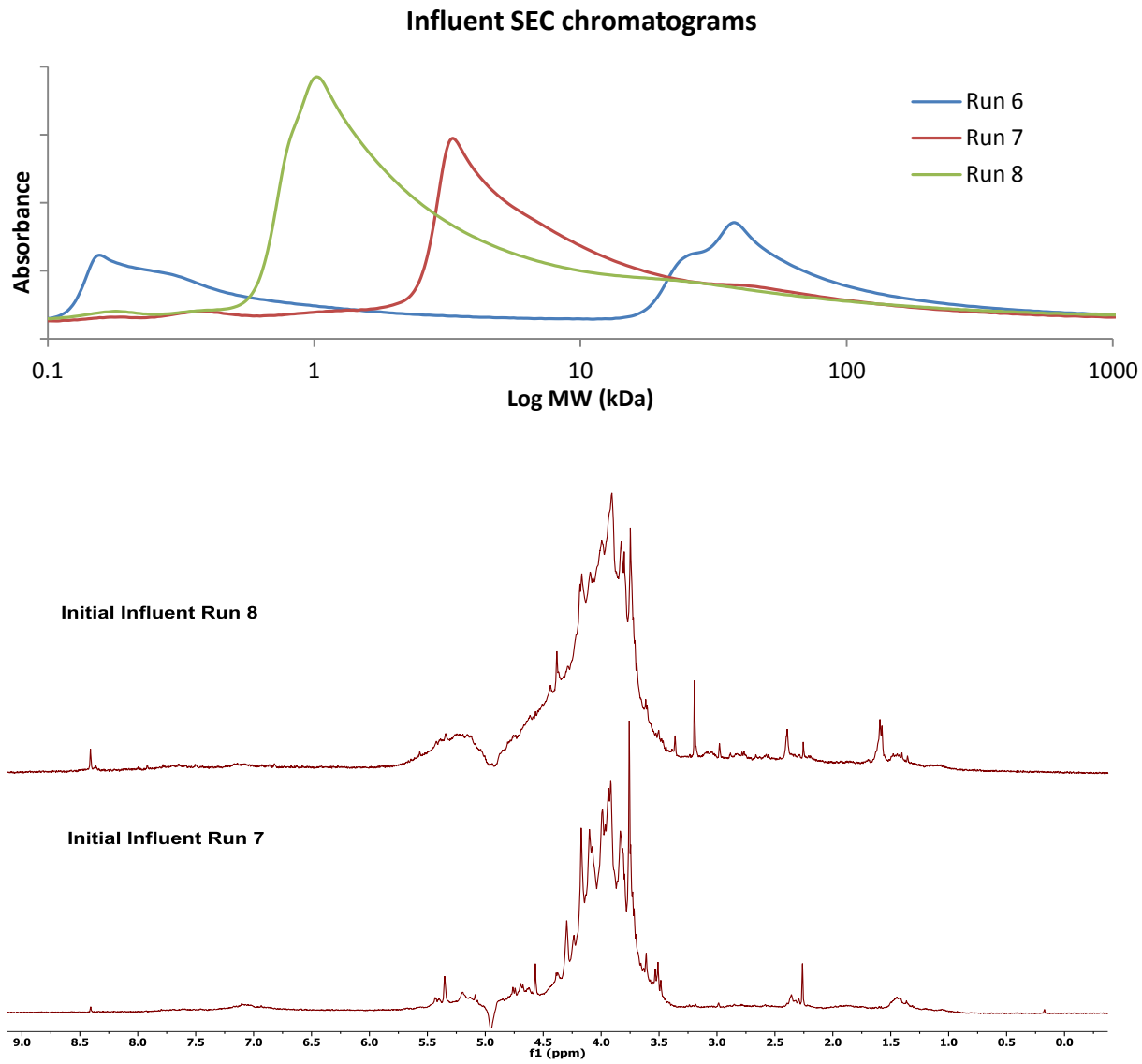


Figure 6.6: Size exclusion chromatograms of un-fractionated influent samples from Run 6, Run 7 and Run 8 and NMR spectra of un-fractionated influent samples from Run 7 and Run 8.

*An NMR influent sample was not prepared from Run 6.

There were two broad envelopes in the NMR spectrum of Run 7 at approximately 1.2 ppm and 2.2 ppm indicating complex structures. Peaks at approximately 1.2 ppm are normally assigned to resonance from methylene protons [141]. Run 8 exhibited sharp single peaks in the chemical shift region between 1.5 ppm and 3.1 ppm, including a peak at 2.1 ppm, indicating the presence of the acetyl functionality [142, 143]. The degradation products from pulping *Pinus radiata* have been found to be carbohydrates with CH – OH moieties and a chemical shift of 3.5 ppm, and phenolic and aromatic ether compounds which give rise to peaks between 6.5 – 8.0 ppm [22]. However, in the

lignin-derived compounds containing carboxylic and hydroxyl groups, the protons exchange with D_2O, which do not resonate in a ^1H NMR spectrum.

The chemical shift region 6.5 – 8.6 ppm is often indicative of protons directly attached to aromatic rings [139]. In both Run 7 and Run 8 spectra broad envelopes of low intensity were detected at approximately 7.0 ppm and 7.5 ppm. In other work significant broad envelopes have been detected in raw pulp and paper effluent samples between 6.5 – 7.2 ppm [144]. The small amount of high molecular weight compounds detected in the influent SEC chromatograms are likely to be complex mixtures of lignin derived compounds, including aggregates of resin acids and fatty acids which comprise tricyclic and long chain aliphatic structures respectively.

The major NMR resonances in Runs 7 and 8 are the envelope between 3.3 ppm and 4.3 ppm indicating an abundance of protons attached to the carbons of hydroxyl, ester and ether functional groups and protons on methyl, methylene and methine carbons directly bonded to oxygen and nitrogen [14, 133]. The methoxyl functional group chemical shift of 3.9 ppm has been found to dominate spectra from pulp and paper effluent samples [25], supporting these assignments.

The overall structures of the major envelopes detected in Runs 7 and 8 spectra were similar, though there was a significant single sharp peak detected in Run 7 at 3.6 ppm. Carbohydrate isomers of glucose and mannose have been detected in pulp and paper wastewater at a resonance of 3.6 ppm [142, 143]. This possibility is supported by a small peak at approximately 180 Da in the SEC chromatogram from the Run 8 influent sample, 180 Da was at the lower detection limit and corresponds to the MW of glucose and mannose. The chemical shift region of 5.1 ppm to 5.3 ppm in pulp and paper wastewater has also been attributed to carbohydrates [142, 143]. Following treatment minimal residual resonance was observed in the region 4.0 ppm to 5.5 ppm, indicating that the compounds were either easily biodegradable or had a high affinity to adsorb to activated sludge.

Formate has been identified in pulp and paper mill effluent samples and from humic fraction samples separated from natural waters, resonating at 8.4 ppm [139, 140, 144]. A singlet peak was detected in most samples at approximately 8.4 ppm, and has been assigned to formate.

6.3.3 Molecular Weight Distribution and NMR Spectroscopic Studies of the Un-fractionated Effluent Humic Substances

The two peaks detected in the SECs of Run 6 un-fractionated effluent samples (Figure 6.7) were also present for the un-fractionated influent sample (Figure 6.6). The molecular weight distribution in Run 6 un-fractionated effluent samples appears to be similar to the influent although there was a significant decrease in ODM. The sub-optimal effluent sample from Run 6 in Figure 6.7 contained 525 mg ODM compared to 159 mg ODM in the optimal sample. With such a significant difference in the mass of ODM there was an expectation that there would be differences in the intensity of the absorbance peaks in the SEC chromatogram. There was also an expectation that peaks in the effluent samples (Figure 6.7) would show peaks in lower molecular weight regions as the ODM in the optimal sample was degraded.

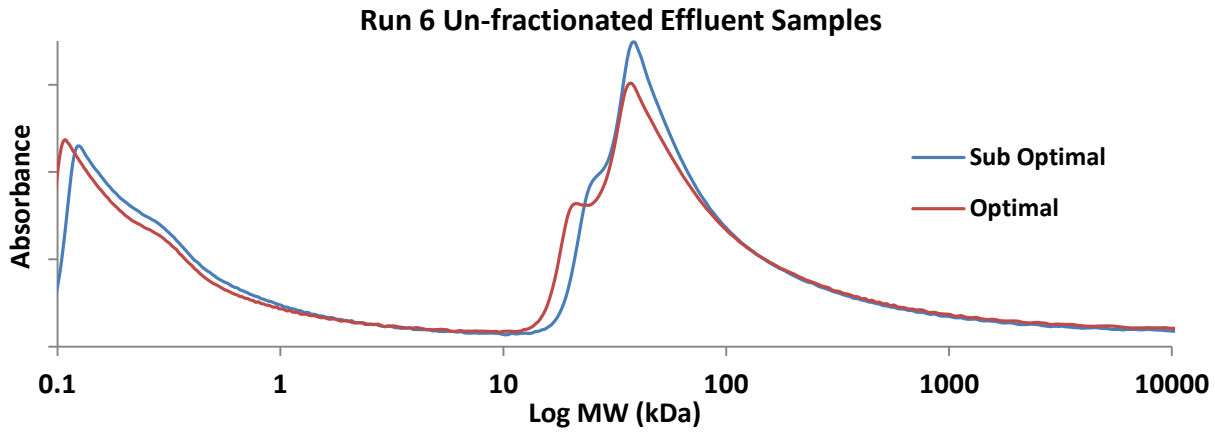


Figure 6.7: SEC comparison of optimal and sub-optimal initial effluent samples from Run 6.

However, Figure 6.7 shows that the molecular weight distribution in un-fractionated Run 6 effluent samples were similar in intensity and shape. The two peaks correspond to molecular weights of approximately 45 kDa and 180 Da, respectively. Some of the difference in the residual ODM in the sub-optimal sample could be attributed to these residual HMW compounds. Although the samples were not quantitative, there was an obvious difference in the peak heights. In the optimal sample the peak corresponding to a molecular weight of 45 kDa was smaller than in the sub-optimal sample, while the peak corresponding to 180 Da was larger for the optimal sample than the sub-optimal sample. This suggests that there were less HMW and more LMW compounds in the optimal sample compared to the sub-optimal, indicating greater degradation of the HMW compounds.

In the Run 7 chromatogram there was a low intensity peak that corresponded to a molecular weight of approximately 180 Da (Figure 6.8). This was in the region of one of the major peaks in the Run 6 un-fractionated effluent samples. In both Run 7 un-fractionated effluent samples the major peaks in the SEC chromatograms corresponded to molecular weights of approximately 800 Da and 2 kDa in the optimal and sub-optimal samples, respectively (Figure 6.8). The shoulder of the two peaks in both samples started at approximately 30 kDa, at this point there was a small peak in the sub-optimal sample. There was an increase in the intensity of the absorbance in the optimal sample compared to the sub-optimal sample, indicating greater degradation of HMW compounds. The average MW of the optimal sample was less than that of the sub-optimal sample indicating more degradation. However, there was an additional small peak in the sub-optimal sample at 180 Da indicating that the differences in the residual COD could be partially due to the presence of simple LMW compounds, which in optimal conditions should be easily biodegraded.

Although there were major peaks in the SEC chromatograms indicating different MW distribution in the optimal and sub-optimal samples, the NMR spectra of the two samples was in the same chemical shift region. This is indicative of compounds that have the same NMR functionality but differ in MW. Previous studies of size fractionation through filtration in pulp and paper mill effluents have found that the residual wood extractives were evenly distributed between 100 kDa, 50 kDa, 30 kDa and 3 kDa fractions [27]. The compounds identified were fatty acids, resin acids and sterols, while residual lignin was also found to be evenly distributed between the MW fractions in the effluent [27]. This could be an indication that the abundance of wood extractives in the treated effluent samples was low. Viney's research on effluent samples prior to the commissioning of the Boyer SETP found the major constituents of the humic effluent samples were compounds < 1 kDa [20], supporting the size fraction found in Run 7.

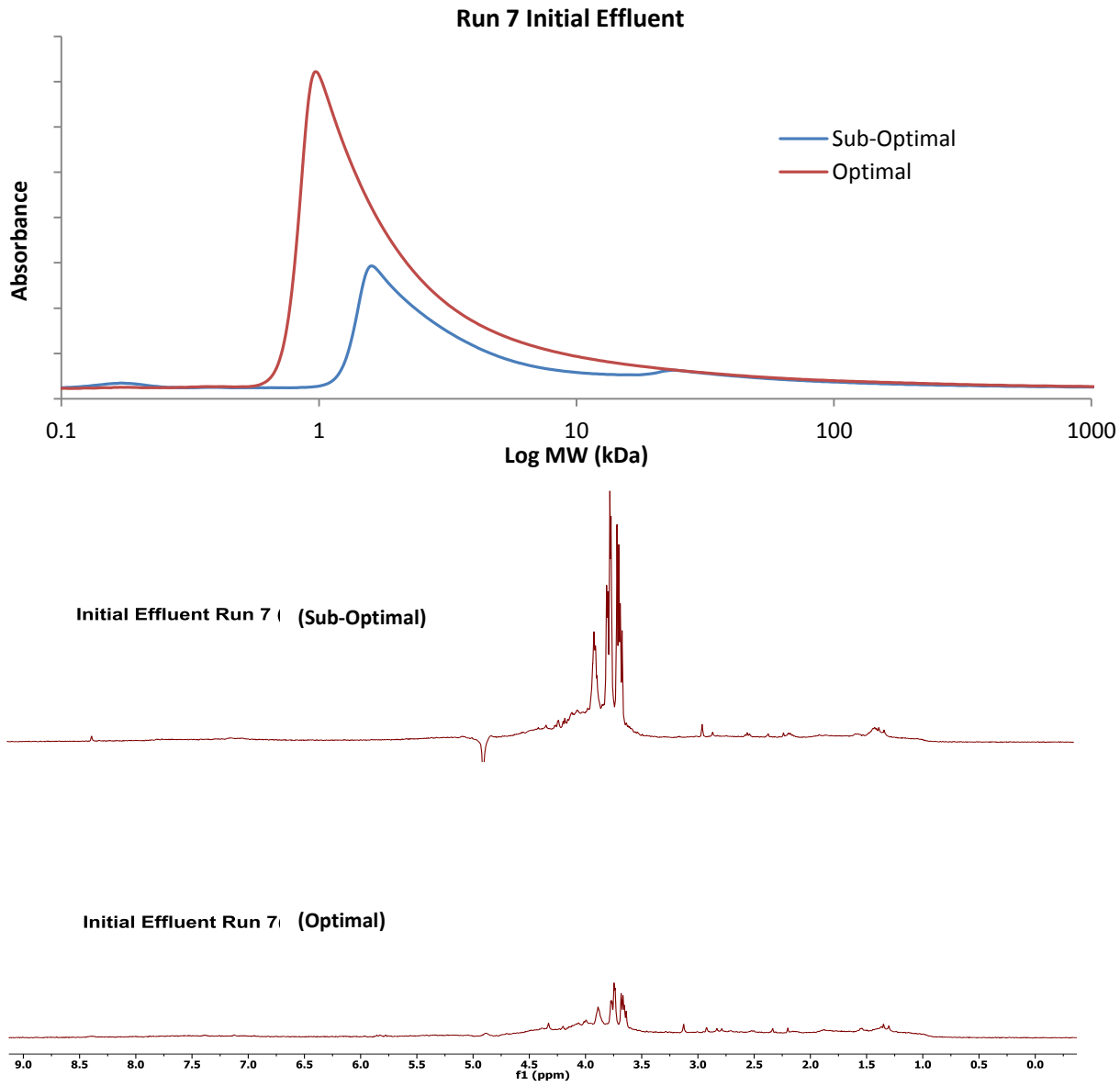


Figure 6.8: SEC and NMR comparison of initial effluent samples from Run 7, comparing optimal and sub-optimal effluent quality.

The NMR spectra of the Run 7 un-fractionated optimal and sub-optimal effluent samples showed there was very little difference in the profile of the resonances with the majority of compounds giving rise to resonances in the region of 3.5 ppm to 4.3 ppm (Figure 6.8). However, the signal intensity in the optimal effluent sample was considerably lower than the sub-optimal sample. This could be an indication that there are similar compounds in each sample though the concentration changes between the optimal and sub-optimal samples. In these initial effluent samples the main envelope is much narrower in comparison to the resonances detected in the influent samples (see Figure 6.6), indicating there has been degradation of the more oxygenated compounds in the region

3.4 ppm to 4.3 ppm. The significant envelope in the porous pot effluent between 3.4 – 4.2 ppm was due to protons attached to electron withdrawing substituents, attached to either alkyl or aryl structures [141]. The envelope in Figure 6.8 had a narrower base with several sharp single peaks that can indicate the presence of LMW compounds. Only very small peaks were detected in the aromatic (6.0 ppm – 8.0 ppm) and aliphatic (0.9 ppm – 1.5 ppm) chemical shift regions.

Spectra of both the optimal and sub-optimal samples had only very small peaks between 1.0 ppm and 3.5 ppm. The peaks at 1.3 ppm were attributed to simple LWM methylene compounds [145] and oxygen attached to methyl groups [141]. These compounds would be expected to be easily biodegradable in the SETP. The un-fractionated effluent NMR spectra in Run 8 were similar to those obtained from Run 7, so similar in fact that this discussion applies directly to them as well.

6.3.4 Molecular Weight Distribution and NMR Spectroscopic Studies of the Hydrophobic Acid Fraction

The three influent chromatograms (Figure 6.9) showed clear differences in the molecular weight distributions of the hydrophobic acid fraction samples of Runs 6, 7 and 8. Unlike the distinct double peaks found in the Run 6 SEC influent and un-fractionated effluent samples, only a single peak was detected in the Run 6 HPhoA fraction. In the un-fractionated Run 6 effluent there were two size fractions, 45 kDa and 180 Da, while the single peak in the HPhoA fraction indicated compounds with a MW distribution of 400 – 4000 Da. The molecular weights observed in the HPhoA fraction were not present in the initial un-fractionated sample, and it has not been possible to establish a reasonable explanation for this.

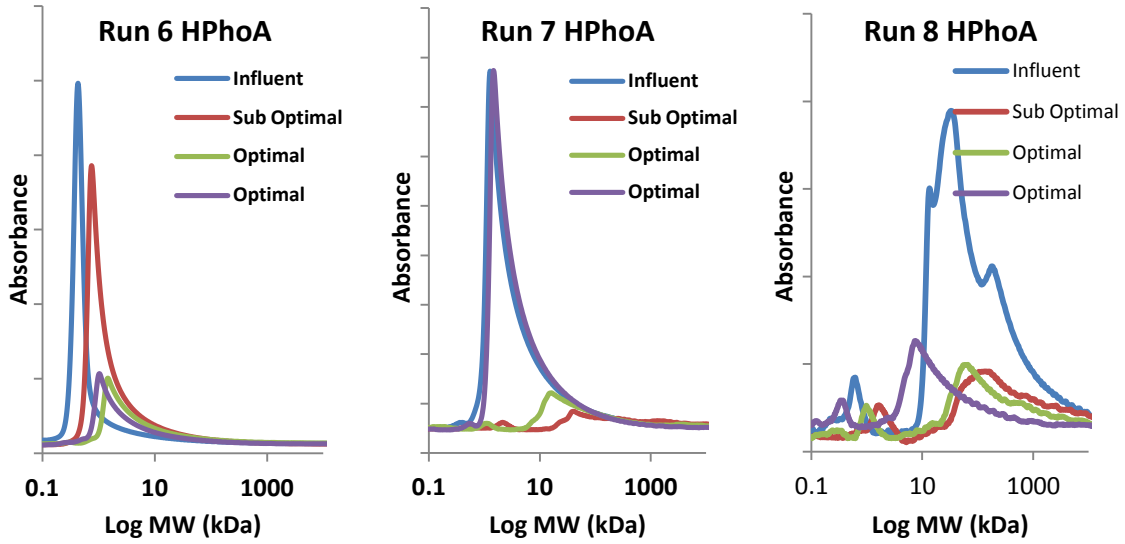


Figure 6.9: SEC chromatograms of the HPhoA fractions of Run 6, Run 7 and Run 8.

Many more peaks were present in the influent HPhoA fraction from Run 8 compared to the other runs, indicating a much more complex size distribution. There is no clear explanation for this as, even though the mean COD of the Run 8 bulk sample was 1048 ± 125 mg/L, compared to 1359 – 1687 mg/L for Runs 1 to 5, the mill was running normally at the time. Run 7, on the other hand, was collected during a PM3 shut down and surge basin cleaning but also had a mean COD 1030 mg/L and yet has a very different MW distribution to Run 8.

The HPhoA fraction has been found to be the most recalcitrant in pulp and paper wastewater, with the majority of compounds derived through the degradation of lignin [37, 132]. The molecular weight range in HPhoA in natural waters and municipal WWTP has been found to be 1 kDa and 50 kDa to < 1 kDa, respectively [37]. The most common size fraction detected in all the HPhoA samples was between 400 – 4000 Da. This corresponded closely to the size of the HS fraction separated from TMP effluent by Antony *et al.* (500 – 1000 Da), which made up approximately 70% of the DOM [135]. It would be expected that resin acids, if present, would be included in this size range that is greater than 200 Da.

Based on previous research, the residual humic matter from the HPhoA effluent samples could also contain recalcitrant lignin based structures [144]. This would support other research results showing pulp mill effluent to have residual lignin derived structures in the effluent [18, 135]. To confirm this hypothesis, UV-Vis absorbance spectra of the HPhoA effluent samples were run. The focus of the UV-Vis absorbance spectra was on the aromatic structures with overlapping π-π* transitions attributable to phenolic structures, expected to be present in degraded lignin [20, 146-148]. There

are two wavelength regions which have been shown to indicate the presence of DOM, firstly the band between $UV_{254-260}$ [148, 149] and secondly $UV_{272-280}$ [20, 147, 148]. As compounds such as carbohydrates, which have low UV absorbance, are easily biodegraded it was expected that their contribution to the UV-vis absorbance would be minimal [134].

In the Run 8 HPhoA samples two UV-Vis absorbance peaks were observed at approximately 245 nm and 290 nm, the region where aromatic structures were anticipated (Figure 6.10). Although UV analysis was not intended to determine the concentration of ODM, the Run 8 samples were prepared for UV-Vis analysis by the same procedure. A 250 mL aliquot of filtered effluent at pH 2 was passed through the DAX-8 resin and the HPhoA fraction was eluted from the resin with 125 mL of 0.1 M NaOH and 125 mL MilliQ water. This would allow at least a semi-quantitative interpretation of the absorbances of these samples. In Figure 6.10 the UV-Vis absorbance of the sub-optimal effluent HPhoA samples was substantially greater than for the optimal samples indicating that HPhoA fraction is considerably more concentrated in the effluent of pot that is performing sub-optimally than in one performing optimally. This also indicates that it UV-Vis could provide a simple measure of effluent quality for the SETP.

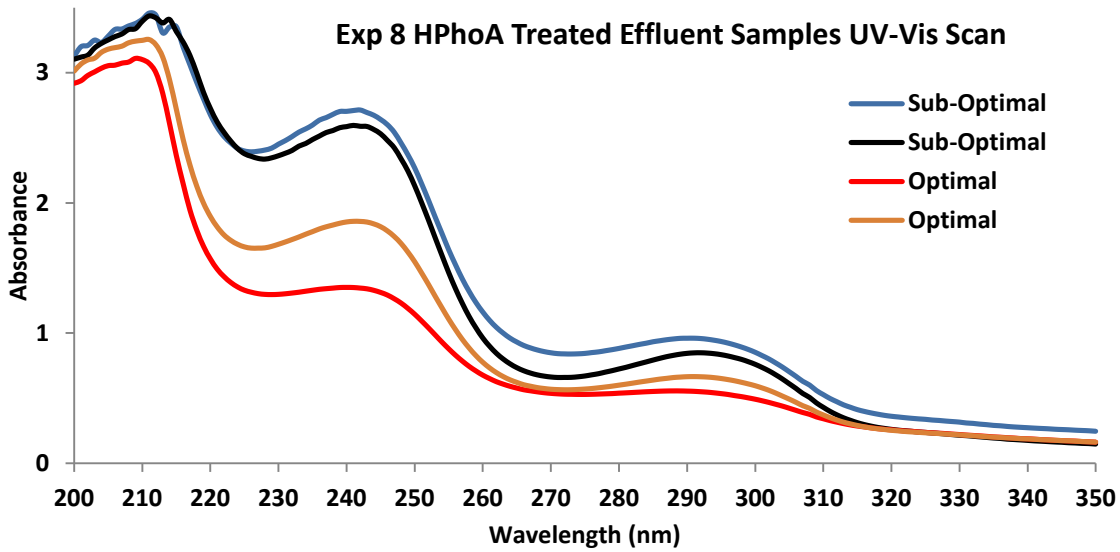


Figure 6.10: UV-Vis absorbance of Run 8 HPhoA fractions, comparing optimal and sub-optimal quality effluent samples.

Unlike the NMR peak intensity in Figure 6.8, where the intensity of the peaks was compared between optimal and sub-optimal effluent samples in Run 7, Figure 6.11 shows HPhoA influent and

effluent NMR spectra for individual pots from Runs 6, 7 and 8. As the axis was adjusted to best show the peaks due to the samples between runs being prepared differently, the peak intensity could not be compared in this case. The peak at 1.8 ppm, which was not present in the un-fractionated influent or the un-fractionated treated effluent samples, could indicate the presence of acetate (CH_3COO^-). Acetic acid, a degradation product of lignin, has been detected previously in treated pulp and paper effluent [144], it could also fit with the LMW compounds detected in the SEC chromatograms at < 180 Da. Lignin derivates in pulp and paper effluent have been reported to have intense sharp peaks in NMR spectra [25], similar to the peak at 1.8 ppm. However, acetic acid should be easily biodegraded in secondary treatment and have only a minimal input to the ODM in the effluent.

It has also been suggested that the aliphatic portion of the HPhoA fraction could be long chain fatty acids which could be assigned to this resonance, although, the peak should be much broader [139]. There was also the potential for contamination, which cannot be ruled out. Acetonitrile, used to clean the resins, has an ^1H NMR resonance at approximately 2.0 ppm, and, although the resins were thoroughly rinsed and the COD of the final rinse water analysed before separations took place, it may still leach off the fractionation columns at trace levels. However, as the COD of the final rinse water ≤ 1 mg/L, it is unlikely that the peaks could be so intense compared to the humic substances in the sample. If the peak was due to contamination it would be expected that it would be larger peak in the first sample through the column and the peak intensity would then decrease in subsequent samples. This was not the case.

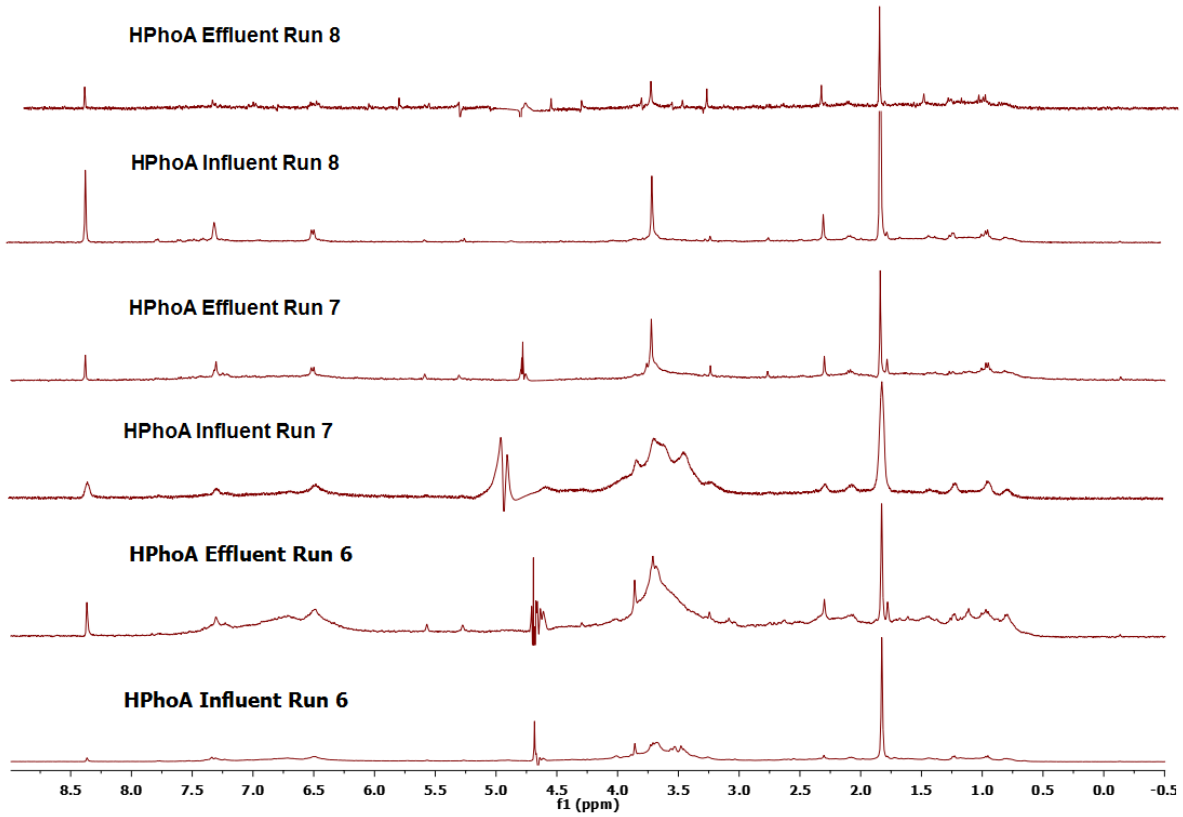


Figure 6.11: ^{1}H NMR spectra of influent and effluent HPhoA fractions from Run 6, Run 7 and Run 8.

A resonance was detected between 3.3 ppm and 3.9 ppm in most HPhoA samples, either as single peaks or broad envelopes (Figure 6.11). This was significantly decreased in the effluent samples of Runs 7 and 8 compared to the respective influent samples, indicating significant degradation of the compounds responsible. In the Run 7 HPhoA fractions there was also a significant decrease in the resonance detected between 3 ppm and 4 ppm in the effluent sample compared to the influent. This region indicates the presence of oxygenated protons that are relatively easily biodegraded. This degradation was clearly reflected in the decrease in size of the peaks in this region.

Formate ($HCOO^{-}$), formed by the hydrolysis of lignin has previously been identified in pulp and paper mill effluent and resonates at 8.4 ppm [139, 140, 144]. However, this does not explain why formate would persist in the fractionated samples as it would be rapidly oxidized to CO_2.

In pulp and paper effluent samples Santos *et al*. found broad bands in the ^{1}H NMR that represent unsubstituted aliphatic carbons in the HPhoA fraction [25]. The Run 6 NMR spectra had broad peaks throughout the chemical shift regions, indicative of complex HMW compounds. However, the SEC chromatograms did not show a significant difference in the size distribution of compounds detected

in the Run 6 samples. The ODM in the Run 6 samples was significantly higher than Run 7 and Run 8, the increase in concentration may have affected the resonance in the NMR spectra.

Resin acids have been identified in pulp and paper effluent including Boyer effluent prior to the SETP commissioning [20, 27] and have been reported to contribute to effluent quality [150]. They also have molecular weights around 300 Da [20, 99], within the most common size fraction seen in the HPhoA samples. However, the removal of resin acids from pulp and paper wastewater through secondary treatment has been reported to be ≥ 95% [28-30], with a residual concentration of approximately 200 and 400 µg/L [29]. Even though resin acids have a tricyclic structure and may contain methyl functional groups [30, 150], that would be seen in the NMR spectra in the chemical shift regions 6.0 to 8.0 ppm and 1.0 to 1.5 ppm, respectively, it would be unlikely to observe these in the NMR spectra at the low concentrations expected here. As can be seen from Figure 6.11, there are indeed only very small resonance peaks in these regions in the effluent HPhoA samples. This almost certainly indicates that resin acids were present at only very low levels in this fraction, as expected from the preceding discussion. However, the peaks downfield in the aromatic chemical shift region 6.0 ppm to 8.0 ppm and upfield at 1.2 ppm to 2.4 ppm may also be due to small aliphatic and aromatic carboxylic acids, and 1 - 2 ring phenols [132].

Though the humic fractions samples were not prepared with the comparison of metals and humic substances absorption in mind, NMR analysis of the Run 7 HPhoA fraction samples indicated that in the presence of copper there was extremely low resonance in the aromatic region of 6.0 – 7.5 ppm. There was also a significant reduction in the 3.1 – 4.1 ppm chemical shift region, where carbohydrates would resonate, compared to the influent sample (see Figure 6.11). Research has shown that copper complexes faster with weak ligands found in the HPhiA and TPhiA fractions (eg low affinity carboxylic functional groups) than those found in the HPhoA fraction [14, 35]. However, other research has indicated that the rate of copper complexation with the HPhoA fraction is determined by the concentration of HPhoA in the wastewater [35]. Due to the retention time in the porous pots and the concentration of humic substances there would be sufficient time and binding sites for copper to complex with the ligands in the HPhoA fraction.

Further research will have to be undertaken to identify the rates of adsorption of metals to humic fractions and their effect on the humic fraction concentration in the effluent.

6.3.5 Molecular Weight Distribution and NMR Spectroscopic Studies of the Hydrophilic Acid Fraction

The residual humic fractions are comprised of degraded lignin and lignocellulose degradation products, with the hydrophilic acid fraction thought to be more degraded than the HPhoA fraction [38]. HMW compounds in the HPhiA fraction have been attributed to biopolymers produced by bacteria [151, 152]. However, these HMW compounds were detected in the influent before bacteria could carry out this conversion. Typically the HPhiA fraction from pulp and paper mill effluent using spruce as the feed stock contains compounds with MW between 3 – 200 kDa [32], and municipal WWTP < 1 kDa [134].

There were three distinct size fractions detected by SEC analysis in all effluent samples: 3 – 6 kDa, 400 – 700 Da and ≤ 180 Da, considerably lower than the effluent from spruce [32]. The dominant fraction was comprised of compounds with MW between 400 – 700 Da, which more closely corresponds to the fraction assigned as building blocks by Antony *et al.* (300 – 500 Da) [135]. No residual HMW compounds were detected in the effluent HPhiA fractions.

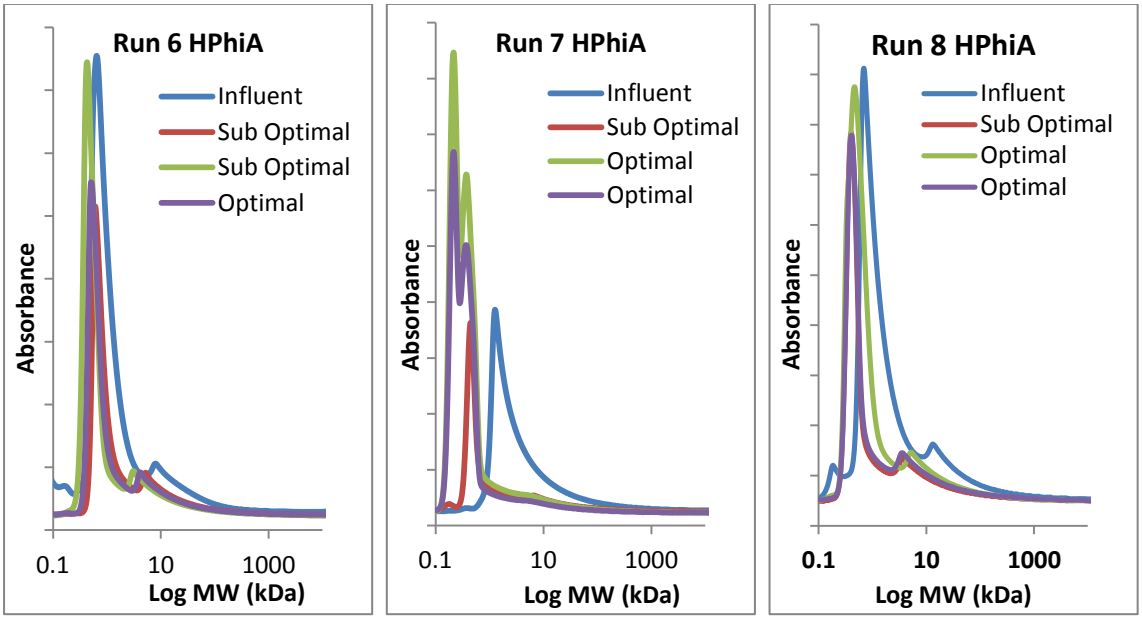


Figure 6.12: SEC chromatograms of the HPhiA fractions from Run 6, Run 7 and Run 8.

It is interesting that the HPhiA from Run 6 (an atypical run) is so similar to the more typical Runs 7 and 8, indicating the differences are in the HPhoA fraction, not the HPhiA fraction. The NMR spectra of the all influent HPhiA samples were very similar (Figure 6.13), and closely resemble the ^{1}H NMR spectra of water-soluble wood polymers extracted from *Pinus radiata* [143]. The main resonance of

the influent samples is within the envelope 3.3 ppm – 5.3 ppm with superimposed single sharp peaks. The hydrophilic compounds detected in untreated pulp and paper wastewater have been identified as mainly glucose, mannose and galactose, and their isomers, that give rise to resonances between 3.2 ppm – 5.5 ppm [90, 91, 142, 143].

There were compounds detected in the SEC chromatograms of influent samples ≤ 180 Da which could also support the presence of simple carbohydrates. The NMR spectra of the effluent samples had sharper peaks, that also indicates the presence of simpler LMW compounds.

Only very small aliphatic peaks were seen in the NMRs of influent HPhiA fraction samples. In the effluent HPhiA fraction samples aliphatic resonances were present, indicating the presence of degradation products from the influent samples with simpler structure [14]. Aliphatic peaks in HPhiA fractions isolated from constructed wetlands have been found to be LMW aliphatic and polyfunctional carboxylic acids [132].

In the NMR of effluent HPhiA fractions there was a marked reduction in the intensity of both shoulders of the major envelope, in the regions of 3.3 – 3.5 ppm and 3.8 – 4.5ppm. This lower resonance implies the reduction in ODM in the HPhiA effluent samples by oxidation of the more highly oxygenated components of this fraction. These carbohydrates would have been easily biodegraded in the SETP, but identifying the residual compounds would aid in understanding how to optimise the operating conditions in the treatment plant. Although the residual compounds in the HPhiA did not significantly contribute to the ODM in the effluent compared to the HPhoA fraction, under optimal conditions, there was approximately 4 – 9% of the influent HPhiA in the effluent. This would make further research into the HPhiA fraction beneficial.

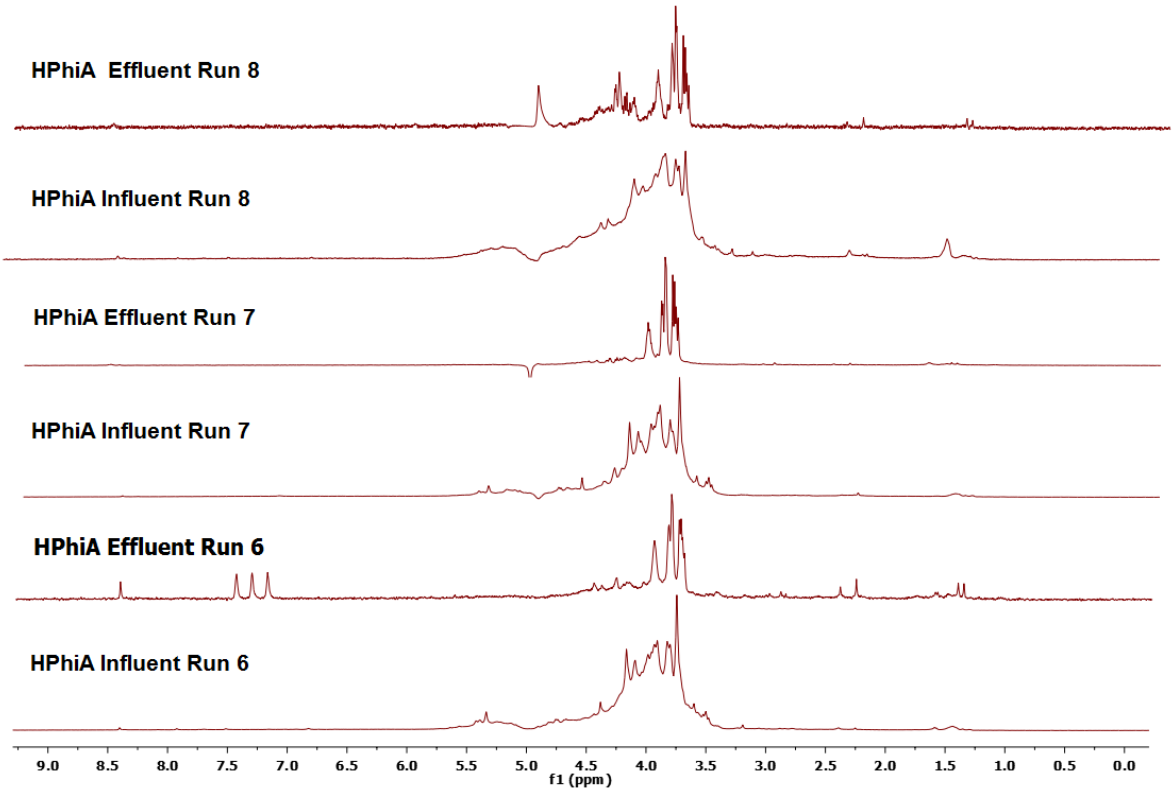


Figure 6.13: ^{1}H NMR spectra of influent and effluent HPhiA fractions from Run 6, Run 7 and Run 8.

Biopolymers which are mainly hydrophilic polysaccharides, could contribute to the residual ODM in the effluent from the HPhiA samples [40, 152]. However, Ciputra *et al*. and Antony *et al*. have reported that in treated pulp and paper mill effluent, there were negligible biopolymers present [21, 135]. To clarify this situation it is necessary to be able to conclusively show the presence, or otherwise, of polysaccharides in this fraction. The ^{1}H NMR resonances from polysaccharides are expected to be between 3 ppm to 6 ppm, which coincides with the most significant peaks observed in the current work (Figure 6.13). However, as biopolymers do not contain an aromatic structure there would be very limited UV absorbance at 254 nm [152], so, though there was minimal UV-Vis absorbance (see Figure 6.14) for any of the HPhiA effluent samples, it is not very informative. Finally, biopolymers consisting of extracellular proteins and polysaccharides have been reported to have molecular weights of 10 to 20 kDa [135] and there were no compounds > 6 kDa detected in the HPhiA effluent samples. This clearly indicates that the residual ODM in the HPhiA fraction was not biopolymers. As the major size fraction from the HPhiA effluent samples was between 400 – 700 Da, the residual HPhiA could have been due to breakdown products of humic substances which have been reported to be between 300 – 500 Da [135]. This size fraction was not expected to contain carbohydrates as they would have been easily assimilated by the activated sludge biomass. The

amount of ODM detected in the HPhiA fraction was relatively low and did not significantly contribute to the total ODM in the effluent.

There were no peaks between 6.5 and 8.1 ppm in the HPhiA fractions indicating the absence of aromatic protons. The HPhiA fractions, with the exception of the Run 6 effluent, did not have a significant resonance in the region of 8.4 ppm where formate has previously been identified. There were also no peaks detected in the region of 1.6 ppm to 1.8 ppm unlike the HPhoA and TPhiA fractions, indicating the absence of aromatic structures and compounds such as formate and acetate in this component. This is not unexpected as the hydrophobic compounds in the effluent samples were anticipated to be retained on the DAX-8 and XAD-4 resins.

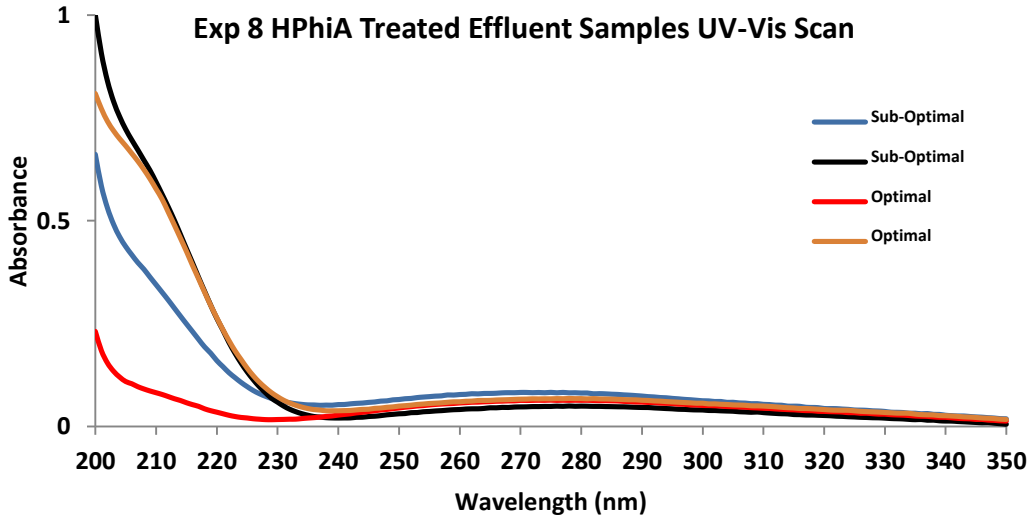


Figure 6.14: UV-Vis absorbance of the Run 8 HPhiA fraction comparing optimal and sub-optimal quality effluent samples.

As the HPhiA fraction is expected to be comprised largely of carbohydrates there should be limited UV absorbance of effluent samples [134]. As can seen from Figure 6.14, this was indeed the case. This is also consistent with the minimal aromatic resonance detected in the ^{1}H NMR spectra discussed above, again indicating that this fraction is largely carbohydrates that should be relatively easily broken down in an efficient SETP.

6.3.6 Molecular Weight Distribution and NMR Spectroscopic Studies of the Transphilic Acid Fraction

In the SEC chromatograms of the transphilic acid fractions there were no peaks in the HMW region. However, the MW of the compounds detected in the effluent TPhiA fractions was greater than in the HPhiA fraction of the effluent samples, especially in Runs 7 and 8 (Figure 6.15). From Table 6.1, the ODM in the effluent samples was between 5 – 20 mg in Run 6, and 4 – 7 mg in Runs 7 and 8. As the TPhiA fraction is slightly hydrophobic, the resistant TPhiA compounds could be from degraded HPhoA not retained on the DAX – 8 resin. However, the reduction of the TPhiA fraction ODM indicates that the majority of the influent TPhiA fraction was easily removed in the SETP through either biodegradation or adsorption to sludge.

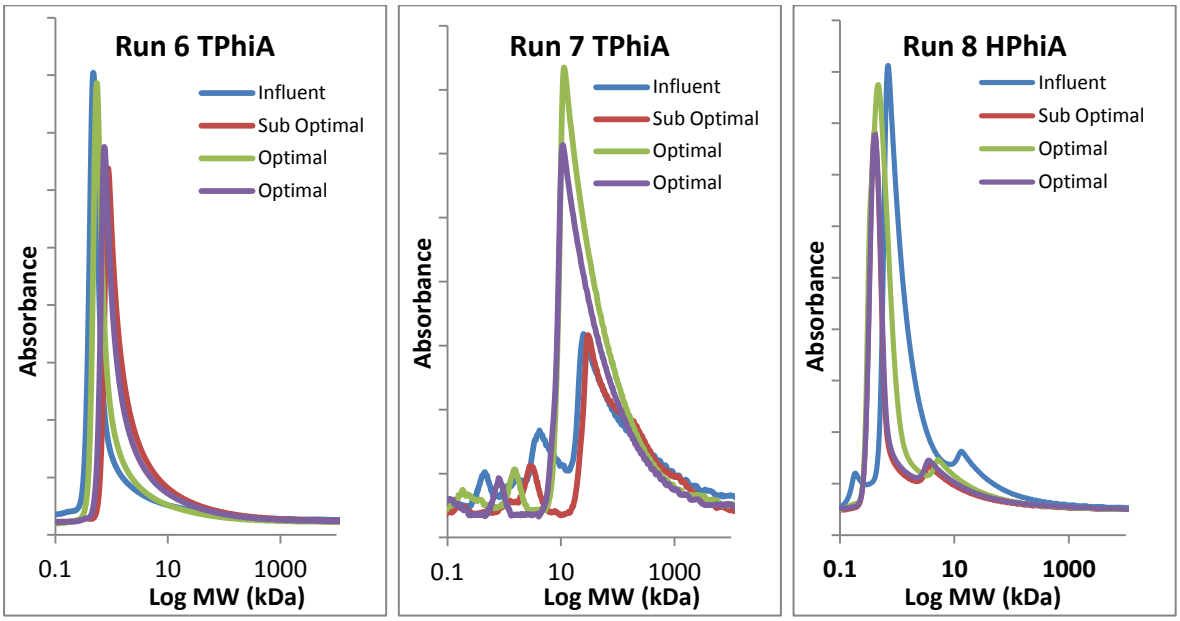


Figure 6.15: TPhiA fraction SEC chromatograms of Run 6, Run 7 and Run 8 samples from the influent, and fractions from optimal and sub-optimal effluent samples

There is limited information about the TPhiA fraction in wastewater treatment and its contribution to effluent quality. The MW of compounds in the TPhiA fraction of Runs 7 and 8 was relatively high compared to the expected < 1 kDa found in municipal WWTPs [37]. The largest peak detected in the effluent Run 7 and Run 8 samples corresponds to MW between approximately 10 – 100 kDa. In the Run 6 effluent samples a single dominant peak represented compounds between 500 – 800 Da, within the expected range. The average MW of the TPhiA fraction was greater than the HPhoA by an order of magnitude, this was unexpected as the XAD-4 resin was employed to retain small

hydrophobic compounds < 20 kDa, while the DAX-8 resin was used to isolate HMW compounds up to 150 kDa.

The SEC chromatograms of the TPhiA were the most complex of all those run, suggesting that there were many different size fractions. The NMR spectra contained sharp peaks indicative of LMW compounds in the effluent and there were some broad peak in the Run 6 and 7 effluent samples which suggest compounds with a higher MW (Figure 6.16).

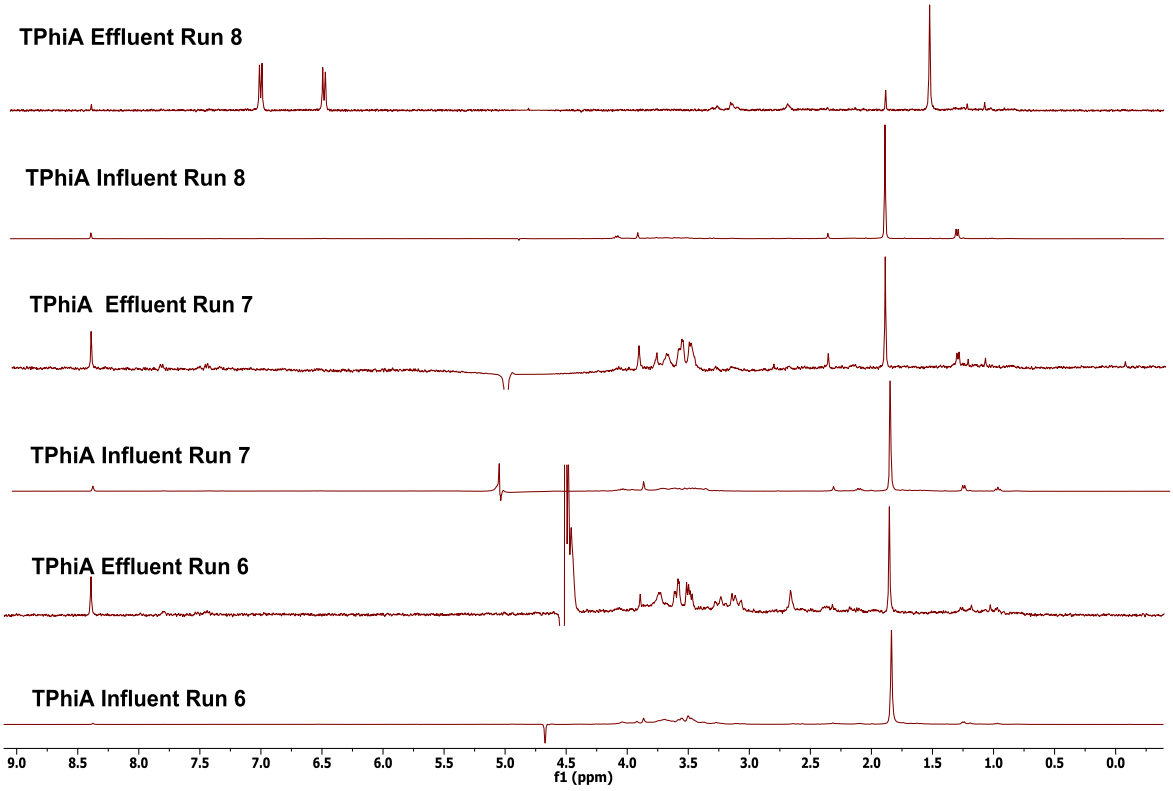


Figure 6.16: ^{1}H NMR spectra of influent and effluent TPhiA fractions from Run 6, Run 7 and Run 8.

Peaks in the NMR spectra of the TPhiA acid fractions were between 6.5 and 8.1 ppm, indicating the presence of aromatic protons. There were also peaks in the 1.7 – 3.0 ppm region, indicating the possibility of methyl substituted aromatic structures [139].

As previously discussed, there were significant sharp peaks in the chemical shift region of 1.8 ppm in most TPhiA samples, corresponding with peaks detected in the HPhoA fraction (Figure 6.16). The single sharp peak at approximately 1.8 ppm was the dominant peak in the TPhiA influent samples, while it was present as residual peaks in the effluent samples. There were also some peaks detected

at approximately 1.1 ppm, especially in the Run 8 TPhiA samples, indicative of simple aliphatic structures.

In Run 8 there were two doublet peaks in the aromatic region at 6.4 and 7.0 ppm, possibly indicating coupled resonances and aromatic methoxy groups. These are similar in structure to syringyl and guaiacyl groups which have been identified in pulp and paper wastewater [18] and are also products of lignin degradation [25]. The UV scan of the Run 8 TPhiA fractions revealed absorbance in both regions ($UV_{254-260}$ and $UV_{270-280}$) where aromatic structures have been detected (see Figure 6.17) [20, 146-148]. Some of the residual compounds in the TPhiA fraction could have an aromatic structure. The material associated with two doublet peaks observed in the NMR spectra of Run 8 TPhiA did not appear to contribute to the UV absorbance, therefore, these compounds were most likely not aromatic. The NMR doublet peaks were detected in one optimal and one sub-optimal sample, and the UV absorbance from two optimal and two sub-optimal samples were comparable. This suggests that the aromatic compounds were at a sufficient concentration to affect the intensity of the UV absorbance in the aromatic regions. The UV absorbance in the TPhiA fraction was most likely due to the presence of the compounds detected in the NMR spectra at 8.4 ppm and 1.8 ppm, these could be formate and acetate respectively, which were discussed previously in Section 6.3.4.

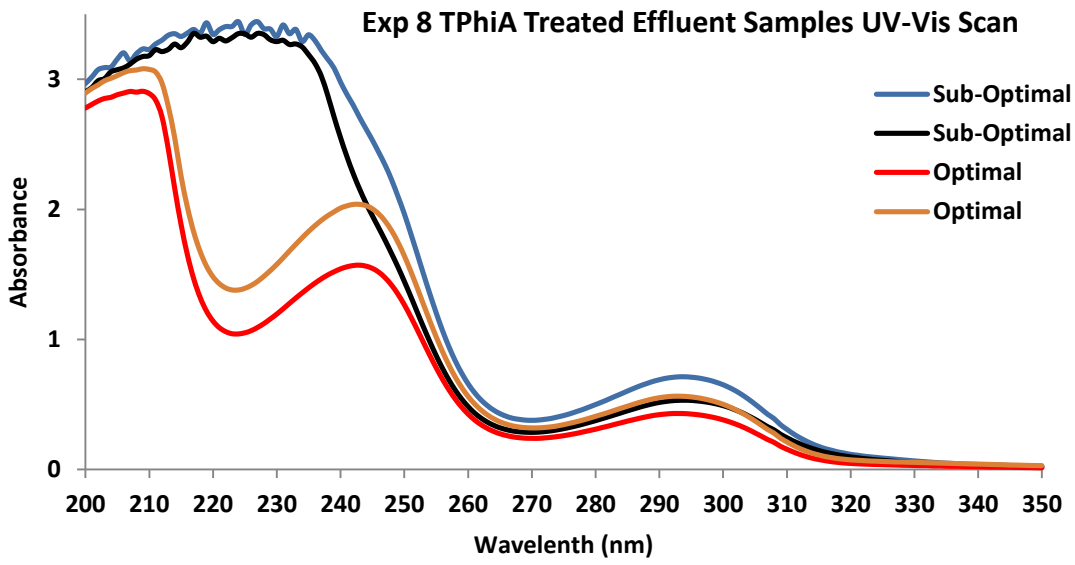


Figure 6.17: UV-Vis absorbance of the Run 8 TPhiA fractions, comparing optimal and sub-optimal effluent samples.

6.4 Conclusion

Though the SETP influent quality at Boyer varied, it generally consisted of four size fractions: > 2000 kDa, 45 kDa, 800 to 3000 Da and ≤ 180 Da. The major size fractions detected by SEC in the influent samples were 45 kDa and 800 to 3000 Da. The major NMR resonance detected in the influent samples was a broad envelope between 3.3 and 5.2 ppm, including some sharp peaks. This was indicative of a complex mixture of HMW and LMW compounds. There were also minor ^{1}H NMR resonances in the aromatic region between 6.0 and 8.0 ppm associated with lignin derived compounds.

The total ODM determined from the bulk samples collected from Boyer (Runs 6 to 8) was comprised of 20 to 30% HPhoA, 15% TPhiA and 25 to 35% HPhiA fractions, which is in agreement with previous research on the Boyer primary clarifier wastewater [20]. By comparison to previous work done by Viney prior to the commissioning of the SETP, it was shown that the secondary treatment through porous pots had the greatest affect on the HPhiA fraction. Prior to the SETP there was 56% residual HPhiA in the effluent, after treatment in the porous pot this was reduced to 4 to 9% residual HPhiA during optimal conditions. This was an indication that a significant amount of the HPhiA fraction was either biologically assimilated or adsorbed to the activated sludge flocs. In the treated HPhiA fractions the main NMR envelope was in the chemical shift region between 3.5 and 3.8 ppm and there were no resonance peaks in the aromatic region 6.0 to 8.0 ppm, indicating that there were minimal aromatic protons in the HPhiA samples. The NMR spectra in the aromatic region were supported by the limited UV absorbance between 230 and 300 nm in the region where aromatic structures were detected. As the majority of the HPhiA fraction was removed through secondary treatment in the porous pots, the residual HPhiA was proposed to be comprised of breakdown products of humic substances.

Comparing the influent and effluent NMR spectra of the HPhiA fraction from the porous pots showed a significant reduction in the resonance of both the upfield and downfield shoulders of the main broad envelope (3.2 ppm to 5.5 ppm). This chemical shift region has previously been associated with carbohydrates in pulp and paper wastewater [142, 143], which would be rightly be included in the HPhiA fraction. The residual resonance was most likely to be from hydrophobic lignin derived compounds in the HPhoA fraction which contributed the most significantly to the residual ODM in the effluent.

The HPhoA fraction was the most resistant to degradation in the porous pots. Before the commissioning of the Boyer SETP the residual HPhoA detected in the effluent was 46% of the influent [20]. Although the porous pots are not a full scale SETP, the residual HPhoA detected in the

porous pot effluents during optimal and sub-optimal operating conditions was 27 to 45% and 30 to 90% of the influents, respectively. In the sub-optimal HPhoA samples the dominant MW fraction was between 400 and 4000 Da. Resin acids have been identified in pulp and paper wastewater, however, lignin derived compounds were most likely to contribute to the residual ODM. There was limited resonance in the NMR spectra to indicate aromatic protons, however, there was significant UV absorbance between 230 and 300 nm indicating the presence of aromatic structures or C=C bonds, such as lignin derived compounds.

In the downfield chemical shift region a peak at 8.4 ppm was present in all HPhoA and TPhiA samples. This has been identified in pulp and paper wastewater as formate. There was a significant residual peak detected at 1.8 ppm, which was also present as the most significant peak in the TPhiA influent fractions. There was minimal residual ODM in the effluent TPhiA fractions. The residual TPhiA before the SETP was 11 to 17% and in the optimal porous pot effluent it was 6 to 15%. The use of porous pots appeared to have limited effect on the removal of TPhiA through secondary biological treatment compared to primary treatment previously employed at Boyer. This suggests that the removal of TPhiA was only partially through biological degradation.

What was not clear was the source of HMW compounds in the TPhiA effluent samples. The 10 to 100 kDa compounds were larger than those detected in the HPhoA samples, with significant UV-vis absorbance between 230 and 300 nm. However, there was only minor resonance in the aromatic region and the peaks detected in the NMR spectra were sharp, indicative of LMW compounds.

There were three size fractions in the HPhiA, 3 to 6 kDa, 400 to 700 Da and ≤ 180 Da, with the dominant fraction being the 400 to 700 Da. The ^{1}H NMR spectra indicated that there was a significant decrease in the HPhiA compounds initially detected in the chemical shift region between 3.3 and 3.5 ppm and 3.8 and 5.2 ppm. In this region carbohydrates such as glucose, mannose and galactose have been identified in the wastewater [142, 143]. As there was efficient removal of the HPhiA fraction it was assumed that the majority of the HPhiA in the influent were carbohydrates and the residual HPhiA compounds could have been due to the presence of biopolymers.

Chapter 7 Effects of Trace Metal and Water-Soluble Vitamin Addition on Activated Sludge Biomass

7.1 Introduction

Activated sludge contains a complex ecosystem of microorganisms that includes single celled organisms, bacteria, protozoa and multi cell metazoa [62]. Bacteria are the main agents involved in the removal of dissolved and colloidal forms of COD found in wastewater. The protozoa and metazoa species act as a clarifier by consuming free bacteria and particulate matter. For optimal growth of the AS biomass inorganic and organic nutrients, such as the trace metals cobalt, copper, iron(III), molybdenum and zinc, are required for cell growth [5, 6, 8, 47]. The concentrations of these trace metals determined in the Norske Skog Boyer (NSB) WWTP were below the theoretical trace metal requirements for cell growth (see Table 1.1). There was, however, an excess of the metals calcium and magnesium in the AS liquor, with levels of 20 mg/L and 41 mg/L, respectively.

The presence of heavy metals at elevated concentrations in the AS is known to alter the species richness and cell density of the AS biomass [64]. The addition of copper to a copper deficient AS changed the rate of COD removal and the cell density and species dominance [63]. Without the correct balance of micronutrients to support diverse microbial growth in the AS, sludge handling and COD removal issues can arise [8]. On the other hand, filamentous bacteria are well suited to sludge conditions with limited nutrients [43]. Balancing the requirements for the AS biomass between the needs for optimal cell growth and toxicity is a challenging enterprise and will vary for each WWTP.

Filamentous bacteria can act as bioindicators for the operational conditions in AS treatment plants, indicating the DO level, the organic loading and nutrient deficiencies. They are also an essential part of the AS biomass and most AS plants throughout the world experience control difficulties generally due to filamentous bacteria. AS flocs are a balance between long chain filaments and small round cell bacteria.

Without filaments dense flocs form that settle rapidly, however, due to the low shear strength of these flocs, small aggregates separate causing an increase in turbidity [52-54]. On the other hand, bulking occurs when there is an excess of filamentous bacteria, which form bridges between flocs, reducing the flocs' ability to settle. Certain filamentous bacteria are associated with bulking. When Type 021N and *Thiorthix sp.* dominate the filament population, bulking is common. To a lesser degree *Haliscomenobacter hydrossis* and *Nostocoida limicola* lead to the same outcome [153]. Bulking has been reported to be controlled though the addition of magnesium, calcium and iron(III)

[107]. Bulking and foaming can affect the efficient removal of COD and suspended solids in the AS treatment plant.

From Australian research into industrial WWTPs the filament species *S. natans*, Type 0914, Type 021N, Type 0041/0675, N limicola, H. hydrossis, Type 1851 and Type 1701 were found to be common to very common [57, 59]. From previous studies the most common filament found in WWTPs across all continents was Type 0041 [60], while Type 021N has commonly been found to predominate in treatment plants in America [59]. Although Type 021N, Type 1701, *N. limicola* and *H. hydrossis* are common in Australian wastewater treatment plants, they have rarely been found to be the dominant filamentous species. However, contrary to the research by Seviour *et al*. [57] the filaments most commonly observed at the Norske Skog, Boyer, WWTP have been Type 021N, Type 0041, *H. hydrossis* and *Sphaerotilus natans* (Rice, S. 2011, pers. comm., 7 March). Type 021N, whose isolation at NSB coincided with the appearance of thick, scum-like foam, is the most significant of these. Although there is frequently minor foaming at NSB, though generally it is thought to be due to the constituents in the wastewater and not filamentous bacteria.

Protozoa and metazoa are higher order organisms that prey on the free bacteria in the AS liquor or the bacteria attached to the edges of the flocs. The predation of the free bacteria contributes to the clarification process and to the removal of COD [51, 64]. Excessive numbers of metazoa can cause a decrease in sludge production through the consumption of whole flocs [51]. The presence and abundance of all forms of protozoa can also indicate the operational conditions of AS treatment plants [43].

7.2 Materials and Methods

7.2.1 Materials

Livingstone slides and cover slips were used for wet mount and fixed smear slides. An Olympus BH-2 microscope with an attached Leica DC 300F camera was used to observe and record the AS and filaments at x 100 and x 400 magnification, Olympus immersion oil was used at magnification of x 1000. Haemocytometer and cover slips supplied by Marienfeld were used to quantify ciliates and rotifers in the AS. A Ratek Instruments TH6P – Thermoregulator heater was used for the temperature trials where the temperature was ≥ 38°C.

Reagents

The reagents used for staining and identifying filamentous bacteria by the Gram stain were: 96% AR ethanol (Merck), AR acetone (AJAX chemicals), safranine (Gurr) and ammonium oxalate, crystal violet, KI and I_2 from BDH. The additional reagents used for the Neisser stain were bismarck brown and methylene blue supplied by BDH and AJAX Chemicals, respectively.

Preparation of Gram Stain Solutions

The methods for preparing the gram stain solutions were adapted from Eikelboom [51] and Jenkins *et al.*[52]. Solution 1 was made in two parts: part A was made by dissolving 2 g of crystal violet in 20 mL of 96% ethanol, part B was made by dissolving 0.4 g ammonium oxalate in 40 mL of distilled water. Part A and part B were combined in equal amounts to make solution 1. Solution 2 was prepared by dissolving 1.7 g of KI and 0.8 g I_2 in 10 mL of distilled water and diluting to 250 mL with distilled water. The decolourizing solution (solution 3) was made using 96% ethanol and acetone at a ratio of 7:3. For solution 4, there was 0.5 g of safranine dissolved in 20 mL of 96% ethanol and diluted to 200 mL with distilled water.

Preparation of Neisser Stain Solutions

There were two solutions prepared for Neisser staining, solution 1 was made in two separate parts. Part A was prepared by dissolving 0.1 g of methylene blue in 5 mL of 96% ethanol and diluting to 100 mL with distilled water. Part B was prepared by making a concentrated crystal violet solution by dissolving 5 g of crystal violet in 50 mL of 96% ethanol, and then adding 1.65 mL of this solution to 3.35 mL of 96% ethanol and before dilution with 50 mL of distilled water. Solution 1 was prepared by combining part A and part B at a ratio of 2:1. A concentrated Bismark brown solution was made by dissolving 1 g of Bismark brown in 100 mL of distilled water, solution 2 was prepared by mixing 33 mL of the concentrated Bismark brown solution with 66 mL of distilled water.

7.2.2 Methods

The methods to calculate the food to mass (F/M) ratio, the sludge volume index (SVI) and the sludge age were discussed in Chapter 3.

Ciliates and rotifers were quantified by using a haemocytometer to count individual cells. Cell counts were undertaken by pipetting a drop of AS liquor onto the haemocytometer counting chamber and placing a cover slide on top. Each cell count was duplicated.

Wet mount slides were prepared for observing living cells and floc structure by pipetting a single drop onto a clean dry slide and placing a cover slip on top of the drop. One edge of the cover slip was first placed on the slide and slowly placed over the drop to minimize the presence of air bubbles and to allow excess liquor to disperse.

Fixed smears were prepared by placing a drop of activated sludge liquor onto a clean dry slide, spreading evenly and allowing to air dry.

Gram Stain Method

An individual fixed smear was prepared for each gram stain. The slides were stained using the solutions prepared for gram staining. The slide was flooded with solution 1 for 60 sec. The excess dye was allowed to run off the slide. The slide was rinsed for 5 sec with tap water before flooding the slide with solution 2 for a further 60 sec where the excess was allowed to run off the slide. The slide was rinsed with tap water for 5 sec and the decolourizing solution applied by a squeeze bottle until the solution running off the slide was clear, the slide was further rinsed with water by applying tap water to the back of the slide. The slide was counter stained by flooding with solution 4 for 120 sec before the back of the slide was rinsed with tap water. The individual slides were blot dried with paper towel and then air-dried.

Neisser Stain Method

Separate fixed smear slides were prepared. The slides were stained using the solutions prepared for gram staining. The slide was flooded with solution 1 for 30 sec and the back of the slide rinsed with tap water for a further 10 sec. Solution 2 was then used to flood the slide for 60 sec before rinsing with water and blot drying.

7.3 Results and Discussion

7.3.1 Effects on protozoa and metazoa

There have been approximately 230 protozoan species identified from AS. Of these there are only a limited number which are frequently observed and that are common to WWTPs throughout the world [63]. The protozoa and metazoa, specifically the ciliates and rotifers, were quantified through counting cells numbers using a haemocytometer in Runs 2 to 8, inclusive, of the porous pot experiments (see Table 7.1). No differentiation between the individual species of ciliates or rotifers was attempted when quantifying the total numbers. The mean cell density of both ciliates and

rotifers observed differed between each run. The mean numbers of total ciliates from each run ranged from 1.0 x 10^2 cells/mL to 6.6 x 10^4 cells/mL (Table 7.1). The high standard deviations were an indication of the variability in the day to day abundance of the protozoan and metazoan species observed. The fluctuations in quantified protozoa and metazoa species have been previously observed in pilot plant trials [66].

Though the most abundant species in activated sludge has been shown to differ between treatment plants, there are a number of similarities. From research on municipal wastewater undertaken by Lee *et al*. [66] and Al-Shahwani *et al.* [154] stalked ciliates *Vorticella sp*., *Carchesium sp*. and crawling species *Chilodonella spp*. and *Aspidisca cicada* were the most prevalent.

There were two dominant ciliate species observed in the AS throughout all the porous pot runs, the stalked ciliate, *Vorticella sp.* and the crawling ciliate, *Aspidisca sp.* (see Figure 7.1). For the porous pot runs only the ciliate species and the total cell density and the individual species abundance were recorded. The total cell density of ciliated protozoa expected to be found in AS plants has been reported to be around 10^4 cells/mL [64, 155]. Further, in municipal pilot plant trials the *Vorticella sp.* was observed at a frequency of 88%, with a maximum cell density of 4 x 10^3 cells/mL [66]. The mean cell density of the *Vorticella sp*. quantified in two separate studies of activated sludge samples collected from European WWTPs has been 4 x 10^3 cells/mL [65] and 9 x 10^3 cells/mL [154]. Protozoa have been used to indicate the operational conditions and effluent quality of WWTPs [43, 65]. In a single permanent municipal pilot plant the presence of *Vorticella sp.* have indicated high effluent quality and good settling of sludge [66]. There was no correlation between the abundance of ciliates and the COD removal or the SS during the porous pot runs. The crawling ciliate, *Aspidisca sp*. was found in comparable numbers to the *Vorticella sp*. at a frequency of 90% with a maximum cell density observed 1 x 10^4 cells/mL [66], and a mean cell density of 2 x 10^3 cells/mL [65, 154]. There was one exception to

Table 7.1: The mean ciliate and rotifer numbers counted from each experimental run. The ciliate counted represent all ciliates, including stalked, crawling and free swimming ciliates.

Mean Cell Density (cells/mL)		Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6	ANOVA Stats
Run 2		**Control**	**molybdenum**	**cobalt**	**zinc**	**copper**	**42°C**	
	Ciliates	6150 ± 16500	8600 ± 20300	6000 ± 10700	1250 ± 3950	7200 ± 14900	0	(P - 0.777; df - 59)
	Rotifers	950 ± 2300	1350 ± 3150	1300 ± 3000	1100 ± 2600	2100 ± 3400	0	(P - 0.893; df - 59)
Run 3		**Control**	**thiamine**	**riboflavin**	**niacin**		**40°C**	
	Ciliates	550 ± 600	550 ± 700	550 ± 750	1250 ± 2350	N/A	0	(P - 0.466; df - 51)
	Rotifers	0	100 ± 200	0	0	N/A	0	
Run 4		**Control**	**pantothenic acid**	**pyridoxine**	**folic acid**		**38°C**	
	Ciliates	3300 ± 3900	3650 ± 4800	2750 ± 5400	7350 ± 8750	N/A	0	(P - 0.263; df - 64)
	Rotifers	200 ± 250	250 ± 200	300 ± 250	250 ± 300	N/A	0	(p - 0.587; df - 64)
Run 5		**Control**	**copper**	**cobalt**	**iron**	**magnesium**	**calcium**	
	Ciliates	**750 ± 900**	200 ± 200*	100 ± 50*	200 ± 250*	250 ± 350*	100 ± 100*	**(P - 0.004; df - 71)**
	Rotifers	250 ± 300	150 ± 350	100 ± 350	100 ± 350	100 ± 350	150 ± 350	(P - 0.559; df - 71)
Run 6		**Control**	**magnesium**	**pantothenic acid**	**folic acid**	**phosphorus**	**phosphorus**	
	Ciliates	20450 ± 28150	27450 ± 24850	29100 ± 24200	36600 ± 47300	66350 ± 190450	13750 ± 13500	(P - 0.669; df - 77)
	Rotifers	350 ± 600	350 ± 600	150 ± 300	400 ± 750	300 ± 650	200 ± 450	(P - 0.907; df - 77)
Run 7		**Control**	**1.0 mg/L copper**	**0.25 mg/L copper**	**0.1 mg/L copper**	**0.1 mg/L cobalt**	**0.025 mg/L cobalt**	
	Ciliates	3500 ± 3850	4500 ± 3950	3788 ± 3350	4800 ± 7100	4550 ± 4050	7650 ± 13550	(P - 0.730; df - 72)
	Rotifers	1050 ± 650	1050 ± 600	800 ± 450	1050 ± 750	650 ± 700	**100 ± 100***	**(P - 0.002; df - 72)**
Run 8		**Control**	**iron / copper**	**iron / copper**	**magnesium/copper**	**calcium / copper**	**calcium / copper**	
	Ciliates	1200 ± 1150	850 ± 900	800 ± 650	1100 ± 1250	900 ± 1600	1050 ± 900	(P - 0.959; df - 65)
	Rotifers	850 ± 950	450 ± 650	850 ± 450	850 ± 900	1200 ± 1500	300 ± 400	(P - 0.290; df - 65)

* Mean cell densities in bold and shaded represent those counts that are significantly different to the Control pot count for that Run.

counting individual ciliate species, the *Chilodonella sp*. crawling ciliate was not frequently observed in the porous pots, and their cell density was approximately 1 x 10^2 cells/mL on the limited occasions they were observed.

Previous studies using municipal wastewater in a pilot plant have found the *Chilodonella sp*. occurred in 79% of the observations at a maximum of 3 x 10^4 cells/mL [66]. On the other hand, free swimming ciliates have an inefficient feeding mechanisms and generally occur in high organic load conditions due to the increase in free dispersed bacteria [52]. Crawling ciliates *Chilodonella sp*. and *Apsidisca sp*. have previously been found to indicate low F/M and higher sludge age [65, 66]. As the mean F/M ratio was between 0.21 and 0.34, the possibility for the abundance of the *Chilodonella sp*. and *Apsidisca sp.* being present due to low F/M ratio is unlikely. The F/M ratio and the sludge age will be discussed in Section 7.3.3.

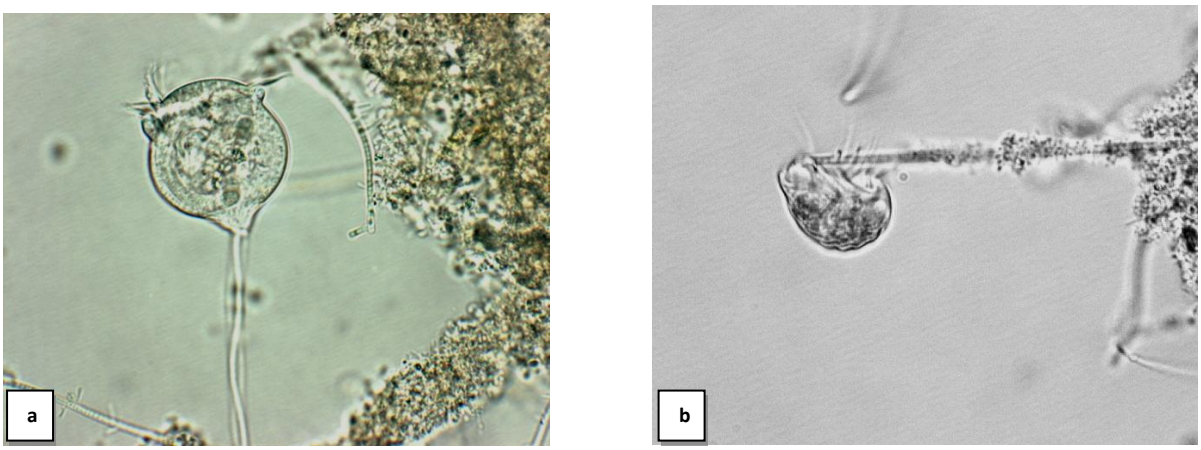


Figure 7.1: A *Vorticella sp*. stalked ciliate (a) and a *Aspidisca sp.* crawling ciliate (b) (400x magnification)

Comparing the mean cell density of ciliates in individual porous pots in each run there was only one statistically significant difference found between the pots in Run 5 (P – 0.004; df – 71). That difference was in the control pot during a metal addition run where there was an increase in the mean ciliate numbers compared to the mean ciliates counted in the trace metal amended pots (Run 5, Table 7.1). This lack of significant difference is largely brought about by the standard deviations of the organism counts due to large differences in operational conditions between pots during the separate runs. The DO, sludge age, F/M ratio and nutrient concentrations as well as the trace nutrients themselves could lead to the large variation in the ciliate numbers observed.

The cell density of the metazoan species was significantly less than the protozoan species. The range of the mean cell density of the rotifers determined in the porous pots was between 1.0 x 10^2 cells/mL and 7.6 x 10^3 cells/mL. There were only three porous pots, excluding the temperature trial pots (see Section 7.3.2), from a total of 37 trial pots where there were no rotifers observed. These pots were the control and the riboflavin and niacin amended pots in Run 3. Rotifers are not usually recognized as bioindicators in activated sludge as is the case with ciliates, so the absence of rotifers in the pots during Run 3 was not considered a performance indicator, even though the pot amended with niacin had a considerable reduction in COD removal. During the porous pot runs there was also no correlation between the abundance of rotifers observed in the activated sludge and the COD removal or the SS. The highest mean rotifer density was 2.1 x 10^3 cells/mL found in a copper trial pot (Pot 5, Run 1, see Table 7.1). During previous pilot plant trials of municipal activated sludge, rotifers have been observed to be about 6% of the total cell density of 40 cells/mL [66]. In a similar trial of municipal activated sludge the mean total cell density was 1 x 10^2 cells/mL [65]. In the current work only a single pot (Pot 6, 0.025 mg/L cobalt addition, Run 7) had a significant statistical decrease (P – 0.002; df – 72) in the mean rotifer cell density compared to the Control pot of that run (Table 7.1). This may have been caused by problems associated with maintaining the DO concentration above 2 mg/L in the 0.025 mg/L cobalt amended pot.

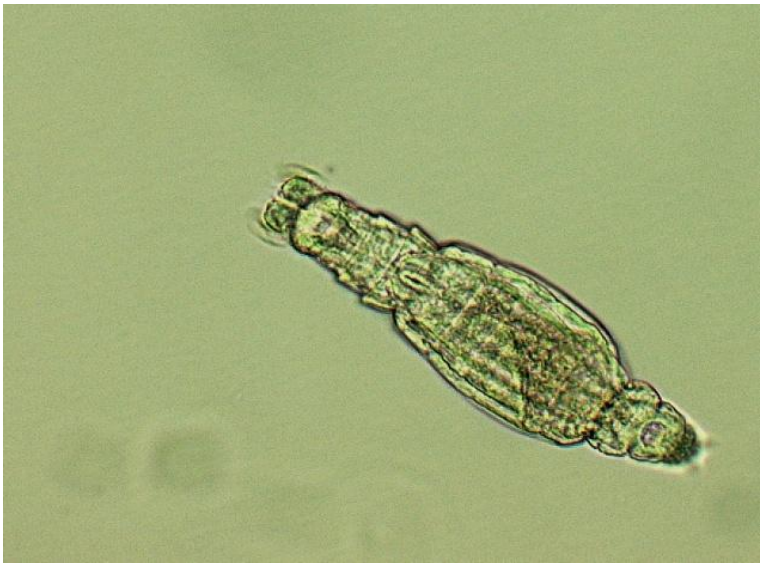


Figure 7.2: A common metazoan rotifer sp., magnification 200x.

The diversity of protozoan and metazoan species in AS can be affected by stress placed on the system through adverse operational conditions and external factors [155]. One external factor that could cause stress and affect the diversity and abundance of microfauna, is the presence of heavy metals [64]. Research has indicated that the nine ciliates commonly identified in AS responded

differently to the concentrations of heavy metals. The range of toxicity reported for copper varied between 0.31 mg/L and 2.05 mg/L and for zinc between < 0.17 mg/L and 84 mg/L [64]. During the porous pot trial with trace metal addition the maximum concentration of copper and zinc found in the porous pot effluent was 0.49 mg/L in Pot 2, Run 7, and 0.19 mg/L in Pot 4, Run 2, respectively. These are just within the range reported to be toxic in both instances, though there were no toxic affects observed in the microfauna. Further, the ciliate and rotifer numbers were not affected by spikes in the copper concentration of 0.95 mg/L or the zinc concentration of 0.19 mg/L. As the duration of the copper spike was three days at a concentration > 0.6 mg/L and there was no inhibition in the COD removal, it was felt that lower concentrations of copper were unlikely to have a toxic affect. Low concentrations of copper in activated sludge have been reported to stimulate the cell density of crawling ciliates, *Acineria uncinata* and *Aspidisca cicada,* although the term "low concentration" was not quantified [63]. As the total number of ciliates was counted and not individual species, it is not clear if there was a significant increase in the crawling ciliates species in the trial pots containing copper, only that there was no significant affect in the total cell density.

Significant differences were found in the cell density of ciliates and rotifers in the trial pot and control pot on two occasions, the first in Run 5 and the second in Run 7. In Run 5 a significantly lower number of ciliates were found in the trial pots involving the individual additions of copper, cobalt, iron, magnesium and calcium. This could have been due to elevated levels of copper or cobalt in Pots 2 and 3 respectively, however, there has been no literature found to suggest that iron(III), magnesium or calcium would have a detrimental effect on the numbers of protozoan species in activated sludge. Even in the current work copper was added at 1.0 mg/L (Pot 2, Run 7) and at 0.5 mg/L, along with with iron(III), magnesium or calcium, in Run 8 with no adverse effects on the protozoan population in either case. Hence, it is unlikely that the trace metal additions were the cause of the reduction in ciliates compared to the control during Run 5.

The second significant difference occurred in the 0.025 mg/L cobalt amended pot during Run 7 where DO dropped below 2.0 mg/L (Table 7.1). In this case a significant decrease in the number of rotifers was identified. Though the target additional concentration of cobalt in the pot was 0.025 mg/L, the concentration in the effluent spiked to 0.16 mg/L on one occasion. In previous additions of cobalt of up to 0.1 mg/L (Run 7) the mean concentration of cobalt in the effluent was 0.08 mg/L, which had no effect on the rotifer population. Cobalt as low as 1.0 mg/L has been reported to inhibit activated sludge biomass and caused deterioration of effluent quality [6, 8]. Although the concentration of cobalt detected in the effluent was well below 1.0 mg/L, the increased cobalt level

coinciding with the reduced DO in the pot could have contributed to the significant decrease in rotifer numbers observed in the activated sludge from the pot amended with 0.025 mg/L cobalt.

7.3.2 Temperature Effects

As an additional study to investigate the possible effects of temperature on the WWTP at Norske Skog NSB three trial pots at elevated temperatures (38°C, 40°C and 42°C) were tested over three separate runs to assess the thermal response of the activated sludge. Temperatures of above 37°C in AS are known to initiate stress responses in the AS biomass and if the temperature at this level for extended periods the floc structure is known to deteriorate and bacteria cells to become dispersed in the liquor, increasing turbidity and SS [52].

In the porous pot trials of elevated temperature (Pot 6 in Runs 2, 3 and 4) adverse effects on the AS were seen in all cases. Following the seeding and settling period there were no ciliates or rotifers observed in any samples, and foaming and flagellates were common. These were all indicators of inhibitory conditions in the porous pots. During the trace metal and water-soluble vitamin trials it was rare to observe flagellates in any pots but their presence generally coincided with a reduction in COD removal. The presence of ciliates and rotifers were observed in all other pots throughout the porous pot trials. At increased temperatures the biomass decreased and floc structure disintegrated, with free bacteria in the effluent affecting the SS and turbidity. The mean COD removal for the three temperature trial pots ranged from 38% to 49%. This was not unexpected as Chakrabarti *et al.* reported that temperatures of 45°C and 50°C in laboratory scale activated sludge reactors treating pulp and paper wastewater significantly decreased the COD removal to 49% and 43%, respectively, after 19 days [7]. Archibald and Young reported that in benchtop experiments of pulp and paper wastewater, that increases in temperature of up to 45°C for a period of 1 h did not have an adverse effect on the BOD removal or inhibit biomass growth [156]. However, at a temperature of 50°C for 1 h the biomass was reduced by 40%, resulting in a reduction of BOD removal [156]. During the porous pot temperature trials the deterioration of the floc and the reduction in biomass and COD removal was not instant, but took up 24 h to take full effect, this could indicate that the activated sludge biomass could withstand a short “shock” increase in temperature of up to ≤ 12 h without adverse affects.

However, even though the 38°C pot was just 3°C higher than the standard porous pot temperature, there was a significant effect on the activated sludge biomass and the effluent quality. This was contrary to findings from Chakrabarti *et al.* where the biomass and COD removal was reported to be

unaffected at 40°C [7]. In Chakrabarti *et al.*'s work this was associated with a reduction in the DO level from approximately 2.0 mg/L in the 35°C control to 1.1 mg/L at 40°C without affecting the COD removal. They also reported that at the higher temperatures of 45°C and 50°C the DO level decreased to between 0.3 and 0.5 mg/L. However, the mean DO in the three porous pots in the current study remained between 1.9 mg/L and 3.0 mg/L at all times. These relatively high DO levels in the porous pots are an indication that the O_2 was not limiting during the temperature trials. Regardless of this it appeared that increasing the temperature to 38°C inhibited biomass growth and COD removal even though it was reported that 40°C should not adversely affect the activated sludge biomass and COD removal in other systems. This is relevant to the summer months at the NSB mill, where the increased atmospheric temperature makes cooling the wastewater a more energy demanding operation. With this in mind, it was proposed that energy could be saved through increasing the wastewater temperature entering the NSB SETP. These results from the porous pots indicate that if they were transferred to the NSB SETP and the temperature was increased to 38°C the activated sludge biota could be inhibited resulting in a reduction in COD removal, a likely increase in SS and sludge handling issues.

7.3.3 Effects of Metal and Vitamin Addition on Operating Conditions

The dominant filamentous bacteria found in AS can also be used to indicate the operating conditions in a WWTP. The dominant filaments identified from the porous pot AS were Type 021N, Type 0041 and *H. hydrossis* which are associated with bulking sludges [157]. When filamentous bacteria dominate in the activated sludge they contribute to bulking though growing beyond the floc structure and bridging between other flocs to produce a more open floc structure which does not settle in the secondary clarification process [52]. In Australia Type 0041 was the most common filament identified in WWTPs [59], while the bacteria associated with bulking and foaming throughout the world are Type 1701, *Thiothrix spp*., *S. natans,* Type 021N and Type 0961 [52, 59].

While filamentous bulking was common during the porous pot trials, it was a major problem during Run 6, the run with the highest organic load. Type 021N filamentous bacteria, a cause of bulking and foaming [52, 53, 56], can be an indicator of nutrient deficiency [52, 158] and often occurs in low DO [52]. After 7 days Type 021N and Type 0041 were observed to be the most dominant filaments in the sludge of Run 6. The residual NH_3 and PO_4^{3-} concentrations were not limiting during Run 6, so the presence of Type 021N and Type 0041 most likely was not due to a nutrient deficiency. *H.hydrossis*, with its characteristic thin straight filaments making it easily identifiable [51], was also observed for the first time after 7 days in Run 6 (See Figure 7.3). Its presence is assumed to be the main influence

on the bulking and foaming issues experienced during this run. *H.hydrossis* generally occurs in treatment plants with a high organic load and low DO [51, 52], which were the prevalent conditions during Run 6.

Bulking has been reported to be commonly caused by low DO, high F/M ratio or deficiencies in the available nitrogen and phosphorus [52]. As the F/M ratio was the same as previous runs and nutrients weren't deficient the likely cause of the bulking was a reduced DO level. The filamentous bacteria associated with low DO are Type 1701, Type 021N, *S. natans* and *H. hydrossis* [56, 60, 61]. Type 021N and *Thiothrix* spp. are also found in DO deficient sludges, where there is a high organic load [159].The organic loading has an effect on the DO level required to prevent bulking: as the organic load increases there is a greater requirement for DO [61]. The target DO concentration used in all porous pot runs was 2.0 mg/L, however, as the organic loading increased the availability of O_2 in the flocs is reduced as the O_2 cannot penetrate to the center of the floc structure [58], leading to localized O_2 deficiency. To overcome this the DO requirement in high organic load conditions can be as high as ≥4.0 mg/L [58]. The mean DO concentration in the porous pots during Run 6 was between 2.6 mg/L and 3.0 mg/L.

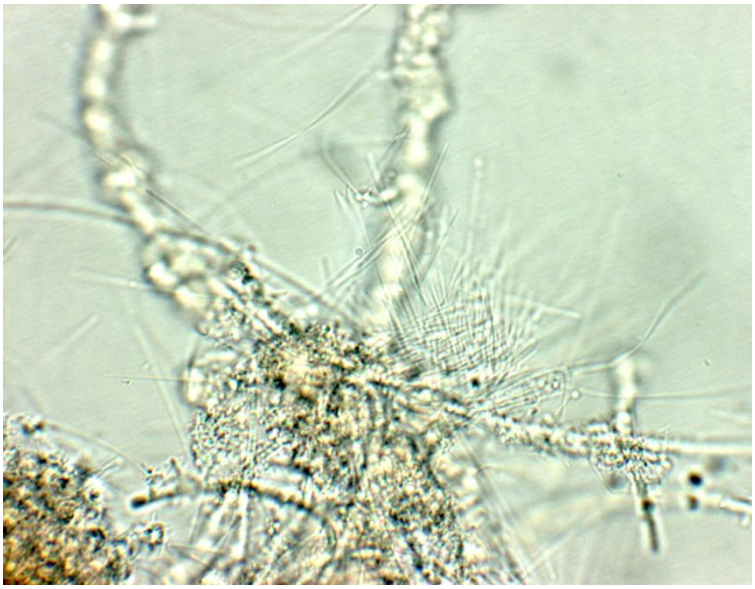


Figure 7.3: *H. hydrossis* from Run 6 observed in Pot 2 amended with 4.0 mg/L magnesium (magnification x 400).

There was some foaming that generally occurred following any low DO excursion in the pots during the porous pot runs. In Australia Type 021N has rarely been associated with foaming [60], while the

filamentous bacteria most strongly associated with foaming have been *Microthrix parvicella*, *Norcardia amarae* and *Nocardia pinensis* [57]. These latter filaments were not observed during any of the porous pot runs.

During Run 6 there was a major foaming problem. Where previously the foam had been a light soapy froth, the foam present during Run 6 was a thick, slimy, dark foam. Thick slimy foam has been reported to occur in nutrient deficient industrial AS, where the foam consists of biopolymers released from the biota in the floc [58]. During this run a floating sludge blanket and gas bubbles were observed in the waste activated sludge (WAS) from all pots, with a thick foam scum in the porous pots, which had not been observed previously. The floating sludge coincided with the first observation of *H. hydrossis* in the sludge on day 7. This was followed by a thick foam scum on day 10. The foam scum can be caused by denitrification if there are anoxic conditions [52]. Denitrification occurs at low DO concentrations when bacteria in the AS respire anaerobically using NO_3^- as a source of oxygen and forming gaseous N_2 [47, 58]. As N_2 has only low water solubility, it forms bubbles that cause the sludge to rise to the surface [52, 58]. There were gas bubbles observed in the WAS from day 14, after settling for 30 min the sludge was floating with a clear liquor solution at the bottom of each beaker.

In hindsight attempting to control the porous pots through the F/M ratio alone was a mistake. In the case of Run 6, with a very high organic load in the influent, an option would have been to reduce the influent flow rate. This was not undertaken at the time due to the assumption that the porous pots would not be operating at the same conditions as the NSB plant in relation to the wastewater retention time, and this would add a further variable to the results between the runs. Through reducing the organic load, the total MLSS in the porous pots would have been reduced through the F/M and lowering the O_2 requirements.

The Sludge Volume Index (SVI) can be a good indicator of how the floc structure responded to the addition of metals and vitamins. The SVI is a measure of the volume of settled activated sludge and the mass of the mixed liquor suspended solids (MLSS). A SVI ≤ 70 mL/g is an indication of pin flocs and dispersed growth [54], while bulking has been defined to have occurred when the SVI ≥ 150 mL/g [54, 58]. However, sludge defined as bulking based on the SVI may not lead directly to increased SS and turbidity in the secondary clarifier and decreased COD removal, due to the bridging filaments and increased bulk of the flocs acting as a filter [58]. In this case the suspended matter and biopolymers are bound to the flocs, which can decrease the SS and increase the COD removal.

As stated, bulking was common during the porous pot runs while the SVI in the pots ranged from 77 to 201 mL/g (Table 7.2). However, this did not have adverse affects on the SS or COD removal. In Run 8 the lowest mean SS and the highest COD removal were recorded at 5 to 10 mg/L and 85 to 87% respectively, while the SVI was between 138 and 167 mL/g. The exception was in Run 6 where there were considerable bulking problems, even though the SVI was between 131 and 151 mL/g well below the SVI levels in some of the other runs where bulking did not cause problems. In the porous pot runs bulking was considered to have occurred when the sludge volume test, a test where the actual volume of the settled sludge is measured, was ≥ 900 mL. It could be assumed that the SVI was not a good indicator for the potential for a floating sludge blanket. Although there were operational problems with bulking and floating sludge in Run 6 there were no statistically significant changes in the SVI in this run.

However, some of changes observed in the SVI in other runs of trace metal and water soluble vitamin additions were statistically significant. Using an ANOVA analysis of means, the mean SVIs of 152 mL/g for Pot 3 (pyridoxine addition) and 174 mL/g for Pot 4 (folic acid addition) were statistically significant increases in comparison to the SVI of the Control Pot (146 mL/g) in Run 4 (P – 0.000; df – 47). In both cases, the SVI was above 150 mL/g, the accepted indication of bulking. Figure 7.4 shows the filamentous interfloc bridging in Pots 3 and 4 of Run 4. Without studying individual filamentous bacteria and their micronutrient requirements, the addition of vitamins pyridoxine and folic acid appears to stimulate the growth of some filamentous bacteria especially Type 021N at a batch experiment scale.

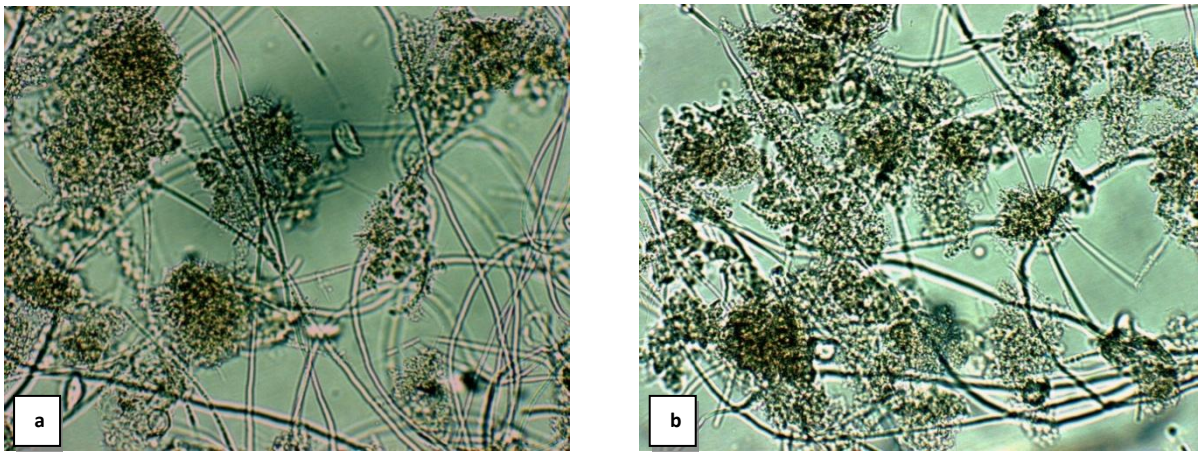


Figure 7.4: Bulking sludge from Run 4 Vitamin pyridoxine (a) and folic acid (b) Type 021N filamentous bulking (Magnification 100x).

During the trace metal additions there was a significant statistical decrease in the SVI on six occasions**.** Two of these occurred in Run 2 from the additions of copper and cobalt at target concentrations of 0.50 mg/L and 0.05 mg/L where the SVIs were 145 mL/g and 147 mL/g, respectively (P – 0.000; df – 44). These were still only marginally below the SVI considered to indicate bulking conditions. In Run 2 the addition of cobalt to 0.05 mg/L was the only occasion where the addition of cobalt to the porous pots caused the SVI to significantly decrease, indicating that cobalt could have an inhibitory effect on the activated sludge. However, in Run 7 there was an opposite effect where there was a significant increase in the SVI recorded in both pots amended with cobalt. The calculated SVI of the 0.1 mg/L and 0.025 mg/L cobalt amended pots was 181 mL/g and 196 mL/g, well above the operational definition of 150 mL/g for bulking (P – 0.000; df – 53). In these latter cases the addition of cobalt could have stimulated the growth of filamentous bacteria through the stimulation of cobalamin production, while during the same run there was the opposite effect through the addition of copper, known to inhibit some micro-organisms [79, 160].

There was a significant statistical decrease in the SVI from the addition of copper at concentrations of 1.0 mg/L and 0.1 mg/L in Run 7 (P – 0.000; df – 53). The SVI calculated from the two pots was the lowest from all eight porous pot runs at 77 mL/g and 99 mL/g, respectively. The effects on the filamentous bacteria can be seen in Figure 7.5 where there are visibly fewer filamentous bacteria in the flocs and no interfloc bridging. There was also a significant decrease in the SVI during Run 8 from the addition of 4.0 mg/L iron(III) with 0.5 mg/L copper and 4.0 mg/L magnesium with 0.5 mg/L copper (P – 0.000; df – 53). With the exception of the pot amended with 0.5 mg/L cobalt in Run 2, all other pots that had a statistically significant reduction in SVI were amended with copper at concentrations between 0.1 mg/L and 1.0 mg/L. In all cases there was no detrimental effect on the COD removal or SS level in the effluent.

Table 7.2: The mean F/M, SVI and sludge age from each pot through all porous pot runs. Sections were left blank when there was no trace metal or water-soluble vitamin amendment.

	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6
Run 1	**Control**	**Iron (III)**	**Calcium**	**Magnesium**		
F/M	0.27	0.24	0.24	0.25		
SVI	176	165	166	173		
Sludge Age	12	7	6	6		
Run 2	**Control**	**Molybdenum**	**Cobalt**	**Zinc**	**Copper**	**Temp 42°C**
F/M	0.31	0.31	0.26	0.29	0.26	0.77
SVI	176	173	**147***	163	**145***	192
Sludge Age	8	6	6	6	6	3
Run 3	**Control**	**Thiamine**	**Riboflavin**	**Niacin**		**Temp 40°C**
F/M	0.23	0.21	0.23	0.28		0.98
SVI	139	123	131	158		314
Sludge Age	10	10	10	12		N/A
Run 4	**Control**	**Pantothenic Acid**	**Pyridoxine**	**Folic Acid**		**Temp 38°C**
F/M	0.21	0.2	0.23	0.24		4.32
SVI	146	145	**152***	**174***		N/A
Sludge Age	6	6	6	6		N/A
Run 5	**Control**	**Copper**	**Cobalt**	**Iron**	**Magnesium**	**Calcium**
F/M	0.34	0.34	0.3	0.32	0.36	0.32
SVI	188	181	162	175	201	180
Sludge Age	11	11	10	11	11	11
Run 6	**Control**	**Magnesium**	**Pantothenic Acid**	**Folic Acid**	**Phosphorus**	**Phosphorus**
F/M	0.31	0.30	0.29	0.30	0.34	0.31
SVI	145	139	138	139	151	131
Sludge Age	23	20	20	20	23	20
Run 7	**Control**	**1.0 mg/L Cu**	**0.25 mg/L Cu**	**0.1 mg/L Cu**	**0.1 mg/L Co**	**0.025 mg/L Co**
F/M	0.21	0.24	0.23	0.21	0.21	0.24
SVI	145	**77***	178	**99***	**181***	**196***
Sludge Age	11	21	21	17	17	21
Run 8	**Control**	**Fe/Cu**	**Fe/Cu**	**Mg/Cu**	**Ca/Cu**	**Ca/Cu**
F/M	0.21	0.22	0.22	0.25	0.24	0.25
SVI	138	**108***	142	**102***	167	137
Sludge Age	13	13	13	31	16	44

*Mean SVI in blue represent a significant statistical decrease. Mean SVI in red represent a significant statistical increase.

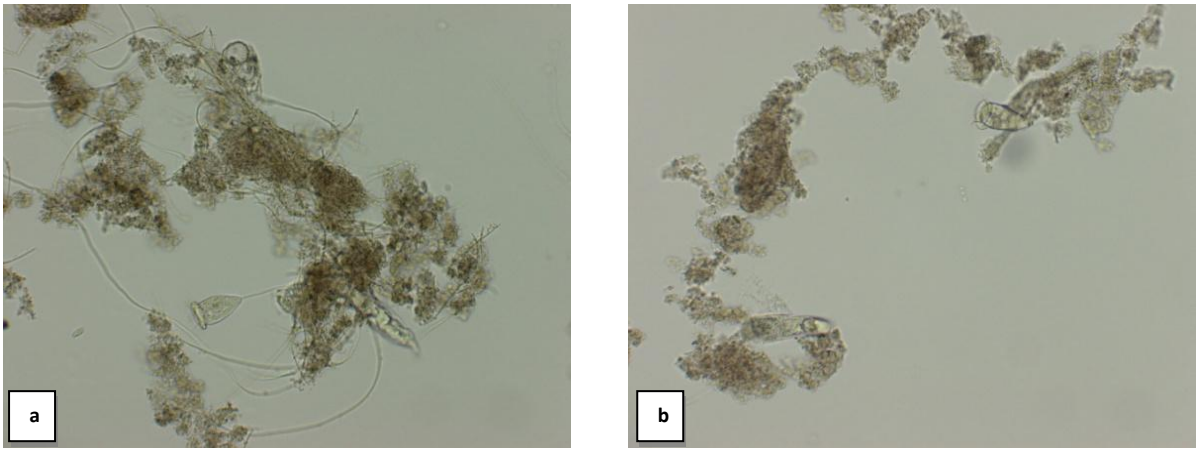


Figure 7.5: Sludge from Run 7, comparing images of Pot 1 (Control) sludge (a) and Pot 2 (copper 1.0 mg/L) sludge (b)(magnification 100x).

Though the addition of magnesium has been reported to decrease the SVI in activated sludge [115], this was not observed during the porous pot runs. Since MgO is used as a pH amendment at NSB there was a relatively high "background" concentration of magnesium in the wastewater and the additional 4 mg/L magnesium had no affect on the floc structure and SVI.

7.3.4 Metals effect on filamentous bacteria

The reduction of the SVI in some of the porous pots amended with copper was created by a significant decline in the abundance of filamentous bacteria observed in these pots (Figure 7.5 and 7.6). Both copper and zinc have been reported to inhibit filamentous bacteria Type 021N at concentrations of approximately 0.32 mg/L and 0.65 mg/L, respectively [160]. Generally copper has been found to be more toxic to filamentous bacteria than zinc [79, 160]. In Run 7 the filament Type 021N was not observed in the porous pots where copper alone was added. However, when it was added with magnesium or calcium in Run 8 there appeared to be no inhibition of 021N or other filaments, even though there was a significantly higher concentration of copper detected in the effluent of the pots amended with multiple metals magnesium/copper and calcium/copper. In Pot 2 during Run 8 there was also a decrease in the abundance of filamentous bacteria, which occurred through the addition of iron(III) and copper. This can be seen in Figure 7.6 where there was no interfloc bridging or growth on filaments protruding from the flocs.

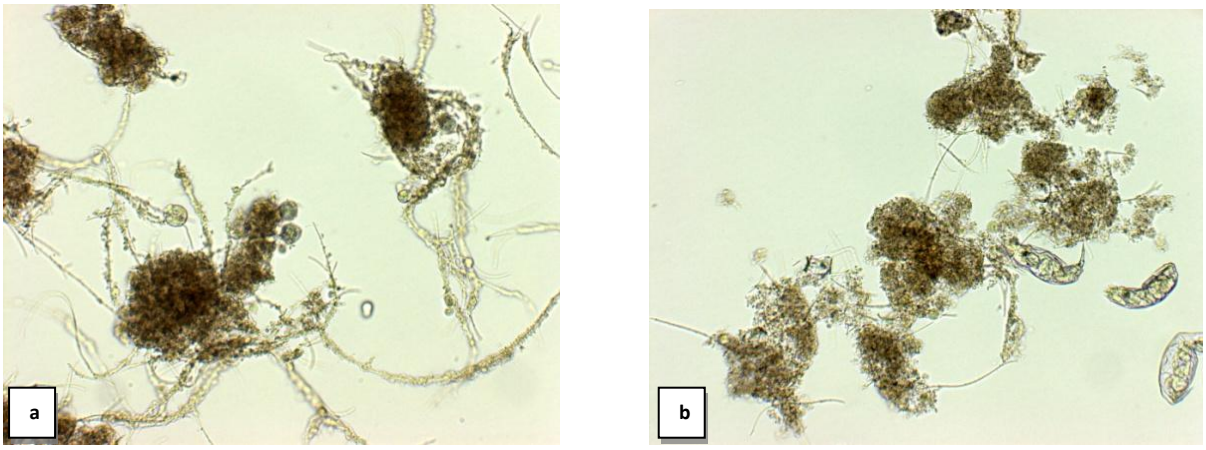


Figure 7.6: Floc structure from Run 8; Pot 1 Control (a) and Pot 2 iron(III) 4.0 mg/L copper 0.5 mg/L (b)(magnification 100x).

There is an understanding that calcium is required for the growth of Type 021N [79, 161], with both Type 021N and *H. hydrossis* growth reportedly enhanced by calcium addition [160]. However, as the concentrations of calcium and magnesium at NSB are already well above those required for optimal biological growth, any additional amount of either metal should not increase bacterial growth unless the metals were not bioavailable in the activated sludge. The presence of calcium is also reported to reduce the toxicity of heavy metals, including copper, to some filamentous bacteria especially Type 021N [79]. Hence the bioavailability and, therefore, the toxicity, of the copper must be affected in the presence of calcium and magnesium. However, the concentration of copper significantly increased in the effluent, implying that it was more bioavailable, when it was added to the porous pots with calcium and magnesium compared to iron(III) (see Table 5.5). This shows that the total copper concentration in the aqueous phase does not necessarily equate to the total bioavailable copper [162] as it is well known that biopolymers produced by bacteria chelate heavy metals, including copper, reducing their toxicity [163].

Further, the growth of some filamentous bacteria is enhanced in the presence of calcium. This could increase the production of biopolymers that would protect the bacteria from the toxic effects of copper, as observed in Run 8. When iron(III) was added it could be acting as a flocculent, binding the biopolymers and reducing the filamentous bacteria's defence against the copper. The addition of iron(III) does not appear to alter the toxic effects of the copper on filamentous bacteria in comparison to Run 7 where copper was added as a single amendment. This could explain why in the pots amended with calcium and magnesium the concentration of copper in the effluent was higher, which could have been due to the solubility of calcium and magnesium. allowing the available copper to chelate with the biopolymers in the liquor [163]. This would reduce the bioavailable

copper and its toxicity. In this case the copper would pass though a glass fiber filter and be detected in solution.

Overall the SVI was significantly decreased in five of ten pots amended with copper, while three that were not affected were those where copper was added with either calcium or magnesium. This indicates that copper as a single amendment at concentrations between 0.1 mg/L – 1.0 mg/L has a significant effect on the abundance of filamentous bacteria in activated sludge. In all the copper amended porous pots the effluent quality as measured by COD was not significantly altered indicating that the overall micro fauna in the activated sludge was not adversely affected by the addition of copper, and its effects were limited to filamentous bacteria.

7.4 Conclusions

With the exceptions of all test pots in Run 5 and a cobalt addition pot in Run 7, the abundance of ciliates and rotifers were not detrimentally affected by the addition of metals during porous pot runs. In Run 5 the mean number of ciliate cells observed in all the trace metal amended pots (1.0 mg/L copper, 0.10 mg/L cobalt and 4.0 mg/L iron(III), magnesium and calcium) was significantly lower than in the control. In Run 7 the addition of 0.025 mg/L cobalt led to the number of rotifer cells being significantly lower that the control and other test pots in the run.

Increasing the temperature to ≥ 38°C in the porous pots had a significant adverse effect on the AS biomass and COD removal in three high temperature trial pots (38°C, 40°C and 42°C). The response in the three pots was similar. Initially the protozoan and metazoan species declined followed by deterioration of the floc structure, that coincided with decreasing effluent quality. At the completion of the temperature trials there were no flocs remaining in the porous pots. The mean COD removal in all trial pots at elevated temperatures was reduced to between 38% and 49%. This indicates that if the porous pot temperature results were transferred to the operation of the NSB SETP, increasing the temperature to ≥ 38°C would have a detrimental effect on the COD removal and increase the SS in the effluent.

In all the porous pot runs bulking occurred to some extent. This was not detrimental to the effluent quality measured by the COD removal and the SS levels. In Run 6, with a high organic loading there was a filament bloom of Type 021N and *H. hydrossis*, and in these conditions there was considerable foaming and an increase in the DO requirements of the flocs. Although Type 021N had been observed previously during the run associated with the high organic load, *H. hydrossis* was only

observed in the high organic loading and low DO conditions. Under these conditions denitrification occurred, though the F/M ratio and the SVI did not indicate changes in the operating conditions. In future studies considerations would have to be made to be more flexible with making alterations to the operating parameters such as the organic loading and the F/M ratio to control bulking and to reduce the potential for a floating sludge blanket.

The addition of copper to the porous pot activated sludge affected the abundance of filamentous bacteria, and this was supported through the statistically significant reductions in the SVI. The SVI was significantly reduced in five of the ten pots where there was copper addition, either as a single or multiple amendment. Although the addition of trace metals has been reported to decrease the SVI in activated sludge, a reduction was achieved only through the addition of copper. Though the mechanism for inhibition of filamentous bacteria was not investigated, there was either direct inhibition through the toxicity of copper to filamentous bacteria or the copper stimulated the growth of other bacteria. The inhibition of filaments through the addition of copper was neutralized in the presence of additional calcium or magnesium. There was also a significant reduction in the SVI from the addition of 0.5 mg/L cobalt during Run 2, where there was no observed difference in the abundance of filamentous bacteria.

Growth of filamentous bacteria appeared to be stimulated by the addition of 1.0 mg/L pyridoxine or folic acid in Run 4, supported by a significant increase in the SVI. The SVI was significantly increased by metal additions on only two occasions, both during Run 7, with the addition of 0.1 mg/L and 0.025 mg/L cobalt.

Chapter 8 Conclusions

Norske Skog commissioned a new secondary effluent treatment plant in January 2008 to treat effluent from its pulp and paper mill at Boyer, Tasmania. At the time this mill produced newsprint and high quality magazine paper from a mixed feedstock of *P. radiata* and *Eucalypt*. During the first two years of operation of the SETP the mean COD and SS removals were 76 ± 6% and 91 ± 8%, respectively. Statistical analysis of the data collected at Boyer from 2008 to 2010 showed that the COD and SS removals were unaffected by the operational and feed stock changes that occur with paper grade changes. A significant relationship between the COD of the primary clarifier and COD removal in the SETP was shown where COD removal was found to be directly proportional to COD input and COD removal was not limited by saturation of the SETP. To drive improvement of the water quality being discharged into the Derwent River, Norske Skog set a COD removal target of 90% to be achieved by 2011.

A survey of the levels of macro and micronutrients was undertaken to identify key deficiencies in the plant effluent that potentially could impact on growth and diversity of the biota in the activated sludge process. The survey showed that copper, cobalt, iron and molybdenum were below recommended levels, magnesium and zinc were within the recommended levels and calcium was well above the recommended minimum level.

In October 2009 the feedstock at Boyer was changed from mixed *P. radiata* and *Eucalypt* to solely *P. radiata* and the cold caustic soda (CCS) plant was decommissioned. No significant differences in the COD, SS and trace metal levels were found between the samples collected before and after the CCS decommissioning, and there was also no change in the COD or SS removal efficiency.

Experiments were undertaken to investigate the effects of micronutrient addition on effluent quality, including the fate of added trace metals and their effects on biota, using porous pots to simulate the SETP. The effect of additions of the micronutrients (copper, cobalt, iron, molybdenum and zinc), macronutrients (calcium and magnesium) and vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and folic acid) were studied individually and in combination.

Of the individual trace metals investigated copper was found to give the greatest improvement in COD removal with a 12% increase, from a mean 70% in a control to 82% COD removal through the addition of 0.5 mg/L copper. Lower additions of copper (0.1 mg/L) resulted in only a 3% increase compared to the control pot while higher additions (1.0 mg/L) showed no significant improvement compared to the control pot. Copper was found to inhibit the growth of filamentous bacteria,

especially Type 021N. The decrease in filamentous bacteria caused a reduction in the SVI which had a beneficial effect on settlability, decreasing the problem of bulking which is detrimental to the operation of the AS process. There was no adverse affect on the ciliate or rotifer populations from any of the copper amendments, however, the continuous addition of 1.0 mg/L copper could have inhibitory effects on all activated sludge bacteria over time.

A study of the fate of the trace metals showed that between 22 – 26% of copper added to porous pots during single metal addition experiments was accounted for in the sludge. Of the metals studied only copper and cobalt have guideline levels set by the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Maximum acceptable levels of these two metals are also given in the Tasmanian Biosolids Reuse Guidelines. Based on the copper concentration in the porous pot effluent during the addition of 1.0 mg/L copper its concentration in the Derwent River was estimated to be 2.2×10^{-3} mg/L, well above the guideline of 1.4×10^{-3} mg/L. It was estimated that addition of copper between 0.1 mg/L and 0.5 mg/L would increase the copper concentration in the Derwent River to 3.0×10^{-4} mg/L and 9.3×10^{-4} mg/L respectively, both below the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. The levels for copper in the sludge (1.9×10^{-3} mg/kg) during a 1.0 mg/L copper amendment was well below the limit of 100 mg/kg copper given in the Tasmanian Biosolids Reuse Guidelines.

Of the other trace metals investigated iron and cobalt were found to improve COD removal by 5% and 4%, respectively, compared to the control with no effect on the SS levels. Approximately 50% of the iron and the majority of the cobalt were found in the effluent. The increase in COD removal during cobalt addition experiments also coincided with an increase in SVI, indicating that the available cobalt could have been used to synthesise cobalamin which stimulated the growth of activated sludge biota, especially filamentous bacteria.

Additions of calcium and magnesium, both macronutrients, were found to improve COD removal by 4% compared to the control. This improvement is expected to be largely through the formation of cation bridges between the divalent cations and negatively charged humic substances leading th the removal of the humic material from solution. The results for magnesium were also supported by the outcomes of the operational change at NSB where MgO was used to replace NaOH for pH amendment. This change led to an improvement in the mean COD and SS removal rates of 6% and 5%, respectively. This result is important as it shows that porous pot COD removal results are directly transferable to the NSB SETP.

The combined effects of the trace metals were also examined. It was expected that the gains from the addition of individual metals would be enhanced by multiple metal additions to trial pots. From the multiple metal trials only 4.0 mg/L iron(III)/0.5 mg/L copper and 4.0 mg/L calcium/0.5 mg/L copper gave significant improvements of 2% in COD removal compared to control pots. With the multiple trace metal additions the biological inhibition of filamentous bacteria from 0.5 mg/L copper was negated when it was added as a component of a multiple amendment with magnesium or calcium. When copper was added with magnesium or calcium the proportion of copper in the effluent increased to between 72% and 96%, respectively, significantly higher than the levels in the effluent when only copper was added (50%). In these cases COD removal was only improved by 2% through the addition of copper and calcium.

The addition of water-soluble vitamins was found to have only a very small effect on improving COD removal in the porous pots. Among the vitamins studied only the addition of 1.0 mg/L folic acid had a statistical significant effect on COD removal (2%). There was direct inhibition of the activated sludge through the addition of 1.0 mg/L niacin. This not only indicated that niacin was not required in the NSB plant, but that if it was used as an amendment the effluent quality would significantly decrease. Based on an increase in the SVI, the addition of pyridoxine (1.0 mg/L) or folic acid (1.0 mg/L) appeared to stimulate filamentous growth, which exacerbated bulking control problems. In the case of pyridoxine the SS levels were also adversely affected. As there were minimal changes in the COD removal through vitamin addition it appeared that increased biological growth, indicated through an increase in SVI, did not affect the recalcitrant humic substances in pulp and paper wastewater.

To better determine the nature of the residual oxygen demanding matter (ODM) contributing to the COD in the effluent, the porous pot influent and effluent samples were fractionated into three acidic fractions: HPhoA, HPhiA and TPhiA. The total ODM determined from the porous pot influent contained 20 to 30% HPhoA, 15% TPhiA and 25 to 35% HPhiA. Of these fractions the total residual ODM in the porous pot effluent after treatment was between 27 to 45% HPhoA, 6 to 15% TPhiA and 4 to 9% HPhiA during optimal conditions. These results had no relationship to the micro-nutrient additions.

The most significant contribution to increasing effluent quality through secondary treatment was from the removal of the HPhiA fraction, where the residual compounds had a MW between 400 – 700 Da. The HPhoA fraction was the most significant contributor to the residual ODM in the porous pot effluent. This fraction had significant UV-Vis absorption between 230 and 300 nm indicating the presence of aromatic structures or C=C bonds which would be resistant to biological degradation,

however, there was minimal NMR resonance in the chemical shift region between 6 – 8 ppm, resonances that would normally be associated with aromatic compounds. There was generally a decrease in the LMW compounds in the HPhoA fraction with the dominant residual compounds being between 400 – 4000 Da.

The addition of magnesium has already led to 6% and 5% improvement in COD and SS removal, respectively, at an industrial level. It is recommended that copper at 0.5 mg/L be added, with an expected 5% improvement in the COD removal from the NSB SETP and that the addition of water-soluble vitamins should not be pursued. The results from the fractionation of humic substances from the porous pot influent and effluent suggest that further research is needed to determine methods for the removal of the recalcitrant HPhoA fraction to increase COD removal from the NSB SETP.

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Appendix

Appendix A Graphite Furnace Temperature Ramps

Table B.1: Cobalt graphite furnace temperature ramp.

Step No.	Final Temp (°C)	Ramp Time (Sec)	Hold Time (sec)	Gas
1	30	1.0	2.0	Off
2 Inject	-	-	-	On
3	90	10.0	35.0	On
4	120	15.0	10.0	On
5	900	10.0	5.0	On
6	900	0.0	1.0	Off
7 Read	2400	0.8	1.0	Off
8	2400	0.0	3.0	On

Table B.2: Copper graphite furnace temperature ramp.

Step No.	Final Temp (°C)	Ramp Time (Sec)	Hold Time (sec)	Gas
1	50	1.0	2.0	Off
2 Inject	-	-	-	On
3	90	10.0	35.0	On
4	120	15.0	10.0	On
5	600	10.0	5.0	On
6	600	0.0	1.0	Off
7 Read	2600	1.0	1.2	Off
8	2600	0.0	3.0	On

Table B.3: Molybdenum graphite furnace temperature ramp.

Step No.	Final Temp (°C)	Ramp Time (Sec)	Hold Time (sec)	Gas
1	30	1.0	2.0	Off
2 Inject	-	-	-	On
3	90	10.0	30.0	On
4	120	55.0	15.0	On
5	1100	10.0	20.0	On
6	1100	0.0	1.0	Off
7 Read	2800	1.2	0.7	Off
8	2800	0.0	3.0	On

Appendix B Boyer Data Analysis and Boyer Metal Data

Table A.1: Mean COD and SS data for different paper grades at Boyer before and after the TMP 3 commission.

% Removal	**Before TMP3**		**After TMP 3**	
	COD	**SS**	**COD**	**SS**
NorBright	76 ± 6	93 ± 6	77 ± 6	90 ± 10
NorStar	76 ± 4	92 ± 8	77 ± 6	91 ± 9
Norstar Super	76 ± 6	91 ± 7	75 ± 6	93 ± 7
NorNews	76 ± 7	91 ± 12	76 ± 6	90 ± 8

Table A.2: Correlation analysis between SETP operating conditions and the mean COD and SS removal.

Correlation (association)		P-Value
%COD removal	Before/After	0.551
	ASR DO	0.399
	BFR DO	0.571
	Sludge Blanket	0.209
	Sludge Age	0.657
%SS Removal	Before/After	0.502
	ASR DO	0.586
	BFR DO	0.639
	Sludge Blanket	0.469
	% COD Removal	0.377

Appendix C Summary Table of Boyer Metals

Table C.1: Concentrations of each trace element from all sample sites, showing the combined (Before & After TMP 3) average concentration and the concentration range. Note the concentrations of the trace elements; Ca, Fe, Mg and Zn are mg/L and Co, Cu, and Mo are µg/L.

Metal		Pri In	Pri Eff	PM 2	TMP	Sec In	Sec Eff	TPM 3	PM 3	Screen	River	Bleach	BFR	RAS	WAS	ASR
Ca (mg/L)	High	42.83	37.80	20.06	26.48	31.14	35.53	39.50	49.51	42.40	40.93	20.64	33.59	33.47	35.42	33.70
	Ave	20.2 ± 9.6	21.0 ± 9.5	11.2 ± 4.1	13.7 ± 3.6	19.2 ± 7.8	19.5 ± 8.0	16.5 ± 10.4	24.2 ± 13.7	12.9 ± 11.8	15.9 ± 8.3	14.8 ± 4.8	19.1 ± 7.6	19.8 ± 7.7	21.4 ± 8.5	19.3 ± 7.8
	Low	6.42	3.32	4.49	8.08	6.51	3.96	4.16	9.57	2.89	0.72	4.57	7.28	8.11	8.93	6.88
Co (µg/L)	High	7.51	8.52	5.58	4.63	5.39	5.86	5.38	6.07	8.94	4.58	6.61	4.98	5.69	5.34	5.55
	Ave	3.9 ± 1.5	3.9 ± 1.5	2.9 ± 1.2	2.8 ± 1.0	3.8 ± 0.85	3.8 ± 1.1	4.0 ± 0.75	3.4 ± 1.4	2.1 ± 2.0	2.4 ± 1.1	3.8 ± 1.3	3.4 ± 1.0	3.7 ± 1.0	3.6 ± 0.8	3.7 ± 0.8
	Low	1.21	1.40	1.03	1.48	2.74	1.82	2.84	0.97	0.24	0.95	1.21	1.23	1.73	2.42	2.45
Cu (µg/L)	High	663.89	67.13	383.54	72.86	63.32	482.49	168.56	780.48	187.85	321.05	415.96	316.38	108.27	90.12	121.87
	Ave	35.6 ± 19.8	27.2 ± 16.5	36.2 ± 21.5	31.9 ± 16.8	24.9 ± 15.4	26.3 ± 21.9	50.9 ± 18.3	36.8 ± 21.6	29.9 ± 34.6	16.7 ± 12.1	45.6 ± 28.5	24.2 ± 15.6	31.9 ± 24.1	29.4 ± 21.3	32.4 ± 28.4
	Low	7.83	5.54	5.12	6.42	5.06	4.35	22.76	4.99	4.58	2.35	5.63	6.04	3.46	4.40	0.00
Fe (mg/L)	High	0.55	0.52	0.40	0.36	0.47	0.58	0.86	0.72	0.54	0.34	0.52	0.45	0.96	1.43	0.57
	Ave	0.32 ± 0.1	0.28 ± 0.12	0.2 ± 0.11	0.20 ± 0.08	0.19 ± 0.14	0.24 ± 0.14	0.39 ± 0.18	0.25 ± 0.16	0.13 ± 0.10	0.15 ± 0.09	0.25 ± 0.15	0.25 ± 0.11	0.38 ± 0.25	0.68 ± 0.35	0.22 ± 0.14
	Low	0.07	0.07	0.00	0.03	0.00	0.00	0.12	0.01	0.00	0.00	0.00	0.02	0.00	0.03	0.00
Mg (mg/L)	High	7.65	7.60	3.74	12.55	7.39	8.14	7.81	6.54	4.24	83.93	7.57	6.69	6.77	7.89	6.89
	Ave	5.3 ± 1.3	5.2 ± 1.3	2.7 ± 0.65	6.2 ± 2.4	5.3 ± 1.3	5.3 ± 1.4	3.3 ± 1.7	4.0 ± 1.2	2.3 ± 0.96	*17.1 ± 19.0	5.0 ± 1.8	4.8 ± 1.2	5.1 ± 1.1	5.6 ± 1.3	5.0 ± 1.2
	Low	2.87	2.77	1.27	3.29	3.10	3.10	1.46	1.95	0.45	2.46	0.80	2.54	2.72	3.14	2.69
Mo (µg/L)	High	10.56	9.19	1203.00	3.90	4.58	4.99	13.03	20.33	13.00	4.99	3.17	6.47	4.67	3.97	5.02
	Ave	3.1 ± 2.7	2.9 ± 2.3	6.9 ± 3.6	1.1 ± 1.2	2.14 ± 1.3	2.5 ± 1.4	2.3 ± 3.2	5.1 ± 4.9	2.3 ± 3.2	2.1 ± 1.3	0.78 ± 0.97	0.21 ± 1.9	1.6 ± 1.3	0.86 ± 1.1	2.0 ± 1.5
	Low	0.00	0.00	0.00	0.00	0.26	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn (mg/L)	High	0.25	0.24	0.28	0.24	0.39	0.31	0.12	0.37	0.13	0.70	0.51	0.39	0.53	0.57	0.39
	Ave	0.18 ± 0.06	0.16 ± 0.03	0.15 ± 0.06	0.14 ± 0.05	0.23 ± 0.06	0.15 ± 0.06	0.09 ± 0.03	0.17 ± 0.08	0.08 ± 0.04	0.10 ± 0.11	0.28 ± 0.12	0.22 ± 0.09	0.27 ± 0.10	0.25 ± 0.09	0.24 ± 0.08
	Low	0.06	0.09	0.05	0.08	0.13	0.10	0.06	0.07	0.02	0.04	0.07	0.10	0.03	0.09	0.07