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MICRONUTRIENTS FOR WASTEWATER TREATMENT

SCHOOL OF WATER SCIENCES
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PhD THESIS

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Abstract

The results of a postal survey strongly suggested that new developments in the optimisation of biological treatment processes would greatly increase the ability of wastewater-treating industries to adapt to Direct Toxicity Assessment (DTA). Trace metals (K, Fe, Mg, Cu, Ca, Mn, Al, Zn, Mo, Co) and vitamins (biotin, niacin, pyridoxine, lactoflavin, thiamine, pantothenic acid) were the micronutrients tested. Respirometry indicated that micronutrient addition could not ameliorate macronutrient deficiencies, but could significantly improve the degradation of hard chemical oxygen demand (COD) in the wastewater (up to 4.24kg COD/kg MLSS/d, i.e. 320% of the control) with no significant effect on the air requirement of the sludge. Complex interactions between trace metals that were dosed simultaneously were evident (e.g. Ca with other metals). Several positive effects led to the conclusion that micronutrients have the potential to optimise the process performance of activated sludge plants treating industrial wastewater. Porous pots were used to trial eight of the micronutrients. The retention of biomass in the pots was increased in all cases. Improvements in the degradation of COD (up to 260% of the control) were observed while biological oxygen demand (BOD) degradation was not affected. This implied the use of recalcitrant substrate components as a food source. Toxicity tests showed that the effluents from the experimental porous pots were less toxic than the control effluents. The effects of niacin addition in activated sludge treatment of industrial waste at pilot-scale were: improved sludge handling, increased COD, ammonia, SS and phosphorus removal. Mean test system COD removal efficiency was 123% of the control. The results of phosphorus and niacin dosing at pilot-scale confirmed the trends observed in the porous pots. The results at all scales indicated that micronutrient addition could be a valuable tool for companies wishing to improve aerobic biological treatment of industrial wastewaters. Interviews were used to assess the potential value of micronutrient addition in responding to DTA. Several industrialists saw micronutrient addition as a route to successful adaptation.

If all is not lost, where is it?

CHAPTER ONE

INTRODUCTION AND SYNOPSIS

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Notation

ANOVA	analysis of variance
BATNEEC	best available technology not entailing excessive cost
BOD	biological oxygen demand
BOD_e	effluent biological oxygen demand
BOD_5	five-day biological oxygen demand
cl	confidence limits
COD	chemical oxygen demand
df	degrees of freedom
DO	dissolved oxygen
DTA	direct toxicity assessment
EA	the Environment Agency
EC	effect concentration
ECP	extracellular polymer
EPA'90	the Environment Protection Act, 1990
GAC	granular activated carbon
HRT	hydraulic retention time
IC	immobilisation concentration
LOEC	lowest observed effect concentration
MCRT	mean cell retention time (sludge age)
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
NOEC	no observed effect concentration
PAC	powdered activated carbon
R&D	research and development
RAS	return activated sludge
RSD	relative standard deviation
SD	standard deviation
SS	suspended solids
SSVI	stirred specific volume index
SVI	sludge volume index
WET	whole effluent toxicity
WRA'91	Water Resources Act 1991

1.1 INTRODUCTION

The possible introduction of direct toxicity assessment (DTA) into the UK has brought about the need for investigating enhanced chemical oxygen demand (COD) and toxicity removal. DTA is the consideration of the effluent as a whole in terms of the impact it may have on receiving waters and is designed to provide a simple and easily understood measure for the protection of aquatic life from potentially harmful effluent discharges. It allows for the control of toxic discharges, the setting of toxicity reduction targets and provides for the assessment of improvements in the quality of receiving waters (Environment Agency, 1996).

Currently, any individual or company that discharges effluent is bound under Section 7 of the UK Environmental Protection Act (EPA), 1990 (HMSO, 1990) to use the *best available technique not entailing excessive cost* (BATNEEC) to "render harmless any materials released into environmental media". In addition, it is an offence under section 85 of the Water Resources Act (WRA), 1991 (HMSO, 1991) "to cause or knowingly permit any poisonous, noxious or polluting matter or any solid waste matter to enter controlled waters". The exceptions to these rules are discharges made into a receiving water with an EPA'90 or WRA'91 authorisation or consent. Discharge consents are set out as numeric targets, usually concentrations of key chemical components. The advantages of this system lie in the ease of measurement, and hence enforcement, as well as in the capacity of the system to diagnose the source of pollutants discovered in the receiving waters. The shortfalls of numeric targets include the physical limitations (no company can measure every component of its effluent), the lack of chemical-specific toxicity data (leading to pointless measurements with no indication of polluting potential), and the complete failure to account for interactions between wastewater components.

The ultimate goal for the UK aquatic environment is the meeting of narrative targets, i.e. use-related water quality objectives (Tinsley, 1998). These targets include consent clauses but do not include any specific methods for compliance. Currently, compliance

with numeric targets does not always equate to compliance with the narrative targets. The aim of the Environment Agency (EA) is to introduce DTA as an additional measure of effluent quality in order to apply a more ecologically relevant test and provide an early warning system to avert pollution incidents. DTA is a measurement of the toxicity of an entire effluent, rather than the concentration of specific effluent components. It can be used as a trigger for action to minimise lethal toxicity from point source discharges. DTA should improve the public image of industries shown to produce effluents of low toxicity but will have major impacts on those whose effluents are toxic, but have previously complied with their numeric targets.

Under the name of Whole Effluent Toxicity (WET), DTA has been implemented in the USA where it has been considered successful (East of Scotland Water, 1996). From its introduction in 1991 it is now fairly well established and the toxicity tests employed vary from region to region according to the indigenous aquatic life. WET testing is regarded as generally sound and representative of small receiving waters such as lakes and narrow rivers. However, problems exist both in the application of tests to estuarine receiving waters, and with the variability of test accuracy caused by the misapplication of tests, misinterpretation of data and a lack of training in laboratory personnel. These may be significant issues for the implementation of DTA in the UK.

Differences between the USA and the UK lie mainly in the roles of the water and sewerage companies. The sewerage undertakers in the UK treat the discharge from a number of industrial operators alongside domestic sewage and road runoff. This makes a great difference to the variability of the influent in the wastewater treatment works in the UK and makes the ten water companies more vulnerable to consent failure and its associated fines. Companies can be categorised according to their ability to adapt to the introduction of new legislation, so the importance of long term process adaptation as opposed to pure optimisation of existing processes has been recognised. Wastewater discharged to sewer is treated at wastewater treatment works comprising several stages of treatment. The majority of treatment works include a biological process, usually aerobic and often as a mixed suspended culture of micro-organisms, or activated sludge.

Activated sludge treatment of waste is an aerated oxidation process. It is one of the best established and widespread biological wastewater treatment processes in the developed world for both domestic and industrial wastewaters (Clark and Stephenson, 1998), and as such its adaptability to accommodate new demands in effluent quality is of great importance. The process relies on the suspension of a microbial population mixed with wastewater under aerobic conditions. Microbial growth brings about the removal of organic matter from the waste as the compounds in the feed are oxidised by the micro-organisms present in the sludge. The end results are microbial biomass and products of oxidation such as CO_2, NO_3^-, SO_4 and PO_4. Activated sludge plants have been used to treat a wide range of industrial wastes by effectively accelerating natural processes involving chemical, biological and physical agents.

A typical activated sludge plant can be schematically represented (Figure 1.1) and includes two phases: an aeration basin and separator or clarifier. In the first phase, aeration, the wastewater is added to the microbial biomass and air added via diffusers or by surface agitation. This aerates and maintains the suspension, allowing maximum contact between the flocs and the waste. Complete mixing ensures an adequate food supply for the microbial cells and maximises the oxygen gradient to optimise mass transfer and disperse the products of metabolism from inside the flocs. Wastewater entry displaces mixed liquor (i.e. mixed water and biomass) into phase two, the clarifier, where the flocculated biomass settles into sludge and clarified final effluent. Some sludge is returned to the aeration tank and the remainder is disposed of. Between 0.4 – 1.0kg dry weight of sludge is produced per 1.0kg biological oxygen demand (BOD) removed from the wastewater (CIWEM, 1997). The floc nature of the biomass is very important as it controls the efficient absorption and adsorption of organics from the waste and the separation of the sludge from the water in the settling tank.

Many bacterial species proliferate in colonies or flocs, which become dense enough to settle out of water. The aeration in an activated sludge plant speeds up the growth of the bacteria present at the outset and increases the number of collisions between flocs and hence their chance of aggregation into larger flocs containing non-living particles. This

process occurs within a set range of environmental conditions, which limit the activity of the organisms responsible for the treatment process. For this reason, biological wastewater treatment requires certain environmental parameters such as dissolved oxygen (DO) levels, mixing regime, provision of nutrition, trace element supply and physical conditions such as temperature and pH. The residence time of the cells within the plant must be sufficient to allow reproduction to occur in order for the influent waste to be treated effectively. As reproduction rates depend on growth and hence on metabolic rate, nutrient paucity (and associated slow cell growth) coincide with poor waste treatment.

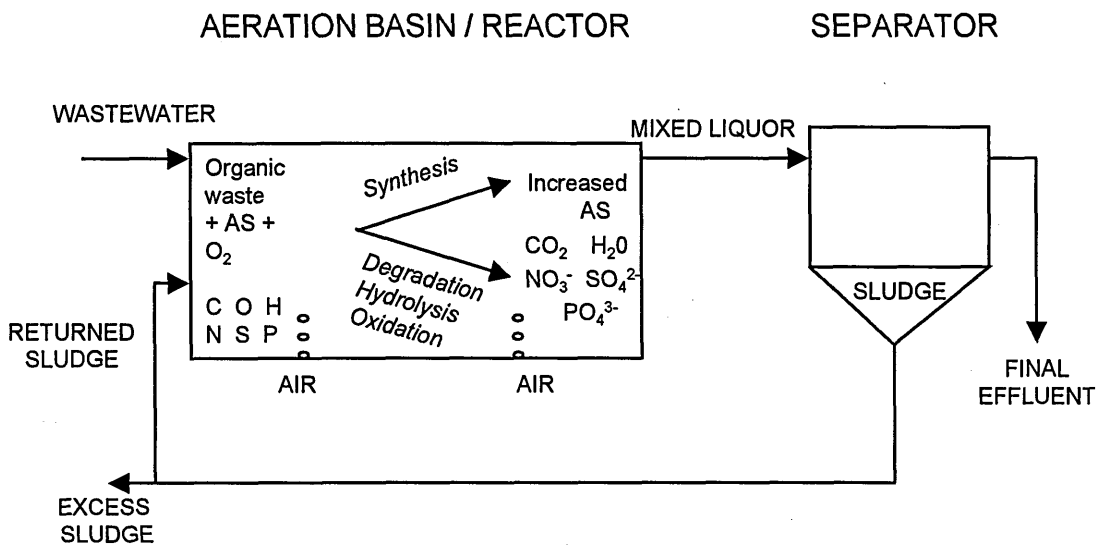

Figure 1.1 Schematic representation of a typical activated sludge plant.

The overall aim of this biological treatment process is to make carbon the limiting factor and hence nutrition must be balanced to achieve the lowest possible quantities of carbon in the effluent (Speitel and Segar, 1995). Until recently, activated sludge process performance has been measured in terms of the minimum effluent BOD and suspended solids (SS). However, increasing emphasis on effluent toxicity and the removal of priority pollutants has highlighted the need to remove COD and recalcitrant organic compounds (or 'hard COD'), in particular from industrial wastewater streams. Enhanced COD removal is possible by changing operating procedures, but such

techniques can often produce inconsistent results (Singleton, 1994). Environmental factors can affect biodegradation by changing the availability of nutrients and target compounds, thus preventing the growth of micro-organisms (Daubaras and Chakrabarty, 1992). Recalcitrance is a problem in industrial wastewater treatment owing to an abundance of xenobiotic compounds which resist degradation; it can arise from inappropriate conditions, inadequate nutrients and the supply of a substrate which does not activate the appropriate enzymes (Eckenfelder and Musterman, 1994). The availability of nitrogen and phosphorus can be limiting factors in the degradation of hydrocarbons and supplements where concentrations are low (*bioaugmentation*) can lead to improved wastewater treatment (Leahy and Colwell, 1990). Bioaugmentation includes the addition of substances such as micronutrients which increase cometabolism or which induce degradive genes (Singleton, 1994).

The role of micronutrients in aerobic biological wastewater treatment is not well defined. To achieve sufficient treatment of industrial wastewater, it is necessary to supply activated sludge with the micronutrients that enable the strains capable of degrading the recalcitrant compounds to thrive and produce a low COD effluent, free of recalcitrant components. COD consists of hard COD, the recalcitrant fraction, and 'soft COD', which is more readily degradable. Enhanced COD removal indicates increased degradation of recalcitrant compounds, which are often responsible for the toxicity of a wastewater stream. Recent research into advanced COD removal has focused on changes in operating conditions (Franta *et al,*. 1994), but this normally results in higher investments and operating and maintenance costs, but not always in lower effluent COD. The overall aim of the work here is to discover how micronutrients may provide a method for establishing low effluent toxicity and COD concentrations, thus allowing wastewater treatment to develop in response to the ever-increasing environmental legislation to which it is subject.

1.2 SYNOPSIS

This research addresses the need for low-cost optimisation of aerobic biological treatment of recalcitrant industrial wastewater. The wastewater used throughout this study was taken from the return activated sludge line of an activated sludge plant that pre-treats pH-balanced, screened wastewater from a chemicals manufacturer and discharges it to a municipal wastewater treatment works. The general aims were to increase process performance, increase and stabilise the maintenance of mixed liquor suspended solids (MLSS) concentrations and to anticipate the future need for improved toxicity removal. Micronutrients dosed directly into the MLSS appeared to be a potential option. A review of previous work on micronutrient addition (Chapter 2) and the results of a postal survey (Chapter 4) supported these initial aims. The survey data suggested strongly that new developments in the optimisation of biological treatment processes would greatly increase the ability of wastewater treating industries to accommodate new, toxicity-based targets for effluent quality.

A series of respirometer screening tests was performed using the parameters of COD removal efficiency and oxygen uptake of activated sludge receiving the chemical wastewater supplemented with doses of micronutrients (Chapters 5 and 6). The tests were looking for micronutrients that showed a potential for maximising COD removal without compromising the condition and sustainability of the biomass. The first phase of testing (Chapter 5) involved balancing the nitrogen and phosphorus content of the wastewater before addition of micronutrients. Six trace metals and six vitamins were used as chemical additives dosed into the mixed liquor. Control sludge batches (receiving no micronutrient supplements) attained an average COD removal rate of 1.941kg COD/kg MLSS/d. Dosing micronutrients into the mixed liquor produced COD removal rates of up to 2.240kg COD/kg MLSS/d. The greatest improvement in wastewater treatment was attained by the addition of 1.0mg/l niacin to the wastewater, resulting in an unchanged oxygen uptake rate (0.011kg O_2/kg MLSS/d) and increased COD removal (2.240kg COD/kg MLSS/d). The results suggested that the vitamins biotin, pantothenic

acid and niacin were required by activated sludge bacteria and that calcium enhanced the stimulatory effects of niacin and manganese.

The second phase (Chapter 6) employed the same wastewater, but without N and P balancing. This meant the effluent was phosphorus-limited, according to the standard ratios for COD:N:P. Six trace metals and three vitamins were used as chemical additives dosed into the mixed liquor. All of the supplements resulted in increased COD removal rates. Control sludge batches (receiving no micronutrient supplements) attained an average COD removal rate of 1.335kgCOD/kg MLSS/d. Dosing micronutrients into the mixed liquor produced improved COD removal rates. The largest improvement in COD removal was attained by the addition of pyridoxine to the wastewater, resulting in a slightly greater oxygen uptake rate (0.036kg O_2/kg MLSS/d) and a COD removal rate of 4.239kg COD/kg MLSS/d, a threefold increase compared with the control. All of the vitamin supplements resulted in improved COD removal. The results suggested that the vitamins niacin, lactoflavin and pyridoxine were required by activated sludge biomass under phosphorus–limited conditions.

In general, the results indicated that while micronutrient addition could not ameliorate macronutrient deficiencies, certain micronutrients were able to improve significantly the degradation of COD in the wastewater with little or no effect on the oxygen demand of the sludge. It was concluded that micronutrient supplements had the potential to optimise biological treatment of industrial wastewaters which are nutrient-limited or changeable.

After the micronutrient screening, it was concluded that areas in which further study was required included longer term, larger scale degradability tests that would ascertain the levels of BOD and COD removal achievable, the effects of micronutrients on the sludge population, and the potential of micronutrient additions to remove toxic components from the industrial wastewater. Small scale activated sludge reactors were used to test trace elements (Chapter 7) and vitamins (Chapter 8) over periods of six sludge ages.

The bench scale work demonstrated how trace elements could provide a method for establishing low effluent COD concentrations and removing recalcitrant constituents from industrial wastewaters (Chapter 7). The research focused on the impact of trace metal supplementation to industrial waste in terms of the performance of activated sludge reactors treating a high-strength, recalcitrant effluent. A four-lane porous pot testing facility fed with industrial wastewater (COD:BOD ratio 4:1; COD 2000mg/l and BOD 500mg/l) was employed to ascertain the optimal trace element type and level of macronutrient availability. Supplements of a wide range of micronutrients were supplied to the reactors, and their ability to improve the biodegradation of the wastewater was assessed in terms of BOD and COD. Retention of biomass in the reactors was increased in all cases, in excess of 100% with specific trace metals. Improvements in the degradation of COD were observed while BOD degradation was not affected. This implies the use of recalcitrant substrate components as a food source. Results from this study demonstrated significant improvements in the process's ability to biodegrade recalcitrant COD. The removal of recalcitrant COD and hence of priority pollutants and toxicity was improved without the need for the expansion of existing wastewater treatment or pre-treatment plants.

Parallel to the trace element work, vitamins were dosed continuously in the bench scale activated sludge simulations (Chapter 8). Measuring BOD_5 and COD removal, oxygen uptake and MLSS monitored the effect on process efficiency. After 48d of chemical treatment the COD removal of the niacin-treated simulation was significantly higher than that of the control (removal rate kg/kg MLSS/d = 260% of control). BOD_5 removal from treated and control samples was not significantly different.

The best results in terms of sustained improvements in BOD_5 removal efficiency were observed in the reactors dosed with phosphorus/pyridoxine and phosphorus/1.0mg/l niacin. The porous pot receiving pyridoxine alone removed less of the influent BOD than the control and the two pots supplied with the lower doses of phosphorus/niacin and phosphorus/niacin/calcium were no more efficient than the control: these additions produced higher maximum BOD removal, but more variable reactor performance. The

trends in COD removal efficiency were similar for each of the test porous pots, indicating that the phosphorus plus niacin dosed reactor performed significantly better than all of the others during the dosing period.

Toxicity tests were performed using an Amtox™ toxicity monitor. As the Amtox™ uses the ammonia removal efficiency of nitrifiers to measure toxicity, the results were indicative of the potential impact the sampled wastewater may have on a sewage treatment works. Amtox™ has been discussed in the literature (Upton and Pickin, 1996) and is under consideration for use as a tool in DTA. Toxicity of the sampled wastewater is represented by a loss of ammonia removal efficiency over the test period. The toxicity of the porous pot effluents was expected to correlate quite closely with the mean effluent COD concentrations, but this was not the case. All of the effluents from the experimental reactors were less toxic than the control reactor effluent samples. Supplements of molybdenum/lactoflavin and phosphorus/0.5mg/l niacin allowed the effluent to be considered to be non-toxic. The results indicated that the two most promising micronutrient additions warranted investigation at pilot-scale, where their effects on sludge handling and nitrification could be assessed in addition to the BOD and COD removal rates. Since effluent toxicity at bench-scale (Chapter 8) was seen to be positively correlated with effluent pH and COD concentrations, and negatively correlated with effluent ammonia, it was reasonable to infer that the same correlations apply at pilot-scale. If this is the case, the higher pH and COD concentration in the effluent from the control rig of the pilot system effluent (Chapter 9) implied greater toxicity in the control effluent than in that of the test plant.

The overall effects of nutrient addition in activated sludge treatment of industrial waste that were demonstrated at pilot-scale (Chapter 9) can be summarised as: improved sludge handling, increased COD, ammonia, SS and phosphorus removal. The results of phosphorus and niacin dosing at pilot-scale confirmed the trends observed at bench-scale, although scale-up problems associated with the methods for oxygen uptake measurement, and the effects of tank geometry on the accuracy of effluent SS data were identified. The results obtained imply enhanced toxicity removal by the activated sludge

and demonstrate the improved degradation of recalcitrant compounds indicated by increased COD removal which is not associated with increases in BOD_5 removal. The net effect was stimulation of the activated sludge micro-organisms with no inhibitory effects from micronutrient addition over a longer test period observed.

The net effect of micronutrient addition at pilot-scale is stimulation of biomass for the enhancement of process performance in terms of recalcitrant substrate removal. The technical work at all scales indicated that micronutrient addition could be a valuable tool for companies wishing to improve the levels of COD and toxicity removal from industrial wastewaters attainable by biological treatment processes. However, technical merit alone is not enough for a technology to become common practice, so an assessment was made of the potential of micronutrient addition for industrial wastewater treatment (Chapter 10). Work was planned to investigate the extent to which UK industry would be prepared to adopt micronutrient addition as an option for reducing wastewater toxicity in preparation for the introduction of DTA.

The work in Chapter 10 examined the role of micronutrient addition in the improvement of wastewater treatment processes to accommodate new legislation. Interviews with industrialists were used to explore the options open to companies to deal with risks, and to assess the potential value of micronutrient addition in removing the risks associated with DTA. All of the respondents had been involved in decision making for the removal of pollutive potential of industrial effluent and had participated in the nationwide process of conference and debate in the run–up to the introduction of DTA.

The work focused on the potential use of micronutrient addition in response to DTA. The results showed that around half of the participants had considered it as an option in the past, and the overwhelming majority of participants numbered micronutrient addition among their list of options for effluent treatment in the future. Among the comments made regarding micronutrient addition were several positive opinions: cost benefits, sustainability and performance featured strongly in the reasons to employ micronutrients, but there were also some negative comments: usually cost. Over 90% of the participants

included micronutrient addition in their lists of options for adaptation to future effluent quality limits. Many participants saw themselves vulnerable to the possible changes brought about by the introduction of DTA. However, with micronutrient addition among their options, companies with the opportunity to treat or pre-treat their own effluents perceived the risk to themselves as being reduced. They saw micronutrient addition as a route to successful adaptation to DTA, based on their companies' processes and the trials performed with micronutrient addition in biological waste treatment that have been carried out in Chapters 5 - 9. If micronutrient addition is made more evident and therefore available, then toxicity-based regulation could be implemented more easily.

CHAPTER TWO

LITERATURE REVIEW: MICRONUTRIENTS IN THE ACTIVATED SLUDGE PROCESS.

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LITERATURE REVIEW: MICRONUTRIENTS IN THE ACTIVATED SLUDGE PROCESS.

2.1 Introduction

Many industrial wastewaters show resistance to the biological treatment processes commonly used to treat municipal and domestic wastes. It had been expected that micro-organisms were theoretically capable of degrading any oxidisable material, provided suitable environmental conditions prevailed (Gale, 1952). However, many industrial processes now exist which result in the release of synthetic compounds unfamiliar to microbial cells and therefore resistant to biodegradation. The xenobiotic nature of these compounds allows them to accumulate in the environment. Industrial effluents often contain solvents and phenolic compounds, which are difficult to remove using conventional wastewater treatment. They vary in volatility, solubility and other properties and behave very differently under variable physical conditions (Singleton, 1994).

The various breakdown products found in activated sludge plants treating industrial effluents include phenols and quinones; some breakdown products are more toxic than the original compound (Allsop *et al.*, 1993; Cerniglia and Heitkamp, 1989; Schöberl *et al.*, 1988). This problem of toxic metabolic products in pure cultures can be surmounted by using mixed cultures, such as those occurring naturally in activated sludge, or a designed microbial consortium having a wider range of metabolic pathways. Mixed cultures are important in waste treatment for the complete mineralisation of organic toxins to carbon dioxide. They often enhance microbial metabolism by degrading toxic metabolic by-products and allowing cometabolism to take place (cometabolism is the degradation of substances by nonspecific enzymes produced by micro-organisms growing on a different substrate) (Atlas and Bartha, 1993). Cometabolism is a particularly useful process for the breakdown of xenobiotics (Entwhistle, 1986).

Activated sludge treatment of waste is an aerated oxidation process and is currently the most widely used biological wastewater treatment process in the developed world for both domestic and industrial wastewaters (Chisti, 1998; Moo-Young and Chisti, 1994; Gray, 1990). Activated sludge plants have been used to treat a wide range of industrial wastes by effectively accelerating natural processes involving chemical, biological and physical agents. Biological oxidation must compete technically and economically with other treatment processes, but is often slower than chemical processes or incineration, and can produce inconsistent end results. An increase in our understanding of the environmental requirements of micro-organisms and the introduction of the use of genetically- manipulated or pure culture organisms has meant that biodegradation has the potential to become an efficient and economically viable option (Singleton, 1994). Environmental factors often limit industrial wastewater treatment by biological processes (Buitrón and Capdeville, 1995; Eckenfelder and Musterman, 1994; Singleton, 1994; Daubaras and Chakrabarty, 1992; Valo *et al.*, 1985; Wood and Tchobanoglous, 1975).

2.2 Optimising the activated sludge process.

The overall aim of the activated sludge process is to make carbon the limiting factor, hence nutrition must be balanced to achieve the lowest possible quantities of carbon in the effluent (Speitel and Segar, 1995). A well-balanced activated sludge community will contain genera of bacteria, fungi, protozoa, rotifers and nematodes mixed in the flocs. *Cyclops, Aelosoma* and dipteran larvae may also be present (Gray, 1990). The material flow within activated sludge is shown in Figure 2.1 and involves three classes of organisms in the sludge, with rotifers and nematodes being the least common organisms and bacteria and saprotrophic protozoa the most common.

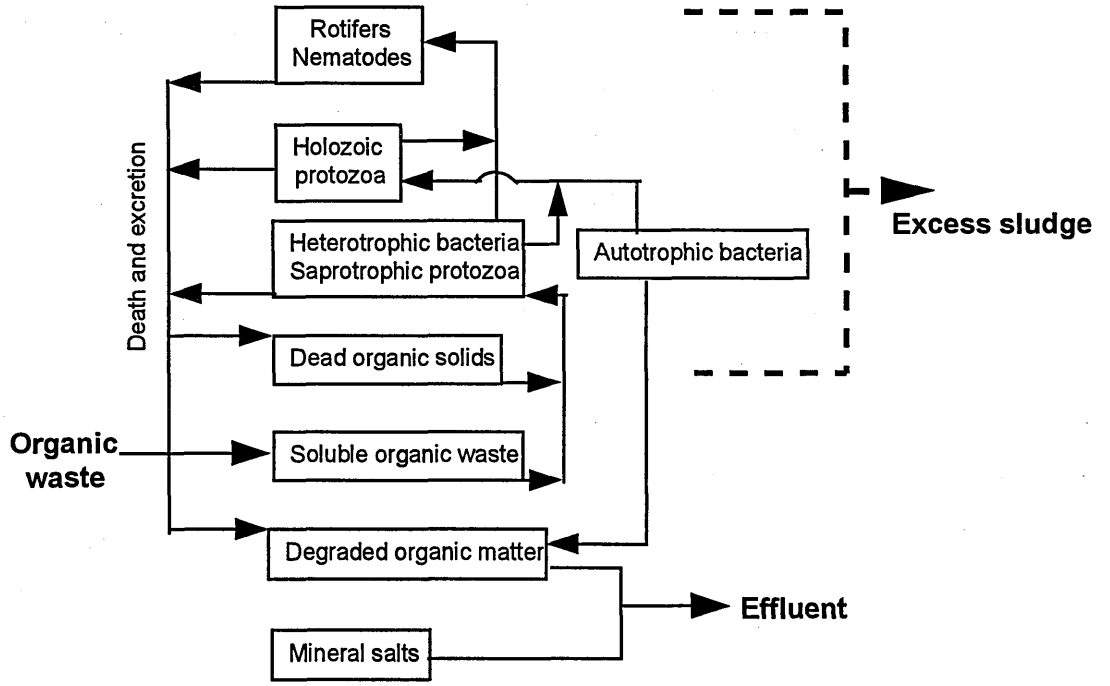

Figure 2.1 Energy diagram of an activated sludge community (adapted from Gray, 1990).

The relative quantities of different organisms in activated sludge indicate the organic loading rate. Ciliated protozoa, for example, generally signify good effluent quality as they regulate the bacterial populations (Beardsley and Coffey, 1985; Curds *et al.*, 1968) and all forms of protozoa are indicators of operating conditions (Madoni *et al.*, 1994; Madoni *et al.*, 1993; Salvadó and Gracia, 1993). Statistical analyses of the frequencies of 19 protozoa versus the BOD, mixed liquor suspended solids (MLSS), sludge volume index (SVI), DO, nitrification, retention time and sludge loading found that certain species were associated with different conditions. For example, shelled amoebae and the ciliates *Coleps hirtus, Plagiocampa metabolica* and *Vaginicola cristallina* were associated with low concentrations of ammoniacal-N, high DO and MLSS values and low sludge loading and SVI (Madoni *et al.*, 1993; Salvadó and Gracia, 1993); it was also seen that the peritrichs *Vorticella microstoma, V.octava, Opercularia coarctata and O. microdiscum* were associated with low MLSS and DO and high effluent BOD (BOD_e) and ammoniacal-nitrogen, contradicting Curds *et al.* (Madoni *et al.*, 1993).

In general, species diversity in activated sludge was found to be inversely proportional to the organic loading rate, except where the results were modified by high mixed liquor volatile suspended solids (MLVSS) (>4000mg/l) or volumetric loading rates >3.8kg five-day BOD (BOD_5)/m^3/d (Salvadó, 1994). The ecology of the activated sludge community is determined by the composition of the incoming wastewater. Variation in the concentrations of macro- and micronutrients can control the population, because the micro-organisms present will adapt to the change rather than resist it (Wood and Tchobanoglous, 1975; Wiggins *et al.*, 1987).

Treatment of industrial waste using activated sludge has often suffered from the inhibition of biological degradation and nitrification, leading to high effluent COD and poor sludge settlement arising from toxic contaminants (Geradi, 1986; Wood and Tchobanoglous, 1975). The reasons given for these problems with all kinds of industrial waste include the presence of toxic concentrations of pollutants, or the opposite problem of a low specialised population due to low substrate concentrations (Kumaran and Paruchuri, 1997; Liessens *et al.*, 1996), inappropriate physical conditions and the associated low viability of cells, a lack of macronutrients and the production of toxic metabolites (Fewson, 1988; Gulyas, 1994).

The loss of process efficiency is usually greater in anaerobic processes because of the limited diversity of micro-organisms found in such processes. The presence of an extremely diverse microbial consortium which includes autotrophs, heterotrophs and organisms at higher trophic levels (e.g. protozoa and nematodes) can allow syntrophic metabolism to occur. Syntrophic metabolism, or cometabolism, allows compounds not normally degraded to be removed from effluents as a result of enhanced biological activity. The compound is partially degraded by one organism, releasing metabolic products which are then degraded by a different organism (Buitrón and Gonzalez, 1996; Liessens *et al.*, 1996; Hickey *et al.*, 1993; Wiegel *et al.*, 1992; Brunner *et al.*, 1985; Venkataramani and Ahlert, 1985).

To maintain a diverse sludge, a wide range of nutrients is required. Limiting concentrations of nutrients shift the population toward the organisms best suited to the conditions. The dominant species are those which either require less of the limited nutrient, are able to synthesise it, or are able to use low concentrations (Wood and Tchobanoglous, 1975). Some activated sludge populations, such as filamentous bacteria, fungi and actinomycetes are able to take up nutrients from dilute solutions and flourish even when nutrient concentrations are low (Wood and Tchobanoglous, 1975; Geradi, 1986). Environmental factors can affect biodegradation greatly by changing the availability of nutrients and target compounds, preventing growth of micro-organisms and less obviously by influencing gene expression in all micro-organisms (Daubaras and Chakrabarty, 1992; Trevors, 1982). Design of the waste-degrading bioreactor influences the environmental conditions which may vary in a cyclic fashion: consequently, the bioreactor design affects the waste degrading performance and may influence also how a culture responds to micronutrients. This aspect has been largely disregarded in most studies (Chisti, 1998; Moo-Young and Chisti, 1994).

Recalcitrance is a fundamental problem in industrial wastewater treatment owing to an abundance of xenobiotic compounds which resist degradation. Recalcitrance can also arise from inappropriate pH, temperature and DO values, resulting in incorrect ionic conditions, inadequate nutrients or cometabolites and the supply of a substrate which does not serve as an activator for the appropriate enzymes (Eckenfelder and Musterman, 1994; Fewson, 1988).

Aerobes have more versatile metabolic pathways than anaerobes (Singleton, 1994), although it is well known that organic waste compounds can be degraded by both aerobic (Chisti, 1998; Moo-Young and Chisti, 1994; Häggbloom, 1992; Commandeur and Parsons, 1990; Neilson, 1990; Reineke and Knackmuss, 1988) and anaerobic (Moo-Young and Chisti, 1994; Fathepure *et al.*, 1988; Tiedje *et al.*, 1987) micro-organisms. Generally, non-chlorinated chemicals are degraded aerobically as they are more easily oxidised than chlorinated compounds, which are more susceptible to anaerobic degradation (Singleton, 1994).

An important factor is pH, which influences growth rates and the bioavailability of compounds (Stanlake and Finn, 1982). The solubility of wastewater components at different pH values can determine the rates of degradation (Singleton, 1994). Most activated sludge plants operate in the range of pH 7.0-7.5 (Beardsley and Coffey, 1985) and laboratory studies are usually carried out at neutral or near neutral pH (Gostick, 1991; Valo *et al.*, 1985), although bioremediation in the environment and wastewater treatment could benefit from research on acid or alkaline environments (Singleton, 1994).

Although alkaline conditions are more inhibitory to most species than acidic ones (Singleton, 1994; Gonzalez and Hu, 1991), organisms exist which are able to thrive at almost any pH. Sometimes waste treatment can be carried out more effectively at pH values other than neutral; one example is the degradation of polycyclic aromatic hydrocarbons by a mixed culture which is most efficient at pH 4.5 (Field *et al.*, 1992). Many useful fungi are acidophiles; *Phanaerochaete chrysosporidium* can be used to remove benzene from soil and water and thrives best at pH 4.5. (Yadav and Reddy, 1993; Mahler and Cordes, 1966). Changing the pH of wastewater streams to the optimum for the species which are able to degrade the major component compounds may be an option for maximising wastewater treatment efficiency.

Microbial cells produce or are associated with extracellular polymers (ECP) which act as adsorption sites for a range of cations. The polymers are usually polysaccharides, proteins, RNA and DNA and they contain anionic functional groups (such as hydroxyl or phosphoryl groups) which act as ligands. Soluble metal ions complex with the ligands and suppress respiration and metabolism. Inside the cell, they can act as catalysts for enzyme systems or block them and interfere with metabolism. Adsorption of metal ions onto the extracellular ligands is maximised when the mixed liquor pH is between 6.0 and 8.0 (Geradi, 1986) a range which overlaps with the optimum operating range (pH 6.5-8.5) for activated sludge plants (Roš, 1993). The extent of toxic effects of metal ions is a function of the metal mass to biomass ratio and hence cannot be predicted from metal concentration in water alone. Acclimation is an important factor in metal toxicity: sludge

communities evolve and micro-organisms adjust their metabolic pathways over time to tolerate most metal ions (Beyenal *et al.*,1997; Hu *et al.*, 1996; Dilek and Yetis, 1992; Wiggins *et al.*, 1987; Chang *et al.*,1986).

Operating temperature is critical to biological processes because it affects the rates of all reactions except biocoagulation (coagulation by extracellular polymers). Temperature control is more important in industrial wastewater treatment than in domestic or municipal waste treatment because there is less material which can be removed by coagulation than is present in domestic wastewater (Dilek and Yetis, 1992). Cumulative respired oxygen and mineralised nitrogen and sulphur have been shown to increase with temperature; however, rate constants estimated from first order models are not consistently related to temperature and alternative strong relationships between the temperature and initial organic carbon supply exist (MacDonald *et al.*, 1995). Most micro-organisms work best at mesophilic temperatures and their varying abilities to degrade pollutants at high or low temperatures are overlooked, because wastewater treatment is traditionally carried out at ambient temperatures rather than in controlled environments. In fact, low temperature has been suggested as the limiting factor in the degradation of pentachlorophenols in natural soil conditions (Cardinaletti *et al.*, 1990), and has been shown to reduce substrate hydrolysis (Tian *et al.*, 1994) and inhibit the degradation of phenolic compounds (Valo *et al.*, 1985). Higher temperatures (>35.5°C) are known to increase rates of reactions, including metabolism and to decrease sludge production (Singleton, 1994; Larsen *et al.*, 1991; Senez, 1962; Ludzak *et al.*, 1961), although this trend is reversed when the temperature exceeds 45°C (Hunter *et al.*, 1966).

Temperature can also influence the metabolic pathways micro-organisms use, the end products formed (Wiesel *et al.*, 1993), and the composition of the activated sludge. Foaming problems follow increases in temperature (Blackall *et al.*, 1991). This is attributed to the increased growth rates of micro-organisms given a certain carbon source. Laboratory studies indicate that the carbon source may also contribute to foaming under these conditions (Blackall *et al.*, 1991).

Biological cells require six macronutrients for metabolic processes including the synthesis of nucleic acids, proteins, lipids and carbohydrates. These are: carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus and the primary nutrients are nitrogen, phosphorus and carbon (Beardsley and Coffey, 1985). An estimation of the nutritional requirements of biological cells can be made using the rate of bacterial growth which depends on the maximum yield coefficient and the rate at which the substrate is consumed. The maximum yield coefficient increases as environmental conditions and nutrient supply approach optimum. This reduces the retention time necessary for complete treatment (Speitel and Digiano, 1988; Benefield *et al.*, 1979). The ratio at which nutrients should be supplied is contentious, with COD:N:P ratios of 100:10:1 and trace sulphur (Beardsley and Coffey, 1985), 250:7:1 (Franta *et al.*, 1994) and 100:20:1 (Metcalf and Eddy, 1991) quoted in the literature. Whichever ratio is used, wastewater streams from industries including chemical and petrochemical manufacturing, sugar refining and paper and cellulose production are low in nutrients (Eckenfelder and Musterman, 1994; Lind *et al.*, 1994).

A lack of macronutrients leads to the most efficient micro-organisms in a culture out-competing the other species in terms of nutrition, so over time a culture with poor diversity will appear. This increases the hydraulic retention time (HRT) required for sufficient treatment of even readily biodegradable substances and compounds which require more than one species for degradation will emerge in the effluent. Some fungi in mixed cultures do not metabolise xenobiotics as their sole carbon source and require added degradable carbon for pollutant removal (Singleton, 1994). The presence of a readily biodegradable carbon source in a wastewater prevents the loss of bacterial viability (Leahy and Colwell, 1990) and the addition of carbon sources such as glucose, glutamate, or organic acids to a wastewater with little nutritional value can increase the degradation of other pollutants (Hendriksen *et al.*, 1992; Gonzalez and Hu, 1991). However, the addition of glucose to a *Pseudomonas* population inhibited substrate degradation in one study (Radehaus and Schmidt, 1992).

Studies on microbial nitrogen and phosphorus nutrition have concentrated mainly on the degradation of oils. The roles of nitrogen and phosphorus as essential nutrients for biological treatment processes are well known (Wood and Tchobanoglous, 1975). The availability of nitrogen and phosphorus can be the limiting factor in the degradation of hydrocarbons and supplements where concentrations are low can lead to improved degradation (Prince, 1992; Leahy and Colwell, 1990). Changes in the sources of carbon and nitrogen can affect the growth rates of the micro-organisms. The chemical nature of the BOD present is a fundamental factor in degradability (Eckenfelder and Musterman, 1994) and the type of nutrient available dictates the extent of the treatment needed to remove the COD. The MLSS sustained in laboratory activated sludge reactors given different sources of carbon and nitrogen showed that nutrients can have combined effects (Table 2.1) and carbon and nitrogen sources should be selected to maintain a population of appropriate micro-organisms for the wastewater present (Blackall *et al.*, 1991).

It is difficult to draw conclusions regarding the type of nutrition required. Adding ammoniacal nitrogen to a mixed culture can improve degradation (Valo *et al.*, 1985), but activated sludge cultures which were starved or supplied with nitrogen showed variable results (Singleton, 1994). The nutrients required to enhance wastewater treatment may not be the usual additions of nitrogen or phosphorus. The practice of analogue enrichment involves the addition of substances which increase cometabolism (such as biphenyl (Brunner *et al.*, 1985)) or which induce degradative genes (Ogunseitan and Olson, 1993). These substances can include micronutrients.

Table 2.1 MLSS in mg/l maintained in activated sludge with different carbon and nitrogen sources (Blackall *et al.*, 1991).

	$(NH_4)_2SO_4$	KNO_3	NH_3	Peptone
Glucose	2130-2600	-	-	-
Acetate	1540-1900	1420	2040	1650
Trihalose	1190	-	-	-
Hexadecane	760	-	-	-
Cooking oil	110	-	-	-

2.3 Micronutrients

The role of micronutrients in biological wastewater treatment is not as well defined as the roles of carbon, nitrogen and phosphorus (Wood and Tchobanoglous, 1975). It is difficult to measure trace quantities and, in addition, complex chemical and biochemical interactions mean that the theoretical requirements for micronutrients have not been established. It is probable that all the micronutrients required for satisfactory waste treatment can be supplied by most domestic wastewaters. Sufficient micronutrition is needed to support all the microbial genera required for treatment, because an unbalanced microbial structure often leads to sludge which fails to settle in subsequent stages. This is a problem at many activated sludge plants treating industrial wastewater. Deficiencies of micronutrients may be alleviated using supplements of the required ionic species, but care must be taken to avoid excess doses, which can inhibit waste treatment.

Appreciation of the role of micronutrients in wastewater treatment has to come from an understanding of their uses in microbial population dynamics, metabolism and growth. Trace elements and vitamins are required by cells in addition to the six macronutrients. The trace elements required include manganese, zinc, cobalt, molybdenum, nickel, copper, vanadium, boron, iron and iodine (Metcalf and Eddy, 1991; Geradi, 1986; Beardsley and Coffey, 1985; Wood and Tchobanoglous, 1975; Luria, 1960) and the vitamins required include K, B_1, B_2, B_6, B_{12}, biotin, niacin and pantothenic acid (Lind *et al.*, 1994; Inamori *et al.*, 1991; Beardsley and Coffey, 1985; Yamada and Kawasaki, 1980; Voigt *et al.*, 1979; Kidder and Dewey, 1949; Schormüller, 1948). Micronutrients are often required at doses of <1mg/l , which means that the demonstration of dosage requirements is technically difficult.

The trace element requirements of bacterial cells can be determined from the composition of the cells (Metcalf and Eddy, 1991). This enables the calculation of the trace element requirements per 1kg BOD influent to produce 100mg/l bacterial cells in the sludge. Care must be taken with trace element requirement estimations, however, as excess micronutrients can be adsorbed onto cell walls, so the concentrations in biomass

ash may exceed the actual required amount (Wood and Tchobanoglous, 1975; Nicholas, 1963). Microbial growth media are developed to contain non-volatile suspended solids two to three orders of magnitude less than the concentrations found in activated sludge (Table 2.2), so media values are probably too low (Wood and Tchobanoglous, 1975). Actual requirements are influenced strongly by the organic and hydraulic loading rates, the cell growth rate (Speitel and Segar, 1995), the nature of the waste, and the mean cell residence time (MCRT) (Wood and Tchobanoglous, 1975).

As microbial growth is fundamental to the activated sludge process, the influent must contain all the specific micronutrients needed to activate the cellular enzymes which degrade the substrate, and the general nutrients required for growth and metabolism. The primary source of micronutrients in wastewater treatment plants is the domestic wastewater stream, so process units receiving mainly industrial waste suffer the most from micronutrient deficiencies. Micronutrients are generally seen as toxic substances and are deliberately minimised in wastewater from industry. Moreover, the composition of any sewage treatment works influent varies with time and location and the majority of works regularly receive wastewater with poorly balanced micronutrients (Wood and Tchobanoglous, 1975). To achieve sufficient treatment of industrial wastewater, it is necessary to enrich activated sludge with a micronutrient supply to enable the strains capable of degrading the non-readily biodegradable fraction of the waste stream to grow in settleable flocs and produce ECPs which do not escape into the bulk liquid (Franta *et al.*, 1994).

These micronutrients must be present in concentrations which are appropriate for the strength of the waste in terms of COD and are also great enough to trigger the metabolic pathways which use them (Wood and Tchobanoglous, 1975). It has long been established that many trace elements must be present in excess of the minimum cell requirements in order for them to be found in cell ash (Wood and Tchobanoglous, 1975). For example, 6 million iron-containing molecules are required for the optimum growth of one cell of *Aspergillus niger* (Lilly and Barnett, 1951). Summaries of the roles of a number of vitamins and trace elements are given in Tables 2.3 and 2.4.

Table 2.2 Theoretical micronutrient requirements of activated sludge.

Vitamins	High (mg/l)	Reference	Low (mg/l)	Reference
B_1	1.2	Schormüller, 1948	0.3	Schormüller, 1948
B_2	2.0	Lind *et al.*, 1994	0.5	Schormüller, 1948
B_6	10	Schormüller, 1948	0.1	Schormüller, 1948
B_{12}	5.0 μgl^{-1}	Lind *et al.*, 1994	-	
Niacin	10.0	Schormüller, 1948	0.1	Schormüller, 1948
Biotin	0.1	Schormüller, 1948	0.05	Lind *et al.*, 1994
Pantothenic acid	2.0	Lind *et al.*, 1994	0.01	Schormüller, 1948

Trace Elements	High (ppm)$^{\otimes}$ (Metcalf and Eddy, 1991)	Low (ppm)$^{\otimes}$ (Metcalf and Eddy, 1991)	Typical (ppm)$^{\otimes}$ (Metcalf and Eddy, 1991)	Typical values from *E. coli* ash (ppm) (Yadav and Reddy, 1993; Luria, 1960)	Typical values from media (ppm) (Yadav and Reddy, 1993)
Ca	0.7	0.4	0.5	1.40	≥ 1.0
K	1.5	0.8	1.0	1.50	≥ 3.0
Fe	0.4	0.1	0.2	0.20	1.0-4.0 as Fe^{2+}
Mg	0.7	0.4	0.5	0.54	3.0-5.0
Na	2.0	0.5	1.0	1.30	≥ 1.0
Cl	0.7	0.4	0.5	0.41	3.0-10.0
Mn	-	-	-	0.01	0.2-0.5
Cu	-	-	-	0.01	0.2-0.5
Al	-	-	-	0.01	0.2-0.5
Zn	-	-	-	0.01	0.2-0.5
Mo	0.5	0.2	0.3	-	-
Co*	5.0	0.1	-	-	-

$^{\otimes}$ From dry weight composition of cells. *Sathyanarayana Rao and Srinath, 1961.

Table 2.3 The role of vitamins in microbial systems.

Vitamin	Requiring organisms	Role	References
Vitamins A, D, E and P	Most micro-organisms	Not mentioned individually but chemical additions including these vitamins have been seen to improve treatment efficiency, reduce biomass production, eliminate reactor disturbances and improve energy efficiency.	Lemmer, 1992; Lind *et al.*, 1994; Sarfert *et al.*, 1990
Vitamin C (Ascorbic acid)	Lactic acid bacteria, spirochaetes	Changes in growth and acidification, varied results. Growth factor.	Schormüller, 1948
Pantothenic acid	All micro-organisms	Growth factor in initial cell growth, fermentation, propagation, respiration and glycogenesis. Most active when other B vitamins are present. Acts synergistically with biotin and pyridoxine. A deficiency results in reduced N and P removal efficiencies.	Schormüller, 1948; Lind *et al.*, 1994; Srinath and Pillai,1966
Biotin	Yeasts and all bacteria, especially lactic acid bacteria	Metabolic activity. Found in large quantities, optimum concentration between $5x10^{-4}$ and $10x10^{-4}$mg/l. Most enterobacteria excrete biotin. Acts synergistically with pantothenic acid and pyridoxine. Large numbers of saprophytes which require biotin are found in municipal wastewater treatment plants. No biotin doses are needed in activated sludge.	Lind *et al.*, 1994; Schormüller, 1948; Voigt *et al.*, 1979; Srinath and Pillai, 1966
Niacin (Nicotinic acid)	Bacteria, especially *Staphylococcus* and *Bacillus*	Growth factor. Takes part in oxidative phosphorylation and production of cozymase. High organic loads select against bacteria which require niacin e.g. *Staphylococcus aureus, Streptococcus casei* and *Lactobacillus arabinosus* which need 1.0mg/l to survive. Enterobacteria synthesise niacin and do not need a supply although dosing stimulates a mixed culture.	Schormüller, 1948; Lind *et al.*, 1994

Table 2.3 Continued.

Vitamin	Requiring organisms	Role	References
Thiamine (Aneurine, Vitamin $B_{1)}$	Yeasts, moulds, protozoa Saprotrophic bacteria	Growth medium constituent. Used for carbohydrate metabolism and cell growth. Requirements range from total dependency on an environmental supply for life (the auxoheterotrophs) to the ability to manufacture all the aneurine required and hence no requirement for an external supply at all (the auxoautotrophs). Activity of codehydrase, codismutase and cocarboxylase	Beardsley and Coffey, 1985; Inamori *et al.*, 1991; Kidder and Dewey, 1949; Lind *et al.*, 1994; Schormüller, 1948; Voigt *et al.*, 1979; Yamada and Kawasaki, 1990
Vitamin B_2 (lactoflavin, riboflavin or vitamin G)	Many bacterial species	Growth, although previous workers found no B_2-dependent micro-organisms. Some can synthesise all the lactoflavin they need. The fungus *Aspergillus niger*, for example, will produce this vitamin in magnesium-deficient media.	Lind *et al.*, 1994; Beardsley and Coffey, 1985
Vitamin B_6 (adermine or pyridoxine)	Bacteria, especially lactic acid bacteria	A specific growth factor (e.g. lactic acid bacteria require 10^{-6}mg/day). One study concluded that no micro-organisms had requirements for pyridoxine. Acts synergistically with biotin and pantothenic acid.	Beardsley and Coffey, 1985; Lind *et al.*, 1994; Voigt *et al.*, 1979; Schormüller, 1948
Vitamin B_{12} (cyanocobalamin)	Bacteria	A complex, cobalt-containing co-ordination compound produced during the normal growth of some micro-organisms. Required for growth.	Beardsley and Coffey, 1985; Sathyanarayana Rao and Srinath,1961
Vitamin B_c	None	Vitamin B_c (folic acid, folate or pteroylglutamic acid) is not required .	Lind *et al.*, 1994
Vitamin K (Quinones)	All bacteria	Constituents of the respiratory chain. Every species has one dominant quinone which enables the identification of the species in a mixed culture.	Hu *et al.*, 1996

Table 2.4 The role of trace elements in microbial systems.

Element	Requiring organisms	Role	References
Iron (Fe^{2+}, Fe^{3+})	Aerobic bacteria, *Aspergillus niger* *Chlorella pyrenoidosa*	Growth factor. Growth factor. Adsorbed in quantities directly proportional to the concentration available.	Mahler and Cordes, 1966; Lilly and Barnett, 1951; Knauss and Porter, 1954
Fe^{3+}	Possibly all organisms Iron reducing bacteria	Electron transport in cytochromes. Synthesis of catalase, peroxidase and aconitase. Ion reduction for floc formation.	Knauss and Porter, 1954; Rasmussen and Neilsen,1996; Neilsen, 1996
Zinc	Bacteria	Metallic enzyme activator. Dissociable on active site of enzymes. Activity of carbonic anhydrase and carboxypeptidase A. Stimulates cell growth. Toxic at low concentrations (1mg/l), especially to protozoa. Can exacerbate toxic effects of other metals and inhibit metabolism.	Mahler and Cordes, 1966; Cardinaletti *et al.*, 1990; Madoni *et al.*, 1996; Shuttleworth and Unz, 1988; Geradi, 1986; Madoni *et al.*, 1994; Beyenal *et al.*, 1997; Gökçay and Yetis, 1996
Cobalt	Bacteria	Metallic enzyme activator. Dissociable on active site of enzymes. Activates carboxypeptidase for synthesis of vitamin B_{12} (cyanocobalamin) but otherwise toxic. Can inhibit metabolism.	Mahler and Cordes, 1966; Geradi, 1986; Hunter *et al.*, 1983; Sathyanarayana Rao and Srinath,1961; Gökçay and Yetis, 1996
Magnesium	Heterotrophic bacteria	Enzyme activator for a number of kinases and phosphotransferase	Stanier, 1966

Table 2.4 Continued.

Element	Requiring organisms	Role	References
Manganese	Bacteria	Activates isocitric dehydrogenase and malic enzymes. Often interchangeable with magnesium in kinase reactions. Lower affinity for binding sites than other metals but still can inhibit metabolism at 1mg/l.	Wood and Tchobanoglous, 1975 Geradi, 1986; Gökçay and Yetis, 1996
	Chlorella pyrenoidosa	Adsorbed in quantities directly proportional to the concentration available.	Knauss and Porter, 1954
Copper	Bacteria	Enzyme activator required in very small quantities. Can inhibit metabolism. Chelates other substances and reduces their toxicity.	Gökçay and Yetis, 1996 Vandevivere *et al.*, 1997
Nickel	*Cyanobacteria* and *Chlorella*, Methanogenic anaerobes, Activated sludge cultures	Stimulates certain enzymes. Methane production. Maintenance of biomass. May inhibit metabolism.	Gökçay and Yetis, 1996
Calcium	All bacteria *Thiothrix, Zoogloea*	Cell transport systems and osmotic balance. Bridging anionic ECP and aiding flocculation. Increase growth rates and improve flocculation. Requirements and effects vary. Interacts with other metals.	Neilsen, 1996 Geradi, 1986 Shuttleworth and Unz, 1988

2.3.1 Vitamins

Vitamins are organic compounds and have much higher toxicity thresholds than trace metals. They are required for the growth of activated sludge (Gray, 1990) because they influence cellular metabolism. The addition of vitamins to activated sludge has yielded some positive results (Lemmer and Nitschke, 1994; Lemmer, 1992; Sarfert *et al.*, 1990). The vitamin requirements of bacteria in activated sludge plants treating both municipal and industrial wastes have been assessed (Lind *et al.*, 1994). Whereas the addition of single vitamins had no significant effects on the activity of cell enzymes, the metered addition of multiple vitamins to different activated sludge plants often led to significant increases in metabolic activity, particularly in highly loaded sludges. Different vitamins and their combinations have different effects on the various bacterial species. Since it is known that our understanding of the vitamin requirements of activated sludge will help to optimise the process ecology and maintain a high diversity of micro-organisms, thereby reducing the chances of problems in sludge settling and improving the quality of the plant effluent (Singleton, 1994; Gostick, 1991; Wood and Tchobanoglous, 1975), the benefits must be considered carefully. The "vitamin B complex" is a group of vitamins originally thought to be a single substance, but it is actually made up of thiamine, riboflavin, niacin, pantothenic acid, biotin, pyridoxine, folic acid, lipoic acid, inositol and vitamin B_2. These substances have different effects on micro-organisms and should be considered separately.

Vitamin shortages can be expected in wastewaters from the chemical, petrochemical, paper and cellulose, sugar, and carcass disposal industries (Lind *et al.*, 1994). Low population densities were found in the absence of thiamine, biotin and niacin, doses of which improved the treatment of wastes from the paper and cellulose industry. Additions of B vitamins to these sludges yielded some results: there was no change in the activity of phosphatases or L-alanine-aminopeptidases, but the activity of esterases and α- and ß-glucosidase increased, particularly in highly loaded sludges. It was also found that increasing the additions gave no further advantage in sludges treating waste from the chemical and animal carcass disposal industries. In spite of these results, however,

higher enzyme activities could be found in the negative controls if the test sludge had not been allowed to acclimate to the waste. All the sludges tested contained vitamin B-requiring species as some proportion of the population (Figure 2.2). The vitamins required were found to be thiamine, niacin and biotin, with no requirement for folic acid, pyridoxine, or riboflavin and the extent of the additions required acts as an indicator of the degree of the vitamin deficiency in the waste.

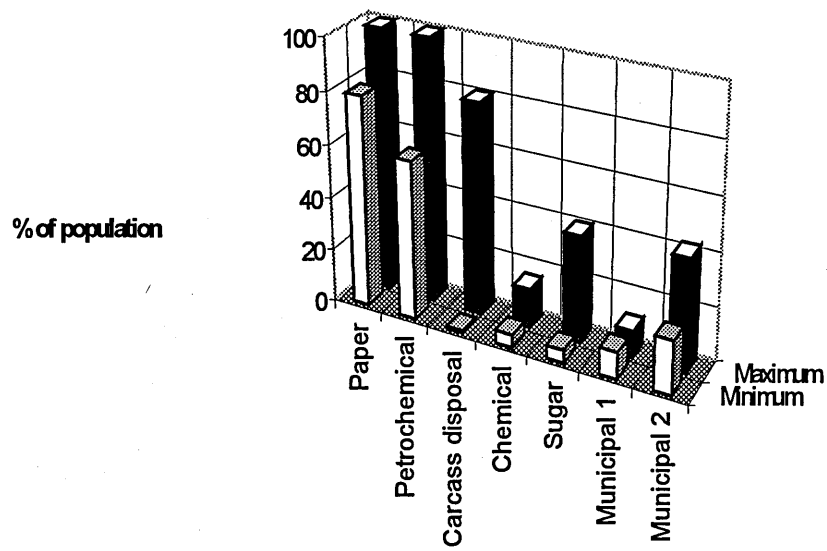

Figure 2.2 Proportions of sludge populations requiring B vitamins (Lind *et al.*, 1994).

Vitamin additions to sludge may not improve the treatment of recalcitrant compounds; this depends on whether the vitamin-deficient species are those which are relevant for waste treatment. It has been shown that B-vitamin addition to sludges degrading nonylphenol ethoxylate, linear alkylbenzene sulphonate, ethylene diamine tetra-acetic acid and 2,4,6-trichlorophenol did not improve degradation or reduce the inhibition of the degradation of casein hydrolysate (Lind *et al.*, 1994). It is thought that the inhibition of the degradation of nonylphenol ethoxylate, linear alkylbenzene sulphonate and 2,4,6-trichlorophenol occurs due to the production of toxic metabolic intermediates (Schöberl *et al.*, 1988). In addition to this, the appearance of, for example, thiamine-requiring organisms may well coincide with that of thiamine producers such as *E. coli*, which

excrete thiamine if provided with an exogenous energy supply (Yamada and Kawasaki, 1980). Additional vitamin supplies may also select for protozoa which are regarded as indicators of high loads and require or are stimulated by thiamine (e.g. *Trithigostoma cucullus* and *Tetrahymena pyriformis*) (Lind *et al.*, 1994; Inamori *et al.*, 1991; Voigt *et al.*, 1979; Kidder and Dewey, 1949; Schormüller, 1948,).

Niacin addition can improve COD removal rates, possibly because niacin is used in oxidative phosphorylation although the exact mechanism of its action is not known. Niacin is essential for the survival of some species of the genera *Bacillus* and *Staphylococcus*, while enterobacteria can synthesise niacin and do not require a supply. Niacin is required for the production of cozymase and as a growth factor in bacterial cells (Lind *et al.*, 1994; Schormüller, 1948). Doses of niacin have produced increased metabolic activity in the industrial sludges investigated (Lind *et al.*, 1994). The optimum dose of niacin is reported to be 0.1mg/l influent (Schormüller, 1948) and it is likely that municipal waste will contain such a small amount. Niacin dosing to a municipal wastewater treatment plant had no effect on the metabolism of the sludge (Lind *et al.*, 1994). This suggests that municipal waste contained sufficient or excess niacin for activated sludge bacteria, but the industrial waste (Lind *et al.*, 1994) did not have sufficient niacin.

Some activated sludges display no requirement for lactoflavin or pyridoxine (Lind *et al.*, 1994). Additionally, although lactoflavin is important for cell metabolism, there is a certain amount of autotrophy in the activated sludge community (for example, by *Candida* species and certain fungi) and the concentration of this vitamin is usually high in bacterial cells (Schormüller, 1948). A magnesium deficiency in the substrate often leads to an increase in lactoflavin formation since either micronutrient can be used for the activity of dehydrases. Pyridoxine is a specific growth factor for most yeasts and members of the genera *Streptococcus* and *Staphylococcus*. Pyridoxine has to be hydrolysed before use and the functional groups on the molecule have to be correct for it to be used in cells. This means that chemical interactions result in complete inactivation and that analogues of pyridoxine are rarely of any value to activated sludge.

The sludges used to treat wastewater from the petrochemical, carcass disposal and sugar industries were found to have a high proportion of biotin requirers, although no biotin deficiency was found in the seven sludges treated (Lind *et al.*, 1994). This may be attributed to the production of biotin by many enterobacteria (Schormüller, 1948) and *Pseudomonas* species (Tsuboi *et al.*, 1967). The work described (Lind *et al.*, 1994) concluded that a diverse sludge population can provide itself with most of the vitamins required, but industrial sludges with less balanced populations are likely to benefit from vitamin additions. This corroborates previous work (Schwarz, 1971) and has been confirmed further by others (Lemmer and Nitschke, 1994).

Persistent sludge bulking has been linked to general nutrient deficiency (Cerniglia and Heitkamp, 1989) and pinpoint flocs, dispersed growth and poor flocculation have been attributed to micronutrient deficiency (Gostick, 1991). Chemical dosing to control sludge settling is documented widely and includes additions of metal salts (Gleisburg, 1980; Vaananen, 1988) and micronutrients (Gostick, 1991). A lack of micronutrients in an activated sludge plant allows filamentous bacteria a competitive advantage over other species, enabling them to proliferate and produce large flocs (Jones and Franklin, 1985). Proprietary mixtures of micronutrients for addition to activated sludge plants exist but are expensive and must be used with caution to avoid toxic excesses of some constituents.

The addition of vitamins does not guarantee bioavailability, as vitamins have to be present in the correct forms; one example is vitamin B_6 (pyridoxine or adermine). Bacterial growth is promoted by the addition of 0.5 – 1.0mg/l adermine hydrochloride, but doses as high as 2.56µg/l of 4,5-bis-deoxyadermine have no growth-promoting effect (Schormüller, 1948). The action of niacin is antagonised by the presence of various metals, particularly iron salts (Schormüller, 1948), showing that the nature of the medium is as important as the micronutrient and that a mixed dose of vitamins will not necessarily produce the sum effect of all the components of the dose.

Previous studies have shown that mixed doses of vitamins caused great increases in the metabolic activity of activated sludge (Lind *et al.*, 1994). It should be noted that high metabolic activity indicated by greater rates of oxygen uptake is also seen in sludges which are not acclimated to the feed composition, and that metabolism alone cannot be taken as an indication of process efficiency. Combinations of pantothenic acid with pyridoxine or biotin act synergistically when added to activated sludge (Schormüller, 1948). Pantothenic acid is antagonised by molecules with similar structure, probably through competition for cell surface binding sites of a particular type, as antagonism is only found in the action of pantothenic acid on certain bacteria (e.g. on the metabolic rate of streptobacteria, but not of yeasts). Thiamine shows no interactions with other vitamins; the presence of pyridoxine antagonises the activity of niacin (Schormüller, 1948). Low MLSS often found in industrial pretreatment plants can be alleviated by supplements of thiamine, biotin and niacin (Lind *et al.*, 1994).

2.3.2 Trace elements

Trace elements are taken up as components or cofactors of enzymes involved in the catalysis of metabolic reactions and the maintenance of enzyme structure. They can also act as metallic enzyme activators which, unlike coenzymes, are not part of the reaction they catalyse, or they are used in electron transport inside the cell (Mahler and Cordes, 1966). Not all micro-organisms require all micronutrients, although almost all of them are required for the growth of at least one common activated sludge organism (Wood and Tchobanoglous, 1975).

Iron (Fe) is an important element in activated sludge in small quantities. Bacteria which reduce Fe^{3+} to Fe^{2+} ions are found in most activated sludge mixed cultures. Fe^{3+} and sulphate reduction are important in floc formation. Fe^{3+} is not as important as oxygen and nitrogen, but is used as an electron acceptor by cytochromes within the cell during aerobic growth (Nielsen, 1996; Rasmussen and Nielsen, 1996) and is used in the synthesis of enzymes such as catalase, peroxidase and aconitase (Wood and Tchobanoglous, 1975). While some divalent cations (e.g., Ca^{2+}), are used in bridging

anionic bacterial ECP which stabilise the flocs (Nielsen, 1996), the reduction of Fe^{3+} has been observed to cause problems with floc strength and effluent quality (Rasmussen and Nielsen, 1996). The Fe^{3+}-reducer *Shewanella alba* can cause deflocculation in activated sludge (Caccavo *et al.*, 1996) even though re-oxidation to Fe^{3+} is rapid (Nicholas, 1963).

Copper is a metallic enzyme activator. It is found in wastewater streams from industrial and domestic sources and exerts a toxic effect on ciliate populations in activated sludge, reducing both total numbers and species diversity when present at concentrations 1–10mg/l (Gracia *et al.*, 1994). However, dosing low concentrations of copper salts into wastewater is beneficial in certain cases. Recent work in which copper was added to detoxify wastewater showed that concentrations above the accepted toxicity threshold can chelate other toxins in the effluent (Vandevivere *et al.*, 1997). Concentrations of 0.6-3.0mg/l influent copper detoxified different effluents from latex production, carpet manufacture and chemical processing plants. Nitrification inhibitors such as allylthiourea and nitrapyrin act by inactivating cellular ammonium mono-oxygenase, so adding copper to bind ammonium mono-oxygenase copper haemes prevents these inhibitors from working. The addition of copper did not lead to high copper concentrations in the treatment plant effluents, although the main disadvantage was that the effects of the copper addition faded after 17h as the copper became adsorbed onto other binding sites. Another disadvantage was that the continuous addition of >3mg/l copper eventually inhibited the COD removal and added to the copper content of the sludge. However, it was concluded that the disadvantages could be avoided and nitrification protected by continually dosing <1.0mg/l copper into a wastewater treatment plant influent.

Cobalt is a metallic enzyme activator required by some bacteria which synthesise cyanocobalamin (vitamin B_{12}) and is also used in the production of carboxypeptidase (Wood and Tchobanoglous, 1975). Cobalt chloride and cobalt sulphate can stimulate the synthesis of cyanocobalamin and some other B_{12}-like factors in aerobically fermenting sludge (Sathyanarayana Rao and Srinath, 1961). Doses of cobalt show potential in optimising the activated sludge process. The amount of cyanocobalamin produced can be increased by up to 150% with the addition of small amounts of cobalt (<1.0mg/l)

(Sathyanarayana Rao and Srinath, 1961). This suggests a stimulatory effect on the metabolic reactions responsible for B_{12} production. However, Sathyanarayana Rao and Srinath (1961) found that the increase in metabolism was not associated with any improvement in BOD or COD removal, nor was there any change in the nitrogen and phosphorus content of the sludge. The combined effects on respiration and COD removal indicate an inhibitory effect rather than stimulation, and cobalt has previously been reported to be toxic to activated sludge and can lead to a deterioration in the effluent quality at a concentration of 1.0mg/l (Geradi, 1986; Hunter *et al.*, 1966).

In some cases the presence of nickel as Ni^{2+} stimulates certain enzymes (Hutchinson, 1973) and cells; examples are *Cyanobacteria and Chlorella* (Madoni *et al.*, 1996) and methanogenic anaerobes (Speece *et al.*, 1983). It has also been shown that the maximum biomass yield and maintenance coefficient for activated sludge plants dosed with 1.0 and 5.0mg/l exceed the maximum biomass yield and maintenance coefficient values in activated sludge plants where no nickel is present (Sujarittanonta and Sherrard, 1981). Conversely, chromium, nickel, silver and zinc have been demonstrated to noncompetitively inhibit bacterial metabolism (Gökçay and Yetis, 1996) and in these instances enzyme inhibition kinetics can be applied to the metabolism of the cells. Michaelis-Menten kinetics can be adapted to describe the effects of substances on sewage micro-organisms and this has been demonstrated on activated sludge bacteria exposed to the chromium ion Cr^{6+} (Lewandowski *et al.*, 1985). Other kinetics may apply in different instances of bacterial degradation of toxins (Allsop *et al.*, 1993).

The addition of trace elements does not guarantee bioavailability. Ions must be soluble to be bioavailable (Amdur *et al.*, 1991). Essential metals such as iron, zinc, copper and cobalt are inadvertently precipitated out of the wastewater as hydroxides if the pH is adjusted using lime (Wood and Tchobanoglous, 1975). Trace elements are cations and can be removed rapidly by adsorption onto the anionic cellular material before the bacteria can assimilate the micronutrients they need (Wood and Tchobanoglous, 1975). Many micronutrients can antagonise each other and result in effective deficiencies (Nicholas, 1963). The concentration may not be toxic, but the micronutrient competes

for entry into cells or binding sites inside. This is the mechanism that allows some metals to remove other toxins by chelation (Vandevivere *et al.*, 1997; Wood and Tchobanoglous, 1975).

More than one metal in a solution can lead to interactions between ions; for example, calcium, potassium and sodium are known to interact with other metals (Geradi, 1986). Interactions are almost impossible to predict because they are influenced not only by metal species and concentration, the operating conditions and strength and type of influent, but also by the species of micro-organisms present, the sludge age and even the order in which the metals are added (Beyenal *et al.*,1997). Toxic effects can also either prevent COD removal or improve it. Consequently, many contradictory results concerning the toxicity of metal mixtures are reported (Beyenal *et al.*,1997; Dilek *et al.*, 1991; Yetis and Gökçay, 1989; Chang *et al.*,1986; McDermott *et al.*, 1963). Microbial kinetics, where mixtures of metal ions and fully acclimated sludges are involved, do not fit the Monod model (Beyenal *et al.*,1997), but an alternative model has yet to be proposed.

Zinc interacts with other metals (e.g., copper) to exacerbate their toxic effects and has been shown to reduce the rates of reactions so that the kinetics of biodegradation in the presence of zinc and copper resemble those of biodegradation without the metals but with a very short sludge age (Beyenal *et al.*,1997). Vitamins and metals may compete for extracellular ligands, or they may chelate with each other. Copper has been shown to antagonise nitrification inhibitors such as allylthiourea and nitrapyrin because these compounds are copper chelators. Small amounts (0.5mg/l) of copper can stimulate nitrification in this way and have been shown to reverse the inhibitory effects of wastewater from carpet manufacturing, chemical production and latex streams (Vandevivere *et al.*, 1997).

As calcium plays a significant role in membrane permeability, it is likely that it may act as an enhancer of the action of any other metal. Calcium has already been shown to interact with other metals (Geradi, 1986). It has also been concluded that the requirements for

calcium and its effects vary greatly between bacteria and that the calcium concentration has a major influence on the toxic effects of other metals (Shuttleworth and Unz, 1988). The presence of calcium due to water hardness has been shown to accelerate acclimation via species selection in the sludge community (Vashon *et al.*, 1982). Activated sludge simulations run on a waste stream based on hard water showed lower SVI and effluent SS, higher MLVSS and greater removal of nitrilotriacetate for the first 30 days of the test. After this period, the soft water reactors began to achieve comparable removal rates. It is unlikely that water hardness affects the ultimate performance of an activated sludge plant but it can influence the speed with which biomass can adapt to new components in the wastewater. This ability could be important to plants that receive wastewaters from industries involving batch manufacturing of different products.

In many cases, excess trace elements have toxic effects (Madoni *et al.*, 1996) and high levels of trace metals can cause reductions in flocculation and nitrification, thus diminishing effluent quality (Geradi, 1986), even though a moderate dose is highly beneficial (Amdur *et al.*, 1991). The influent (pH and strength), the metals involved (species, concentration and the order in which they are added) and the microbial population (type and concentration) are the major factors which influence the toxicity of the elements. Toxic effects become more acute with decreasing species diversity in the sludge (Dilek and Gökçay, 1996; Gökçay and Yetis, 1996; Madoni *et al.*, 1996; Yetis and Gökçay, 1989; Chang *et al.*,1986; Tsuboi *et al.*, 1967). Toxicity mainly depends on metal species and concentration and partly on the prevailing pH, the type and strength of the influent and the extent of the system acclimation (Gökçay and Yetis, 1996; Madoni *et al.*, 1996; Tian *et al.*, 1994; Dilek and Yetis, 1992; Vaananen, 1988). In addition to these factors, many metals interact and give effects which can be non-interactive, antagonistic, or synergistic (Ting *et al.*, 1991). Some metals are antagonistic even at non-toxic concentrations, as they compete for binding sites either within cells or on particles (Wood and Tchobanoglous, 1975).

The components of an industrial treatment plant influent rarely include all the chemicals required by a cell; therefore, microbial cells alter their activity in response to their

environment (Prescott *et al.*, 1990). However, the physical lack of nutrients or the unavailability of nutrients often leads to poor waste treatment, as mentioned previously. The transition elements, for example, often form insoluble salts or complex with organic chemicals and as such are unavailable to cells (Amdur *et al.*, 1991). It is possible that only soluble species are available to activated sludge microbiota (Sujarittanonta and Sherrard, 1981). Even so, micro-organisms may adapt to the presence of metals. Most research is based on unacclimated cells and neglects the acquired tolerance of potentially toxic levels of trace elements. Acclimated sludges are reported to maintain the efficient treatment of wastewater in spite of high metal concentrations (Geradi, 1986; Chang *et al.*,1986), although it has also been reported that shock loads are toxic to activated sludge whether it has been acclimated or not (Battistoni *et al.*, 1993). Acclimation is finite, as energy is required, and some bacteria such as the nitrifying species *Nitrobacter* and *Nitrosomonas* cannot acclimatise to heavy metals; hence failure to nitrify is often the first sign of metal poisoning (Geradi, 1986).

2.4 Concluding remarks

Micronutrient addition to activated sludge plants offers advantages other than effective recalcitrant COD removal and improved sludge handling. An increase in the vitamin content of waste sludge can widen the choice of disposal routes and make the process more cost effective. This is often an important consideration due to the volume of sludge that the activated sludge treatment of waste generates and the magnitude of sludge disposal costs, which can account for approximately 50% (Hall, 1996; Kalinske, 1978) or even up to 65% (Gray, 1990) of the total running costs of a plant.

Toxicity can arise from excess micronutrients present in the wastewater to be treated and from overdosing with chemical additions into the activated sludge. Micronutrient doses have to be calculated very carefully and tailored to each situation because toxic thresholds are extremely variable. The sensitivity of microbial species to one chemical can differ by up to seven orders of magnitude (Kenaga, 1987) and the sensitivity of one species to several chemicals is also variable (Mayer and Ellersieck, 1986). This makes

the production of a model of micronutrient toxicity very difficult. Many toxicity series exist for heavy metals alone, depending on the type and structure of the microbial community (Beyenal *et al.*,1997; Cimino and Caristi, 1990; Beaubien and Jolicoeur, 1984; Speece *et al.*, 1983).

However, micronutrient dosing offers a real optimisation strategy for biological processes treating industrial wastewaters. The addition of vitamins and trace elements to biological treatment systems to improve their performance shows great potential as an economical alternative to physical or chemical treatment methods. It is known that a balanced activated sludge community can at least partially maintain its own supply of micronutrients and in doing so provide adequate wastewater treatment. Improvements to COD removal in industrial wastewater treatment by the activated sludge process can be achieved using acclimation and nutritional supplementation to maintain a diverse sludge community.

In spite of the potential harmful effects of excess micronutrients, optimal dosing remains an option for process enhancement. Particular areas for further study are the effects of other pollutants on degradation and the biodegradation of mixtures of pollutants. A central point in the future may be the need for toxicity testing to determine the effectiveness of microbiological treatment processes.

2.5 PRELIMINARY SUMMARY AND CONCLUSIONS

Literature work was carried out to investigate the history of micronutrient addition to assess its potential as a method for enhanced wastewater biotreatment. The work focused on the activated sludge process because this type of system is currently the most widely used biological wastewater treatment process in the developed world for both domestic and industrial wastewaters.

The literature surveyed revealed that the factors limiting the performance of activated sludge plants are often environmental and that many industrial processes can produce xenobiotic wastewaters which resist biological treatment. The availability of nutrients determines the community structure of biological systems and hence the efficiency of the degradation process. Micronutrients influence the bacteria involved in waste degradation and also the species diversity within the community. The requirements for, and toxicity of, different micronutrients vary according to the nature of the waste and the ecology of the system. Adding micronutrients to biological treatment processes is one possible approach to upgrading an existing facility in order to deal with increasing volumes and strengths of industrial wastewater and the tightening discharge legislation.

Micronutrient addition is a potential method for improving the performance of industrial wastewater treatment by the activated sludge process. Chapter 2 indicates that experimental work into micronutrient addition as an option for industrial wastewater treatment is required.

CHAPTER THREE

OBJECTIVES

3. OBJECTIVES

1. Establish whether current operating procedures and levels of wastewater treatment in activated sludge plants will be sufficient for operators to accommodate toxicity–based consents.

2. Test a wide range of micronutrients for their potential to enhance COD removal from industrial wastewater using respirometry.

3. Investigate the most promising micronutrients using porous pots to determine longer-term effects on process stability and performance.

4. The effects of the most technically and economically effective micronutrient additions on nitrification and sludge handling are also to be investigated through the use of a pilot scale activated sludge plant.

5. Investigate whether micronutrient addition would be used in industry as an option for process improvement in response to DTA.

CHAPTER FOUR

DIRECT TOXICITY ASSESSMENT AND THE NEED TO ADAPT BIOLOGICAL WASTEWATER TREATMENT.

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DIRECT TOXICITY ASSESSMENT AND THE NEED TO ADAPT BIOLOGICAL WASTEWATER TREATMENT.

4.1 Introduction

The techniques available for classic ecotoxicity testing cover several trophic levels in fresh and marine water. Trophic levels refer to the hierarchy of species in the food chain. Ecotoxicity tests are an established approach with good foundations, previous use, and they are internationally defined. These tests have registered methods with strict validation to ensure reproducibility. Receiving waters are tested by bioassay: a set of biological tests that are applied to numbers of samples for comparison to a control. The most common ecotoxicity tests used in water analysis are outlined in Table 4.1. However, certain barriers to conclusive toxicity testing for DTA exist. Validation procedures are extremely important but they raise the costs of testing. Laboratories that can not afford validation do not have the ability or resources to handle a wide range of tests and waters. The theory of the standard tests is sound, but interferences are numbered and varied. Controls are hard to find and large sample volumes are required. Therefore, the EA DTA demonstration programme is an excellent plan. There is a need to concentrate on the reliability and reproducibility of the test methods to ensure they deliver data which answer the questions posed.

In the UK, the EA has issued a consultation document (Environment Agency, 1996) on the proposed introduction of toxicity-based conditions to discharge licences. The consultation period lasted from 16 July to 30 September 1996 and was open to written comments from any interested party. The document discusses the ecotoxicological methods to employ and the protocols required for quality assurance, the licensing procedure, effluent characterisation and toxicity reduction. A response compendium was produced in March 1997 which contained the consultees' replies and a breakdown of the numbers of consultees concerned about the core issues raised in their comments

(Environment Agency, 1997). There was also a workshop (Taylor, 1996) which detailed more information regarding the companies' responses.

This study set out to understand the potential impacts of applying this new concept in effluent standards. A further aim was to identify whether these impacts could be characterised in a model that recognised specific attributes of those operators concerned. The model was intended for use as a predictive tool for use with potentially affected companies at a later date. This was achieved by meeting two objectives:

1. Identifying the defining characteristics of operators that are exposed to higher risks
2. Identifying the options open to such operators to allow them to minimise the impact that DTA may have on them

4.2 Who might be affected by the introduction of DTA?

The first section of the study consisted of a review of earlier research that indicates the responses of various industrial sectors to legislative change. This allowed some consideration of the approach to take in order to make the data obtained useful. The consultation report document has shown that the two major concerns of UK companies are:

- dealing with cost and benefit
- the choice of technical procedures used to determine ecotoxicity targets

However, the document gives no indication of the individual concerns of small companies, water companies etc., and so is of limited use in attempting to predict the potential impacts of the scheme. In order to assess the implications of the introduction of DTA for UK companies, a number of steps were taken:

Table 4.1. Outline of common ecotoxicity tests.

Test	Test organism	Method	Output	Interferences
Algae test	*Scenedesmus subspicatus* (fw) or *selanastrum* (m)	Range of concentrations added to media. Aliquots tested at 24hr intervals. Nutrition standardised so poor nutrition does not emulate pollution.	IC_{50} and IC_{10}	Spectroscopic analysis sensitive to colour pollution. Dyes and suspended solids lead to artificially high results. Filtering solids means that the water is no longer a representative sample. Receiving waters may already hold algae.
Invertebrate test	*Daphnia magna* (fw) Pacific oyster embryo (m)	Concentrations of pollutants prepared in aqueous media	24 and 48hr immobilisation EC_{50} and EC_{10}. Highest NOEC and LOEC	*Daphnia* death hard to see. Also the organisms are sensitive to change and die in a change of water regardless of its polluting potential. The species used may not be relevant to the receiving water. No accounting for toxicity in sediments.
Invertebrate test	Oyster embryo (m)	16-42 cell stage embryos placed in various concentrations of sample water. At 24hrs add formaldehyde. Study the embryo shapes for correct development.	24hr EC_{50} and EC_{10}. Highest NOEC and LOEC	Sudden death (as with *Daphnia*). Control water hard to find. Suspended solids and other fauna affect development. Other species may be more appropriate to the test waters.
Vertebrate test	Rainbow trout (fw) Turbot (m)	Internationally accepted method. Juvenile fish in concentrations of test samples. Exposure can be static, semi-static or flow-through to cater for volatility, spontaneous degradation etc. in test compounds.	24hr LC_{50} and LC_{10}. Highest value for NOEC and LOEC	High background fatality. Huge sample volumes required (10-20l per test) and ∴ high costs of transport and storage. *In situ* testing is more appropriate. One big advantage though: lowest background fatality of all the tests.

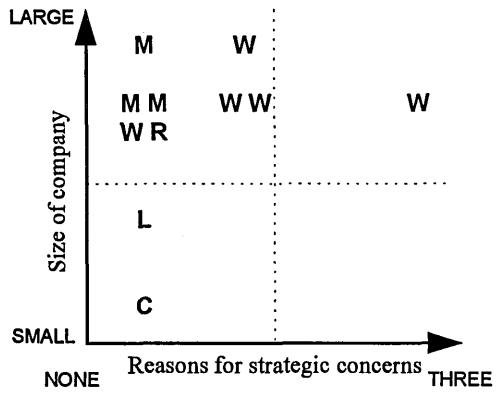
fw = freshwater, m = marine, IC_{50} = 50% immobilisation concentration, EC = effect concentration, NOEC = no observed effect concentration, LOEC = lowest observed effect concentration

- compile a questionnaire to find out the views of informed UK companies about the implications of implementing DTA for their processes
- assess the potential effects of DTA on industry
- identify the options available to industry to accommodate DTA

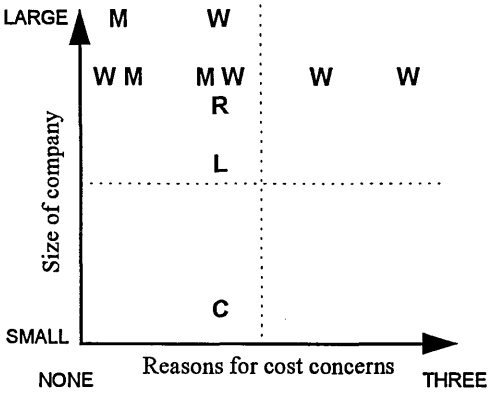
The question, *who might be affected?* was addressed using a survey. A set of questions was designed to elicit sources of concern for individuals with responsibilities in various effluent-producing UK industrial sectors. Respondents were also asked to grade the gravity of their concerns and identify their underlying reasons. Recipients of the questionnaires were chosen for their prior awareness of the issue of developing DTA and previous willingness to be involved in discussions such as workshops or seminars. Most were identified from EA documents, as there is no value in questioning respondents with no knowledge of the subject. The sample was designed to include regulators and the regulated, researchers and industrial workers. Recipients were also chosen to try and represent as many stakeholding industrial sectors as possible, including the water and wastewater treatment industry, manufacturers and waste disposal operators.

The questionnaire was piloted using two recipients to ensure that the information gained would be of value then sent out to a number of recipients with a covering letter stating the aims of the questionnaire. Many of the respondents agreed to take part in further discussion. The results (Appendix A) led to interpretation in the form of a set of matrices whose axes define each respondent (Figure 4.1).

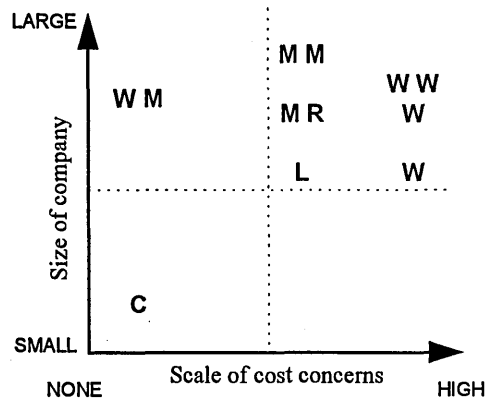
The level of control a company has over the inputs to its processes was assessed according to the amount of choice available to that company. This acts as a reflection of the level of control over the companies' waste outputs. An example of a respondent with a large amount of control over their inputs would be a manufacturer who is able to decide what to produce and how to do so. Respondents with little control include waste managers and wastewater treatment operators who are able to refuse an input to their systems only in the most extreme circumstances and who have no control over the rates of input.



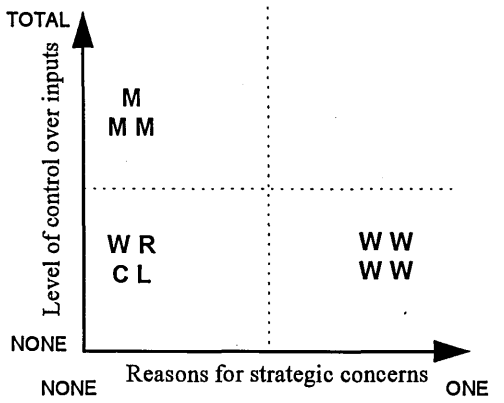
1. Size of company versus number of stated causes for concern reference managerial issues.



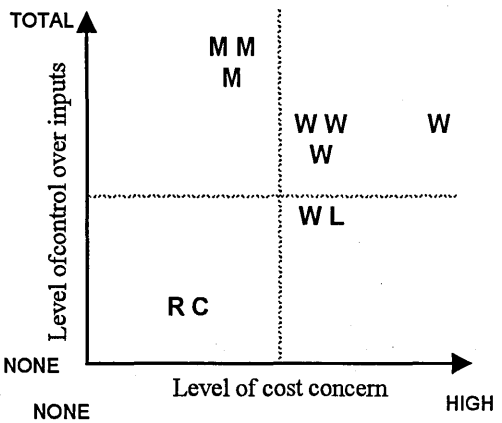
2. Size of company versus number of stated causes for concern reference financial costs.



3. Size of company versus scale of concern reference financial costs.



4. Control over process inputs versus managerial issues.



5. Control over process inputs versus cost concerns.

Legend

M	=	manufacturer
C	=	consultancy
W	=	water company
R	=	regulatory body
L	=	landfill/waste operator

Figure 4.1. Matrices mapping attributes of respondents' companies with their concerns.

The definitions of the remainder of the axes for each matrix were defined during data sorting, e.g. the level of concerns regarding the expected costs to the company of implementing DTA. The respondents could choose from very, slightly and not at all concerned, with no differentiation between operating and maintenance costs and capital outlay. 'Reasons for management concerns' includes issues such as keeping up to date with technical and legislative developments and overcoming staff objections to additional or replacement roles. The matrices were based on the attributes of the respondents, as set out in Table 4.2. A wide range of matrices was produced but only those resulting in clusters of respondents are presented here.

Matrix 1. Size of company versus number of stated causes for concern reference managerial issues. Only the large and medium sized companies showed managerial concern, possibly related to their more complex workplace hierarchy. Dissent is almost impossible in the consultancy, a company with very few staff. The medium to large companies in the left hand cluster are water companies whose staff already possess many of the skills required for the proposed new tests. Some mentioned a fear of prosecution by the EA or stated concern over the possible introduction of DTA as a legal compliance parameter, but did not class these as issues to be addressed by management staff in the same way as certain other causes for reservation, listed below. Few of the reasons arose in the comments of more than one respondent, showing that the perceived problems of implementing DTA are highly speculative rather than being based on firm statements of intent by the EA. A similar distribution of responses is obtained in the following matrix, company size versus cost issues. In many cases, the same respondent expressed concern in both areas, perhaps belying general concern rather than real problems.

Matrix 2. Size of company versus number of stated causes for concern reference financial costs and Matrix 3. Size of company versus scale of concern reference financial costs. In matrix 2 the X-axis indicates the number of topics regarding cost which are seen as possible causes for concern. A maximum of three issues was raised by any one respondent, including a general 'overall cost' in spite of the

acceptance of DTA as a 'good principle'. The larger companies expressed the least number of causes for concern. The medium - large companies are spread across the X axis and the waste operator plans to send test samples out-of-house, avoiding some variation in testing costs and the outlay involved in setting up ecotoxicity laboratories for the small number of samples envisaged. The X-axis in matrix 3 represents the level of concern (very, slightly, and not at all). The largest respondent is a regulator whose workload will alter in nature and size, and the large companies on the left include a distributor of goods, and a water company which is a strong supporter of DTA. This respondent can foresee extra costs for the company via its suppliers but not in relation to the processes this respondent actually carries out.

Matrix 4. Control over process inputs versus managerial issues. The Y-axis on this matrix indicates the level of control the respondent company has over inputs to its processes (as in matrix 1). The X-axis indicates the number of topics causing concern in terms of managerial issues. The maximum number of topics identified by any one respondent was one, although these topics were diverse. The reasons stated included a desire to avoid legal prosecution, overcoming changes in staff responsibilities and keeping up with new developments in technical and legal issues, given by respondents in the bottom right hand group. Smaller water and waste companies included in the bottom left hand cluster felt that no managerial problems were foreseen, possibly in relation to their small number of hierarchical layers. The manufacturers in the sample (top left cluster) stated that they would change the inputs to their processes rather than deal with technical issues in wastewater testing. Many said that the number of management issues arising would depend upon the role of DTA (i.e. legal tool or trigger for action).

Matrix 5. Control over process inputs versus cost concerns. The X-axis represents the number of reasons for concern about cost, and the Y-axis indicates the level of control over process inputs. Therefore Matrix 5 is an indication of the level of control the respondent is able to exert over the nature and/or quantity of process inputs. The regulators are not concerned about cost of implementation, so this matrix is not relevant to them. The consulting companies whose process is a service have inputs that

are out of their control, but they will not be affected more than usual in terms of capital costs and their running costs will not be affected at all. In fact, the introduction of DTA measures could bring more work for such companies, leading to financial benefits.

Table 4.2. Defining characteristics of questionnaire respondents.

Characteristics	**Variations**
Size of company (number of employees)	0-199, 200-499, 500-999, 1000-1999, 2000+
Stated reasons for concern regarding managerial issues	The reasons reported from a total of eight that occurred: • capital outlay justification • operational costs justification • fear of legal action • public relations problems • staying up to date with technical advances • potential staff objections (especially re. extra duties) • decision making re. changes to process inputs • uncertainty over the use of DTA as a legal tool
Stated reasons for concern over financial issues	The reasons reported from a total of five that occurred: • unnecessary extra laboratory work • application of inappropriate toxicity tests • general concern despite approval of DTA in principle • fear of legal action resulting from lack of control over inputs to processes • concerns arising from uncertainty re. the industries and/or parameters to which DTA will be applied

4.3 Summary of Matrix Results

The results can be summarised as follows:

1. Control over process inputs reduces the level of concern felt by a company with respect to the cost of introducing DTA. Notable exceptions are companies whose process is a service and who therefore produce no effluent.

2. Predominantly the medium sized companies (500-2000) expressed concern regarding management issues. Respondents from larger companies differed in opinion, indicating that the level of concern is linked more to the nature of the respondents' business.

3. Company size does not directly determine the level of concern regarding financial costs. Small companies were at opposite ends of the axis, and the others showed an almost even spread.

4. Most respondents chose one of the two main reasons for cost concern (compulsory use of unnecessary and/or inappropriate tests). DTA is accepted in principle, concerns arise mainly from its application.

5. Most of the water companies in the sample were more concerned about managerial issues than costs, with the exception expressing no concern in either area. The manufacturers, regulators and consultancies do not foresee managerial problems arising from the introduction of DTA.

In general, the operators at risk include every industrialist that discharges wastewater - food processing plants, petrochemical refineries, textiles industry, tanneries, sewerage undertakers and waste disposal operators whose facilities produce leachate. However, the water industry is the major player in the aquatic environment business, therefore arguments arising from the model focus on this sector. Interesting points to note were

found in the responses to a set of yes/no questions (Table 4.3). The answers to these questions indicated a widespread approval of the principle of DTA in spite of a number of concerns regarding the techniques to be employed and a high level of optimism for its use as a valuable additional tool for pollution prevention. The positive attitude towards implementation of toxicity-based consents is the most important conceptual change required for the universal use of DTA to be realised.

The results from the matrices led to a generic common model (Figure 4.2) which states that certain "quick fix" options are available to companies with the need to adapt to new quality control on their effluents but that different options are needed for long term, sustainable compliance. The introduction of DTA requires a change in opinions and attitudes to water testing. Evidence of effluent toxicity will be enough for the EA to serve the dischargers with a notice to act, alongside advice and guidelines on how to investigate (with BATNEEC in mind). While DTA will not be a legal pass/fail consent, failure to act on EA guidelines can prompt legal action under the current legislation. DTA will be an easier way to gain environmental benefit from the UK discharge consents system and will prevent the rapid growth of numeric chemical targets, easing the load in terms of numbers of tests the water monitoring industries will be required to perform.

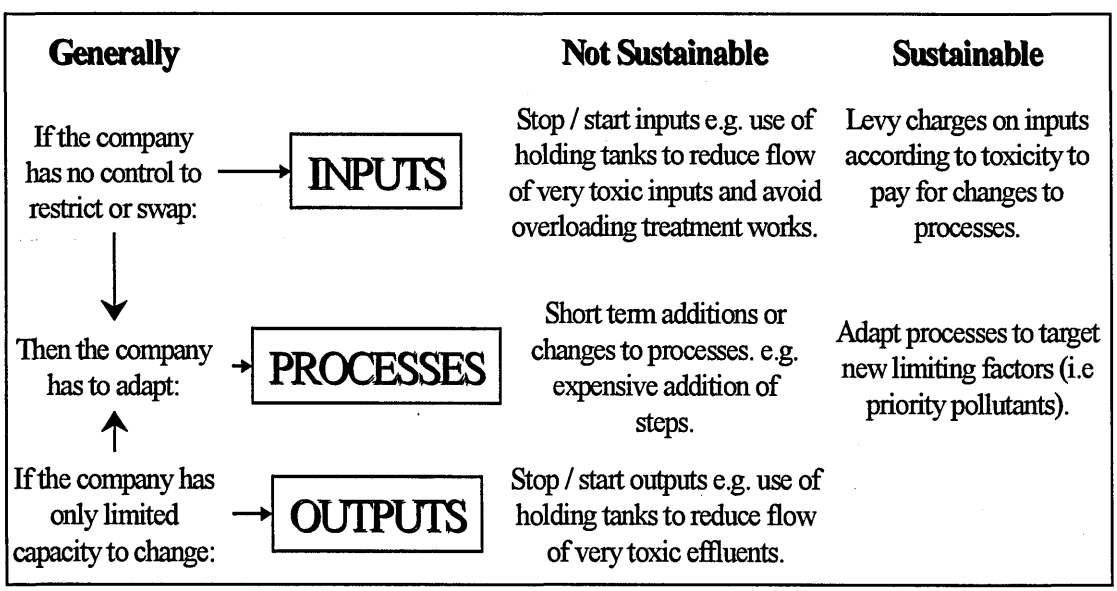


Figure 4.2. Generic common model of adaptation opportunities.

Table 4.3. Responses to conceptual questions.

WOULD YOU CONSIDER THE FOLLOWING TO BE ISSUES WITHIN YOUR COMPANY IF DTA WERE TO BE INTRODUCED?			
	Yes	**No**	**Comments**
Misapplication of tests	64%	36%	National protocols may not be appropriate to local receiving waters. Fish tests under consideration are not appropriate.
Misinterpretation of test data	73%	27%	Concerns about technical difficulties. Concern that mixing zones may not be interpreted correctly. National protocols may not be appropriate to specific samples.
Lack of training in personnel	45%	55%	As with many EA issues. Keeping up to date will be an issue.

DOES YOUR COMPANY SHARE TECHNICAL CONCERNS REGARDING THE FOLLOWING?	**Yes**	**No**	**Case dependent**
Level of variation in test procedure	73%	27%	0%
Use of alternatives to *Daphnia* e.g. Amtox™	73%	27%	0%
Use of ecotoxicology data in legal matters	73%	27%	0%
Are current controls adequate for control of discharge consents?	9%	73%	18%
Do you believe it could be possible to use DTA as a tool for pass/fail compliance?	18%	64%	18%
In your opinion, would the introduction of DTA as a trigger for action enhance current environmental practice?	100%	0%	0%
Do you think that DTA should supersede current tests used to determine discharge consent compliance?	27%	55%	18%
Would DTA be a worthwhile addition to the range of effluent tests used at present?	91%	9%	0%

4.4 What are the options for companies that need to adapt?

Discussion of the matrices with the EA led to the identification of the options for water companies or other industries with pre-treatment plants (Table 4.4).

4.4.1 Changing inputs

One of the options open to water companies is the refusal to accept highly toxic wastewater. This is technically one of the simplest options; it sorts out the trouble at source for water companies accepting traders' effluents. Pursuit of this option will reduce the number of discharges to sewer, but it may lead to more discharges directly into receiving waters and hence to more environmental pollution. It may also lead to closure of manufacturing companies who can not afford to operate wastewater treatment plants. Water companies could also levy charges for discharges to sewer, or implement pre-treatment regulations according to toxicity. This system is in place in parts of the USA (East of Scotland Water, 1996). New charges may lead to an increase in the number of companies operating their own wastewater pre-treatment plant. Any company treating composite effluents could segregate component wastewater streams; this leads to overall cost savings and treatment optimisation (Looney, 1996). Product substitution by manufacturers could be a route to reducing by-product/wastewater toxicity, but this option is difficult for industries such as pharmaceuticals manufacture to take.

4.4.2 Changing wastewater treatment processes.

This can be simply put as the adaptation of treatment processes to target new effluent quality targets. Techniques available include the use of activated carbon (non-renewable), activated sludge and biofilms (self-renewing and therefore more sustainable). The use of online toxicity monitors as far upstream in the sewer system as possible, to identify toxic effluent streams and pass the responsibility of toxicity reduction back to the polluter would promote toxicity minimisation by the producers of effluents.

Manufacturers of wastewaters have options of wastewater minimisation (Wang and Smith, 1994; Hamilton and Dowson, 1994), reclamation of toxic wastewater components, and the use of pre-treatment works open to them.

4.4.3 Changing outputs

End–of–pipe solutions to toxicity include pH neutralisation, dechlorination of treatment works effluents having tertiary treatments, and chelation (of metals especially).

Table 4.4. Options for adaptations for compliance with ecotoxicological discharge consents.

Changing inputs	Changing wastewater treatment processes.	Changing outputs
• Refuse to accept highly toxic wastewater • Charge for discharges to sewer according to toxicity. • Implement pre-treatment regulations • Segregate component wastewater streams • Product substitution • Recycling of toxic wastewater components	• Use of online toxicity monitors. • Use nitrification / denitrification treatment processes to remove ammonia, a strong pollutant. • Remove known toxins using activated carbon. • Use more pre-treatment upstream of sewage treatment works to stabilise effluent. • Use more biological processes. Avoids toxins from treatment chemicals, good for removing organics and metals (Musterman *et al.*, 1996).	• pH neutralisation • Dechlorination of sewage treatment works effluents having tertiary treatments • Chelation (of metals especially)

4.5 Conclusions

In the light of the information obtained, especially reference the recommendations for wastewater-treating industries, it can be concluded that company size does not determine its level of concern regarding financial and managerial costs. Companies can be categorised according to their perceived ability to adapt to the introduction of DTA. The importance of long term process adaptation as opposed to pure optimisation of existing processes has been recognised. Ongoing work will explore methods of process adaptation such as the use of micronutrient addition in the activated sludge process rather than the optimisation of existing operating parameters (such as pH and temperature).

4.6 INTERIM SUMMARY AND CONCLUSIONS

The literature review (Chapter 2) indicated that the addition of vitamins and trace metals to industrial wastewater could increase the number of waste streams that can be treated using activated sludge. It also suggested that the performance of existing treatment systems could be enhanced by micronutrient addition.

Questionnaires were sent to a number of people involved in different industries that are stakeholders in the aquatic environment, to investigate the need for increased and enhanced use of biological wastewater treatment in response to the possibility of implementing DTA in the UK. The questionnaire responses led to the drawing of a number of conclusions. The information gathered from the respondents, particularly the details about the recommended means of wastewater treatment optimisation, showed that traditional measurements, such as the size of a company, or its membership to a particular industrial sector, do not determine its level of concern regarding financial and managerial costs. The ability of a company to adapt to new environmental legislation appears to be based on the ability, perceived or real, of that company to alter three key stages of their process, i.e. inputs, processes and outputs.

Companies can be categorised according to their perceived ability to adapt to the introduction of DTA. The results led to a generic common model of "quick fix" options available to companies with the need to adapt to new quality control on their effluents, but that different options are needed for long term, sustainable compliance. The importance of long term process adaptation as opposed to the alteration of existing process operating parameters was recognised, and the need for work to explore possible methods of bioprocess expansion and adaptation was established.

CHAPTER FIVE

SCREENING OF MICRONUTRIENT SUPPLEMENTS.

EDITIONS OF THIS CHAPTER ARE PUBLISHED AS:

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Burgess JE, Quarmby J and Stephenson T (1999). Micronutrient supplements for optimisation of the treatment of industrial wastewater using activated sludge. Water Research, 33,18: 3707-3714.

SCREENING OF MICRONUTRIENT SUPPLEMENTS.

5.1 Introduction

Activated sludge treatment of industrial waste is used to treat a wide range of wastewaters worldwide (Clark and Stephenson, 1998). Many industrial waste waters show resistance to the biological treatment processes commonly used to treat municipal and domestic wastes. Performance figures vary greatly, but typically 85-95% BOD removal is expected of an activated sludge plant treating general municipal waste (Nicoll, 1988) and plants treating industrial wastewaters are expected to achieve 75-95% BOD removal (Metcalf and Eddy, 1991) and 80-85% COD removal (Henze *et al.*, 1995). It had been expected that micro-organisms were theoretically capable of degrading any oxidisable material, provided suitable environmental conditions prevailed (Singleton, 1994) but a number of industrial processes now exist which result in the release of synthetic compounds unfamiliar to microbial cells and therefore resistant to biodegradation.

Various breakdown products are found in activated sludge plants treating industrial effluents, including phenols and quinones, and some products are more inhibitory than the original compound (Schöberl *et al.*, 1988). This problem of toxic products can be surmounted by using mixed cultures, such as those occurring naturally in activated sludge, or a designed microbial consortium having a wide range of metabolic pathways. Mixed cultures are important in waste treatment for the complete mineralisation of organic toxins to carbon dioxide and often enhance microbial metabolism by degrading toxins produced by metabolism and allowing cometabolism to take place (Entwhistle, 1986). Many industrial wastewaters are lacking in the nutrients required for microbial growth. A number of nutrients is essential for metabolic activity. The macronutrients required are carbon, oxygen, hydrogen, nitrogen, phosphorus and sulphur. Nitrogen and phosphorus may need to be added to the influent in treatment plants dealing with industrial wastewater, as the availability of nitrogen and phosphorus can limit the

degradation of organics (Valo *et al.*, 1985, Singleton, 1994). It is common for wastewater treatment plants in this situation to experience problems associated with sludge handling and poor biological waste treatment (Pala and Sponza, 1996). A wide range of micronutrients including copper, manganese and B-vitamins are required by activated sludge (Lemmer *et al.*, 1998; Lind *et al.*, 1994). Sufficient micronutrition is needed to support all the genera required for activated sludge to treat wastewater as an unbalanced activated sludge community can lead to sludge handling problems and less than 50% BOD removal.

Biological oxidation must compete technically and economically with other treatment processes, but is often slower than chemical processes or incineration and can produce inconsistent end results. A vast increase in our understanding of the nutritional and environmental requirements of micro-organisms has meant that biodegradation has become an efficient and economical option (Singleton, 1994). Until recently, process performance has been measured in terms of BOD and solids removal. However, increasing emphasis on effluent toxicity and priority pollutants has highlighted the need to remove COD, recalcitrant organic compounds that impart toxicity to wastewater streams. The aim of this study was to investigate the optimisation of the capacity of an activated sludge mixed culture to remove COD via micronutrient additions.

5.2 Materials and methods

Atomic emission spectrophotometry using an Inductively Coupled Plasma Atomic Emission Spectrophotometer (*Thomas Jarrell Ash, Plasma 300)* was used to identify the missing or deficient trace elements in the industrial waste as compared to theoretical trace metal and vitamin requirements of activated sludge micro-organisms (Table 5.1). The concentrations of available nitrogen, phosphorus and sulphur were also measured and compared with the theoretical requirements of bacteria for these nutrients stated in the literature. The theoretical vitamin requirements and their doses were taken from the literature (Table 3.2). This procedure produced a list of micronutrients requiring supplementation (Table 5.1).

A series of tests was performed using a closed cell aerobic respirometer (*CES Ltd. Aerobic Respirometer*). This model operates in a similar way to the Warburg respirometer (Heddle, 1980). Respiration is the key biochemical process of life. Heterotrophic organisms obtain their energy form oxidation/reduction reactions, i.e. reactions in which electrons are removed from one chemical as it is oxidised and passed on to another to reduce it. Organisms obtain energy from the oxidation of reduced molecules (substrate). In aerobic respiration, oxygen is finally reduced to produce water as a waste product of the metabolic process. The respiration rate is the rate at which oxygen is used as the terminal electron acceptor. When the respiration rate of a sludge sample is taken it indicates the condition of the sludge in terms of metabolic activity.

The closed cell respirometer uses a manometric cell coupled to an oxygen generating system which is held at a constant temperature. Oxygen consumption is measured by monitoring the pressure reduction in a closed vessel. The CO_2 produced is absorbed in an alkali trap (10M NaOH) so as not to mask the effect of oxygen removal, and the reduction in cell pressure can be related to oxygen consumption by the biomass present. The pressure change is counteracted by the electrolysis of an aqueous solution of $CuSO_4$ (25% w/v) and the amount of oxygen consumed is directly proportional to the power required to produce replacement oxygen by electrolysis. Six trace elements and six vitamins, as identified in literature (Table 5.1), were tested in single supplements and pairs of micronutrients. Multiple supplements of three or more micronutrients were not performed to facilitate clear data interpretation.

The activated sludge was taken from the RAS line of an actiavted sludge pretreatment plant receiving effluent from the fine chemicals industry that contains a high proportion of organic components (e.g. toluene, chlorobenzene) and has high COD, of which 81 ± 11% is soluble (COD:N:P of 880:20:1; BOD:N:P of 212:20:1; COD:BOD of 4:1). The wastewater was given a phosphorus supplement in the form of orthophosphoric acid (*AnalaR, BDH Merck*) such that the COD:N:P ratio of the wastewater was 100:10:1. The pH of the wastewater was 8.2 ± 0.2. Several experimental runs were performed in which ten (nine tests and one control) 50ml aliquots of the activated sludge were placed

in respirometry cells to which predetermined quantities of micronutrient supplements had already been added. The volumes in the cells were made up to 55cm^3 with deionised water as required to standardise the partial pressures within the cells and make the tests comparable.

Table 5.1 Nutrient requirements of activated sludge and the doses used.

Nutrient	Range of theoretical micronutrient requirements (mg/l)†	Concentration of micronutrients detected in the wastewater (mg/l)	Dose added (mg/l)
Macronutrients			
Nitrogen	15.0 minimum[1]	32.00	none
Phosphorus	3.0 minimum[1]	1.69	1.30
Sulphur	1.0 minimum[1]	100.0	none
Trace elements			
Calcium	0.4 - 1.4	0.44	1.0
Potassium	0.8 - ≥3.0	95.0	none
Iron	0.1 – 4.0	1.20	none
Magnesium	0.4 - 5.0	10.0	none
Manganese	0.01 - 0.5	<1.0	1.0
Copper	0.01 - 0.5	<1.0	none
Aluminium	0.01 - 0.5	0.02	1.0
Zinc	0.01 – 0.5	<1.0	1.0
Molybdenum	0.2 - 0.5	<1.0	0.5
Cobalt	0.1 - 5.0	<1.0	1.0
Vitamins			
Biotin	0.05 – 0.1	-	1.0
Niacin	0 – 10	-	1.0
Thiamine (B_1)	0.3 – 1.2	-	1.0
Lactoflavin (B_2)	0.5 – 2.0	-	1.0
Pyridoxine (B_6)	0.1 – 10	-	1.0
Pantothenic acid	0.01 – 2.0	-	1.0

[1] From suggested COD:N:P ratio of 100:10:1, Beardsley and Coffey, 1985 † Table 2.2

The respirometer cells were stabilised at 20°C and stirred continually for six hours. Oxygen uptake was recorded automatically at five minute intervals. A sample of the original activated sludge was filtered for influent COD (*Whatman GF/C* filter) and measurements of effluent COD were made after six hours in the same way (*Hach* system, *Camlab*, adapted from APHA, 1992). The MLSS of the sludge was determined using standard methods (APHA, 1992). The data were converted to mean oxygen uptake rate

over the six hour period, and COD removal rate per unit biomass. Tests were replicated to ensure reproducibility. The data were analysed using Analysis of Variance (ANOVA): Two-Factor Without Replication. This analysis tool performs a two-factor ANOVA that does not include more than one sampling per group, testing the hypothesis that means from two or more samples are equal. This technique expands on tests for two means, such as the t-test (Berthouex and Brown, 1994).

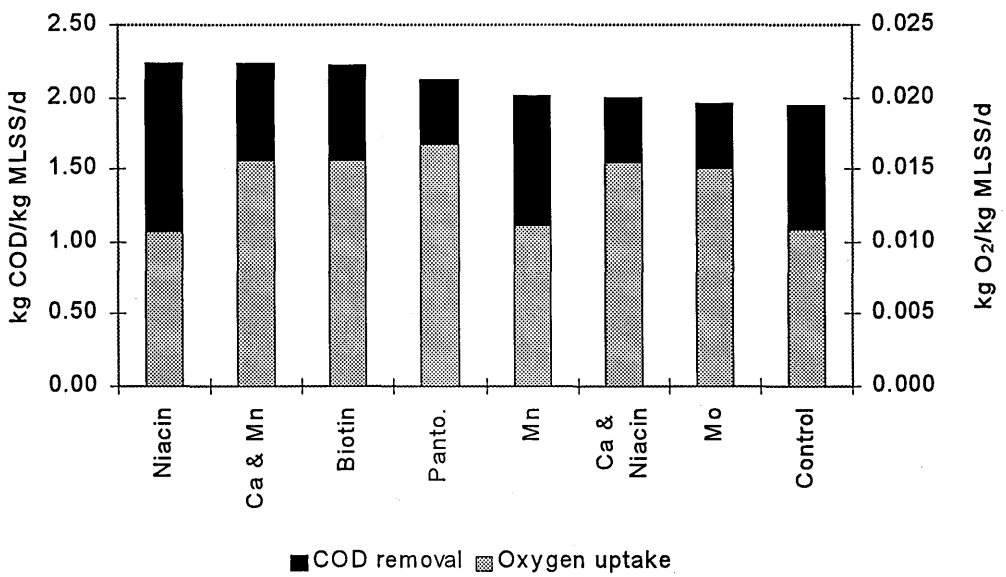
5.3 Results

The data can be divided into three sections, according to their effects on the activated sludge. Seven of the dosing regimes resulted in stimulation of the sludge, indicated by increased COD removal rates and either no changes, or increases in oxygen uptake rates compared with the control values of 0.01kg O_2/kg MLSS/d oxygen uptake and COD removal of 1.94kg COD/kg MLSS/d (Figure 5.1). The greatest improvement in wastewater treatment was attained by the addition of 1.0mg/l niacin to the wastewater, resulting in an unchanged oxygen uptake rate (0.01kg O_2/kg MLSS/d) and increased COD removal (2.24kg COD/kg MLSS/d), a significant change (ANOVA F=0.02, P=0.90) (Figure 5.1). A drop in respiration coupled with a rise in COD removal indicated the adsorption of particulate COD onto cell wall binding sites, but as the change in oxygen uptake rate is not statistically significant (ANOVA F=0.09, P=0.90) the net effect of niacin dosing can be considered stimulation. The results suggested that the vitamins biotin, pantothenic acid and niacin are required by activated sludge bacteria and that calcium enhanced the stimulatory effects of niacin and manganese.

Thirty-six of the supplements resulted in increased oxygen uptake and decreased COD removal, indicating inhibitory effects (Figure 5.2). This group includes some metals, which can prompt accelerated respiration by acting as catalysts in microbial kinetics (e.g. cobalt). These results showed that calcium and zinc dosed individually stimulated respiration but when combined they inhibited the treatment (Figure 5.3). The data indicated that dosing with any pair of metals which included calcium exhibited extremely

varied COD removal rates, closely linked to the rates produced by the additive of the other metal (i.e. not calcium) alone.

Thirty-five of the micronutrient supplements had inhibitory effects on the sludge as reflected in the reduced COD removal and low respiration rates (Figure 5.3). The dosing of pairs of vitamins consistently reduced COD removal rates although the effects on the rate of oxygen uptake were varied. It was difficult to determine how the micronutrients interacted. However, 20 of the 36 metal/vitamin combinations resulted in a reduction in the respiration rate and/or COD removal, suggesting that most micronutrient complexes are not as beneficial as additions of single supplements. Only one combination, calcium and niacin, yielded better COD removal rates than the control. It is clear that vitamin dosing did not reduce any inhibitory effects of the metals added to the sludge, and it can not replace acclimation. The addition of a mixture of cobalt and lactoflavin had a detrimental effect on COD removal. The respiration rate rose but the COD removal rate dropped to 0.13kg COD/kg MLSS/d (Figure 5.3), indicating a strong inhibitory effect.



(B_1 = thiamine, B_2 = lactoflavin, B_6 = pyridoxine)

Figure 5.1. Mean oxygen uptake and COD removal rates of supplements whose effects were unchanged or increased O_2 uptake and increased COD removal.

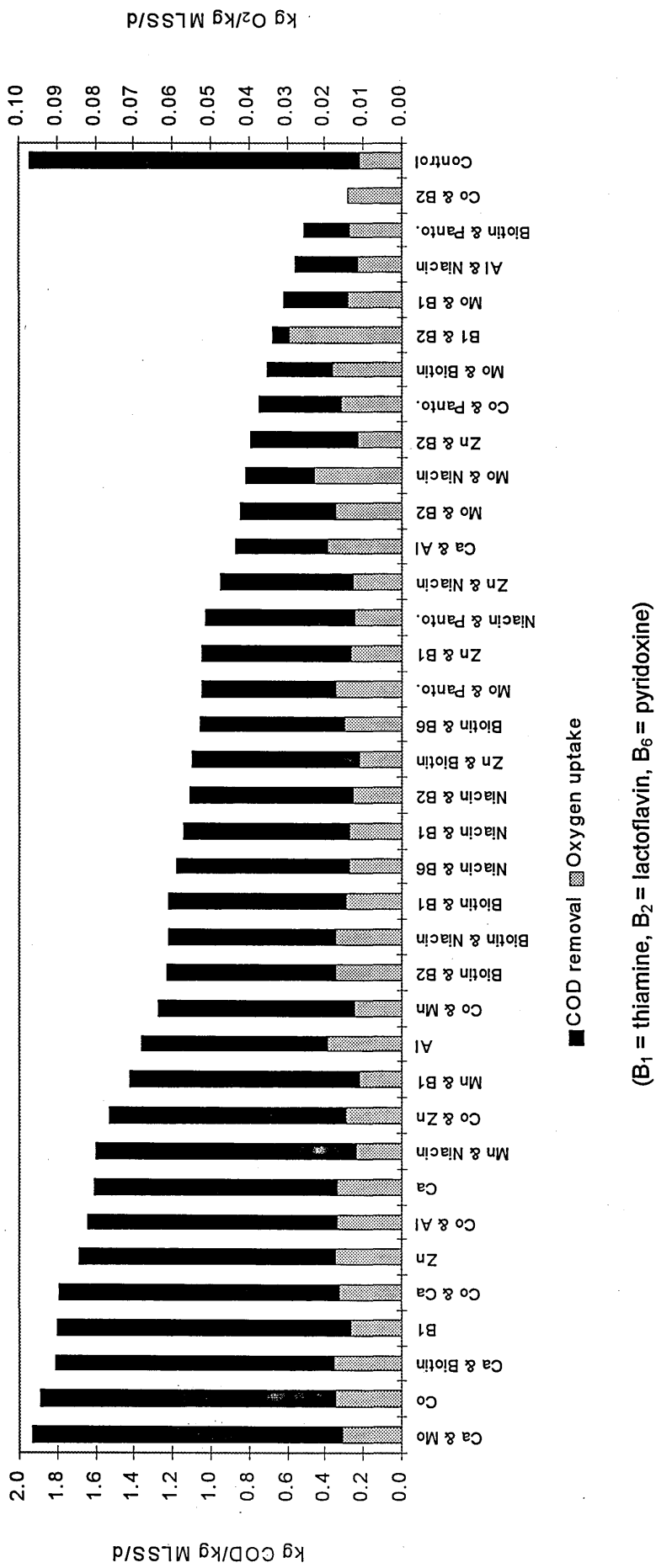


Figure 5.2. Mean oxygen uptake and COD removal rates of supplements which exerted inhibitory effects (increased O_2 uptake and decreased COD removal).

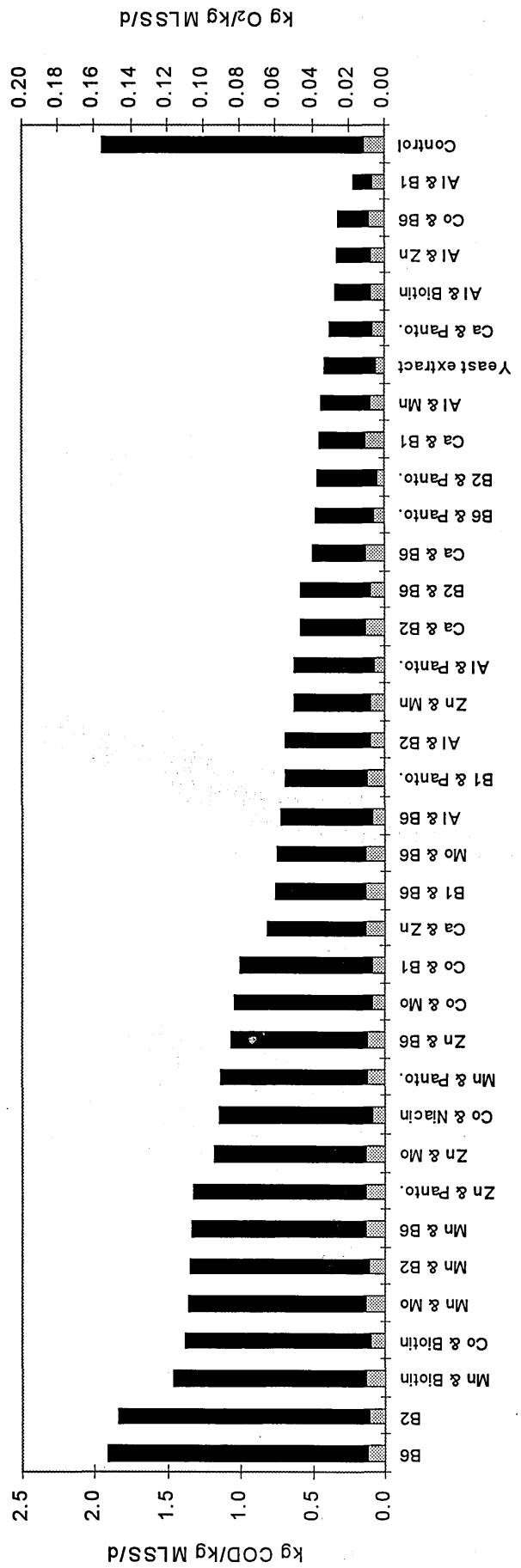


Figure 5.3. Mean oxygen uptake and COD removal rates of supplements which exerted inhibitory effects (decreased O_2 uptake and COD removal).

5.4 Discussion

There is limited information available in the literature regarding respiration rates of sludge measured using comparable operating regimes and respirometry methods, however, the range of respiration rates reported here (0.0039 kg O_2/kg MLSS/d for lactoflavin/ pantothenic acid to 0.0293 kg O_2/kg MLSS/d for thiamine/ lactoflavin dosed sludge) is lower than the previously published values using this model of respirometer (Table 5.2).

Table 5.2 Respiration rates of activated sludge

Respirometer	Process	Waste water	Loading rate g BOD/g SS/d	Respiration rate kg O_2/kg MLSS/d
Current study (control sludge aliquot)			0.16	0.011
Not known [1]	Aerobic batch digestion	Synthetic	0.31	0.079
Current model [2]	Activated sludge, $AlSO_4$ coagulant	Domestic*	0.21	0.096
Current model [2]	Activated sludge, polyelectrolyte coagulant	Domestic*	0.27	0.24
Current model [3]	Activated sludge, iron (II) sulphate coagulant	Domestic*	0.26	0.29
Current model [3]	Activated sludge, iron (III) chloride coagulant	Domestic*	0.21	0.206
Current model [4]	Activated sludge	Domestic*	0.30	0.084
Not known [5]	Oxidation ditch	Municipal	Not known	0.094

[1]Matsuda *et al.*, 1993; [2]Clark *et al.*, 1999a; [3]Clark *et al.*, 1999b; [4]Mayhew and Stephenson, 1998; [5]Barnes *et al.*, 1983

A number of biological factors influence respiration rates of biomass, including the species of bacteria present, the presence or absence of protozoa, the source of wastewater and the degree of acclimation of the biomass to its substrate (Montogomery, 1967), long acclimation periods causing low oxygen uptake rates (Lind *et al.*, 1994).

Physicochemical factors such as the composition of the wastewater, its temperature and hence its ability to contain DO also affect the respiration of biomass. The low rates observed here may be attributed to the acclimation of the activated sludge to the wastewater and the low levels of nutrients contained in this chemical wastewater. The high COD:BOD ratio of the wastewater caused the loading rate in terms of BOD load applied per unit MLSS to be low in comparison to the COD load; in addition, the BOD:N:P ratio of this industrial wastewater was 212:20:1 so the availability of N and P was lower than the other wastewaters listed in Table 5.2. Clark *et al.*, using the same respirometer obtained respiration rates twenty times higher than those in the current study from activated sludge supplied with domestic wastewater with BOD:N:P of 100:17:2 (assuming 90% of available P was removed by chemical precipitation from original ratio of 100:17:16).

Stimulants (Figure 5.1) included pantothenic acid, biotin and niacin, corroborating previous literature (Lemmer *et al.*, 1998). Other authors have reported that activated sludge has no requirement for pantothenic acid (Lind *et al.*, 1994) or biotin (Srinath and Pillai, 1966). Most enterobacteria can produce sufficient biotin to supply the sludge, accounting for the conflicting reports. Niacin addition improved COD removal rates (Lind *et al.*, 1994), but as the optimum dose is 1.0mg/l (Lemmer *et al.*, 1998), it is possible that there is sufficient niacin in municipal wastewaters. This study showed that the industrial wastewater did not contain sufficient niacin and therefore dosing is highly likely to be required. Molybdenum is a common limiting nutrient (Grau, 1991). In this study, manganese and molybdenum were the only two single additives which gave enhanced COD removal results and stimulated respiration.

Some of the supplements led to increased oxygen uptake and decreased COD removal (Figure 5.2). Three of the trace metals tested act as enzyme activators (zinc, cobalt and manganese). Metabolic uncouplers have this effect on respiration and process performance (Mayhew and Stephenson, 1997). Additions of calcium to wastewater have been shown to improve flocculation and zinc is known to stimulate cell growth (Shuttleworth and Unz, 1988). As calcium plays a significant role in membrane

permeability, it may intensify the action of other metals (Shuttleworth and Unz, 1988), which accounts for the effect seen with the addition of calcium and manganese. The improvement in COD removal by activated sludge dosed with calcium and manganese may be owing to the ability of Ca^{2+} ions to increase membrane permeability and allow manganese better access to the bacterial cells, speeding up metabolism. However, dosing with calcium and other micronutrients together resulted in a reduction in COD removal, often associated with increased respiration. Calcium supplements altered the effects of all the other micronutrients (Table 5.3).

Thiamine showed a stimulatory effect concurrent with previous literature (Lind *et al.*, 1994). These results indicate that thiamine is the most important vitamin to activated sludge, but as certain bacteria can excrete thiamine, supplements may not be necessary (Schormüller, 1948). Zinc at 1.0mg/l was found to be inhibitory to the activated sludge biota, also observed previously (Geradi, 1986; Chua and Hua, 1996). This concentration of zinc does not actually enter the bacterial cells, but competes for extracellular binding sites thus preventing the adsorption and metabolism of organics. Zinc interacts with other metals to exacerbate their inhibitory effects and has been shown to reduce the rates of biodegradation reactions (Beyenal *et al.*, 1997).

Cobalt addition prompted an increase in respiration, but a small decrease in COD removal. A dose of 1.0mg/l cobalt added to activated sludge has been shown to produce a 50% increase in vitamin B_{12} synthesis, suggesting a stimulatory effect on metabolism. However, the increase in activity was not associated with any improvement in BOD or COD removal (Sathyanarayana Rao and Srinath, 1961). The combined effects on respiration and COD removal indicate a inhibitory effect rather than stimulation, and cobalt has previously been reported to be inhibitory to activated sludge, leading to a deterioration in effluent quality (Hunter *et al.*, 1983).

Table 5.3 Changes to the effects of micronutrient addition on the addition of calcium.

Micronutrient dosed.	Percentage change on addition of calcium.	
	COD removal rate	Oxygen uptake rate
Al	-36.5	-2.1
Biotin	-19.0	14.1
Co	-5.2	-5.7
Lactoflavin	-68.6	29.4
Mn	11.2	39.8
Mo	-1.1	0.7
Niacin	-10.8	45.5
Pantothenic acid	-82.4	-58.0
Pyridoxine	-74.3	9.0
Thiamine	-75.1	-23.3
Zinc	-51.2	-41.4

The final group of additives (Figure 5.3) exhibited inhibitory effects by decreasing COD removal and oxygen uptake. The results from dosing with lactoflavin and pyridoxine corroborate a previous study where the sludge tested had no requirement for either vitamin (Lemmer *et al.*, 1998). It has also been reported that although lactoflavin is important for cell metabolism, there is a certain amount of autotrophy in the activated sludge community and supplements are not normally necessary (Schormüller, 1948). This contradicts previous work where mixed vitamin doses increased the metabolic activity of activated sludge (Lind *et al.*, 1994). Combinations of pantothenic acid with pyridoxine or biotin have been seen to act synergistically when added to activated sludge, although the presence of pyridine antagonises the activity of niacin (Schormüller, 1948).

More than one micronutrient in a solution leads to interactions between ions. Interactions are almost impossible to predict, as they are influenced not only by metal species and concentration, the operating conditions and influent characteristics, but also by the species of micro-organisms present, the sludge age and even the order in which

the micronutrients are added (Beyenal *et al.*, 1997). Sublethal inhibitory effects can also either prevent COD degradation via the suppression of metabolism, or improve it by altering metabolism so that bacteria need to degrade a greater amount of substrate to obtain sufficient energy. It should be noted that high metabolic activity indicated by greater rates of oxygen uptake is also seen in sludges which are not acclimated to the feed composition and that metabolism alone can not be taken as an indication of process efficiency. Consequently, many contradictory results concerning the inhibitory properties of micronutrient mixtures are reported (Chang *et al.*, 1986, Yetis and Gökçay, 1991, Dilek *et al.*, 1991, Beyenal *et al.*, 1997). The samples were not acclimated to the micronutrients tested so supplements applied to a full scale reactor may produce different results to these data, which were acquired over test periods of six hours. Further study into the effects of some of the micronutrients is required to confirm long term stimulatory or inhibitory effects.

5.5 Conclusions

The vitamins niacin, biotin and pantothenic acid, the metals manganese and molybdenum, and the combinations of calcium/manganese and calcium/niacin exerted stimulatory effects on the sludge. Increased COD removal and decreased oxygen uptake indicate the physical removal of the substrate rather than biodegradation. This can mean the adsorption or absorption of COD although this is usually negligible with such high proportions of soluble COD, and therefore this effect is more likely to be due to interactions between the additives. Of the 13 combinations showing this effect, 8 were vitamin/metal pairs. This indicates that metal adsorption is antagonistic to vitamin utilisation.

The inhibitory effects indicated by decreased COD removal and increased oxygen uptake common to uncouplers, inhibitors and xenobiotics were exhibited by many pairs of micronutrients. This may be a result of synergism of inhibitory properties or antagonism of beneficial ones.

Supplements of micronutrients have the potential to ameliorate deficiencies in activated sludge. Many factors influence the effects that supplements have on activated sludge, and combinations of micronutrients must be tailored to individual treatment plants. These results apply to a wastewater that has been characterised, and greater understanding would be required when adding micronutrients to industrial wastewater of variable flow and composition.

5.6 INTERIM SUMMARY AND CONCLUSIONS

Chapter 4 showed that there is a need for UK water companies to investigate means of adaptation to new effluent controls, via enhancing COD and toxin removal from wastewaters, and the information in Chapter 2 suggested that micronutrient addition was a potential method for the required adaptation of the activated sludge process. The concluding remarks indicated that experimental work into micronutrient addition as an option for industrial wastewater treatment was required.

The process performance and metabolic rates of several samples of activated sludge which were dosed with micronutrient supplements have been compared in Chapter Five. It was confirmed experimentally that a wastewater stream from a chemicals manufacturing plant did not contain a sufficient supply of macronutrients or micronutrients for efficient biological treatment. This was concluded from the observation that control sludge batches (receiving no nutrient supplements) attained a lower average COD removal rate than any of the test sludge batches. It was found that activated sludge biomass could be stimulated to degrade more COD by the addition of phosphorus and certain micronutrients. Some of the supplements increased the metabolic rate of the sludge while some decreased it, indicating a range of stimulatory and inhibitory effects. The vitamins niacin, biotin and pantothenic acid, the metals manganese and molybdenum, and the combinations of calcium/manganese and calcium/niacin exerted stimulatory effects on the sludge. The results indicated that metal adsorption is antagonistic to vitamin utilisation, and that synergism of inhibitory properties or antagonism of beneficial ones may lead to detrimental effects (decreased COD removal and increased oxygen requirements).

The data obtained, compared with results obtained by previous workers made it clear that many factors influence the effects that supplements have on activated sludge, and combinations of micronutrients must be tailored to individual treatment plants. Complex interactions between micronutrients that were dosed simultaneously were evident.

Several positive effects led to the conclusion that micronutrient supplements have the potential to optimise the process performance of activated sludge plants treating industrial wastewater. No measurement of effluent toxicity was made, although the increased performance of the sludge in terms of respiration (indicating bacterial activity) and improved rates of COD removal imply that a positive effect on toxicity is likely to have occurred. It was proposed that supplements of micronutrients might have the potential to ameliorate macro- or micronutrient deficiencies in activated sludge, and to investigate this idea further, some of the tests that had been performed were repeated, but with no phosphorus addition to the wastewater. This method places the activated sludge under phosphorus–limited conditions.

CHAPTER SIX

MICRONUTRIENTS AND MACRONUTRIENT BALANCING.

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MICRONUTRIENTS AND MACRONUTRIENT BALANCING.

6.1 Introduction

Changes in UK and European legislation over recent years have shifted the emphasis of wastewater treatment from the removal of BOD and suspended solids to the removal of the less readily degradable COD and specific priority pollutants. Many of the industrial wastewater streams that require treatment are not easily biodegradable owing to extremes of pH, the concentration of organic components or deficits in nutrients such as nitrogen and phosphorus.

Chronic phosphorus deficiencies can arise from a physical lack of phosphorus in an available form, and in this situation a simple orthophosphate supplement can alleviate process performance difficulties. Other cases arise, however, in which the activated sludge plant is usually capable of dealing with the organic loading, but batch processing of products in industrial pre-treatment plants or rainfall variations in municipal plants can cause changes to the nature of the organic loading, raising the phosphorus demand of the activated sludge. In these cases, nitrite but no nitrate is produced, and BOD and COD removal efficiencies are affected (Nowak *et al.*, 1996). The treatment of industrial waste using activated sludge is expected to achieve between 95 and 99% BOD removal and 80 to 90% COD removal (Eckenfelder and Grau, 1992), but the process is frequently reported to suffer from high effluent COD arising from a low density of specialised populations in the sludge, inhibition of biological degradation and nitrification by inappropriate conditions (pH, temperature, DO, nutrition, etc.), and poor sludge settling associated with an imbalanced sludge community (Pala and Sponza, 1996; Gulyas, 1994; Fewson, 1988).

Certain nutrients are essential for metabolic activity. In treatment plants dealing with industrial wastewater, nitrogen and phosphorus may be added to the influent as the availability of these macronutrients can limit the degradation of organics (Singleton, 1994). Adequate micronutrition is required to support all the genera present in activated sludge in order for a suitably diverse community to survive, as an unbalanced mixed culture leads to poor quality effluent and bulking or foaming sludge (Soddell and Seviour, 1996; Blackall *et al.*, 1996). Pinpoint flocs, dispersed growth and poor flocculation have been attributed to micronutrient deficiency and have been shown to be ameliorated by micronutrient dosing (Gostick, 1991). Maximising population diversity in activated sludge, thus making full use of cometabolism, can prevent the acute effects of a change or rise in influent COD.

The aim of this study was to investigate the optimisation of the capacity of an activated sludge mixed culture to remove COD from a nutrient-limited industrial wastewater stream using micronutrient supplements.

6.2 Materials and methods

The wastewater used in Chapter 5 was used here, but no phosphorus was added to the wastewater. A series of tests was performed using a closed cell aerobic respirometer (*CES Ltd. Aerobic Respirometer*). This model operates in a similar way to the Warburg respirometer (Heddle, 1980). The respiration rate of a sludge sample indicates the condition of the sludge in terms of metabolic activity. The respirometer employed uses a manometric cell coupled to an oxygen generating system which is held at a constant temperature. Six trace elements and three vitamins were tested (Table 6.1).

The COD:N:P ratio of the phosphorus–limited wastewater was 880:20:1 (compared with the ideal ratio of 100:10:1 (Beardsley and Coffey, 1985)) and it was known to contain a number of organic components, including phenols, amines and anilines. The activated sludge was taken from a wastewater treatment plant treating waste from the fine chemicals industry and batch fed daily on industrial wastewater. An MCRT of six days

was maintained. 50ml aliquots of the activated sludge were taken from the same source, at the same time, and placed in respirometry cells to which predetermined quantities of micronutrient supplements had already been added (Table 6.1). Additional samples which received phosphorus supplements (Orthophosphoric acid, AnalaR) to alter the COD:P ratio to 100:1, and control samples receiving deionised water in place of supplements, were also tested. The volumes in the cells were made up to 55ml with deionised water as required to standardise the partial pressures within the cells and make the tests comparable. The respirometer cells were stabilised at 20°C and stirred continually for six hours. An additional sample of the original activated sludge was filtered for influent COD. Measurements of effluent COD (*Hach* system, *Camlab*, adapted from APHA, 1992) and the MLSS concentration (APHA, 1992) were determined for each individual aliquot of sludge. The tests were replicated and the mean values for each micronutrient supplement addition were used in the data analysis. The data were converted to oxygen uptake rate and COD removal rate per unit MLSS. The results were interpreted using a t-Test: Paired Two Sample for Means (Berthouex and Brown, 1994). This analysis tool and its formula perform a paired two-sample student's t-test to determine whether a sample's means are distinct. This t-test form does not assume that the variances of both populations are equal. You can use a paired test when there is a natural pairing of observations in the samples, such as when pairs of samples are taken daily.

Atomic emission spectrophotometry using an Inductively Coupled Plasma Atomic Emission Spectrophotometer (*Thomas Jarrell Ash, Plasma 300)* was used to identify the trace element deficiencies in the wastewater as compared to theoretical requirements of activated sludge. The concentrations of available nitrogen and phosphorus were also measured and compared with the theoretical requirements of bacteria for these nutrients stated in the literature (Table 6.1).

Table 6.1. Nutrient requirements of activated sludge and the doses used.

Nutrient	Range of theoretical micronutrient requirements (mg/l)†	Concentration detected in the wastewater (mg/l)	Dose added (mg/l)
Macronutrients			
N	15.0 minimum[1]	32.00	None
P	3.0 minimum[1]	1.69	None
Trace metals			
Calcium	0.4 – 1.4	0.44	1.0
Manganese	0.01 – 0.5	<1.0	1.0
Aluminium	0.01 – 0.5	0.02	1.0
Zinc	0.01 – 0.5	<1.0	1.0
Molybdenum	0.2 – 0.5	<1.0	0.5
Cobalt	0.1 – 5.0	<1.0	1.0
Vitamins			
Niacin (Nicotinic acid)	0 – 10	-	1.0
Pyridoxine (Adermine, Vitamin B_6)	0.1 – 10	-	1.0
Lactoflavin (Riboflavin, Vitamin B_2 or Vitamin G)	0.5 – 2.0	-	1.0

[1] From suggested COD:N:P ratio, Beardsley and Coffey (1985)

† Table 2.2

6.3 Results

The vitamin doses added to the influent resulted in stimulation, indicated by increased COD removal and either increased or unchanged oxygen uptake rates (Figure 6.1). The small changes observed in some uptake rates were not statistically significant (ANOVA F=0.11, P=0.95). The control values were 0.027kg O_2/kg MLSS/d oxygen uptake and COD removal of 1.335kg COD/kg MLSS/d (Figure 6.1). The largest improvement in wastewater treatment was attained by the addition of pyridoxine to the wastewater, resulting in a slightly greater oxygen uptake rate (0.036kg O_2/kg MLSS/d) and a threefold increase COD removal (4.239kg COD/kg MLSS/d), compared with the control (Figure 6.1). All of the vitamin supplements resulted in improved COD removal. The results suggested that the vitamins niacin, lactoflavin and pyridoxine are required by activated sludge biomass under phosphorus–limited conditions.

Sludge that was dosed with phosphorus exhibited a similar oxygen uptake rate and higher COD removal rate than those of the control. However, comparison of these results with the data from the samples receiving vitamin supplements showed that vitamin supplements had a greater stimulatory effect on the biomass than phosphorus addition.

The trace element supplements can be divided into those that stimulated the metabolism of the activated sludge (Figure 6.2) and those that inhibited it (Figure 6.3). Stimulatory action is indicated by increased COD removal associated with increased oxygen uptake rates. The stimulants included four of the single metal doses: aluminium, calcium, cobalt and manganese, some of which have well documented biochemical roles (e.g. calcium, cobalt) but the actions of others, such as aluminium and manganese are less understood. The addition of aluminium alone stimulated the activated sludge and its combination with manganese, molybdenum and zinc resulted in even better process performance. The greatest effects were seen when supplements of aluminium and manganese were dosed simultaneously (0.032kg O_2/kg MLSS/d oxygen uptake and COD removal of 3.606kg COD/kg MLSS/d).

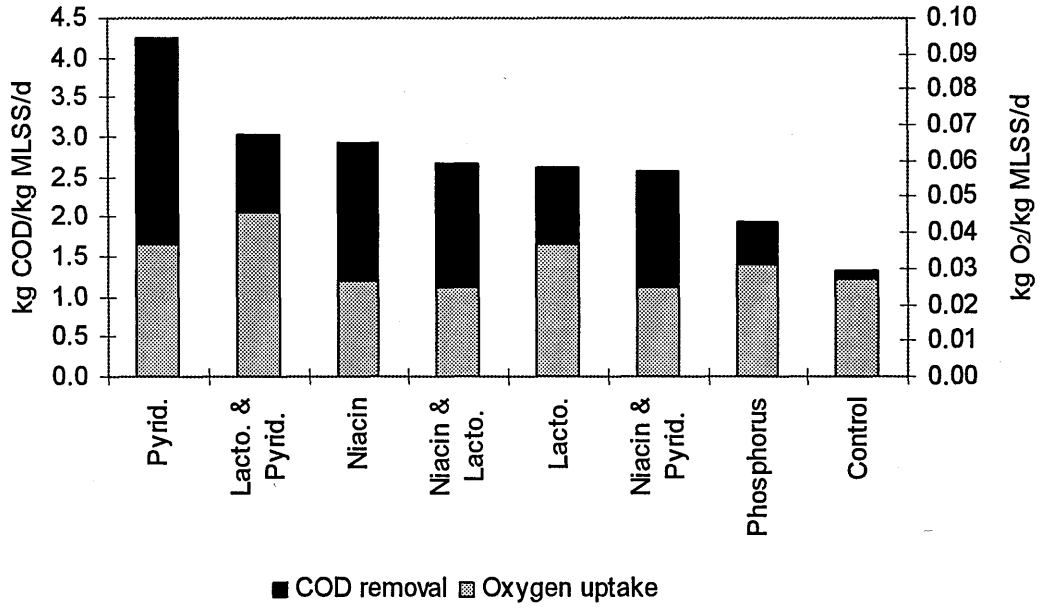

Figure 6.1. COD removal rates and oxygen uptake rates of activated sludge receiving vitamin supplements.

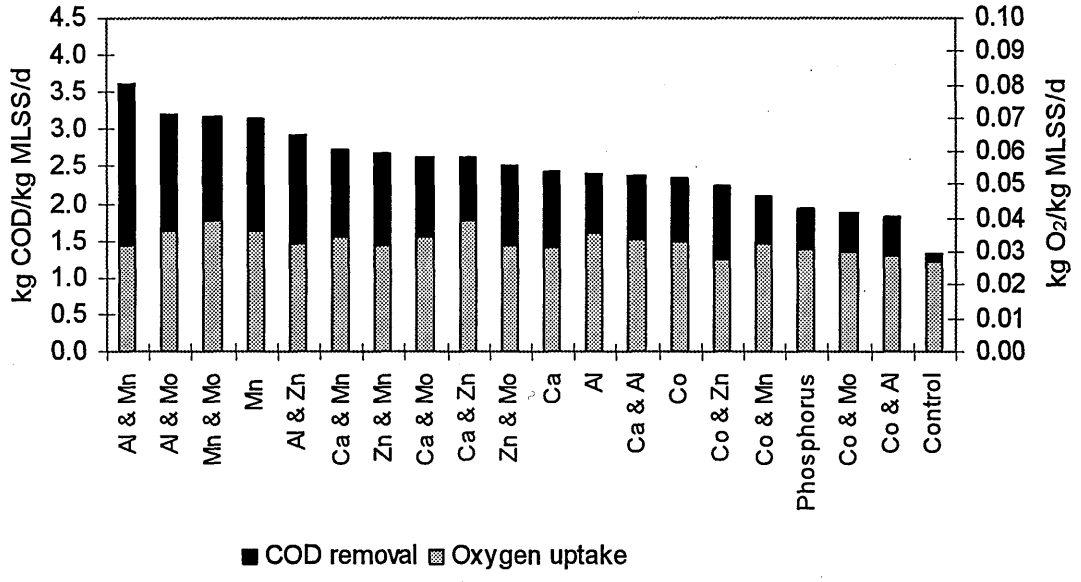

Figure 6.2. COD removal rates and oxygen uptake rates of activated sludge receiving metal supplements which acted as stimulants.

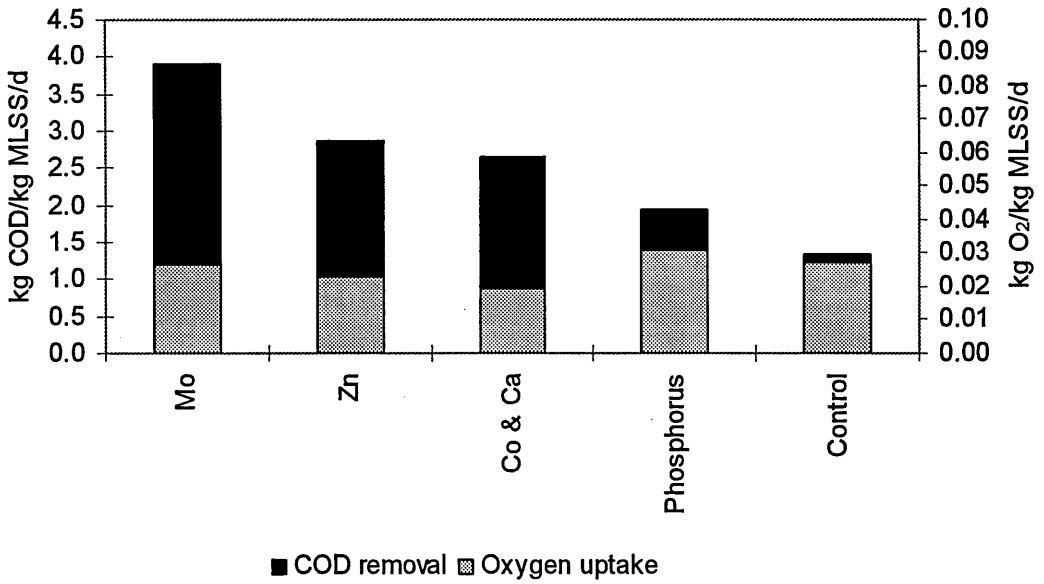

Figure 6.3. COD removal rates and oxygen uptake rates of activated sludge receiving metal supplements which exerted toxic effects.

Inhibitory effects on sludge metabolism can be seen either as overall suppression of metabolism (i.e. decreased oxygen consumption and substrate degradation) or as disruption of metabolism (opposite changes to the rates of substrate and oxygen uptake, i.e. one rate increasing and the other decreasing, or disproportionate changes e.g. increased rate of oxygen uptake with no associated alteration to the rate of substrate degradation). Single doses of molybdenum and zinc and a combined supplement of cobalt/calcium showed toxic effects (Figure 6.3).

Combinations of trace metals and vitamins were dosed into the activated sludge. Almost all of these supplements stimulated the metabolic rate of the sludge (Figure 6.4), increasing the rates of COD removal and oxygen uptake compared with the control. The greatest improvement to COD removal was attained by the addition of manganese and pyridoxine, resulting in a removal rate of 3.677kg COD/kg MLSS/d. Three of the combined vitamin/metal supplements were less effective than the addition of phosphorus in improving COD removal, indicating that all of the combinations tested except cobalt plus pyridoxine, cobalt plus niacin and aluminium plus niacin have the potential to

counteract low phosphorus levels in this industrial wastewater. The addition of molybdenum plus pyridoxine to activated sludge produced a slower rate of oxygen uptake (0.0269kg O_2/kg MLSS/d compared to 0.0271kg O_2/kg MLSS/d in the control) associated with higher COD removal, but as the change in oxygen uptake rate is not significant (Paired t-test, t=1.04, df=72, cl=1SD), the observed effects can be considered as indicative of stimulation.

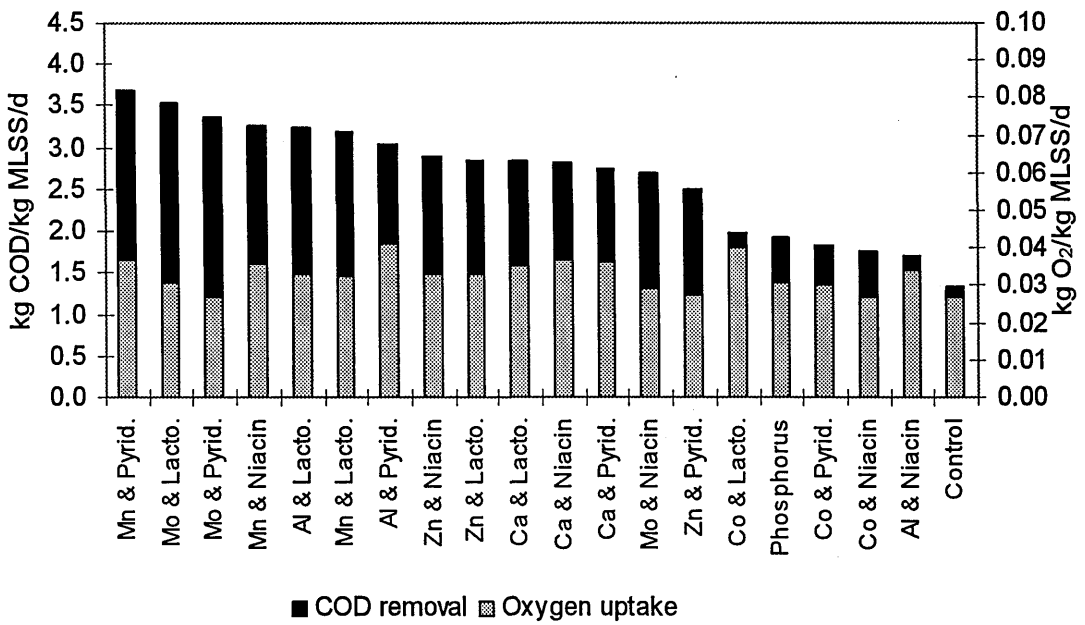

Figure 6.4. COD removal rates and oxygen uptake rates of activated sludge receiving combined supplements of metals and vitamins.

6.4 Discussion

All of the vitamins acted as stimulants (Figure 6.1), corroborating previous reports in which microbial requirements for niacin, lactoflavin and pyridoxine were demonstrated (Lemmer *et al.*, 1998; Voigt *et al.*, 1979). The vitamin supplements stimulated the biomass more than the addition of phosphorus, suggesting that the accepted COD:N:P ratio is too simple a summary of the nutritional requirements of activated sludge. Niacin supplements have been shown to improve COD removal and ameliorate low MLSS in various industrial pre-treatment plants (Lind *et al.*, 1994). Niacin is synthesised from ammonia by intestinal species of bacteria, so sewage treatment works accepting domestic wastewater receive a good supply of niacin (Voigt *et al.*, 1979), well above the optimum dose of 1.0mg/l (Schormüller, 1948). However, a chronic ammoniacal-N deficit in industrial wastewaters, which also carry fewer synthesising bacteria, can lead to niacin deficiency in activated sludge biomass, and supplements are beneficial in these cases. Some of the impacts of nitrogen insufficiency may actually be the effects of niacin deficiency, and a low dose would improve the treatment of nitrogen-lacking wastewater.

Lactoflavin and pyridoxine are both required by certain bacteria (Schormüller, 1948; Voigt *et al.*, 1979; Lind *et al.*, 1994). Some activated sludge populations have shown no requirement for these vitamins (Schormüller, 1948; Venkataramani and Ahlert, 1985; Lind *et al.*, 1994), but previous workers used sludges that were supplied with municipal wastewater, and it is probable that in this situation yeasts and fungi in the biomass can provide an adequate lactoflavin and pyridoxine supply (Schormüller, 1948). Previous work has demonstrated the ability of lactoflavin supplements to increase the population of heterotrophs in activated sludges from industrial wastewater treatment plants (Lemmer *et al.*, 1998). This study showed greater increases in sludge metabolism in response to lactoflavin than to phosphorus, indicating a certain amount of counteraction of the macronutrient deficiency. In spite of the high COD removal obtained by adding pyridoxine to the sludge, its ability to alleviate phosphorus deficiency over a long period is debatable. Pyridoxine is hydrolysed into pyridoxal phosphate for use in metabolism, so supplements in the absence of a continuous phosphorus supply may be of little use. In

addition, pyridoxine is susceptible to chemical interactions (Schormüller, 1948), indicated by the lower levels of COD removal attained by sludge samples dosed with pyridoxine in combination with other vitamins. It has been reported that doses of pyridoxine are not required by bacteria degrading industrial effluent (Venkataramani and Ahlert, 1985), but it is likely that the level of available phosphorus and the number of interacting chemicals are the deciding factors. As the mechanisms of the interactions between vitamins are not understood, it is difficult to state which ones antagonised or acted synergistically with which others.

The data from the addition of trace metals indicate that many metals are more useful in process optimisation than phosphorus supplements (Figures 6.2 and 6.3). Many of the metals employed have known biochemical uses, but the combinations of metal ions are more difficult to characterise. For example, additions of calcium to industrial wastewater improve sludge handling, and zinc is known to stimulate cell growth (Shuttleworth and Unz, 1988). Aluminium has been quoted as one of the trace elements required by micro-organisms (Wood and Tchobanoglous, 1975), but little is known about its role. These results showed that calcium and zinc combined interacted to produce greater COD removal than was attained with either metal alone. Calcium plays a significant role in increasing membrane permeability, and it may enhance the action of substances which act inside the cell wall (Shuttleworth and Unz, 1988). However, it is probable that this mechanism reduces the toxic effects of other cationic metals, such as Zn^{2+}, which compete with substrate molecules for extracellular binding sites thus preventing their adsorption and metabolism (Geradi, 1986; Chua and Hua, 1996). The action of calcium on the transport system improves the access of organic substrate molecules to the bacterial cells, so reducing the toxic effect of the adsorbed metals.

The observed decreases in oxygen uptake rates not associated with decreased substrate degradation (Figure 6.3) indicate disruption of metabolism. Metabolic action of bacteria may be suppressed in its entirety (Singleton, 1994) or inhibition occurs on only one side of the anabolism/catabolism equation which disrupts the metabolic equilibrium. This disruption can be caused by metabolic uncouplers such as nitrophenol, which inhibits

anabolism; the effects are seen as increased oxygen uptake by activated sludge with no associated increase in substrate degradation but reductions in biomass growth (Low and Chase, 1996; Mayhew and Stephenson, 1997). The results here indicate the opposite effect, i.e. inhibition of the catabolic processes in the activated sludge biomass. These supplements generated higher COD removal rates than the phosphorus supplements, but while the increases in COD removal rates may be seen as beneficial, the stress exerted on the biomass may lead to long term damage rather than actual process optimisation (Geradi, 1986), although investigations into biomass yield reduction via metabolic uncoupling have taken place (Mayhew and Stephenson, 1997). Sublethal toxic quantities of metals dosed over long periods of time have led to the loss of nitrification and unbalanced sludge populations with associated sludge handling problems (Geradi, 1986; Vandevivere *et al.*, 1997).

More than one trace element in a solution also leads to direct interactions between ions (Ting *et al.*, 1991). Interactions are almost impossible to predict, as they are influenced not only by metal species and concentration, the operating regime and nature of the influent, but also by the biological species present and the order in which the elements are added (Geradi, 1986; Beyenal *et al.*, 1997), so a number of contradictory reports exists (Chang *et al.*, 1986; Yetis and Gökçay, 1989; Dilek *et al.*, 1991; Beyenal *et al.*, 1997). These data suggest that the use of selected trace metal supplements can improve poor process performance of activated sludge treating nutrient-deficient wastewaters. A range of micronutrients can be used to maintain a diverse biomass that is more able to adapt to changeable or adverse conditions, owing to its lack of specialist use of metabolic pathways. All of the combined vitamin/metal supplements produced stimulatory effects on the activated sludge (Figure 6.4). Clearly, the interactions at work here are very complex. For example, lactoflavin and niacin are electron carriers and therefore have anionic binding sites (Stryer, 1988). If the metallic cations bind to them, the net effect could be the reduction of the action of that metal (toxic or beneficial effects) or of the vitamin, depending upon the factors mentioned above. Whatever chemical interactions exist, a clear trend of improved COD removal by activated sludge receiving micronutrient supplements emerges. Only five cases, all of which were

combined supplements involving cobalt or aluminium, showed less improvement than the sludge samples dosed with phosphorus. All of the supplements produced COD removal rates which were greater than those of the controls.

6.5 Conclusions

Micronutrient supplements have the potential to improve biological wastewater treatment process performance through the maintenance of a diverse population. The results indicate that dosing activated sludge which is treating phosphorus-limited industrial wastewater with any of the micronutrients tested produced better COD removal than the same sludge with no supplements at all. All three of the vitamins had beneficial effects, as did four of the metals. However, care must be taken with dosing trace metals to avoid excess doses that can cause toxic effects; the concentrations of micronutrients already in the influent must be taken into account when doses are calculated. Also, as some of the micronutrients are involved in the metabolism of nitrogen or phosphorus, the limiting macronutrients, longer term testing on a larger scale is required to provide information about the effects of micronutrient supplements on nitrification and sludge handling.

6.6 INTERIM SUMMARY AND CONCLUSIONS

In response to the need for enhanced COD and toxin removal by activated sludge treatment (Chapter 4), the process performance and metabolic rates of several samples of sludge which were dosed with micronutrient supplements have been compared. In Chapter 5, it was confirmed experimentally that a wastewater stream from a chemicals manufacturing plant did not contain a sufficient supply of macro- or micronutrients for efficient biological treatment. It was also found that activated sludge biomass could be stimulated to degrade more COD by the addition of phosphorus and certain micronutrients. Some of the supplements increased the metabolic rate of the sludge while some decreased it, indicating a range of stimulatory and inhibitory effects, and complex interactions between micronutrients that were dosed simultaneously were evident.

Several positive effects led to the conclusion that micronutrient supplements have the potential to optimise the process performance of activated sludge plants treating industrial wastewater. It was proposed that supplements of micronutrients might have the potential to ameliorate nutrient deficiencies in activated sludge, and to investigate this idea further, some of the tests that had been performed were repeated in Chapter 6, but with no phosphorus addition to the wastewater. This method placed the activated sludge under phosphorus–limited conditions.

The COD removal and respiration rates of activated sludge, which was dosed with micronutrient supplements, were compared. Six trace metals and three vitamins were used as chemical additives dosed into the mixed liquor. Again, some of the supplements increased the metabolic rate of the sludge while some decreased it, indicating a range of stimulatory and toxic effects; although under phosphorus–limited conditions, all of the supplements resulted in increased COD removal rates. Control sludge batches (phosphorus-limited, and receiving no micronutrient supplements) attained an average COD removal rate of 1.335kg COD/kg MLSS/d. Dosing micronutrients into the mixed liquor produced COD removal rates of up to 4.239kg COD/kg MLSS/d. It was

concluded that micronutrient supplements have the potential to enhance biological treatment of industrial wastewaters which are nutrient limited or changeable, but as the COD removal rates were lower than those observed in Chapter 5, it was concluded that micronutrient additions can not ameliorate macronutrient deficiencies. This shows that the best way to enhance activated sludge performance in terms of COD removal is by balancing the COD:N:P ratio and adding small concentrations of key micronutrients. The effects observed so far have included COD removal and respiration rate only, owing to the scale of the experiments; other parameters indicative of process performance include BOD_5 removal, MLSS and levels of effluent toxicity. These long-term effects on degradation can be simulated using porous pots (Chapter 7).

CHAPTER SEVEN

BENCH – SCALE TREATABILITY TESTS: TRACE ELEMENTS.

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Treating recalcitrant industrial wastewater: the role of trace metals.

Proceedings of the Water Environment Federation / Purdue University Industrial Wastes Technical Conference, June 27 – 30, Indianapolis, Indiana, U.S.A. Water Environment Federation, CD-ROM.

BENCH – SCALE TREATABILITY TESTS: TRACE ELEMENTS.

7.1 Introduction

Environmental factors can affect biodegradation by changing the availability of nutrients and target compounds and preventing the growth of micro-organisms (Daubaras and Chakrabarty, 1992). Many industrial effluents show resistance to the biological treatment processes used commonly to treat wastewater. Recalcitrance can arise from inappropriate conditions, inadequate nutrients and the supply of a substrate which does not activate the appropriate enzymes (Eckenfelder and Musterman, 1994). The availability of nitrogen and phosphorus can be limiting factors in the degradation of hydrocarbons and bioaugmentation (controlled supplements where concentrations are low) can lead to improved wastewater treatment (Leahy and Colwell, 1990). Bioaugmentation can also involve the addition of substances which increase cometabolism or induce degradative genes, such as trace metals.

The potential role of trace elements in biological wastewater treatment is not clear, although the biochemical roles of many trace metals are understood. To achieve sufficient treatment of industrial wastewater it is necessary to supply activated sludge with the micronutrients that enable the strains capable of degrading the recalcitrant compounds to thrive and to produce a low COD effluent. Recent research into advanced COD removal has focused on changes in operating conditions, but this normally results in higher investments and operating and maintenance costs, but not always in lower effluent COD. Biological oxidation must compete technically and economically with other treatment processes, but can be slower and less consistent than chemical processes. A vast increase in our understanding of the nutritional and environmental requirements of micro-organisms has meant that biodegradation has become an efficient and economical option (Singleton, 1994). The objective of the work here is to discover how trace elements may provide a method for improving the biodegradation of industrial

wastewater and establishing low effluent COD concentrations free of recalcitrant organic constituents.

7.2 Materials and methods

An activated sludge simulation was carried out using porous pots (USEPA, 1996; Painter and King, 1978). Each pot contained 3000ml of activated sludge. The pots were covered to minimise evaporation and insulated to prevent acute temperature changes (Figure 7.1). A 70:30 mixture of industrial wastewater and domestic sewage was supplied at 0.25l/h, thus maintaining a 12h hydraulic retention time (HRT). After a 24d acclimation period, the influent was changed to 100% industrial effluent (2000mg/l COD, COD:N:P of 20:5:1), supplied at 0.25l/h. The porous pots were also given predetermined doses of orthophosphate (*BDH Merck, Orthophosphoric acid, AnalaR*) and trace elements (Table 7.1). The tests proceeded for a further 48d. MCRT was 6d. DO levels were maintained at 1.0-5.0mg/l.

Solids were brushed off the inner walls and pH, DO and temperature were monitored daily using hand-held probes (*Jenway, Model 3071 pH and temperature meter; Model 9071 portable DO_2 meter*). MLSS and influent and effluent BOD_5 and COD were measured three times a week according to *Standard Methods* (APHA, 1992), and aliquots of sludge were analysed respirometrically to obtain oxygen consumption data (*CES Ltd. Aerobic Respirometer, Series 17*). Species diversity of the sludge was examined using a light microscope (*New Quodmaster, Model M4000-D, Swift, Japan*) and haemocytometer (*Neubaeuer improved deep cell haemocytometer*) with three repetitions of 1ml MLSS samples. Species number and type were recorded according to methods described by Eikelboom and van Buijsen (1981). The tests were performed in a series of three experimental trials, each including one control. To make the results comparable, the data were normalised so that the measurements from the controls were always equal to 100% and the values from the test pots can be presented as a percentage of the control. ANOVA (described in Chapter 5, section 5.2) was used to compare the data.

Table 7.1 Nutrient supplements used in the treatability tests.

Porous pot no.	Supplement	Dose added (mg/l)
1	None	-
2	Phosphorus and manganese	1.3 and 1.0
3	Phosphorus, calcium and manganese	1.3, 1.0 and 1.0
4	Phosphorus and molybdenum	1.3 and 0.5
5	Molybdenum	0.5
6	Manganese and aluminium	1.0 and 1.0

Figure 7.1 Porous pot setup.

7.3 Results

A total of five supplements were tested at concentrations between 0.5 and 1.3mg/l. MLSS, oxygen consumption data and influent and effluent BOD_5 and COD were used to calculate oxygen uptake rates per unit MLSS, BOD_5 and COD removal rates per unit MLSS and BOD_5 and COD removal efficiencies (Appendix B).

Successful stimulation of activated sludge to degrade the recalcitrant components of the wastewater was illustrated by increased COD removal associated with increased oxygen uptake and little or no change in either the concentration of MLSS retained in the reactors or in the removal of BOD_5. The activated sludge simulations were run under conditions as similar to those in a full scale pre-treatment plant as possible. The target MLSS was 3240mg/l and target MCRT was 6d. However, for the operation of the small scale test facility, the MCRT was able to be maintained at 6d whilst allowing the MLSS to vary. Maintenance of 6d MCRT resulted in MLSS between 2980mg/l and 720mg/l. The control pots in each trial maintained the lowest MLSS, a trend visible in the comparison of MLSS concentrations maintained in the porous pots (Figure 7.2). The data suggest that micronutrient supplements allow a greater mass of micro-organisms to thrive in recalcitrant wastewater.

After 10 days of dosing with the micronutrients, the pots supplemented with PO_4/Mn, Mn/Al and PO_4/Ca/Mn removed much more COD than the control (Figure 7.3). The BOD_5 removal by the sludge supplied with these supplements was variable. The sludge dosed with Mn/Al showed increased BOD_5 removal, indicating a general stimulation of the metabolism of the sludge, and the other two pots (PO_4/Mn and PO_4/Ca/Mn) removed BOD_5 at a lower rate than the control (Figure 7.4). This indicated the alteration of the sludge metabolism to use the recalcitrant fraction of the wastewater as the main substrate.

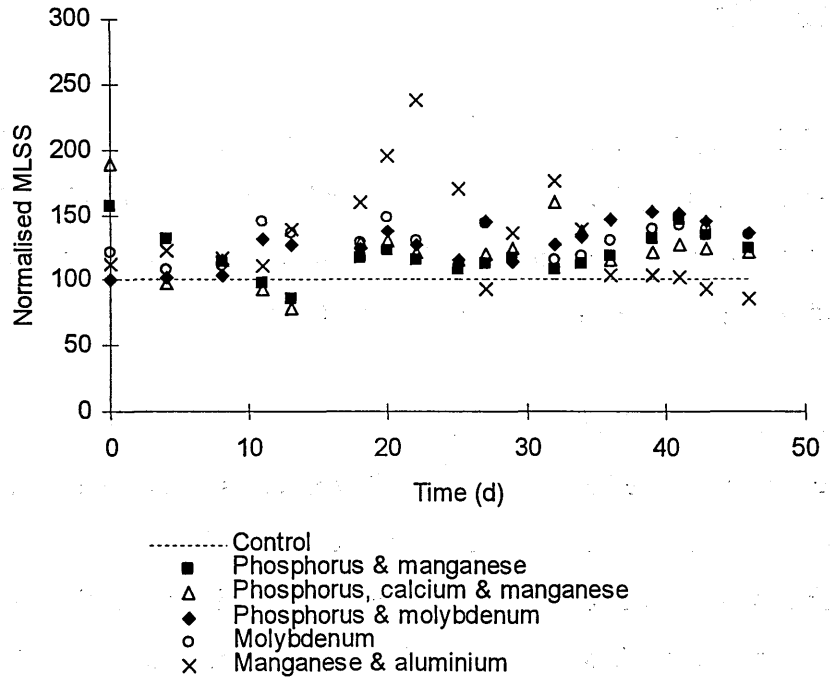

Figure 7.2 MLSS concentrations as a percentage of the control.

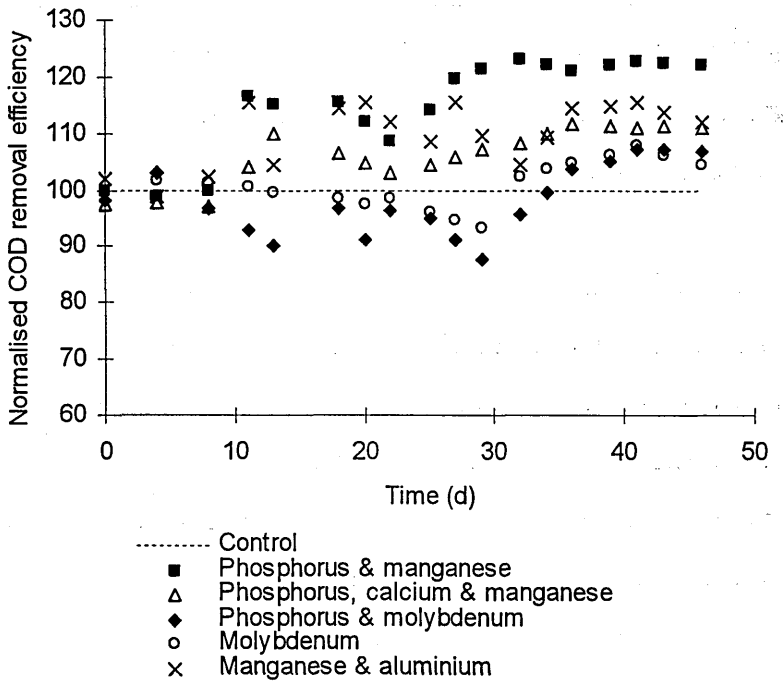

Figure 7.3 COD removal efficiency as a percentage of the control.

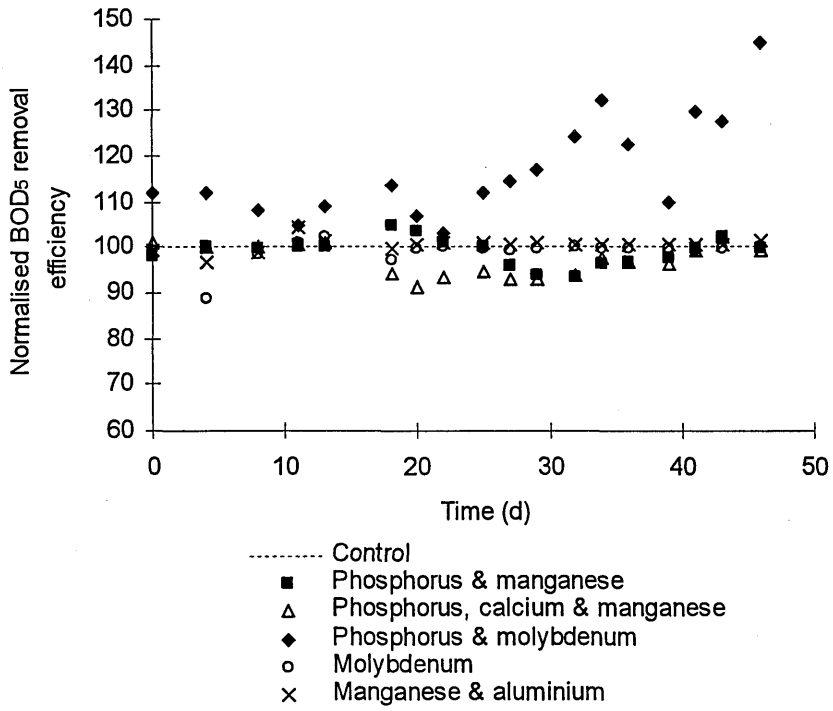

Figure 7.4 BOD_5 removal efficiency as a percentage of the control.

Oxygen uptake rates of activated sludge can be used as an indication of the level of activity of cell metabolism. Increases in oxygen uptake associated with increased substrate degradation generally indicate stimulation of metabolism. However, some metals have toxic or inhibitory effects on biomass when present in excess quantities. Inhibitory effects are seen either as the suppression of substrate degradation and oxygen uptake, or as inhibition of degradation accompanied by increased oxygen uptake. The latter effect arises from the interruption of the link between anabolism and catabolism in bacterial cells (i.e. uncoupling). The addition of PO_4/Mo, and to a lesser extent, the addition of Mo alone led to the inhibition of the activated sludge. The oxygen uptake by the sludge receiving these supplements was higher than the control, and the COD removal was lower (Figure 7.5).

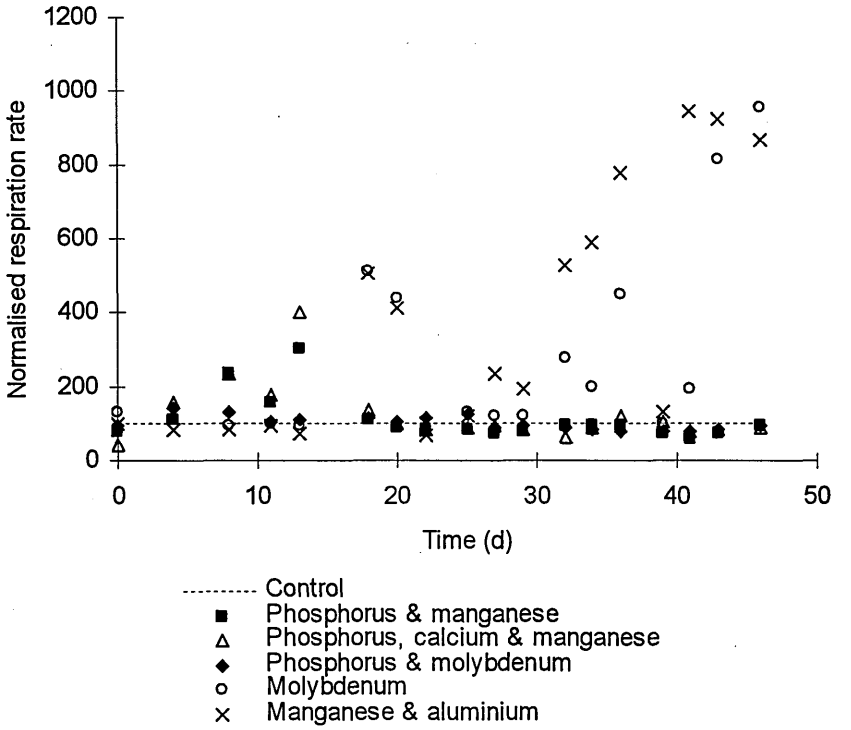

Figure 7.5 Normalised oxygen uptake by activated sludge supplied with trace elements.

A summary of the results (Table 7.2) shows that the additions of PO_4/Mn and Mn/Al were useful in terms of significantly improved COD removal (ANOVA F=0.003, P=0.9). The additions of PO_4/Mn, PO_4/Ca/Mn and Mn/Al resulted in increased removal of BOD_5, although the variation in the performance of the pots receiving PO_4/Mn and PO_4/Ca/Mn is great enough to negate the improvement, and the differences in these pots are not statistically significant (ANOVA F=1.34, P=0.9). The variation in MLSS concentrations is not significant owing to the large standard deviations in each data set, nor is the effect on oxygen uptake rate (ANOVA F=2.19 and F=1.97, respectively, P=0.9).

Table 7.2 Means and SDs for percentage COD removal, percentage BOD_5 removal, MLSS concentration and oxygen uptake rates.

	Mean Control	PO_4/Mn	PO_4/Ca/Mn	PO_4/Mo	Mo	Mn/Al
% COD removal	60.7 ± 1.2	68.4 ± 6.0	63.0 ± 3.88	60.5 ± 5.0	62.2 ± 4.2	67.6 ± 2.8
% BOD_5 removal	70.7 ± 5.1	79.1 ± 14.0	78.2 ± 14.4	45.4 ± 5.3	42.7 ± 5.6	93.0 ± 1.5
MLSS (mg/l)	1372.2 ± 101.5	2115.8 ± 246.5	2156.9 ± 228.4	1294.4 ± 202.4	1313.3 ± 308.3	1714.4 ± 565.1
Oxygen uptake (kg/kg MLSS/d)	0.19 ± 0.06	0.21 ± 0.07	0.24 ± 0.07	0.22 ± 0.05	0.25 ± 0.06	0.19 ± 0.08

Microscopically, the activated sludge in all four porous pots was the same at the beginning of the experiments, having originated from the same source. Very little change was seen under the microscope from week to week, although by the end of the trial some differences became apparent. Table 7.3 shows that the most diverse community was found in the porous pot receiving industrial waste, manganese and calcium. The least diverse was found in the control pot (Figure 7.6); all of the sludge samples from porous pots supplied with nutrients showed greater species diversity (Figure 7.7) . The diversity seemed to reflect the COD removal rates achieved by the pots. Although the measurement of the sludge volume index was not possible owing to the small volume of the simulations, it was seen that all of the sludge samples usually settled well, but the samples taken from the control pot and the pot supplied with phosphorus alone occasionally bulked. All of the sludges contained free cells and were made up of compact flocs of a similar size.

Table 7.3 Micro-organisms present in trace element-dosed activated sludge.

Organisms	Control 1	Control 2	Control 3	Control 4	Phosphorus	Phosphorus & Mn	Ca & Mn	Phosphorus & Mo	Mo	Mn & Al
Carchesium	0	0	0	0	++	0	+	+	0	0
Epistylis	0	0	0	0	++	+	++	+	+	+
Opercularia	+	+	+	+	+	0	+	+	+	+
Vorticella	+	+	+	+	++	+	+	++	++	++
Aspidisca	+	+	+	0	++	++	++	+	+	+
Blepharisma	+	0	0	0	+	+	+	0	+	+
Chilodonella	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	+	0	+	0	0	0	0
Euplotes	0	0	0	0	0	0	+	0	0	0
Paramecium	0	0	0	0	++	+	+	+	+	+
Bodo	0	0	0	0	+	+	+	0	0	0
Thecamoebae	0	+	0	0	0	0	0	+	0	0
Nematodes	0	+	+	+	0	0	+	+	+	+

Key: 0 = absent; + = 5-10 per 0.1ml: ++ = >10 per 0.1ml

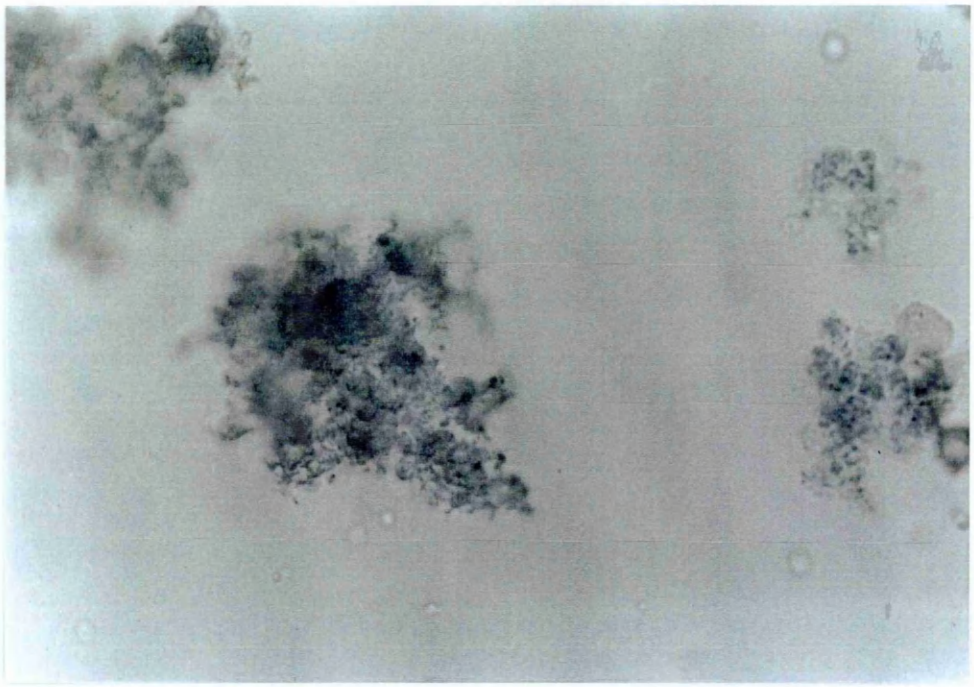

Figure 7.6 Activated sludge floc from a control pot (×100)

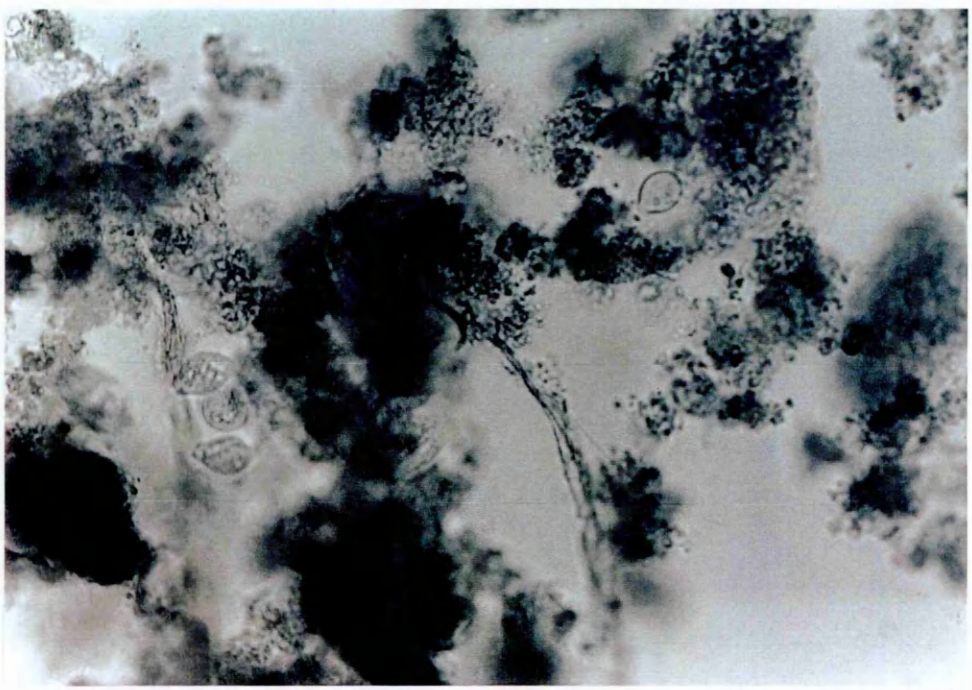

Figure 7.7 Activated sludge floc from a porous pot supplied with manganese and aluminium (×100)

7.4 Discussion

Micronutrient addition slightly increased the MLSS concentration in some of the experiments. Trace elements are taken up as components of enzymes and their cofactors to act in the catalysis of metabolic reactions and the maintenance of enzyme structure. They can also act as metallic enzyme activators or they are used in electron transport inside the cell (Mahler and Cordes, 1966). While these roles are fundamental to the life of the cell, not all micro-organisms require all micronutrients, although almost all of them are required for the growth of at least one common activated sludge organism (Wood and Tchobanoglous, 1975), so the addition of trace metals in cases of low concentrations could be expected to lead to increased biomass concentrations. Calcium increases the growth rates of *Thiothrix* and *Zooglea*, for example (Geradi, 1986).

The increases in oxygen uptake, BOD_5 and COD removal by sludge receiving Mn/Al indicate general stimulation of cell metabolism. PO_4/Mn and PO_4/Ca/Mn increased COD removal but did not increase BOD_5 removal, indicating the increased use of the wastewater components imparting COD as the substrate. As calcium plays a significant role in membrane permeability, it is likely that it may act as an enhancer of the action of any other metal. Calcium has already been shown to interact with other metals (Geradi, 1986) and it has also been concluded that the requirements for calcium and its effects vary greatly between bacteria, and that the calcium concentration has a major influence on the toxic effects of other metals (Shuttleworth and Unz, 1988). The presence of calcium owing to water hardness has been shown to accelerate acclimation via species selection in the sludge community (Ting *et al.*, 1991). Manganese and magnesium activate isocitric dehydrogenase and malic enzymes in bacteria (Wood and Tchobanoglous, 1975).

In spite of the metabolic roles of manganese and aluminium, there was no change to the oxygen consumption by sludge receiving supplements of Mn/Al. This may be because the addition of micronutrients does not guarantee bioavailability. Ions must be soluble to be bioavailable (Amdur *et al.*, 1991). Trace elements are cations and can be removed

rapidly by adsorption onto the anionic cellular material before the bacteria can assimilate the micronutrients they need (Wood and Tchobanoglous, 1975).

Results from trials in which multiple trace metals are dosed into biomass are often difficult to interpret, as more than one metal in a solution can lead to interactions between ions, for example, calcium, potassium and sodium are known to interact with other metals (Geradi, 1986). Interactions are almost impossible to predict, as they are influenced not only by metal species and concentration, the operating conditions and strength and type of influent, but also by the species of micro-organisms present, the sludge age and even the order in which the metals are added (Beyenal *et al.*, 1997). Inhibitory effects can also either prevent COD degradation or improve it. Consequently, many contradictory results concerning the toxicity of metal mixtures are reported (Beyenal *et al.*, 1997, Chang *et al.*, 1986, Dilek *et al.*, 1991, McDermott *et al.*, 1963, Yetis and Gökçay, 1989). Many micronutrients can antagonise each other and result in effective deficiencies (Nicholas, 1963). Some metals are antagonistic even at non toxic concentrations as they compete for binding sites either within cells or on particles (Wood and Tchobanoglous, 1975). This mechanism allows some metals to remove other toxins by chelation (Vandevivere *et al.*, 1997, Wood and Tchobanoglous, 1975). Inhibitory effects become more acute with decreasing species diversity in the sludge (Chang *et al.*, 1986, Dilek and Gökçay, 1996, Gökçay and Yetis, 1996, Madoni *et al.*, 1996, Ting, 1991, Yetis and Gökçay, 1989).

The components of an industrial wastewater rarely include all the chemicals required by a cell, therefore microbial cells alter their activity in response to their environment (Prescott *et al.*, 1990). However, the physical lack of nutrients or the unavailability of nutrients often leads to poor effluent treatment.

7.5 Conclusions

Supplements of a selection of trace metals were supplied to the reactors, and their ability to improve the biodegradation of the wastewater was assessed in terms of BOD_5 and COD removal. Retention of biomass in the reactors was increased in all cases, in excess of 100% with specific trace metals. Improvements in the degradation of COD were observed while BOD_5 degradation was not affected. This implies the use of recalcitrant substrate components as a food source. The practice of bioaugmentation - the tailored addition of micronutrients into wastewater in which the measured micronutrient concentrations are low - can be a useful tool in optimising biological treatment of difficult waste streams. The removal of recalcitrant COD and hence of priority pollutants and toxicity can be improved without the need for expansion of existing wastewater treatment or pre-treatment plants.

7.6 INTERIM SUMMARY AND CONCLUSIONS

The experiments carried out in Chapters 5 and 6 were short term, laboratory–based tests. To study the longer–term effects of micronutrient dosing on activated sludge performance, treatability tests to be performed over several sludge retention times were proposed. This was intended to take bacterial acclimation into account. The work presented in Chapter 7 demonstrated how trace elements can provide a long term method for establishing low effluent COD concentrations and removing recalcitrant constituents from industrial wastewaters. The research focused on the impact of trace metal supplementation to industrial waste in terms of the performance of activated sludge reactors treating a high-strength, recalcitrant effluent.

Supplements of trace elements were given to activated sludge in the reactors, and the biodegradation of the wastewater was assessed by measurements of influent and effluent BOD_5 and COD removal. COD removal was improved in the test pots and BOD_5 removal remained the same. The MLSS concentration was increased. As COD and BOD_5 do not equate to toxicity, further tests were designed to include testing the pot effluents for toxicity.

Legal limits regarding the output of metals in treatment works effluents and sludge applied to agricultural land may cause companies to discount metal addition as a long term option for wastewater treatment, although the doses employed here are low. Conversely, vitamin addition may not suffer from such negative popular opinion, and can widen the choice of sludge disposal routes open to operators. Therefore, porous pot tests using vitamin additions were scheduled, in spite of the higher chemical costs associated with vitamin dosing. As COD does not always equate to toxicity, the vitamin experiments include testing the pot effluents for toxicity to nitrifying bacteria in order to assess the potential of micronutrient dosing as a means of complying with DTA and protecting municipal wastewater treatment works downstream of pre-treatment plants treating industrial wastewaters.

CHAPTER EIGHT

BENCH – SCALE TREATABILITY TESTS: VITAMINS.

AN EDITION OF THIS CHAPTER IS PUBLISHED AS:

Burgess JE, Quarmby J and Stephenson T (1999).

Vitamin addition: an option for sustainable activated sludge process effluent quality.

Journal of Industrial Microbiology and Biotechnology. In press.

BENCH – SCALE TREATABILITY TESTS: VITAMINS.

8.1 Introduction

Any individual or company that produces wastewater in the UK is legally bound to render it harmless, except when wastewater is discharged under consent. Current discharge consents are set out as numeric targets, easy to measure and hence enforce. The shortfalls of numeric targets include the lack of chemical-specific toxicity data and the failure to account for interactions between wastewater components. In response to this situation, the EA is piloting DTA as an additional measure of effluent quality in order to apply a more ecologically relevant test and avert pollution incidents. DTA is a measurement of the toxicity of an entire wastewater, rather than the concentration of specific chemical components.

The role of vitamins in biological wastewater treatment is not well defined, but it is known that in order to achieve sufficient treatment of industrial wastewater, it is necessary to supply activated sludge with the micronutrients that enable the strains capable of degrading the toxins to thrive and produce a non-toxic, low-COD effluent, free of recalcitrant components. The aim of the work here is to discover whether micronutrient dosing can enhance COD and toxicity removal as measured by Amtox™ - an ammonia toxicity monitor designed to detect toxins that are damaging to nitrification processes.

Amtox™ has been described in the literature (Upton and Pickin, 1996) and is under consideration for use as a monitoring tool in DTA. The toxicity of the sampled wastewater is represented by the loss of ammonia removal efficiency over the test period. A non-toxic wastewater will allow the removal efficiency to remain above the baseline for the duration of the test.

8.2 Materials and methods

The screening of potential micronutrients was carried out using activated sludge from an industrial wastewater treatment plant. An activated sludge simulation was carried out using the porous pot method (USEPA, 1996; Painter and King, 1978). Each pot contained 3l of activated sludge. The pots were covered to minimise evaporation and insulated to prevent acute temperature changes (Figure 7.1). Wastewater from a fine chemicals factory (1500-2000mg/l COD, COD:BOD_5 of 4:1, COD:N:P of 880:20:1) was supplied at 0.25l/h, thus maintaining a 12h HRT. The MCRT was 6d. DO levels were maintained at 1.0-4.0mg/l. Solids were brushed off the inner walls and the pH, DO and temperature were monitored daily using hand-held probes (*Jenway Model 3071 pH and temperature meter; Model 9071 portable DO_2 meter*).

MLSS and influent and effluent BOD_5 and COD were measured three times a week according to *Standard Methods* (APHA, 1992), and samples of the sludge were analysed respirometrically (*CES Ltd. Aerobic Respirometer, Series 17*). Species diversity of the sludge was examined using a light microscope (*New Quodmaster, Model M4000-D, Swift, Japan*) and haemocytometer (*Neubaeuer improved deep cell haemocytometer*) with three repetitions of 1ml MLSS samples at ×400 magnification. Species number and type were recorded according to methods described by Eikelboom and van Buijsen (1981). After a 24d acclimation period on 70:30 industrial:domestic mixed feed, the pots were fed 100% industrial wastewater and the influents to all but one pot per trial were supplied with phosphorus (*BDH Merck, Orthophosphoric acid, AnalaR*) and micronutrients at set doses (Table 8.1), predetermined by the composition of the wastewater and the theoretical requirements of activated sludge micro-organisms (Table 5.1). The trials proceeded for 48d.

The experiments were performed in a series of tests, each with its own control porous pot. The data were normalised so that each of the controls was reported as 100%, with the experimental pot data reported in proportion to the concurrent control.

After primary data analysis, composite samples of selected effluents were taken over three days and tested for toxicity to nitrifying bacteria. Some of the effluents produced were subjected to 2h toxicity tests, using an Amtox™ (*PPM Ltd., Ammonia Toxicity Monitor Version 1.01F*), with 80ml immobilised nitrifying cultures (*PPM Ltd., Wild Type Immobilised Nitrifyers*) and a baseline removal limit of 60%. A new culture was used to test each effluent to avoid artefacts arising from the acclimation of the bacteria to the effluent. As the Amtox™ uses ammonia removal efficiency of nitrifyers to measure toxicity, the results are indicative of the potential impact the sampled wastewater may have on a sewage treatment works.

Table 8.1 The nutrient doses employed in the study.

Trial no.	Pot no.	Regime	Doses added (mg per l of influent)
Run 1	1	Control	-
	2	Phosphorus and niacin	1.3 and 1.0
Run 2	1	Control	-
	2	Phosphorus, calcium and niacin	1.3, 1.0 and 1.0
Run 3	1	Control	-
	2	Pyridoxine	1.0
Run 4	1	Control	-
	2	Phosphorus and pyridoxine	1.3 and 1.0
	3	Molybdenum and lactoflavin	1.0 and 1.0
Run 5	1	Control	-
	2	Phosphorus	1.3
	3	Phosphorus and niacin	1.3 and 0.5
	4	Phosphorus, calcium and niacin	1.3, 0.5 and 0.5

8.3 Results

A total of eight supplements were tested at concentrations between 1.0 and 1.3mg/l. MLSS, oxygen consumption data and influent and effluent BOD_5 and COD were used to calculate oxygen uptake rates per unit MLSS, BOD_5 and COD removal rates per unit MLSS and BOD_5 and COD removal efficiencies (Appendix C). The MLSS maintained in the treated porous pots varied (Table 8.2). The treated pots maintained more biomass than the controls, with the exception of the pots receiving supplements of pyridoxine alone and phosphorus/niacin or a phosphorus/niacin/calcium mix at the lower dose of 0.5mg/l. Reducing sludge production is an important cost consideration, and it was seen that a combined phosphorus/niacin or phosphorus/niacin/calcium low dose in particular allowed the reactor to run at a lower MLSS concentration while maintaining COD removal, although the reduction in MLSS was not significant (paired t-test, df = 26, t=7.91 and t=2.33, respectively). The variation in MLSS must also be taken into account during the interpretation of the oxygen uptake rates (Figure 8.1).

The respiration rate data were recorded as rates per mass of MLSS, and hence do not reflect the oxygen demand of a fixed volume of mixed liquor, unless MLSS concentration is also specified. However, as most activated sludge plants are operated to maintain a fixed MLSS, it is probable that the results are applicable to full-scale operations. Activated sludge supplied with the phosphorus/1.0mg/l niacin mixture showed a significantly lower mean oxygen uptake rate than the control (paired t-test, df=26, t=0.17). The sludge receiving molybdenum/lactoflavin had the same mean uptake (paired t-test, df=26, t=3.32) as the control but showed more variation, and the rest of the pots showed higher mean respiration rates then the concurrent controls. Increased oxygen uptake associated with improved substrate degradation indicates metabolic stimulation of the biomass, whereas increases associated with reduced substrate degradation suggest disruptive or inhibitory effects on cell metabolism, so the results have to be considered in conjunction with the substrate degradation data.

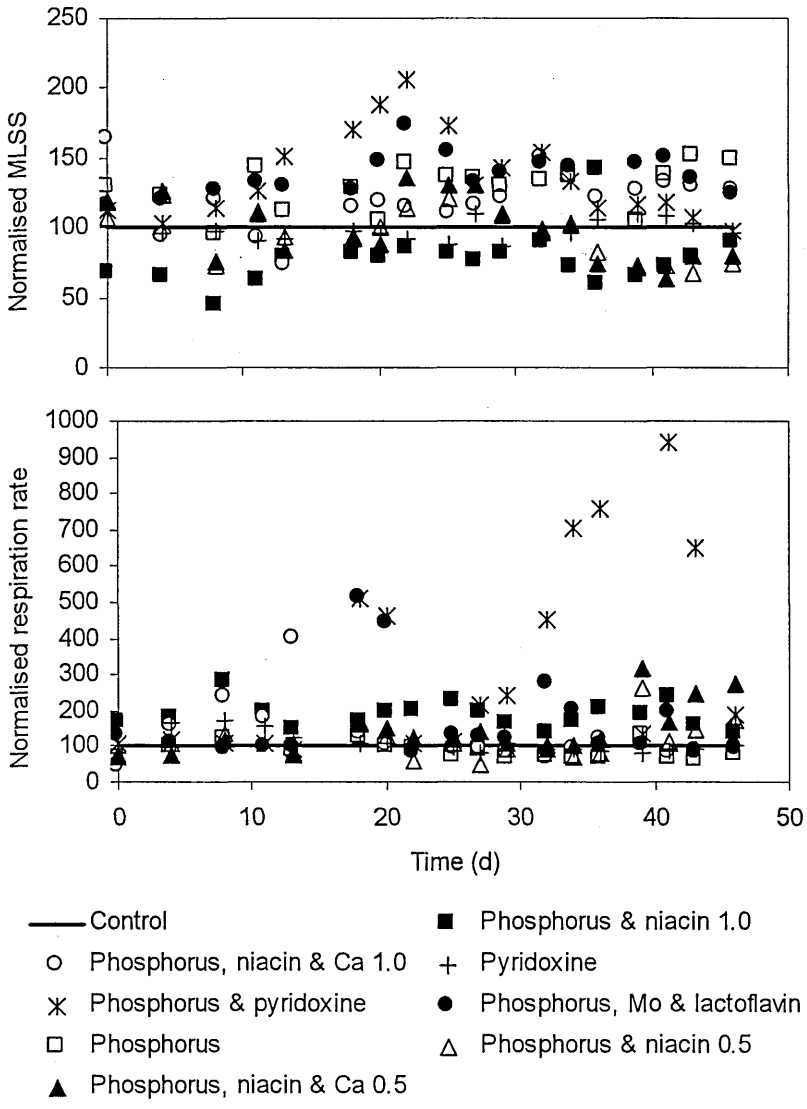

Figure 8.1 Respiration rates per unit MLSS and MLSS concentrations (expressed as a percentage of the concurrent control).

The ongoing reactor performance was assessed in terms of the removal of BOD_5 and COD per unit MLSS and as percentage removal of the influent oxygen demand. Before dosing commenced (on day 0) and up to day 20 after dosing began, no significant difference was seen in the removal of BOD_5 by any of the reactors. After 20 days of micronutrient supplements, some scatter was seen in the BOD_5 removal efficiencies of

the porous pots (Figure 8.2). The best results in terms of sustained improvements in BOD_5 removal efficiency were observed in the reactors dosed with phosphorus/1.0mg/l niacin (Figure 8.2) (paired t-test, df=26, t=0.43). The porous pot receiving pyridoxine alone removed slightly less of the influent BOD_5 than the control (Table 8.2), so pyridoxine-treated sludge effluent was not selected for toxicity testing. The two pots supplied with the lower doses of phosphorus/niacin and phosphorus/niacin/calcium produced higher maximum BOD_5 removal, but similar mean values and a more variable reactor performance (therefore no significant difference was detected using t-test analysis) (Figure 8.2). These effluents were tested for toxicity to investigate possible relationships between BOD_5 and toxicity removal.

The reactor supplied with phosphorus/niacin/calcium at 1.0mg/l performed significantly better in terms of COD removal than the others during the dosing period (paired t-test, df=26, t=0.36; Figure 8.3): the maximum, minimum and mean COD removal efficiencies were the highest, and the standard deviation was among the smallest of the values computed for the data sets (Table 8.2). The COD removal rates per unit MLSS did not vary significantly (according to paired t-tests) between the dosing regimes in spite of the wide range of percentage influent COD removed by the reactors. This may be due to the variation in MLSS concentrations.

Table 8.2 Summary data for-bench scale vitamin tests.

		Mean control	Phosphorus and niacin 1.0mg/l	Phosphorus, calcium and niacin 1.0mg/l	Pyridoxine.	Phosphorus and pyridoxine	Phosphorus, molybdenum and lactoflavin	Phosphorus	Phosphorus and niacin 0.5mg/l	Phosphorus, calcium and niacin 0.5mg/l
MLSS (mg/l)	Max	2164	3260	2690	1580	2670	2160	2860	1780	1810
	Min	1353	1020	1680	730	1340	1450	1230	1190	1040
	Mean	1636	1833	2107	959	1764	1762	1975	1426	1449
	SD	298.9	715.8	227.3	208.3	478.4	183.1	435.8	199.0	248.2
Respiration rate (kg O_2/kg MLSS/d)	Max	0.21	0.02	0.35	0.37	0.38	0.34	0.31	0.35	0.46
	Min	0.11	0.00	0.14	0.18	0.07	0.02	0.09	0.16	0.20
	Mean	0.16	0.01	0.25	0.24	0.21	0.15	0.20	0.25	0.32
	SD	0.03	0.00	0.06	0.07	0.10	0.12	0.08	0.06	0.07
% BOD_5 removal	Max	78	92	94	52	97	94	95	89	93
	Min	64	89	36	34	91	86	25	38	22
	Mean	72	90	78	38	94	91	75	74	69
	SD	3.9	0.9	14.4	4.3	1.2	1.7	17.6	15.0	21.2
% COD removal	Max	64	72	75	71	68	65	71	72	70
	Min	52	44	59	54	57	59	34	46	42
	Mean	58	61	69	60	64	63	61	64	63
	SD	3.5	7.2	3.9	4.4	3.4	1.7	9.1	7.6	7.8

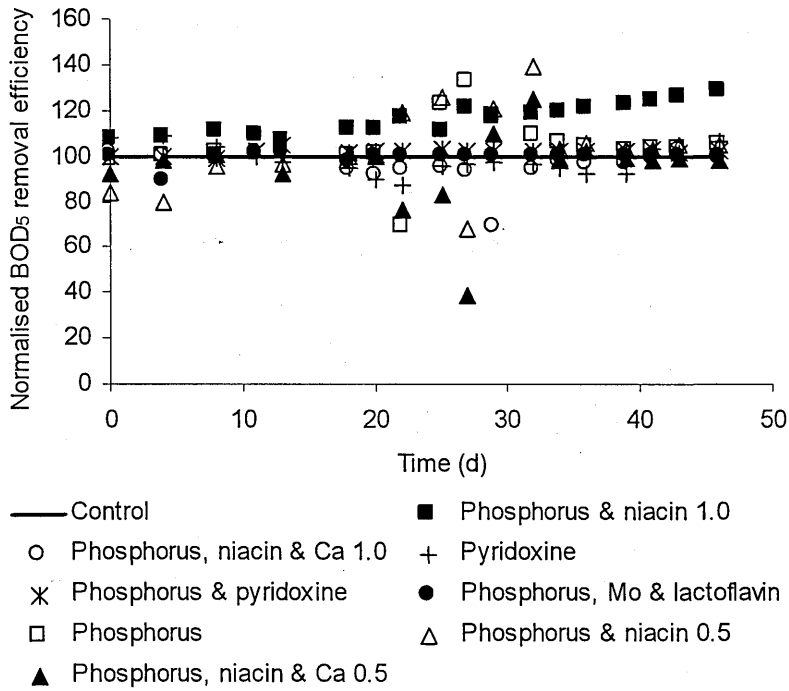

Figure 8.2 BOD_5 removal efficiencies attained by the reactors (expressed as a percentage of the concurrent control).

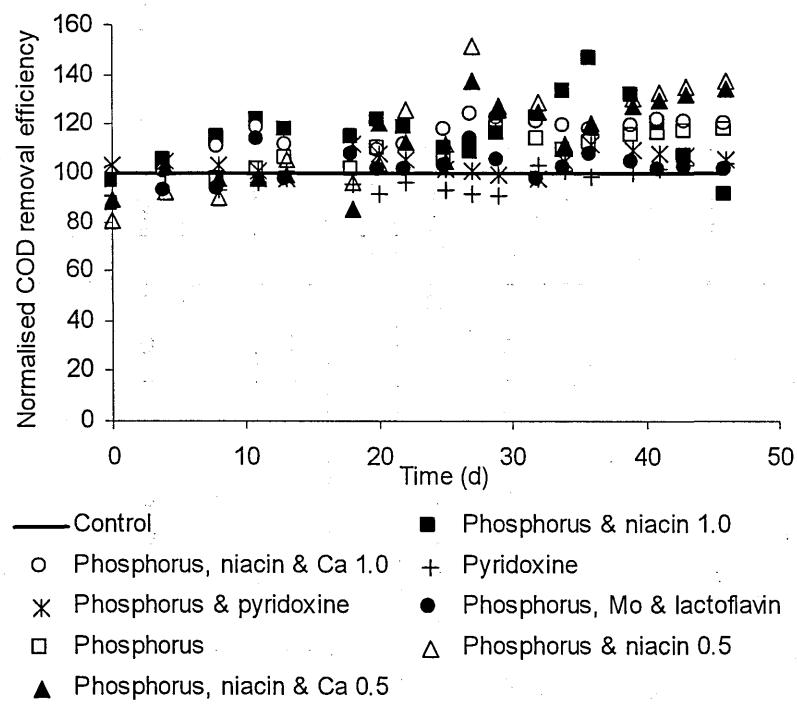

Figure 8.3 COD removal efficiencies attained by the reactors (expressed as a percentage of the concurrent control).

After 48d testing, similar differences as described in Chapter 7 appeared in the species diversity of the sludges. Table 8.3 shows that the most diverse community was found in the porous pot receiving supplements of phosphorus and niacin. Again, the least diverse was found in the control pots; all of the sludge samples from porous pots supplied with nutrients showed greater species diversity, firm flocs with ciliates and low levels of filaments present (Figures 8.4 and 8.5). As with metal additions, species diversity reflected the COD removal rates achieved by the pots. All of the sludge samples usually settled well, but it was seen that the samples taken from the control pot and the pot supplied with phosphorus alone bulked occasionally.

Table 8.3 Micro-organisms present in vitamin-dosed activated sludge.

Organisms	Control 1	Control 2	Control 3	Control 4	Control 5	Phosphorus and niacin 1.0mg/l	Phosphorus, calcium and niacin 1.0mg/l	Pyridoxine.	Phosphorus and pyridoxine	Phosphorus, molybdenum and lactoflavin	Phosphorus	Phosphorus and niacin 0.5mg/l	Phosphorus, calcium and niacin 0.5mg/l
Carchesium	0	0	0	0	0	+	+	0	0	0	0	0	0
Epistylis	0	0	0	0	0	++	+	0	0	+	0	+	+
Opercularia	+	+	+	+	+	+	+	+	+	+	+	+	+
Vorticella	+	+	+	+	+	++	+	+	++	++	+	++	++
Aspidisca	+	+	+	0	0	++	++	+	+	0	+	+	+
Blepharisma	+	0	0	0	0	+	0	+	0	+	0	+	+
Chilodonella	0	0	0	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	+	+	0	0	0	0	+	+	+	+
Euplotes	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramecium	0	0	0	0	0	++	++	+	+	+	+	+	+
Bodo	0	0	0	0	0	0	0	0	0	0	0	0	0
Thecamoebae	0	+	0	0	0	+	+	0	0	0	0	0	0
Nematodes	0	+	+	+	+	+	+	0	+	+	+	+	+

Key: 0 = absent; + = 5-10 per 0.1ml: ++ = >10 per 0.1ml

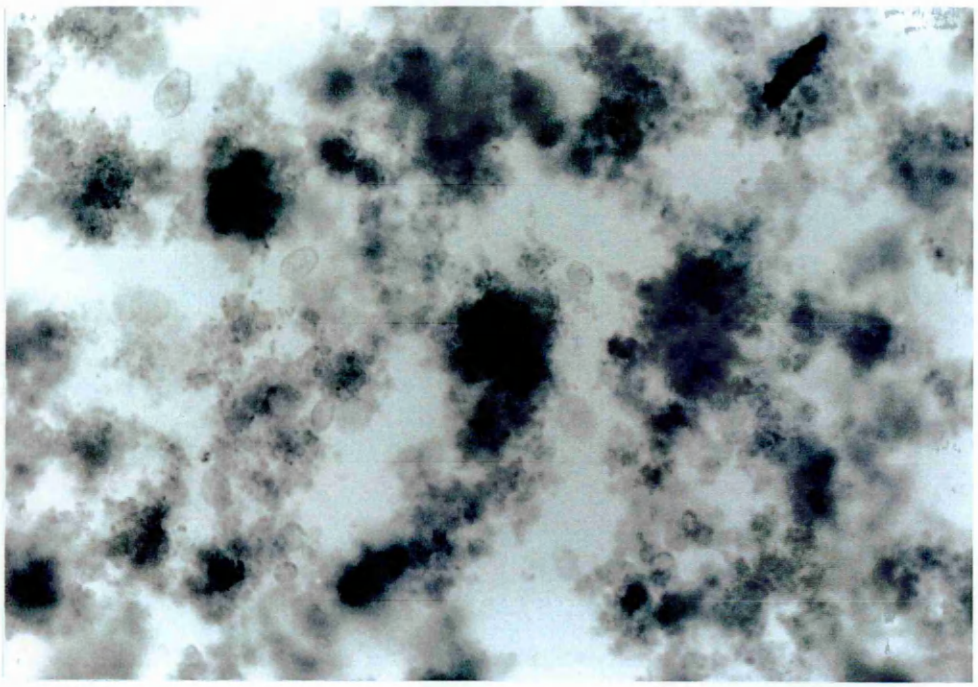

Figure 8.4 Activated sludge floc from a porous pot receiving phosphorus (×50)

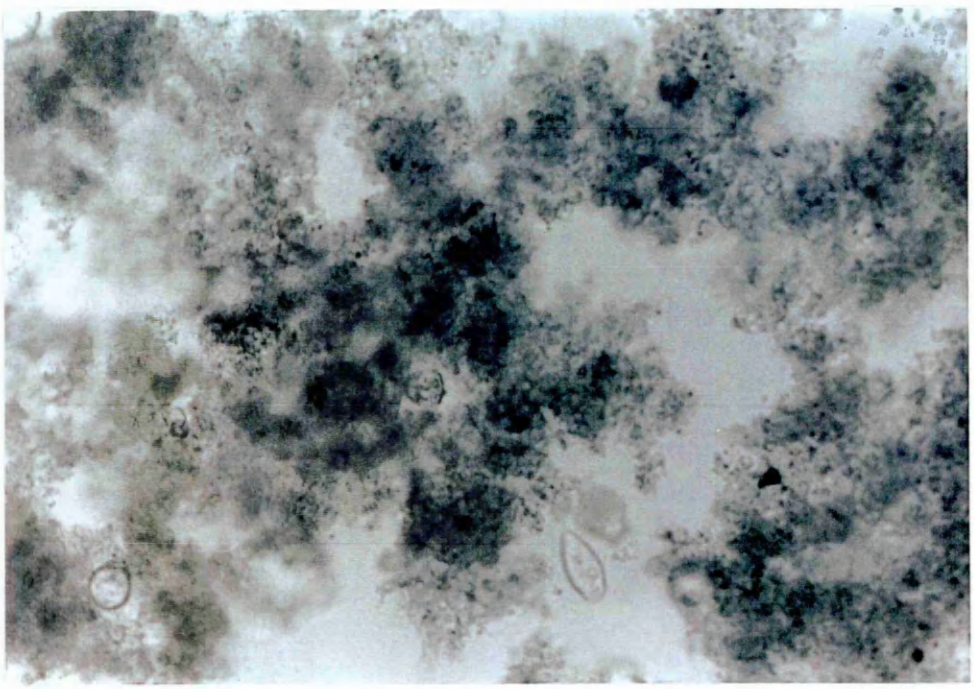

Figure 8.5 Activated sludge floc from a porous pot supplied with phosphorus, niacin and calcium (×100)

The toxicity of the porous pot effluents was expected to correlate closely with the mean effluent COD concentrations, but this was not the case. The inhibitory effects the effluent samples had on the nitrifying bacteria do not correlate with the concentrations of BOD_5 present. A certain amount of negative correlation was observed between toxicity reduction and ammonia concentration (Table 8.4), and stronger positive correlation was seen between toxicity and effluent values for COD and pH. All of the effluents from the experimental reactors were less inhibitory to the Amtox™ bacteria than the control reactor effluent samples (Figure 8.6). Supplements of molybdenum/lactoflavin and phosphorus/0.5mg/l niacin allowed the efficiency removal of the cultures to remain above the 60% removal efficiency baseline throughout the duration of the test.

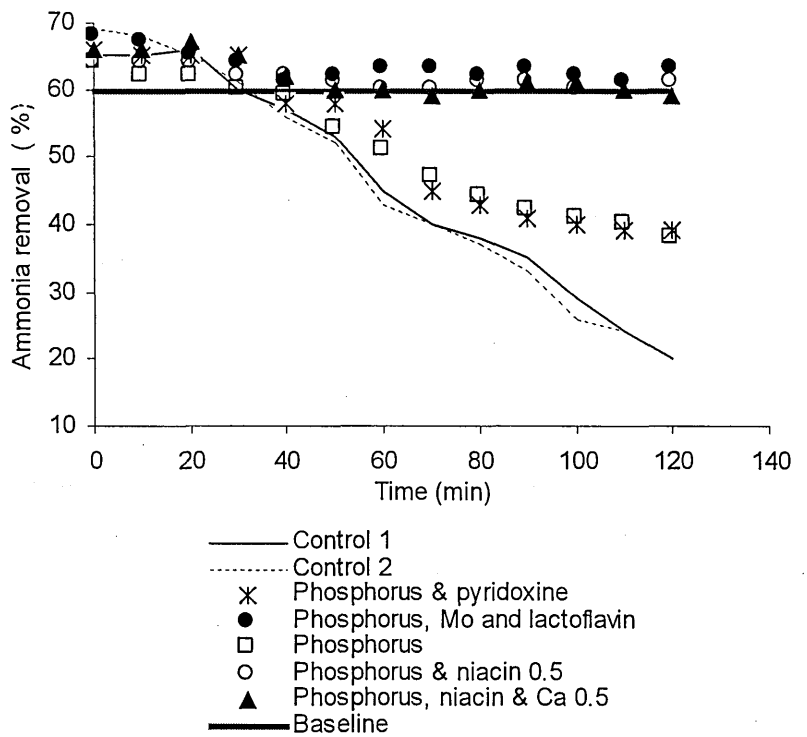

Figure 8.6 Toxicity of the reactor effluents plotted as ammonia removal efficiency of an immobilised nitrifying culture.

Table 8.4 Characteristics of the effluents tested for toxicity.

Porous pot	Inhibition (%)	Ammonia (mg/l)	COD (mg/l)	BOD_5 (mg/l)	pH
Control 1	40	19.93	771	39	8.05
Control 2	40	25.45	832	193	8.11
Phosphorus	22	20.07	796	177	8.01
Phosphorus & pyridoxine	21	19.50	670	28	8.07
Phosphorus & niacin & Ca 0.5mg/l	1	24.14	737	221	7.86
Phosphorus & niacin 0.5mg/l	-1	21.59	677	163	7.95
Phosphorus, Mo & lactoflavin	-3	26.24	699	40	7.87
Correlation coefficient	-	-0.42	0.73	0.01	0.79

8.4 Discussion

Vitamins are required for the growth of activated sludge (Lemmer, 1992) as they influence cell mechanisms. Vitamins and their combinations have different effects on varying bacterial species. The "vitamin B complex" is a group of vitamins thought originally to be a single substance, but is actually made up of thiamine, riboflavin, niacin, pantothenic acid, biotin, pyroxidine, folic acid, lipoic acid, inositol and vitamin B_{12}. These substances have different effects on different micro-organisms and should be considered separately.

Niacin addition can improve COD removal rates, possibly because it is used in oxidative phosphorylation, the production of cozymase and as a growth factor in bacterial cells (Lind *et al.*, 1994, Schormüller, 1948). Doses of niacin produced a greater metabolic activity in the industrial sludges investigated (Lind *et al.*, 1994). The optimum dose of niacin is reported to be 0.1mg/l influent (Lemmer *et al.*, 1998, Schormüller, 1948), and it is likely that municipal waste will contain such a small amount, but the industrial waste used here does not contain enough niacin for activated sludge bacteria. In this study,

niacin acted as a stimulant, increasing oxygen uptake and COD removal, corroborating previous work on industrial wastes (Lemmer *et al.*, 1998, Lind *et al.*, 1994).

Some activated sludges display no requirement for added lactoflavin or pyridoxine (Lind *et al.*, 1994, Schormüller, 1948). Pyridoxine has to be hydrolysed for use and the functional groups on the molecule have to be correct for it to be used in cells. This means that chemical interactions result in complete inactivation, and that analogues of pyridoxine are rarely of any value to activated sludge (Stryer, 1988). This accounts for the results here, in which the addition of pyridoxine and phosphorus/pyridoxine did not enhance COD removal.

Vitamin shortages can be expected in wastewaters from certain industries (Lind *et al.*, 1994), although vitamin additions to sludge may not improve the treatment of recalcitrant compounds; this depends on whether the vitamin-deficient species are those which are relevant for waste treatment. It has been shown that B-vitamin addition to sludges degrading nonylphenol ethoxylate, linear alkylbenzene sulphonate, ethylene diamine tetraacetic acid and 2,4,6-trichlorophenol did not improve degradation or reduce the inhibition of the degradation of casein hydrolysate, although the effects on COD removal were not reported (Lind *et al.*, 1994). In this case, the COD removal efficiencies of the experimental reactors were all improved by micronutrient additions, although this did not correlate with the removal of toxicity from the wastewater concerned. This illustrates the point that COD does not equate to the toxicity of wastewaters to nitrifying bacteria, and the operation of unit processes discharging to nitrification systems should not focus only on BOD_5 or COD removal.

Sublethal inhibitory effects can also either prevent substrate degradation via the suppression of metabolism, or improve it by altering metabolism so that bacteria need to degrade a greater amount of substrate to obtain sufficient energy. Consequently, many contradictory results concerning the properties of micronutrient mixtures are reported.

8.5 Conclusions

The vitamins niacin and pyridoxine resulted in improved COD removal by the activated sludge. The inhibitory effects indicated by decreased COD removal and increased oxygen uptake common to uncouplers, inhibitors and xenobiotics were not exhibited by any of the vitamins. Micronutrient additions that are tailored to wastewater streams have the potential to become a very useful tool for the optimisation of biological treatment processes. The results in the current study displayed significant reductions in effluent COD and toxicity without the need for capital investment. All of the vitamins had beneficial effects. The practice of bioaugmentation - the tailored addition of micronutrients into wastewater in which the measured micronutrient concentrations are low - can be used in optimising biological treatment of difficult waste streams. The removal of recalcitrant COD and hence of priority pollutants and toxicity can be improved without the need for expansion of existing wastewater treatment or pre-treatment plants. As the target of future legislation alters to prioritise on toxicity and priority pollutant removal, the improvement of existing wastewater treatment plants in ways such as this will gain an important place in the technologies available to industry involved in the minimisation of aquatic pollution.

8.6 INTERIM SUMMARY AND CONCLUSIONS

The objective of Chapter 8 was to discover whether vitamins can reduce industrial wastewater toxicity and enhance COD removal. After 48d of chemical treatment, the COD removal of one simulation, supplied with phosphorus and niacin at 1.0mg/l of wastewater, was significantly higher than that of the control (removal rate as kg COD/kg MLSS/d = 260% of control). As in Chapter 7, the BOD_5 removals from treated and control samples were not significantly different, indicating the degradation of a wider range of substrate components. The addition of two mixed supplements, molybdenum/lactoflavin and phosphorus/niacin, removed the wastewater components that were toxic to nitrifying bacteria as indicated through toxicity testing, thus protecting downstream nitrification/denitrification treatment processes and showing the potential for micronutrient addition to become an operating strategy for DTA compliance.

No one supplement emerged in Chapters 7 and 8 as an overall best option for enhanced process performance. There were several contenders for further investigation, depending on the weighting of the performance criteria set out in Table 8.5.

Table 8.5 Qualitative performance of micronutrient supplements at bench-scale.

Criterion	Highest maximum	Highest minimum	Highest mean
Percentage COD removal	P & niacin	P & niacin & Ca	P & Mn = P & niacin & Ca
COD removal per unit MLSS	P & niacin	P & niacin	P & niacin
Percentage BOD_5 removal	P & Mo	P & niacin	P & niacin
BOD_5 removal per unit MLSS	P & niacin	P & niacin	P & niacin
Oxygen demand	P & pyridoxine	P & pyridoxine	P & pyridoxine
Maintenance of MLSS	P & Ca & Mn	Mo & lactoflavin	Mo & lactoflavin
Reduction in effluent toxicity	P & Mo & lactoflavin	N/A	N/A

Percentage COD, BOD_5 and toxicity removal were the main priorities for assessing process efficacy, but other aspects of the process were taken into account. The maintenance of sufficient concentrations of biomass is often difficult in certain activated sludge units dealing with industrial wastewater, so the maintenance of MLSS and the removal rates attained per unit MLSS were considered. Oxygen uptake (and hence air requirement) is an important cost consideration in activated sludge process operation, thus a micronutrient which could provide sufficient biomass and facilitate high levels of COD, BOD_5 and toxicity removal without increasing oxygen uptake rates represented the best technical option.

It was decided to trial addition of phosphorus and niacin at pilot-scale, in spite of the cost of the vitamin, as it removed more toxicity than any other dosing regime involving just one micronutrient (Table 8.3) and produced significant improvements in the other performance criteria (Table 8.5). The respirometry and porous pot experiments demonstrated that micronutrient addition has the potential to enhance activated sludge process performance, but only at laboratory scale. The pilot plant work was designed to provide information about scaling-up micronutrient addition, and its impacts on sludge handling as this information had not been available previously.

CHAPTER NINE

PILOT-SCALE TRIAL OF NIACIN ADDITION.

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Chemosphere. In preparation.

PILOT-SCALE TRIAL OF NIACIN ADDITION.

9.1 Introduction

9.1.1 Scale-up of the activated sludge process

Although process engineering employs mathematical simulation models in many situations, observations at laboratory and pilot-scale are considered to be more reliable, particularly when industrial wastes are to be treated, as each effluent is unique. In order to draw meaningful inferences from pilot-scale data, the process unit design must be considered in terms of biological, chemical, environmental and hydraulic conditions. Small-scale activated sludge plants can be scaled up, provided hydrodynamic conditions remain the same, as biological coefficients are not affected by scale (Cooper and Boon, 1983).

Lack of similarity between bench, pilot and full-scale plants has a significant impact on process performance. Relationships between different scales can be established by using the theory of similarity that, in water or wastewater treatment technology, applies in the following ways (Horváth and Schmidtke, 1983):

1. **Geometric similarity:** exists when one reactor is a scale model of another, but the similarity can not exist simultaneously on a linear and a volumetric scale.
2. **Kinematic similarity:** applies to fluids and solids in motion during a period of time. In geometrically similar systems where particles have corresponding velocities, e.g. where HRT is maintained, kinematic similarity is achieved. This is of particular importance, since if flow patterns are similar, then heat or mass transfer rates in the systems will also be in relation to one another.
3. **Dynamic similarity**: relates to pressure, inertia, gravity, viscosity and surface tension in fluid systems. It is of indirect importance to heat and mass transfer rates, but is very difficult to achieve as different depths of fluids apply varying pressure.

4. **Chemical similarity**
5. **Biological similarity**
6. **Thermal similarity:** Chemical, biological and thermal similarity occurs when there are fixed concentrations of chemicals, biomass and heat that prevail at all scales.

There are inherent limitations to any bench- or pilot-scale system. At best, only an approximation of mixing conditions is achieved. Mixing by aeration in small-scale reactors produces turbulence closest to the mixing provided by fine bubble aeration at full-scale, and least like surface mechanical aerators (Jenkins *et al.*, 1983). Clarification of sludge in such a unit results in lower effluent turbidity than in a system with surface aeration or impeller mixing. Only large-scale pilot plants can begin to simulate full-scale turbulence and its effects on effluent SS values (Jenkins *et al.*, 1983). Sludge settling rates are affected by the concentration of solids and the physical conditions prevailing. Stirred specific volume index (SSVI) can be scaled up to within 20% accuracy as the slow stirrers minimise the surface effects of the cylinder wall (Cooper and Boon, 1983). Results obtained using clarifiers designed with stirrers similar to those used in SSVI measurement can therefore be expected to scale up more accurately than those without.

A problem specific to industrial waste treatment is that the scale-up of systems to remove priority pollutants is not so well understood. Air stripping, biodegradation or adsorption onto the surface of the flocs can remove organic pollutants. Most organics will undergo partial if not complete biodegradation, but in many cases long acclimation times are required to achieve maximum degradation rates (Eckenfelder and Quirk, 1983). Lastly, even the best designed simulation is only an approximation of full-scale operation as aeration system turbulence and vessel surface:volume ratios can not be simulated and their consequent effects always vary between bench, pilot and full-scale systems (Horváth and Schmidtke, 1983; Jenkins *et al.*, 1983). Parameters such as molecule, bacterium, bubble and floc size can not be altered according to scale, so the resulting distortions affect rates of mass, heat and oxygen transfer (Horváth and Schmidtke, 1983). Diurnal changes in sewage or industrial waste flow are not reproduced at bench or pilot-scale, and this must be borne in mind when considering the results (Jenkins *et al.*,

1983). Hence complete similarity between two systems is impossible to attain, but approximate modelling at pilot-scale can achieve realistic predictive results.

The vitamin niacin, or nicotinic acid, is required as a growth factor in activated sludge. Many vitamins, including niacin, have been shown to increase substrate degradation, reducing biomass yield, producing consistent effluent quality and reducing running costs, but have not been able to produce statistically significant advantages from vitamin dosing (Lind *et al.*, 1994).

9.1.2 Niacin (Nicotinic acid)

Niacin is active after conversion to codehydrases which are made up of niacin amide, phosphoric acid, adenine and pentoses and termed as diphospho- and triphospho-pyridine nucleotide (Stryer, 1988). The active molecular part of the molecule is the pyridine ring which, under partial and reversible hydration, supplies dihydrocodehydrases. These are not auto-oxidisable and therefore require an acceptor which, still under dehydration, reoxidises them ready for reconversion. The importance of the dehydrogenases is demonstrated by the fact that almost all fermentative dehydrations occur only in the presence of diphospho-pyridine or triphospho-pyridine, and so such dehydrations can be classified as pyridine catalyses (Schormüller, 1948). As well as their action as hydrogen-transferring activators, the codehydrases are essential to phosphate transport. Isomerism or extracelluar hydration of the diphospho-pyridine or triphospho-pyridine molecule and modifications to the pyridine ring prevent the hydration of the codehydrases, rendering them biologically inert.

Niacin, its amide and its ethyl ester are active growth factors for several industrially important bacteria in which niacin and the ethyl ester are first converted to the amide for use in the cell. Dependency on a niacin amide supply ranges from total (the influenza bacillus *Haemophilus parainfluencae* cannot use niacin in place of its amide, as it is not able to synthesise the codehydrase from a supply of its components (Schormüller, 1948)) to none (yeasts and normal intestinal flora are capable of synthesising niacin amide from

carbon, ammonia and nutrient salts (Schormüller, 1948; Lind *et al.*, 1994)). Within the range lie micro-organisms that require niacin for success, but not for survival (Kidder and Dewey, 1949), suggesting that niacin may not only be a building block for the codehydrases already known, but a constituent of other important enzymes (Schormüller, 1948).

The aim of this trial was to confirm the data obtained at bench-scale, identify scale-up problems/effects and demonstrate the effects of phosphorus and niacin addition for enhanced biological wastewater treatment of industrial wastes.

9.2 Pilot plant design and operation

The factors involved in design of reactors for the activated sludge process are:

1. substrate concentrations
2. temperature
3. pH
4. degree of dilution
5. DO concentration
6. presence of inhibitory substances
7. nutrient balancing
8. sludge age (MCRT)

Substrate degradation rates are affected by these eight parameters and since none of them is scale-dependent, it is possible to determine biological kinetics at any scale. (Cooper and Boon, 1983). Substrate uptake and oxygen uptake per unit solids per hour can be obtained with confidence from bench-scale units provided continuous flow (not batch-fed) reactors are used (Jenkins *et al.*, 1983). Factors which pose problems in scaling up are those that relate to the hydrodynamics involved in mixing, aeration and separation of solids. Oxygen transfer rates at pilot-scale are not the same as in full-scale plants employing the same methods of aeration and/or mixing. The ratio of wall area to

unit volume causes changes in mixing intensity and efficiency, and the numbers of diffusers per unit volume vary in aeration basins of different sizes. Fine bubble aeration in pilot plants produces higher rates of oxygen transfer than the same technique in larger plants, partly owing to the differences in circulation of the mixed liquor brought about by the wall:volume ratio (tank geometry). If a similar mixing pattern can be achieved then it is desirable, as it can affect microbial ecology and flocculation (Cooper and Boon, 1983).

Clarifier design is concerned with efficient removal of SS from the effluent. The hydraulic characteristics of full-scale plants can not be simulated at pilot-scale owing to typical hydraulic overflow rates and solids loading rates (Jenkins *et al.*, 1983), but even so, some aspects of design are worth considering. Protrusion of the return activated sludge (RAS) intake up into the clarifier and the use of a conical base hinders sludge removal from the very bottom of the clarifier and leads to rising sludge. The MLSS output should be set well down in the clarifier wall, so that the distance the sludge has to fall to the bottom of the clarifier is reduced (Jenkins *et al.*, 1983).

The wall:volume ratio modifies hydraulic, physicochemical and biological conditions, as the surfaces may act as catalysts, support for fixed biofilms and influence the boundary layer in turbulence patterns. For geometric similarity, the wall:volume ratio should be constant between systems. However, ratio of aeration basin size to clarifier size is more important as experiments with unmatched units never yield reliable results (Horváth and Schmidtke, 1983).

The pilot plant was designed based on previous work (Clark *et al.*, 1999a) and modified to mimic a full-scale activated sludge plant treating the industrial waste employed. Aeration basin design recreated the full-scale in terms of number of compartments, completely or partially mixed etc. in order to produce volume-geometric, kinematic, chemical, biological and thermal similarity. DO concentration is of critical importance; changing the DO from <0.5 to >0.5mg/l has a pronounced effect on sludge settleability (Jenkins *et al.*, 1983), so the pilot-scale system was designed to use the same DO range

as at full-scale. The pilot plant clarifier operated to satisfy the scaling requirements of the aeration basin, as recommended in the literature (Horváth and Schmidtke, 1983).

The pilot plant was located on the site treating the industrial wastewater at full-scale. It consisted of two lanes, each comprising a 300l aeration basin and a 100l clarifier (Figure 9.1). Each basin was initially filled with 150l industrial wastewater and 150l activated sludge taken from the RAS channel of the pre-treatment activated sludge plant dealing with the wastewater. For an acclimation period of eight days, the industrial wastewater used for the experiments in Chapters 5 - 8 was supplied at 25l/h, thus maintaining the HRT at 12h (mean COD 1739 ± 275mg/l, mean BOD_5 804 ± 287mg/l). After acclimation, one of the aeration basins was supplied with 0.06l/h mixed orthophosphoric acid and niacin in solution (at concentrations providing 3.0 and 1.0mg/l influent respectively) in addition to the wastewater feed. The other aeration basin received tap water at 0.06l/h, and acted as a control. The control served to account for any micronutrients remaining in the activated sludge after the initial inoculation, and to negate observations arising from the additional fluid volume entering the test aeration basin.

The aeration basins and clarifiers were covered to minimise liquid loss by evaporation (Figure 9.2). The DO was maintained at 1.4-2.4mg/l and the pH remained within 7.4-8.5 without control. Solids were brushed off the basin walls weekly, and sludge wasted from the aeration basins at a rate to maintain a MCRT of 6d. Measurements of the influent and effluent COD, BOD_5, ammonia, SS and total phosphorus concentrations were made; MLSS, SSVI and respiration rate of the sludge were also measured according to *Standard Methods* (APHA, 1992). Data were analysed using paired t-tests (Berthouex and Brown, 1994), as described in Chapter 6.

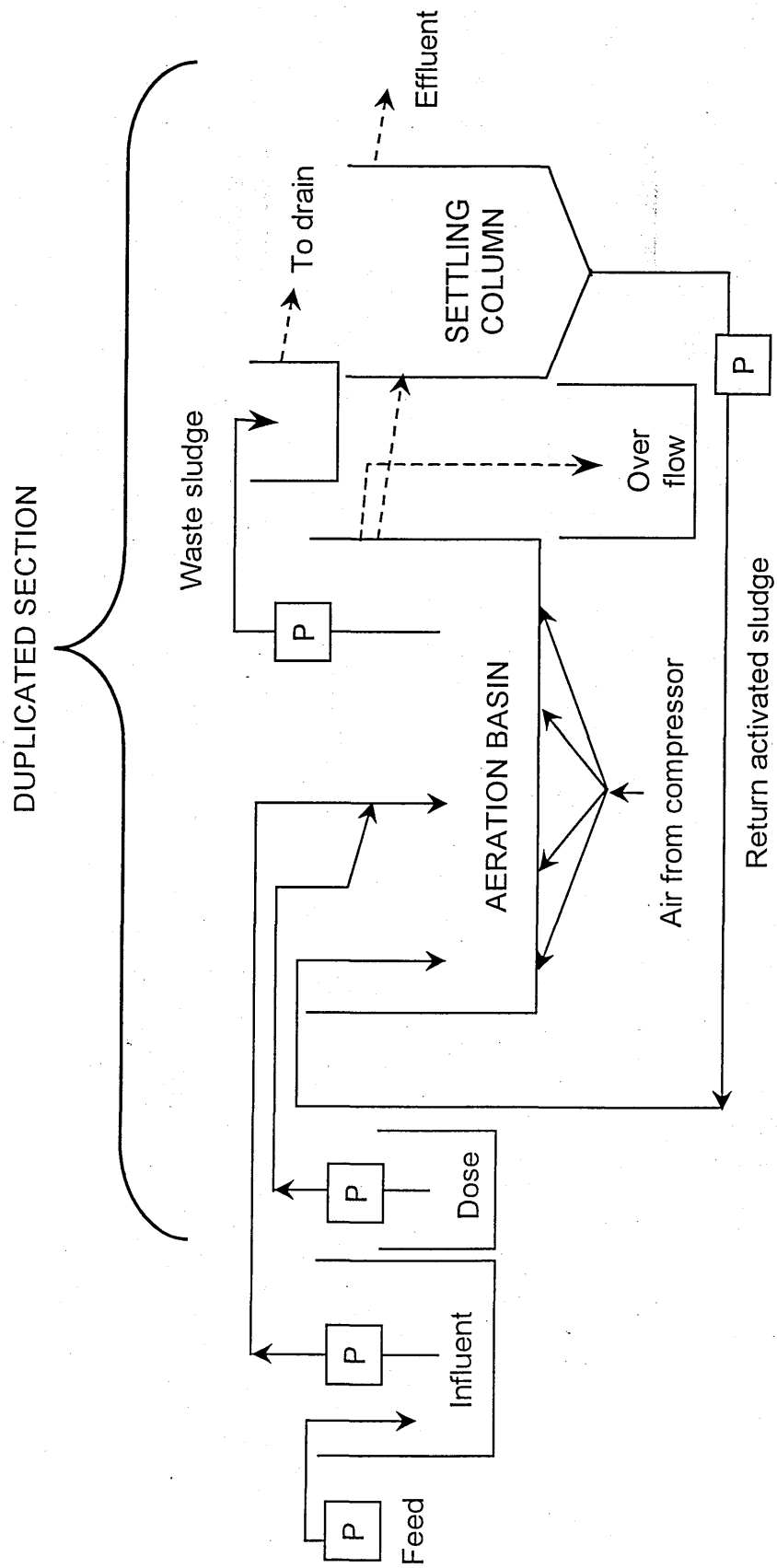

Figure 9.1. Schematic representation of the pilot plant.

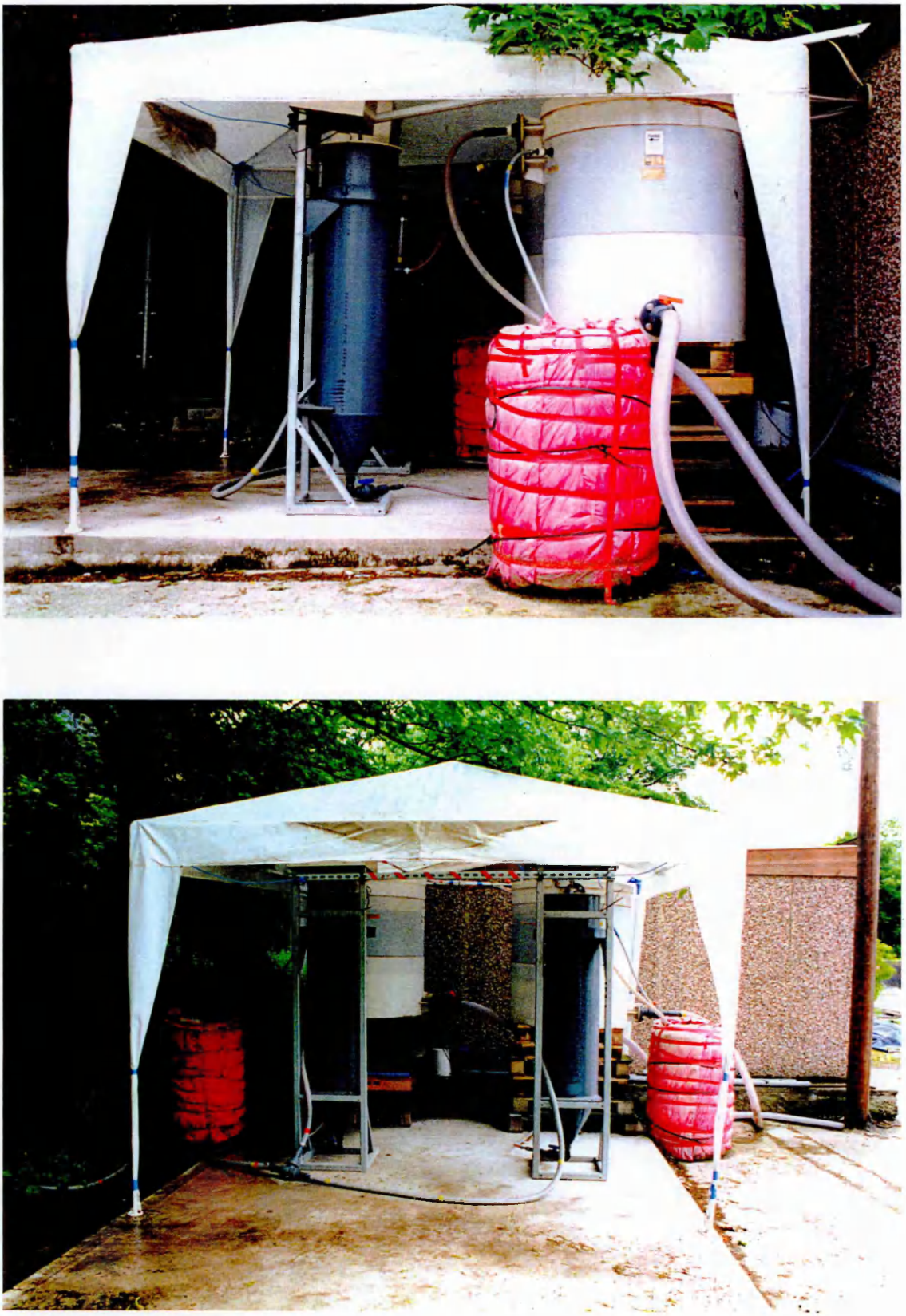

Figure 9.2 Pilot plant layout.

9.3 Results

The measurements of oxygen uptake, MLSS, BOD_5, COD, ammonia, SS and phosphorus were used to calculate removal rates per unit MLSS and removal efficiencies in the pilot plant (Appendix D). Niacin dosing in the test aeration basin had a direct impact on the sludge, resulting in an increased MLSS concentration and lower SSVI (Figure 9.3). Although the mean values for MLSS do not appear to be different (Table 9.1), the difference in MLSS between the test and control was statistically significant (t=4.58, df=20), as was the change in SSVI (t=1.67, df=17) when examined using a t-test with samples paired by date (paired two-sample for means, 1 standard deviation confidence limit). A summary of the sludge data shows that the test tank maintained an average of 50% more MLSS than the control system (Table 9.1), and that the variability in the MLSS data (i.e. relative standard deviation) was similar between the two systems. The SSVI data showed more variability in the test basin than in the control, although the difference noted was negligible.

The oxygen uptake rates of sludge samples taken from the aeration basins were converted to rates per unit MLSS per day, and it can be seen from the summary data that the control sludge showed a higher oxygen demand than that of the test system (Table 9.1). The reduction in air requirement per mass of MLSS was not statistically significant (t=1.34, df=8; paired two-sample t-test for means, 1 standard deviation confidence limit) and the variability in the measurements taken from the two systems was the same.

Table 9.1 Summary data of activated sludge characteristics.

	MLSS (mg/l)		SSVI (ml/g)		Oxygen uptake (kg/kg MLSS/d)	
	Control	Test	Control	Test	Control	Test
Maximum	2562	3200	250	207	0.80	0.64
Mean	971	1486	113	96	0.47	0.39
Minimum	320	386	44	44	0.25	0.18
Relative standard deviation	62%	61%	51%	57%	36%	36%

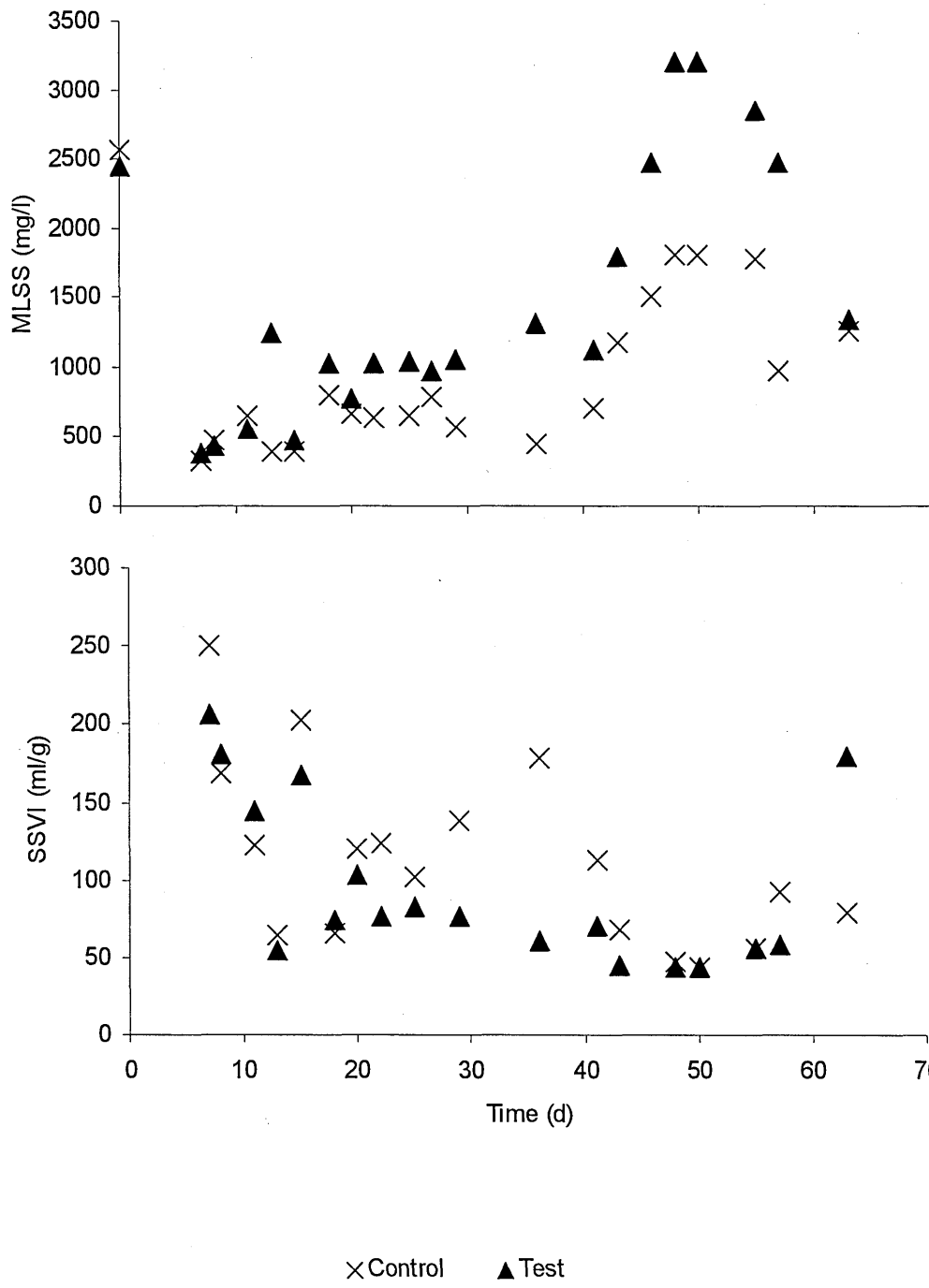

Figure 9.3 MLSS and SSVI of the activated sludge in the pilot plant.

The MLSS concentrations in the aeration basins reached a sharp peak on day 40, possibly attributed to fluctuations in influent SS concentration (day 36, 50 mg/l; day 40,

600 mg/l; day 44, 314 mg/l, Figure 9.6) and corresponding variation in influent phosphorus levels (day 36, 24.4 mg/l; day 40, 67.7 mg/l; day 44, 19.6 mg/l). The increase in phosphorus supply could account for the observed increases in MLSS. The increases in influent SS and total-P were not associated with increased influent ammonia, BOD_5 or COD levels, and the COD and BOD_5 removal efficiencies in both systems dropped, perhaps as the result of a change in the chemical composition of the wastewater as opposed to its organic load.

Process performance was assessed in terms of the removal of effluent quality determinants (Table 9.2). The data were tested for significant differences between the control and the tests system using the paired two-sample t-test for means with 1 standard deviation confidence limit. The removal efficiencies of BOD_5 from the influent were not significantly different, although this may be due in part to the small number of degrees of freedom in the BOD_5 t-test. The data showed variation (Figure 9.4), partly attributable to air supply interruptions at days 8 and 24 and the peak in influent SS and P at day 40.

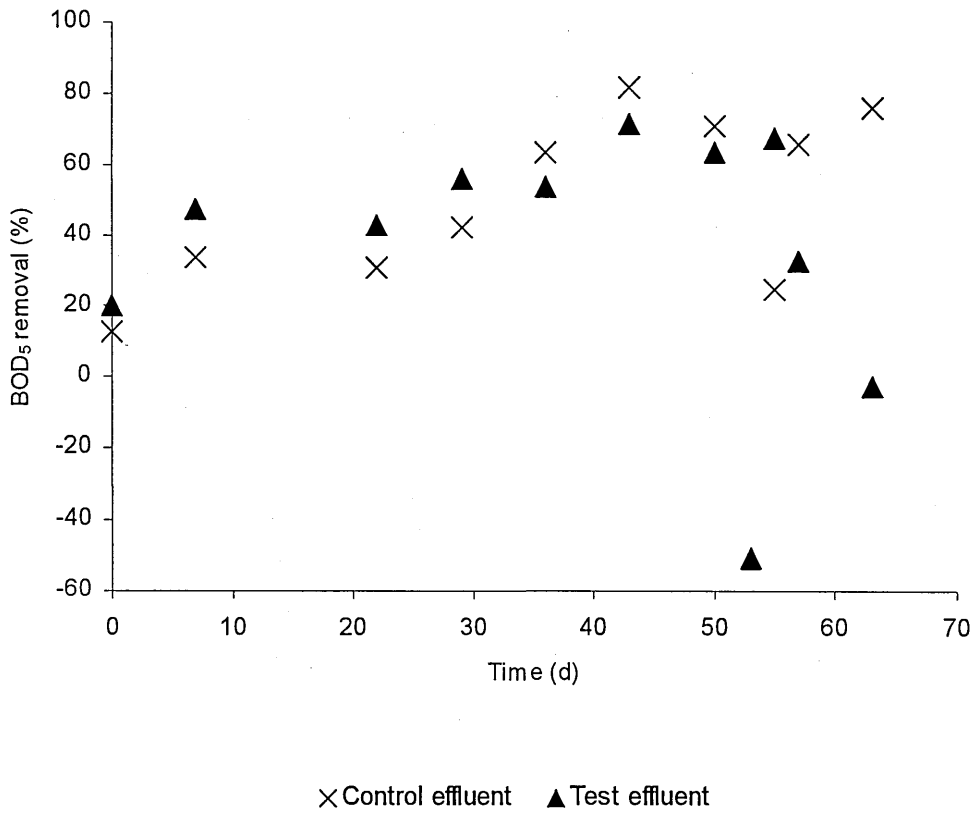

Figure 9.4 BOD_5 removal efficiency of the two pilot-scale systems.

The percentage COD removal attained by the test system was not significantly different to that of the control (Table 9.2). The trends in COD removal show the same disturbances as the BOD_5 data in Figure 9.4. It can also be seen that the test system recovered from these perturbations in the conditions before the control (Figure 9.5). COD removal rates per unit MLSS were significantly higher in the test system than in the control (Table 9.2).

Table 9.2 Summary data and t-test results for process performance determinants.

		BOD_5			COD			Ammonia			SS			Total Phosphorus		
		Feed	Control effluent	Test effluent	Feed	Control effluent	Test effluent	Feed	Control effluent	Test effluent	Feed	Control effluent	Test effluent	Feed	Control effluent	Test effluent
DATA (mg/l)	Max	1100	890	863	2290	2260	1890	57	58	54	600	484	308	72	28	26
	Mean	805	422	449	1739	1314	1212	30	31	31	188	183	151	36	15	13
	Min	278	175	122	1370	777	776	8	15	15	32	80	80	20	3	4
	RSD (%)	36	55	46	16	29	23	45	32	31	88	66	41	50	58	62
	t-test	t=0.20 df=10, cl=1 SD No significant difference			t=0.55, df=17, cl=1 SD Significant difference			t=-0.06, df=19, cl=1 SD No significant difference			t=1.04, df=12, cl=1 SD Significant difference			t=2.70, df=11, cl=1 SD Significant difference		
		BOD_5			COD			Ammonia			SS			Total Phosphorus		
		Control	Test		Control	Test		Control	Test		Control	Test		Control	Test	
Removal efficiency (%)	Max	82	71		57	57		55	52		77	75		87	87	
	Mean	26	37		25	30		-34	-42		-81	-41		52	60	
	Min	-220	-51		-10	-3		-309	-382		-448	-300		-15	-2	
	RSD (%)	331	99		87	56		-263	-265		-210	-287		66	50	
	t-test	t=-0.59, df=10, cl=1 SD No significant difference			t=-1.33, df=16, cl=1 SD No significant difference			t=0.69, df=19, cl=1 SD Significant difference			t=1.44, df=12, cl=1 SD Significant difference			t=-2.01, df=11, cl=1 SD Significant difference		
Removal rate (kg/kg MLSS/d)	Max	0.08	0.03		0.13	0.06		0.00	0.00		0.02	0.01		0.00	0.00	
	Mean	0.03	0.00		0.03	0.01		0.00	0.00		0.00	0.00		0.00	0.00	
	Min	0.00	-0.03		-0.02	0.00		0.00	0.00		-0.02	0.00		0.00	0.00	
	RSD (%)	70.38	-1199.13		119.03	177.39		1687.54	-2308.22		-1292.63	332.64		71.32	185.88	
	t-test	t=0.42, df=10, cl=1 SD No significant difference			t=1.63, df=16, cl=1 SD Significant difference			t=1.26, df=19, cl=1 SD Significant difference			t=0.49, df=12, cl=1 SD No significant difference			t=2.22, df=11, cl=1 SD Significant difference		

t = critical t ratio; df = degrees of freedom; cl = confidence limit; SD = standard deviation; RSD(%) = relative standard deviation.

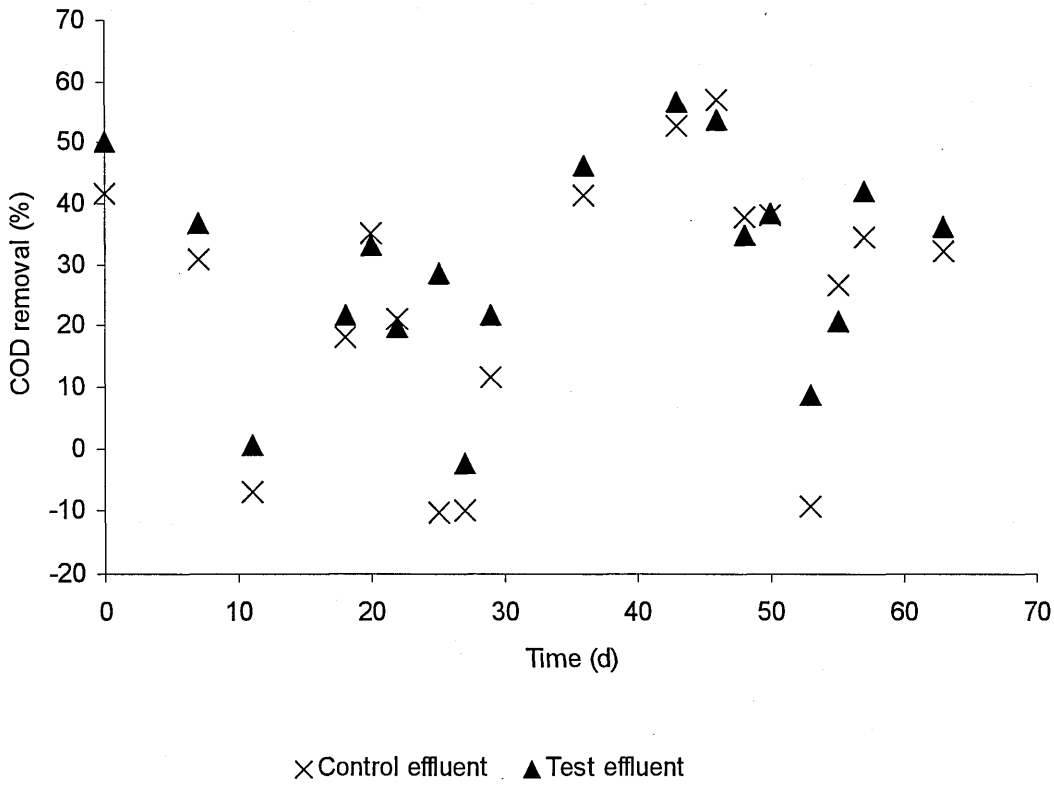

Figure 9.5 Percentage COD removal by the pilot plant.

Mean ammonia removal efficiency was a negative value for both control and test tanks. There were signficant differences in the removal efficiencies and rates per unit MLSS; the test tank removed more ammonia than the control (Table 9.2). The negative values for ammonia removal arise from the presence of amines in the influent wastewater, which break down to ammoniacal nitrogen during treatment and negate any degradation of ammonia that occurs.

Removal of suspended solids was greater in the test system than in the control (Figure 9.6). In general, effluent SS values exceeded influent values so removal data were negative values in spite of the mean effluent SS concentrations being lower than the mean influent concentration (Table 9.2). This may be due partly to poor settling in the clarifier, and partly to the variability in SS concentrations in the samples. The variability (RSD) in influent SS was approximately 80%; in the control effluent it was 65% and in the test effluent only 40%. These values show that the test system performed much

more consistently than the control in terms of SS effluent quality, and it can be seen from the plot of percentage removal of SS against time that the change in influent concentration at day 40 had a smaller effect on the test effluent SS than the control (Figure 9.6).

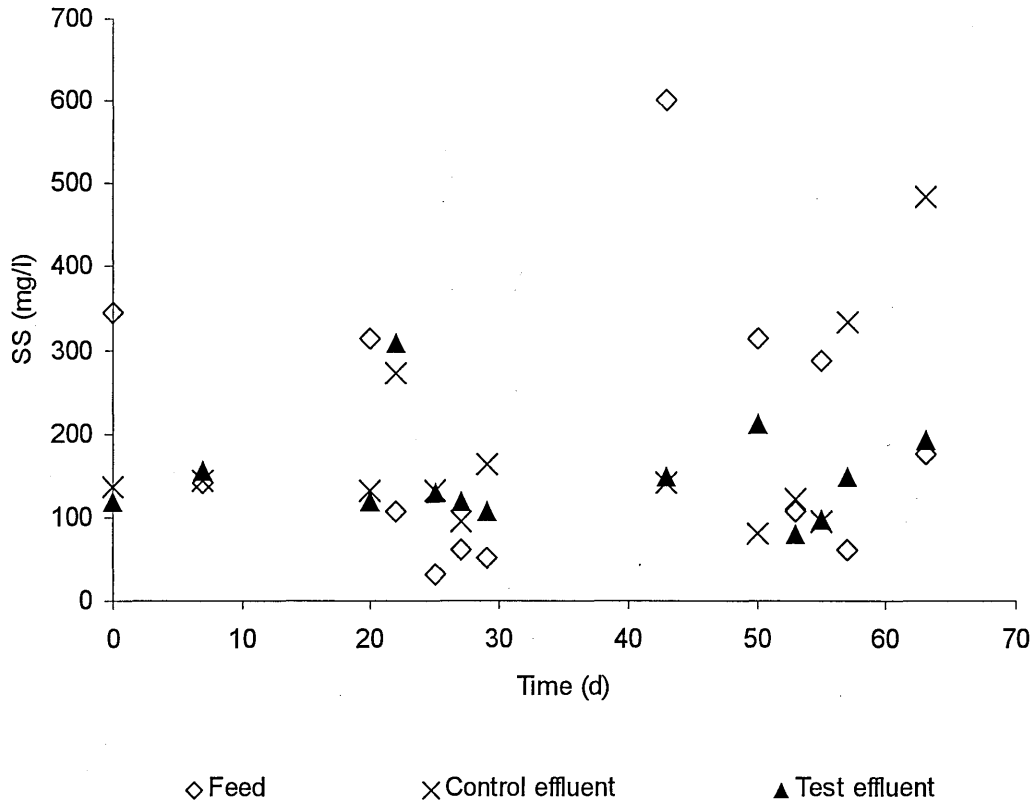

Figure 9.6 Influent and effluent SS concentrations.

The MLSS in the clarifiers bulked instead of settling well, a characteristic reflected in the high SSVI values obtained. The possible causes of this include the low levels of nutrients available selecting for filamentous bacteria in the biomass and the composition of the wastewater, as bulking in this case can not be attributed to low temperatures, short HRT or low DO concentrations. Bulking MLSS was seen to break through into the plant effluent at times during the trial, and this had an effect on effluent COD values. A plot of COD and SS removal efficiencies on the same axes (Figure 9.7) shows that percentage removal of COD and SS in both systems followed similar trends, and that effluent SS contributed to effluent COD. Measurement of filtered influent and effluent COD could have given more information on soluble COD removal.

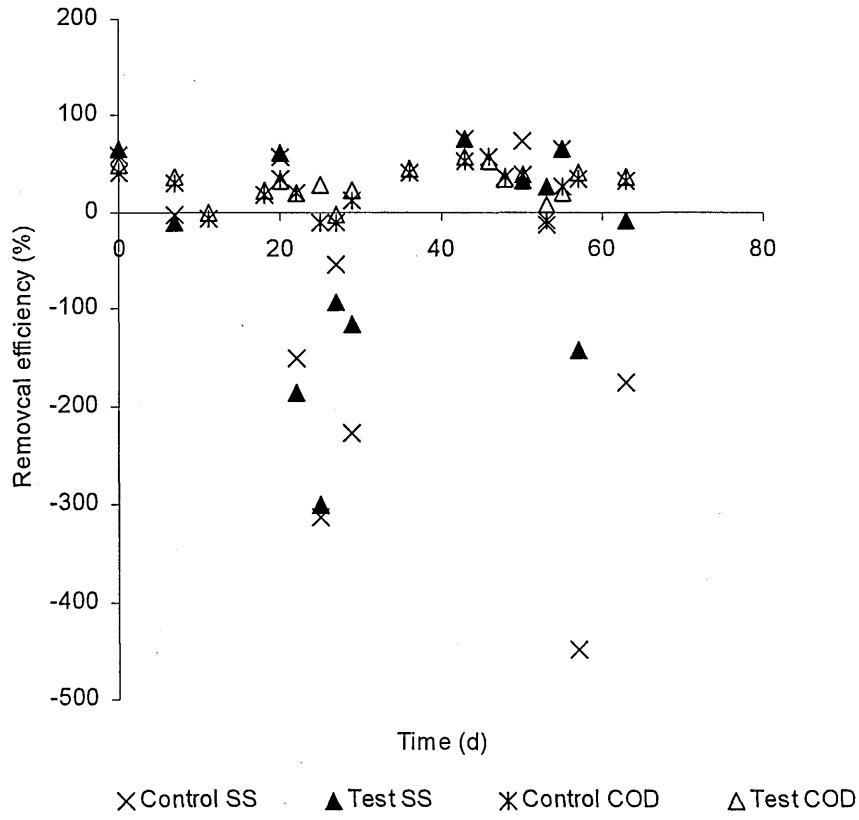

Figure 9.7 COD and SS removal efficiencies (percentage removal).

The total phosphorus data showed that the mean test effluent phosphorus concentration was 2.0mg/l less than the control (Table 9.2). The total phosphorus in the influent averaged approximately 40mg/l, but not all of the phosphorus was present in bioavailable forms and the control system operated under phosphorus–limited conditions. Although 3.0mg phosphorus was added to the test system per 1000ml influent, the effluent phosphorus concentrations were significantly lower and the removal rate per unit MLSS were significantly higher than that of the control.

9.4 Discussion

The increase in MLSS is explained by the action of niacin as a growth factor and the provision of phosphorus to the sludge. Missing thiamine, biotin or niacin leads to low population densities in industrial biological waste treatment systems, (Lind *et al.*, 1994; Lemmer *et al.*, 1998). Increased population densities of heterotrophic saprophytes were found in tests of supplemented biotin, niacin and thiamine in industrial wastewater treatment works (Lemmer *et al.*, 1998), however, municipal sewage sludge has no requirement for added niacin (Lind *et al.*, 1994). Increasing the biomass yield is not desirable in general, but in cases where recalcitrant wastewater results in population densities that are too low for sufficient treatment (the control system maintained just 971 ± 603mg/l), increased MLSS is an advantage.

The decrease in SSVI was due to changes in floc structure and the increased MLSS, as sludge settling rates are affected by the concentration of solids and the physical conditions prevailing. SSVI can be scaled up to within 20% accuracy as the slow stirrers minimise the surface effects of the cylinder wall (Cooper and Boon, 1983), therefore it is reasonable to expect that the observed improvements in sludge settling would also be seen at full-scale.

The reduction in oxygen demand per unit biomass represents an economic advantage, but the results here must be treated with caution. The respiration rate data were recorded as uptake per mass of solids, and hence do not reflect the oxygen demand of a fixed volume of mixed liquor, in which the solids content may vary. As most activated sludge plants are operated to maintain a fixed MLSS, it is probable that the results are applicable to full-scale operations. Oxygen utilisation is not subject to scale-up effects, but the results were obtained using samples withdrawn from the reactor to obtain data in closed vessels and the values obtained may be artificially low owing to substrate-limiting conditions developing during the test (Eckenfelder and Quirk, 1983). Oxygen uptake rates may also be affected by the level of turbulence in the reactor as this affects the flocs' architecture and hence microbial ecology.

No significant variation in BOD_5 removal was observed. Requirements for niacin are widespread and include even soil bacteria, but in most cases associations of heterotrophic bacteria can at least partially provide their own supply (Lind *et al.*, 1994). Exchanges between vitamin-excreting and vitamin-requiring organisms negate the use of metered doses in municipal and domestic wastewater treatment works, but industrial wastewater treatment works should be considered individually for requirements for extra vitamin dosing (Lind *et al.*, 1994). Nitrifiers (*Nitrosomonas, Nitrobacter*), denitrifiers (*Hydrogenophaga, Acidovorax*), *Clostridium* and *Bacillus* species are niacin auxoautotrophs (Lemmer *et al.*, 1998). Auxoautotrophs exist in activated sludge populations from a range of sources, including chemical wastewater treatment works (Lind *et al.*, 1994; Lemmer *et al.*, 1998), so the activated sludge was potentially able to provide enough niacin for survival and metabolism of the readily degradable substrate components that represent BOD_5.

The removal of COD was enhanced by phosphorus and niacin addition. Previous workers have reported that vitamin addition did not enhance the degradability of certain partially-degradable compounds (nonylphenol ethoxylate, linear alkylbenzene sulphonate, ethylenediamine tetraacetic acid and 2,4,6-trichlorophenol) or reduce the inhibitory effects of their intermediates on the degradation of other, degradable substances (casein hydrolysate) (Lind *et al.*, 1994). However, heterotrophic bacteria requiring biotin, thiamine and niacin are found in all activated sludge populations (Lind *et al.*, 1994). In situations with low F/M ratios, including the chemical industry (Lemmer *et al.*, 1998), micronutrients can be deficient and biological wastewater treatment can benefit from supplements (Lemmer and Nitschke, 1994). The observed increases in COD removal were not associated with a small (not statistically significant) increase in oxygen uptake by the activated sludge. This indicates stimulation of the sludge biomass rather than COD removal via chemical reactions or the increased adsorption of substrate components onto the cell walls.

Effluent SS values showed significant reductions in the test system, although there was no difference in the rates of SS removal per unit MLSS. Only very large pilot plants can

begin to simulate full-scale turbulence and its effects on effluent SS values (Jenkins *et al.*, 1983), so these data can not be considered to be a reliable indication of probable SS removal performance by a full-scale plant.

The phosphorus removal efficiency was greater in the test than the control and the effluent phosphorus concentration was significantly lower, although the increased MLSS led to lower removal rates per unit MLSS. The lower effluent concentrations indicate that the added phosphorus was completely taken up, and may have acted to raise the concentration in the aeration basins above a threshold level needed to trigger certain metabolic pathways which require phosphorus for non-essential life processes.

The oxygen uptake rates and BOD_5 and COD removal rates per unit MLSS obtained at bench-scale (Chapters 7 and 8) and at pilot-scale were compared using a paired t-test for measurements taken at the same time in each trial. Insufficient numbers of data points fell at the same time for the examination to be absolutely conclusive, as the df values are all below 9 (except COD removal rates in the test units), but all of the tests performed showed statistically significant differences (Table 9.3).

This can be attributed to major differences between the two systems. Firstly, the bench-scale reactors had no clarifier, the effluent being obtained by filtration through the porous pot inners, so the COD and BOD_5 values measured were effectively those of coarsely-filtered effluent, not subject to the influence of changes in sludge settleability as in a pilot- or full-scale plant. Secondly, the mixing in the bench-scale aerated reactors was via one sintered glass diffuser at the base of the conical vessel, and via four air nozzles on the bottom of the tank at pilot-scale. This will have caused differences in mixing in the two systems, and means that the DO in the porous pots was between 1.0-5.0 mg/l, while the DO in the pilot plant was 1.4-2.4mg/l. Lastly, the sludge samples used to measure oxygen uptake rates were transported to the same laboratory for analysis. At bench-scale this was a journey of just a few minutes; at pilot-scale the journey took approximately four hours, during which period the sludge was not aerated, mixed or fed.

Substrate limitation will have therefore been a major factor in oxygen uptake rates before the respiration tests even began.

Table 9.3 Descriptive statistics for rates comparisons at bench- and pilot-scale.

		Control		Phosphorus & niacin 1.0	
		Bench-scale	Pilot-scale	Bench-scale	Pilot-scale
O_2 uptake rate (kg/kgMLSS/d)	*Max*	0.21	0.80	0.02	0.64
	Mean	0.11	0.47	0.01	0.39
	Min	0.16	0.25	0.00	0.18
	SD	0.03	0.17	0.00	0.14
	t-test	t = 1.12, df = 7, cl = 1 SD Significant difference		t = 1.13, df = 6, cl = 1 SD Significant difference	
BOD removal rate (kg/kgMLSS/d)	*Max*	1.77	0.08	0.64	0.02
	Mean	1.17	0.03	0.39	0.00
	Min	0.80	0.01	0.18	0.01
	SD	0.30	0.02	0.14	0.00
	t-test	t = 1.55, df = 5, cl = 1 SD Significant difference		t = 1.16, df = 5, cl = 1 SD Significant difference	
COD removal rate (kg/kgMLSS/d)	*Max*	4.42	0.13	0.03	2.48
	Mean	2.59	0.03	-0.01	1.88
	Min	1.51	-0.01	-0.03	1.14
	SD	0.70	0.04	0.02	0.45
	t-test	t = 1.13, df = 6, cl = 1 SD Significant difference		t = 1.01, df = 11, cl = 1 SD Significant difference	

The porous pot method is described as an economic and convenient means of collecting biodegradability information that is not significantly different from data obtained using other bench-scale apparatus that resembles an activated sludge plant. However, it is also stated that higher rates of degradability could be achieved as porous pots are not subject to fluctuations in sludge growth rates and settling characteristics (Painter and King, 1978). The results obtained in this study corroborate the latter observation, and the evidence suggests that data obtained using porous pots are comparable only to other porous pot data, and extreme care should be taken in using them predictively for full-scale operations. The pilot plant suffered several interruptions to the air supply, and the mean values reported include data points from periods of start-up and operation problems in addition to data from steady operation. The outliers have not been removed

from the data set for two reasons: to allow t-tests to be performed, as removal of the outliers would have reduced the df to less than 9, and because the results were intended mainly for a comparative study of the test rig in relation to the control. As both systems were subject to the same influences, it was felt that a comparison could still be drawn. In some cases, the greatest differences in process performance occurred during operational problems around days 25 and 51 (Figures 9.4-9.6), suggesting that the addition of niacin allowed a buffering effect that counteracted the loss of oxygen in the test system.

9.5 Conclusions

The effects of nutrient addition in activated sludge treatment of industrial waste that were demonstrated at pilot-scale can be summarised (Table 9.4). The results of phosphorus and niacin dosing at pilot-scale confirmed the tabulated general effects observed at bench-scale, although scale-up problems associated with the methods for oxygen uptake measurement, and the effects of tank geometry on the accuracy of effluent SS data were identified.

Table 9.4 Summary of the effects of nutrient dosing at pilot-scale.

Parameter	Observed effect	Significance
MLSS	Increase	Significant
SSVI	Decrease	Significant
Oxygen uptake rate	(Decrease)	Not significant
BOD_5 removal	(Increase)	Not significant
COD removal	Increase	Significant
Ammonia removal	Increased removal efficiency rate Decreased removal rate per unit MLSS	Significant Significant
SS removal	Increased removal efficiency rate Decreased removal rate per unit MLSS	Significant Not significant
P removal	Increased removal efficiency rate Decreased removal rate per unit MLSS	Significant Significant

The results obtained imply enhanced toxicity removal by the activated sludge and demonstrate the improved degradation of recalcitrant compounds indicated by increased COD removal which is not associated with increases in BOD_5 removal. The net effect was stimulation of the activated sludge micro-organisms with no inhibitory effects from micronutrient addition over a longer test period observed.

9.6 INTERIM SUMMARY AND CONCLUSIONS

Bench-scale models such as the porous pots employed in Chapters 7 and 8 represent a compromise between pilot plant production and use of data from the literature that can be used for waste treatability studies, biodegradability and toxicity determinations and determination of biological process kinetics (Jenkins *et al.*, 1983). However, the applications of such small systems are generally limited to four objectives: (1) establishing process feasibility, (2) developing basic relationships for design, (3) defining broad range of performance, (4) demonstration for teaching or sales promotion (Schmidtke and Smith, 1983). In bench-scale systems where air diffusion is used for mixing, the DO is almost at saturation, as more air is required for mixing than for respiration. This means DO control is not possible, and any measurements of sludge handling properties that are made are not valid.

Pilot-scale models are larger, generally operating with continuous flow of 20-100l/min. Their applications are much broader and include confirmation of bench-scale data, identifying scale-up effects / problems, studying process features, resolving engineering problems, identifying and resolving operational problems, identifying long term effects, providing a convincing demonstration, confirming design criteria for prototype, and examination of system economics (Schmidtke and Smith, 1983).

Bench-scale activated sludge simulations may be used for waste treatability studies, biodegradability and toxicity determinations. Activated sludge plants can be scaled up provided hydrodynamic conditions remain the same, as biological coefficients are not affected by scale. The eight process configurations listed in section 9.2 were maintained in the pilot plant as they are at full-scale, and as none of them is scale dependent, it is possible to determine biological characteristics at pilot-scale.

Since effluent toxicity at bench-scale (Chapter 8) showed positive correlation with effluent pH and COD concentrations, and negative correlation with effluent ammonia

(Table 9.5), it is reasonable to infer that the same correlations apply at pilot-scale. If this is the case, the higher pH and COD concentration in the control pilot system effluent imply greater toxicity in the control effluent than in that of the test plant.

Table 9.5 Characteristics of porous pot and pilot plant effluents.

	Inhibition (%)	NH_4 (mg/l)	COD (mg/l)	BOD_5 (mg/l)	pH
Porous pot Control	40	25.45	832	193	8.11
Porous pot P & niacin 0.5mg/l	-1	21.59	677	163	7.95
Correlation with inhibition of nitrifyers	-	-0.42	0.73	0.01	0.79
Pilot plant control	-	31.5	1312	457	8.11
Pilot plant P & niacin 1.0mg/l	-	31.2	1230	445	7.62

The overall effect of micronutrient addition at pilot-scale is the stimulation of biomass, thus enhancing process performance in terms of recalcitrant substrate removal. Work was planned to investigate the extent to which UK industry would be prepared to adopt micronutrient addition as an option for reducing wastewater toxicity in preparation for the introduction of DTA.

CHAPTER TEN

INDUSTRIAL APPLICATION OF MICRONUTRIENT ADDITION.

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INDUSTRIAL APPLICATION OF MICRONUTRIENT ADDITION.

10.1 Introduction

An earlier chapter on process improvement focused on a framework for process adaptation from the operators' perspective (Chapter 4). This chapter will explore adaptation and the proactive role of the regulator, from the regulators' perspective. The focus is the method that can be used when attempting to understand the adaptive response of a sector.

10.1.1 Direct Toxicity Assessment (DTA)

DTA is the consideration of the effluent as a whole in terms of the impact it may have on receiving waters. This indicator is designed to provide a simple and easily understood measure for the protection of aquatic life from potentially harmful effluent discharges. It allows for the control of toxic discharges, the setting of toxicity reduction targets and provides for the assessment of improvements in the quality of receiving waters. The EA National Centre for DTA, in Oxfordshire, has been instrumental in selecting and publicising toxicity test methods for regulatory purposes. The centre provides a protocol for the introduction of a toxicity-based consent and has established a research and development (R&D) strategy for the continued development of DTA procedures (Environment Agency, 1997).

Differences between the USA, where toxicity-based consents are already established, and the UK lie mainly in the roles of the water and sewerage companies. In the USA no industrial waste is sent to sewer alongside domestic waste, which means that the water and waste companies are under no threat from unexpected toxic discharges into the sewage treatment works. The sewerage undertakers in the UK are in a very different situation, as they treat the discharge from a number of industrial operators alongside

domestic sewage and road run-off water. This makes a great difference to the variability of the influent in the sewage treatment works in the UK and makes the water companies more vulnerable to consent failure.

The development of strategies to accommodate legislation occurs differently in many companies. Hill and Westbrook (1997) identify three levels within a company at which this may occur:

1 Corporate level. Decisions at this level address issues such as *what sector of the industry to be in, which sectors might facilitate sustained growth* and *how to enter such sectors.*

2 Business unit level. At this level, managers address questions like, *what market sectors do we serve? What are the needs of serving these sectors, and how are they changing? How can we best compete?*

3 Functional level. Individual functions such as product or service supply and research and development. In the case of the water treatment industry, issues such as, *what actions are needed to meet targets? What developments are needed to improve the efficiency of our operation? What technologies or techniques are best able to assist us in this aim?*

R&D can provide the answers to the questions asked at the business unit and functional levels, either in-house or from academia. In many cases, the answers to the questions facing business units do not readily present themselves. Tightening environmental legislation requires increasing effluent treatment. However, a familiar aspect of business is that it is often hard to justify financial investment in an infrastructure where environmental benefits are undervalued in the short term. In addition, the challenge facing companies is not simply one of improving performance in an existing task, or a more stringent standard for restricted chemicals, but a completely new target to meet, i.e. toxicity reduction.

10.1.2 Micronutrient Addition

Many industrial wastewaters show resistance to the biological treatment processes commonly used to treat municipal and domestic wastes. A great number of industrial processes exist which result in the release of synthetic compounds unfamiliar to microbial cells and therefore resistant to biodegradation (Buitrón and Capdeville, 1995). These compounds accumulate in the environment.

Activated sludge plants have been used to treat a wide range of industrial wastes by effectively accelerating natural processes involving chemical, biological and physical agents. Biological treatment must compete technically and economically with other treatment processes, but is often slower than chemical processes or incineration and can produce inconsistent results. Recent increases in our understanding of the environmental requirements of micro-organisms and the introduction of the use of genetically manipulated or pure culture organisms has meant that biodegradation has the potential to become an efficient and economical option. Environmental factors often limit industrial wastewater treatment by biological processes (Singleton, 1994).

Variation in the concentrations of macro- and micronutrients can control the population because the micro-organisms present will adapt to the change rather than resist it (Valo *et al.*, 1985). Treatment of industrial waste using activated sludge has often suffered from the inhibition of biological degradation, leading to high effluent polluting potential. The reasons given for these problems with all kinds of industrial waste include nutrient imbalances. Biological cells require six main nutrients for survival - carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus.

Adding the main nutrients to activated sludge can improve effluent degradation (Singleton, 1994) but the results of nutrient addition are sometimes variable. Enhancing wastewater treatment may not only be through the usual additions of nitrogen or phosphorus based nutrient supplements. The practice of analogue enrichment involves

the addition of substances that increase the uptake of unusual sources of nutrition, such as chemicals to be removed from the effluent. These substances include micronutrients.

10.2 Research Methodology

The purpose of the study reported here was to analyse the extent to which research into the use of micronutrient additions has an application in the context of adaptation of UK industry to DTA.

10.2.1 Research Context

This study is part of a wider programme of research into process development in UK industrial wastewater treatment and the reason for the focus on water companies is the importance of that industrial sector to the UK aquatic environment. The research used three stages: (1) a postal questionnaire exploring the perceived risks of DTA implementation to UK companies involved in effluent treatment (Chapter 4), (2) technical research into the application of micronutrient addition for removal of toxic components from industrial effluent (Chapters 5 to 9), and (3) interviews with companies in the UK and USA to explore the number and types of options open to them to deal with risks, and to assess the potential value of micronutrient addition in removing the risks associated with DTA.

In Chapter 4, questionnaires were sent to a number of people involved in different industries that are stakeholders in the aquatic environment. The responses led to the drawing of a number of conclusions. The ability of a company to adapt to new environmental legislation appears not to be based on such simple attributes such as company size or membership to particular industrial sectors, but to the ability, perceived or real, of that company to adapt at three key stages of their process, i.e. inputs, processes and outputs. Briefly, it was observed that:

1. Control over process inputs reduces the level of concern felt by a company.

2. Predominantly the medium sized companies expressed concern regarding management issues.
3. Company size does not directly determine the level of concern regarding financial costs.
4. DTA is accepted in principle, concerns arise mainly from its application.
5. Most of the water companies were more concerned about managerial issues than costs.

The results from the matrices led to a generic common model which stated that certain "quick fix" options are available to companies with the need to adapt to new quality control on their effluents but that different options are needed for long term, sustainable compliance. The importance of long term process adaptation as opposed to pure optimisation of existing processes was recognised and the current work explores methods of process adaptation such as the use of micronutrient addition in the activated sludge process (adaptation) rather than the optimisation of existing operating parameters (such as pH and temperature).

10.2.2 Methodology

Questionnaire respondents from Chapter 4 were invited to participate in follow–up interviews that focused on the options they perceived are available to them to adapt to toxicity removal. To widen the range of industrial sectors represented, additional participants were asked to read and discuss the questionnaire and the discussion was extended to include options for toxicity removal. The postal questionnaire had enabled data to be gathered from a larger sample, at a general level, whilst the interviews facilitated a more detailed understanding of the specific organisational and technical factors influencing the adaptation processes available. All the companies in the sample are involved in some way in industrial effluent treatment. That is, they have manufacturing processes which produce liquid effluents that they treat or part–treat themselves, or their business function is to accept liquid effluent and render it harmless to the receiving environment. All participants had been involved to some extent in decision

making for the removal of pollutive potential of industrial effluent and had participated in the nation-wide process of conference and debate in the run–up to the introduction of DTA. Further details of the respondent sample are contained in Table 10.1.

Table 10.1. Respondent sample details.

	% of respondents
Nature of main business	
Oil refining	7.7
Regulation	7.7
Consultancy	7.7
Industrial research	15.0
Waste disposal	7.7
Water treatment	23.1
Dairy processing	7.7
Chemical manufacture	23.1
Number of employees	
0 - 199	15.3
200 – 500	23.1
499 – 999	38.5
1000+	23.1
Location	
UK	92.3
USA	7.7

This paper focuses on just one aspect of the study: the potential use of micronutrient addition in response to DTA. This was approached from two angles in the interviews. First, the interviewees were asked to indicate freely what their preferred methods of toxicity removal were, and a list of possibilities were suggested if prompting was required. For example:

> Q. "What technologies do you think would be options for you to use in toxicity removal?"
>
> A. "Our first option is always to prevent toxicity, by product substitution. Then we would minimise it, with recycling. Solvents are cleaned and returned. Then if we have to treat it we would use biological treatment. We've got an onsite pre-treatment plant."
>
> Q. "So if you had to treat toxic effluents, what do you think you would do? If I read you a list, could you tell me what you think would be applicable in your case?"
>
> A. "Yes."

The prompt list contained thirteen options, some rephrased reiterations of previous choices, and the interviewees would respond, *yes*, *no*, *in some cases*, etc. and be drawn out on their responses. Then the interviewees were asked whether they had previous knowledge and/or experience of micronutrient addition. This second questioning process is represented diagrammatically (Figure 10.1).

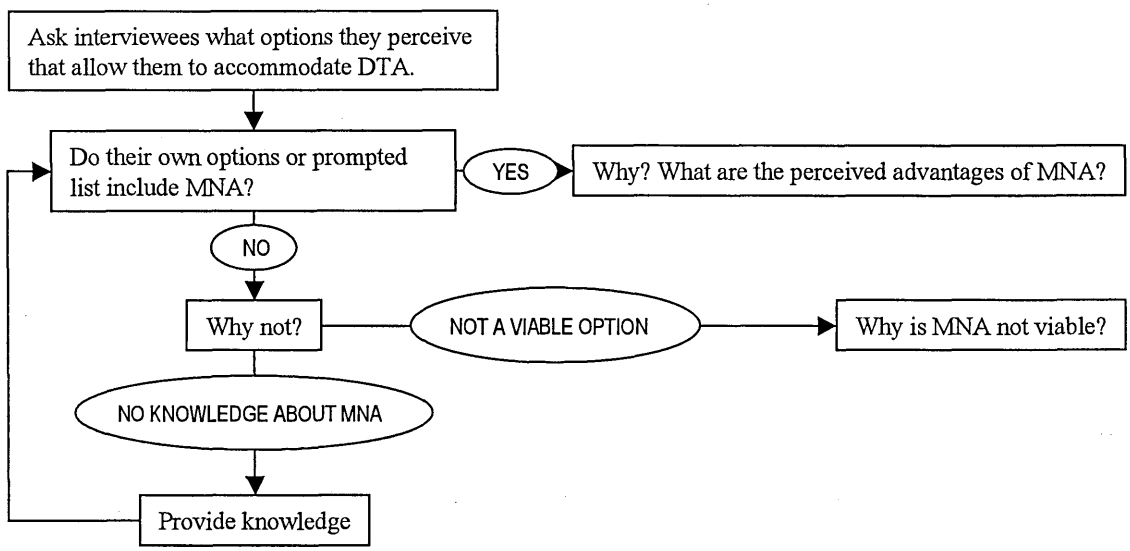

Figure 10.1. Questioning procedure.

An *aide-mémoire* was prepared (Appendix E) and used as a basis for the open-ended interviews with managers and personnel from R&D and technical areas, who were particularly involved with water use or wastewater output and treatment in their company. Sample size was small, thus limiting the reliability, but enabling greater validity through the in-depth nature of the research. Maximising the sample diversity was more important than obtaining a representative sample to be averaged. Representatives from as many different industries as possible were interviewed to gain a full account of the options for companies to adapt to DTA from different perspectives.

The main questions related to experience in effluent treatment process optimisation in general, and biological treatment such as the activated sludge process in particular: management of process optimisation for toxicity removal and comparisons of more and

less successful means of toxicity reduction using the process. The interviews lasted 30 to 60 minutes and notes made during the discussion were offered to the participants for checking and clarification. The interviews reveal the richness of the options available to companies to accommodate DTA by capturing the complexities of the decision-making process, and thereby providing insights into the procedures and practices affecting the validity of micronutrient addition as an option for responding to DTA.

10.3 Results and Discussion

10.3.1 Perceptions – the effects of DTA

The information obtained (Appendix E) gave rise to a set of secondary data. The broad effect of DTA on effluent treatment was considered. The interviewees were asked:

"Do you foresee any problems for your company in adapting to DTA?"

Interviewees were requested to expand on their comments and indicate the basis for any expected problems (i.e. technical, financial or managerial) and the extent of their concern. From Table 10.2, it is clear that many interviewees regarded responses to DTA as issues, not necessarily as problems. Whilst these are perceptions, they suggest that industrialists are accustomed to differentiating challenges and problems, and that DTA is widely perceived as an excellent principle with questions regarding its implementation surrounding its technical applications. These results were backed up by comments made by interviewees on the potential risks of toxicity in effluents.

Notably, the water companies were waiting to see how manufacturers would react; the same manufacturers were speculating on the likely actions of the water companies they dealt with, and the steps they may have to take themselves, in response. However, the reservations expressed had not deterred the participants from accepting the principle of DTA as sound, and recognising the need for an ecologically relevant test of effluent quality.

Table 10.2. The effect of DTA on effluent treatment.

We asked: *Do you foresee any problems for your company in adapting to DTA?*				
Nature of business	**In general**	**Technical**	**Managerial**	**Financial**
Oil refining (already subject to DTA)	None expected, but some arose from ignorance of available options.	No – plenty of options exist.	Some, to do with new tasks for technical staff.	Very little, although many problems were expected.
Consultancy	None.	No.	No.	No.
Industrial research	None.	No.	No.	There will be a financial impact, but no problems.
Water treatment	Only minor. Some changes are bound to occur.	Tracing the sources of toxic inputs. Learning new skills.	The number of sites needing extra work may be a cause for concern.	No training issues; technical work will be contracted out. Or cost may have to be borne.
Dairy processing	Very worried.	Don't know. Possible training issues.	Don't know.	Money is not usually a problem.
Chemical manufacture	Possibly. Most new developments extend from established methods.	No.	Maybe. Charges for discharge to sewer or limits that force us to begin treating our own waste would be a problem.	No. Progress always carries a cost.
Waste disposal	Not in the short term, although long term expectations are vague.		The cost of extra technical work.	
Regulation	Not for companies that want to adapt. Some challenges to meet, but nothing insurmountable.			

10.3.2 Options for industry to adapt to DTA.

Table 10.3 contains a list of 13 options which, after a review of previous work and technical literature, were considered by the researchers as possible options available to industry wishing to reduce the toxicity of effluent discharges into the aquatic environment. Interviewees were asked to indicate the extent to which each of the options was available to their own company. Several interviewees volunteered opinions on the relevance of the options to other industrial sectors. It can be seen from Table 10.3 that the most common option was the general one of optimising treatment processes.

Table 10.3. Options perceived as available to participants for effluent toxicity reduction.

We asked: ***Which of these technologies do you see as options for toxicity removal?***		
	For selves %	**For others %**
Refuse to accept highly toxic wastewater.	31	23
Charge for discharges to sewer according to toxicity.	23	23
Implement pre-treatment regulations.	23	31
Segregate component wastewater streams.	46	46
Use product substitution to reduce by-product/wastewater toxicity.	31	23
Optimisation of treatment processes.	69	15
Include activated carbon, activated sludge or biofilms.	62	23
Use wastewater minimisation.	38	23
Start/increase recycling of toxic wastewater components.	38	15
Use more biological treatment processes to remove ammonia.	46	8
Use more pre-treatment upstream of sewage treatment works.	46	38
Use more biological processes.	62	15
Use online toxicity monitors and pass back the responsibility of toxicity reduction.	38	23

At first this appears to contradict the hierarchy of options expressed by the majority of the interviewees, which can be summarised:

1. First choice: avoid causing toxicity (e.g. product substitution)
2. Second choice: minimise toxicity (e.g. toxic component recycling)
3. Third choice: render toxins harmless (e.g. optimise treatment of effluent)

the reasons for which were given as moral or social responsibility, economic pressures and common sense (*"prevention is better than cure"*). However, the lack of availability of the options falling into the first and second choice categories is clear. Product substitution, for example, listed as the first choice in the hierarchy, was available to only 30% of participants and effluent recycling to 40%, leaving only effluent treatment as a commonly open option (available to 70% of interviewees).

10.3.3 Micronutrient addition for DTA

Using the procedure represented in Figure 10.1, the attitude of the interviewees towards micronutrient addition as a means of adapting biological processes to enhance toxicity removal was explored. Table 10.4 shows that most of the interviewees had heard of micronutrient addition, (though one of these had encountered it only on the researchers' web site). Around half of them had considered it as an option in the past, and the overwhelming majority of participants numbered micronutrient addition among their list of options for effluent treatment in the future. Among the comments made regarding micronutrient addition were several positive opinions (Table 10.4), including one detailed observation:

> "Return on capital investment is very important – activated carbon is a big capital cost, micronutrient addition is no capital, so an initial advantage. Also PAC [powdered activated carbon] increases sludge production, which is a cost, and GAC [granular activated carbon] needs regeneration then eventual replacement, another cost. Micronutrient addition made no difference to sludge production. We use it to treat emulsions. It's good for activated sludge, lagoons, biofilms...mostly we use activated sludge. Some lagoons for very low flows."

Table 10.4. The perceived potential of micronutrient addition for effluent toxicity reduction.

We stated: *Technical research shows micronutrient addition to be effective for toxicity reduction.* We then asked:

Have you heard of it?		**Have you considered it in the past?**		**Would you consider using it in the future?**		**Please give reasons.**	
Yes	No	Yes	No	Yes	No	Why?	Why not?
76.9	23.1	46.2	53.8	92.3	7.7	Low cost Performance Sustainability Adaptability Simplicity	Unfamiliarity Ongoing cost Unsuitability

Positive comments:

"It's sustainable. No capital cost, so no instant financial impact. We tend towards biological treatment anyway, as it's the most adaptable."

"Easy. It's cheaper and simpler than GAC. If it works, we use it."

"It's got small capital cost, though it's an ongoing cost. But still the cheapest option."

"We'd rather remove toxins at source, but if we have to treat them then micronutrient addition is one option we consider."

"We're very keen to optimise, especially with ammonia consents dropping. Ideally, we'd have our own plant on just our own waste, add things, have more control over what goes down the drain"

Negative comments:

"We usually add PAC to remove COD, which may or may not be toxic. We might do it if a contractor suggested it."

"No need for it in most sewage works. We might try it for more toxicity/COD removal, but it's more likely we'd try changing operating parameters, temperature, pH and so on."

"The plant works OK as it is. If the performance needed enhancing in the future, we'd look at micronutrient addition in more detail."

"Micronutrients are very expensive, but less cost than G/PAC and less handling issues, so some advantages. We'd get in a company that had tailored products, though, not do it ourselves, and these companies are very expensive."

Cost benefits, sustainability and performance featured strongly in the reasons to employ micronutrients. However, there were also some negative comments (Table 10.4) in which cost was often cited as a reason **not** to use micronutrients. Some interviewees focused on the low or non-existent capital expenditure involved in beginning micronutrient addition, while others were reluctant to commence an option that involves the ongoing purchase of chemicals. Most comments were conditional, balancing cost against the cost of other options available to meet the same need, the cost of not taking action at all, and considering micronutrients only in certain cases:

> "As long as it reduces toxicity and provides an advantage, that is, it is as good as activated carbon, or better, for less cost, then we use it."

> "We've never needed it for normal sewage. We could use it for odd wastewaters, like we add ammonium nitrate sometimes. This is the same principle. We don't use it if the wastewater contains enough [micronutrients]. A need exists if there is not enough – it's the obvious choice to optimise biological systems."

> "We often add N or P where wastewaters aren't balanced. On the same basis we'd add micronutrients. I prefer activated sludge, it's simple and cheap, no regeneration like GAC. We tend to tailor micronutrient addition to the waste streams, buy pre-made mixtures from companies and dose it directly. It works, but it's quite expensive. We don't use it as standard, though we might use it more when DTA comes in. But if the water board levy charges on toxicity we might just pay them instead – it depends on the cost so I don't know yet."

> "I think we would use it. We might. We'd rather adapt our own pre-treatment plant than keep on paying for discharges – I expect [the water company] will bring in charges to cover their own costs. Ideally we'd stop producing toxicity, then treat it ourselves, then if we have to we'll pay [the water company]."

> "DTA means new ideas, maybe we need new solutions. Sewage doesn't need micronutrient addition, maybe our trade wastes will. It will have to be cost effective to be a real option, but the fact that you just dose your existing plant is a big advantage. The cost of micronutrients may be too high, I'd need to look at it more to say if it's an option or not. Technically, it's an option. Economically, I don't know."

One interviewee stated that:

> "DTA is no different from tighter chemical limits. We just think it is because we haven't done it before."

indicating that much of the concern expressed during earlier research (Chapter 2) has been allayed, as nationwide consultation and discussion has taken place - the DTA workshop in Torquay, for an early example (Taylor 1996). This opinion, that DTA will have no more effect on treatment process improvement than increasing chemical quantity controls, was reiterated by one interviewee whose company is already subject to toxicity based discharge consents, and who uses micronutrient addition extensively. Their comments supported the use of micronutrient addition for toxicity reduction:

> "Micronutrient addition is a biological principle, applicable to lagoons, activated sludge and biofilms – all biological wastewater processes. The nature of the wastewater is the deciding factor on the level of success, not the type of biological process."

10.4 Conclusions

It is evident from the discussions about micronutrient addition to reduce toxicity in industrial effluent that many interviewees have carefully considered the practical and financial implications of adopting such a plan. Over 90% of the participants had made the informed decision to include micronutrient addition in their lists of options for adaptation to future effluent quality limits, some of whom had used micronutrient addition in the past and found it successful. None of the participants with previous experience of micronutrient addition had found it to be ineffective, although clearly cases exist where micronutrient addition is not required (i.e. 'normal sewage'). Negative views on the principle of micronutrient addition were clearly not held in this study, and analysis pointed to the possibility that some of the disadvantages of micronutrient addition are lessened as alternatives become less feasible; for example, if water companies were to levy charges on the toxicity of manufacturers' effluent, many manufacturers would then include micronutrient addition in their list of possible courses of action, in spite of the cost of micronutrient supplies. The research was not primarily

oriented to generating a formula for rendering micronutrient addition viable, but a number of factors were highlighted as discriminating between situations in which micronutrient addition was and was not considered practicable. Key factors included a strong requirement to remove toxicity from effluents, a cost implication of failure to do so and in many cases the need for further knowledge of the technique, from external R&D, from experience or through contractors or consultancies. Some participants saw micronutrient addition as an option only for new treatment facilities, where wastes could be characterised and segregated for tailored micronutrient addition.

Many participants saw themselves being vulnerable to the possible changes brought about by the introduction of DTA. However, with micronutrient addition among their options, companies with the opportunity to treat or pre-treat their own effluents perceived the risk to themselves as being reduced. They saw micronutrient addition as a route to successful adaptation to DTA, based on their own knowledge of their companies' processes and the trials performed with micronutrient addition in biological waste treatment that have been carried out externally. If micronutrient addition is made more evident and therefore available as an option, then regulation is implemented more easily. Under this premise, micronutrient addition may be of particular interest to regulators wishing to inform companies of not just the targets that are set, but how to attain them during the process of implementing new controls or standards. The implications for legislators are that they should assess the scope for adaptive response of operators before implementing change. Clearly, an opportunity to implement change is through the impositions of legislative restrictions. However, even when these changes are considered reasonable and fair, they can only be accommodated by attention to the ways in which operators can adapt their operations or move to a new 'position', or level of performance. Similarly, companies need to be alert to their vulnerability from processes that can have a limited capacity to be adapted or substituted. Both the regulator and the operator have a strategic responsibility to scan for future implementation and directions of legislation.

10.5 INTERIM SUMMARY AND CONCLUSIONS

Actions by the Environment Agency to advance effluent testing towards more ecological relevance have resulted in the development of innovative standards compared to those currently relied upon for environmental protection. Chapter 10 presents the method used to understand company responses for innovating to meet these new standards (successful treatment process development) and the scope for the contributions from the regulators. The implications for the commercial prospects of UK manufacturing and wastewater treatment are explored and discussed in the wider context of future legislative compliance. There is a need for UK industries to begin to adapt to DTA and this paper offers a technique for process adaptation and the scope for operator/legislator collaboration. Emergent key concepts were: (1) legislators should assess the scope for adaptation of operators before implementing change, (2) opportunities for change include new restrictions reinforced by evidence of methods for adaptation; i.e. if options are made obvious, regulation is implemented more easily, (3) companies should be alert to their vulnerability from processes that are not easily adapted, and (4) both the regulator and the operator have a strategic responsibility to scan for future implementation and directions of legislation.

Strong support for the use of micronutrient addition in enhanced biological treatment of industrial wastewaters was evident, in spite of the widespread knowledge that domestic wastes would not benefit. The interviews showed that the laboratory and pilot plant work on micronutrient addition has direct relevance to industrialists treating trade and industrial wastewaters at full-scale, especially as a means of reducing effluent toxicity in the future in order to meet any effluent toxicity standards that may be introduced. Demonstrating the potential value of macronutrient balancing and micronutrient supplements in enhancing the treatment of a chemical wastewater was shown to be useful work with relevance to UK wastewater-treating industries.

CHAPTER ELEVEN

GENERAL DISCUSSION AND CONCLUSIONS

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11.1 Discussion.

11.1.1 General discussion.

The activated sludge process is one of the oldest, best-established methods of wastewater treatment, and as such is very well understood. However, micronutrient addition to this process for enhanced industrial waste treatment is an area in which little published work is available, as the majority of chemical additions into activated sludge are metal co-precipitants, to improve sludge settleability or biological phosphorus removal (Clark and Stephenson, 1998). The activated sludge process has been optimised in a number of ways, mainly with respect to sludge handling and the removal of BOD_5 and SS from municipal wastewater (Eckenfelder and Musterman, 1994; Metcalf and Eddy, 1991), and so has probably reached its full potential in these areas. The imminent introduction of DTA to effluent standards monitoring represents a new direction in which the operation of activated sludge plants may be improved, and the potential of this versatile process to accommodate new priorities in effluent treatment and thus remain one of the most useful methods of effluent treatment in the world is enormous. The aim of the research was to investigate the potential of micronutrient addition for enhanced biological treatment of industrial wastewater, and determine whether such a technique could have a place in the future of the UK wastewater treating industry.

The objectives of the research (Chapter 3) can be summarised:

1. Establish whether operators expect to be able to accommodate DTA.
2. Screen micronutrients for their potential to enhance industrial wastewater treatment at laboratory-scale.
3. Investigate the most promising micronutrients at bench-scale.
4. Study the effects of the best micronutrient addition at pilot-scale.
5. Investigate whether micronutrient addition would be accepted by industry as a viable option for toxicity reduction.

The initial literature work investigated the potential of micronutrient addition in activated sludge wastewater treatment as a method for enhanced COD removal. The factors limiting the performance of activated sludge plants are often environmental, and many industrial processes can produce xenobiotic wastewaters, which resist biological treatment (Section 2.1). The availability of nutrients determines the species diversity and community structure of biological systems and hence the ability of that community to undertake cometabolism. In the case of xenobiotics, cometabolism can be the primary mechanism for biodegradation. Adding micronutrients to biological treatment processes is one possible approach to upgrading an existing facility in order to deal with increasing volumes and strengths of industrial wastewater and the tightening discharge legislation. As the requirements for, and toxicity of, different micronutrients vary according to the nature of the waste and the ecology of the system, such additions should be tailored to individual cases.

11.1.2 Can UK wastewater treatment accommodate DTA?

Questionnaires sent to a number of people in different industries that are involved in the aquatic environment showed that traditional measurements, such as the size of a company, or its membership to a particular industrial sector, do not determine its level of concern regarding financial and managerial costs with respect to future legal requirements (Chapter 4). The ability of a company to adapt to new environmental legislation appears to be based on the ability, perceived or real, of that company to alter three essential stages of their process, i.e. inputs, processes and outputs. The results led to a generic common model of "quick fix" options available to companies with the need to adapt to new quality control on their effluents, for example, wastewater minimisation (Wang and Smith, 1994; Hamilton and Dowson, 1994) or segregating different wastewater streams (Looney, 1996). However, different options are needed for long-term, sustainable compliance. The importance of long-term process adaptation as opposed to alteration of existing process operating parameters was recognised by the questionnaire respondents and the need to explore methods of bioprocess expansion and adaptation was established.

Actions by the EA to advance effluent testing towards more ecological relevance have resulted in the development of innovative standards compared to those currently relied upon for environmental protection. Chapter 10 presents the company responses for successfully developing treatment processes and the scope for the contributions from the regulators. There is a need for UK industries to begin to adapt to DTA and this paper offers a technique for process adaptation and the scope for operator/legislator collaboration.

Strong support for the use of micronutrient addition in enhanced biological treatment of industrial wastewaters was evident, in spite of the widespread knowledge that domestic wastes would not benefit. The interviews showed that the laboratory and pilot plant work on micronutrient addition has direct relevance to industrialists treating trade and industrial wastewaters at full-scale, especially as a means of reducing effluent toxicity in the future in order to meet any effluent toxicity standards that may be introduced. Demonstrating the potential value of macronutrient balancing and micronutrient supplements in enhancing the treatment of a chemical wastewater was shown to be useful work with relevance to UK wastewater-treating industries.

11.1.3 Scale-up of the lab-scale results.

Activated sludge process configurations are: substrate concentrations, temperature, pH, degree of dilution, DO concentration, presence of inhibitory substances, nutrient balancing and sludge age. Substrate degradation rates are affected by these 8 parameters, and since none of them is scale dependent, it is possible to determine biological kinetics at bench scale (Cooper and Boon, 1983). In addition, oxygen utilisation is not subject to scale-up effects and although lower results are obtained where samples are withdrawn from the porous pot to obtain data in closed vessels such as respirometer cells (Eckenfelder and Quirk, 1983), the data were obtained using the same materials and methods in the respirometry and porous pot trials and should therefore be subject to the same interferences. This suggests that the rates of oxygen uptake and COD removal per unit MLSS for specific micronutrient dosing regimes obtained by

respirometry (Chapters 5 and 6) would be observed in the porous pots (Chapters 7 and 8). However, this was not the case. The porous pot data did not match the respirometry data numerically, either as rates of uptake per unit MLSS or as percentages of the concurrent controls. If the micronutrients tested are ranked by oxygen uptake or COD removal, the lists for respirometry and porous pots are not the same. A set of t-tests was performed on the respirometer and porous pot results paired by dosing regime, which showed significant differences between the values obtained (Table 11.1). The results were then tested for correlation, independent of numerical value, and no correlation between the data sets was found (Table 11.1).

Table 11.1 Comparisons of lab-scale (respirometry) and bench-scale (porous pot) results.

Parameter	Correlation coefficients between porous pot and respirometer data	t-stat (t=1.09, df=11)
Substrate degradation rate (kg COD / kg MLSS/d)	0.1983	4.2918
Oxygen utilisation rate (kg O_2/ kg MLSS/d)	0.2231	9.2541
Substrate degradation rate as % of control	-0.1501	2.3356
Oxygen utilisation rate as % of control	0.4599	1.1760

Although activated sludge plants can be scaled up, provided hydrodynamic conditions remain the same (as biological coefficients are not affected by scale) (Cooper and Boon, 1983), and activated sludge systems involve the yield coefficient, which is independent of size or geometry of the reactor and depends only on the operating conditions and the nature of the substrate (Eckenfelder and Quirk, 1983), oxygen uptake rate may be affected by the level of turbulence in the reactor (Eckenfelder and Quirk, 1983). In addition, substrate uptake and oxygen uptake per unit solids per hour can be obtained with confidence from bench scale units provided continuous flow reactors are used (Jenkins *et al.*, 1983), and as the sludge used for respirometry was maintained in a batch-

fed reactor, the rates calculated from this work can not be expected to scale up to reactors under continuous-flow conditions.

In any case, scale-up of systems to remove priority pollutants is not so well understood (Eckenfelder and Quirk, 1983). Organic pollutants can be removed by air stripping, biodegradation or adsorption onto the surface of the flocs. Most organics will undergo partial if not complete biodegradation, but in many cases, long acclimation times are required to achieve maximum degradation rates. Off-gas analysis is needed to relate stripping and biodegradation and determine the fate of organic pollutants in industrial wastewater.

11.1.4 Interactions between micronutrients

Some negative effects of micronutrient dosing were observed during respirometry (Chapters 5 and 6), in which micronutrient additions resulted in decreased COD removal and oxygen uptake. In the case of single supplements, this can be attributed to the toxicity of excess doses. The extent of toxic effects of trace element ions is a function of the metal mass to biomass ratio and hence cannot be predicted from the metal concentration alone. Deficiencies of micronutrients may be alleviated using supplements of the required ionic species, but care must be taken to avoid excess doses, which can inhibit waste treatment (Wood and Tchobanoglous, 1975). Acclimation is also an important factor in metal toxicity: sludge communities evolve and micro-organisms adjust their metabolic pathways over time to tolerate most metal ions (Beyenal *et al.*, 1997; Hu *et al.*, 1996; Dilek and Yetis, 1992; Wiggins *et al.*, 1987; Chang *et al.*, 1986).

The effects of dosing two micronutrients simultaneously are strongly influenced by the interactions between the micronutrients. Complex chemical and biochemical interactions between metals (Geradi, 1986) and with organic compounds (Erk *et al.*, 1998) mean that the theoretical requirements for many micronutrients have not been established (Wood and Tchobanoglous, 1975). More than one metal in a solution can lead to interactions between ions; for example, calcium, potassium and sodium are known to interact with

other metals (Geradi, 1986). Interactions are almost impossible to predict because they are influenced by the operating conditions and strength and type of influent, the metal species and concentration, the species of micro-organisms present, the MCRT and even the order in which the metals are added (Beyenal *et al.*, 1997). In addition to these factors, many metals can be non-interactive, antagonistic, or synergistic (Ting *et al.*, 1991). Some metals are antagonistic even at non-toxic concentrations, as they compete for binding sites either within cells or on particles (Wood and Tchobanoglous, 1975). Many contradictory results concerning the biological effects of metal mixtures are reported (Beyenal *et al.*, 1997; Dilek *et al.*, 1991; Yetis and Gökçay, 1989; Chang *et al.*, 1986; McDermott *et al.*, 1963).

As calcium plays a significant role in membrane permeability, it is likely that it may act as an enhancer of the action of any other metal. Calcium has already been shown to interact with other metals (Geradi, 1986). It has also been concluded that the requirements for calcium and its effects vary greatly between bacteria and that the calcium concentration has a major influence on the toxic effects of other metals (Shuttleworth and Unz, 1988). The presence of calcium due to water hardness has been shown to accelerate acclimation via species selection in the sludge community (Hartz *et al.*, 1985; Vashon *et al.*, 1982). It is unlikely that water hardness affects the long-term performance of an activated sludge plant, but it can influence the speed with which biomass acclimates to new wastewater components.

In order to investigate the interactions between micro- and macronutrients, the results obtained dosing micronutrients with and without phosphorus should be compared at both lab- and bench-scales. However, as only pyridoxine and molybdenum were dosed into activated sludge with and without phosphorus at bench-scale, there are not enough data to make a meaningful comparison. A comparison can be made using only the respirometry data. A t-test is not appropriate, as the comparison is not looking for numerical similarity, so the data are arranged according to dosing regime and examined for correlation (Table 11.2). No correlations were found between the substrate degradation and oxygen utilisation rates per unit MLSS or as a percentage of the

controls, indicating that the combined effects of macro- and micronutrients are not simply additive at lab-scale.

Table 11.2 Comparisons of phosphorus-balanced and phosphorus-limited results.

Parameter	Correlation coefficient
Substrate degradation rate (kg COD / kg MLSS/d)	0.1691
Oxygen utilisation rate (kg O_2/ kg MLSS/d)	0.1901
Substrate degradation rate as % of control	0.1691
Oxygen utilisation rate as % of control	0.1737

11.1.5 Micronutrients in other applications.

Organic pollutants are degraded under both aerobic (Häggbloom, 1992; Commandeur and Parsons, 1990; Neilson, 1990; Reineke and Knackmuss, 1988) and anaerobic conditions (Evans and Fuchs, 1988; Tiedje *et al.*, 1987). More is known about micronutritional requirements in anaerobic systems than in activated sludge. Failure to provide trace elements to a two-phase anaerobic digester treating coffee waste results in a rapid decrease in the volatile fatty acids produced in the pre-acidification tank, a decrease in overall gas production and methane yield and an increase in volatile fatty acids present in the final effluent (McDougall *et al.*, 1993). Nitrogen-limited conditions in an upflow anaerobic sludge blanket system result in decreased VSS yield, COD removal and ammonia removal, and the structural degradation of sludge granules (Sam-Soon *et al.*, 1990).

Manganese, iron and cobalt are significant growth factors in a mesophilic upflow anaerobic sludge blanket reactor (Goodwin *et al.*, 1990). Other reported effects of trace metal additions include: increased gas production but decreased methane percentage (with no associated change in COD or ammonia removal) on addition of 7.5mg/l cobalt to a thermophilic anaerobic fixed film reactor, COD removal increased from 50% to 90%

on the addition of 50mg/l iron and 10mg/l cobalt to a mesophilic anaerobic filter treating volatile acid wastes and a 20% increase in gas production of a cattle dung reactor dosed with 15mg/l cobalt (Seif *et al.*, 1993). This indicates that cobalt is an important requirement in anaerobic biodegradation. However, iron deficiency causes reduced COD removal rates in an upflow anaerobic sludge blanket, while depletion of nickel and cobalt had no effect (Shen *et al.*, 1993), showing that results of trace metal deficiencies are inconsistent in anaerobic as well as in aerobic systems. Interactions between nutrients also exist under anaerobic conditions: both phosphorus and calcium improve granulation when added singly to an upflow anaerobic sludge blanket reactor, when added simultaneously granules of greater stability are produced (Goodwin *et al.*, 1990).

The micronutrient doses employed in anaerobic wastewater treatment are higher than in activated sludge systems, 7.5-50mg/l in the examples above compared with 0.1-1.5mg/l for most trace metals (Table 2.2). The reported improvements in process performance from the addition of trace metals to anaerobic systems are much greater than in this study (from 50% to 90% removal in anaerobic reactors (Seif *et al.*, 1993) compared with increased COD removal from 60% to 68% (Chapter 7) in an aerobic reactor). This may be due to overcaution in avoiding toxic excess doses, as the requirements of aerobic microbial consortia involved in wastewater treatment biomass are not well known (Curtis and Craine, 1998). The micro-organisms involved in anaerobic processes are better understood than aerobic mixed cultures. The most important anaerobic microbial groups have been characterised, and their nutritional requirements documented (Bergey, 1989). It may be that in order to achieve similar improvements in aerobic process performance as in anaerobic processes, the most important degrading bacteria should be identified, and their nutritional requirements determined at a biochemical level, as this type of detailed information on the properties of aerobic mixed cultures for wastewater treatment is lacking (Curtis and Craine, 1998).

This study found the vitamin niacin and the trace metals calcium and manganese to be the most important micronutrients for an aerobic mixed culture treating industrial wastewater. These conclusions may not be transferable to other types of wastewater,

biomass or environment. To estimate the transferability of the results, the bacteria involved in this particular case should be identified, and the extent to which they are found in other situations determined. This would allow us to assess the extent to which the results obtained here are applicable to other wastewaters and types of process.

11.2 Final Conclusions.

1. Current operating levels in activated sludge plants are not perceived to be sufficient for operators to accommodate DTA.

2. Respirometry screening can produce a comparative measure for predicting the potential of micronutrient additions, although the rates of respiration and substrate degradation can not be assumed to prevail at all scales of operation. The dosing regimes: phosphorus/biotin, phosphorus/pantothenic acid, phosphorus/manganese, phosphorus/calcium/manganese and phosphorus/ calcium/ niacin stimulated the biomass to remove COD. The addition of several mixed and single micronutrient doses to phosphorus-limited wastewater improved COD removal, but micronutrient supplements can not replace phosphorus balancing.

3. At bench-scale, improvements in the degradation of COD and removal of toxicity were observed while BOD_5 degradation was not affected, implying the increased use of recalcitrant substrate components as a food source when a balanced phosphorus and micronutrient supply is provided. The removal of recalcitrant COD and hence of priority pollutants and toxicity can be improved without the need for expanding existing wastewater treatment or pre-treatment plants.

4. Phosphorus/niacin dosing at pilot-scale showed that maintenance of biomass, COD, SS, phosphorus and ammonia removal can be improved. The increased COD removal is not associated with increases in BOD_5 removal and therefore demonstrates the improved degradation of recalcitrant compounds. This implies enhanced toxicity removal by the activated sludge.

5. Micronutrient addition is considered to be a viable option by industrialists for accommodating DTA legislation and compliance.

11.3 Recommendations for Further Work.

Demonstrating the potential value of macronutrient balancing and micronutrient supplements in enhancing the treatment of a chemical wastewater was shown to be useful work with relevance to UK wastewater-treating industries. However, the following issues have arisen from the work completed here:

The action of the micronutrients at the cell wall has not been studied, and issues surrounding the effects on wall permeability and the action on intracellular enzymes have not been addressed. Currently there is little information more detailed than "growth factor" on the action of many nutrients, and an understanding of the mechanisms of micronutrition is required before the technology can be used to its full potential. As a biological principle, it is possible that micronutrient addition could be used in other unit processes such as biological aerated filters, trickling filters and rotating biological contactors to increase the performance of wastewater treatment works without the need for physical expansion.

There is a need to identify the microbes and pollutants involved in this project to start to characterise wastes and describe the microbial consortia/nutrient combinations appropriate for the degradation of specific pollutants. Little is known about the variation in micronutrient requirements between micro-organisms. To assess the extent to which the technology can be transferred to other processes, the requirements of bacteria for specific nutrients should be determined. The technique of micronutrient supplementation appears intuitively to be transferable to many other applications, such as *in situ* degradation of organic pollutants (e.g. oil, nerve gas, and organochlorines including DDT). A potential end result in the future could be the maintenance of data on microbial species, nutrient mixtures, physical conditions and contact times required to deal with pollution incidents involving organic pollutants.

Some metal ions have been demonstrated to non-competitively inhibit bacterial metabolism (Gökçay and Yetis, 1996) and in these instances enzyme inhibition kinetics

can be applied to the metabolism of the cells. Michaelis-Menten kinetics can be adapted to describe the effects of substances on sewage micro-organisms and this has been demonstrated on activated sludge bacteria exposed to chromium (Lewandowski *et al.*, 1985). Zinc interacts with other metals (e.g., copper) to exacerbate their toxic effects and has been shown to reduce the rates of reactions so that the kinetics of biodegradation in the presence of zinc and copper resemble those of biodegradation without the metals but with a very short sludge age (Beyenal *et al.*, 1997). Other kinetics may apply in different instances of bacterial degradation of toxins (Allsop *et al.*, 1993). Microbial kinetics, where mixtures of metal ions and fully acclimated sludges are involved, do not fit the Monod model (Beyenal *et al.*,1997), but an alternative model has yet to be proposed. As the kinetics of microbial growth are used to design and operate biological unit processes, the development of a model applicable to processes treating wastewater with a significant metal content and simulating the effects of trace metal addition would be valuable.

CHAPTER TWELVE

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APPENDIX A

QUESTIONNAIRE RESULTS.

QUESTIONNAIRE RESULTS

1 What is the nature of your business?

Water/sewerage services	45%	Regulation	9%
Manufacturing	18%	Research	0%
Other	28%		

2 Number of employees

0 - 199	9%	200 - 500	0%
499 - 999	9%	1000-1999	64%
2000+	18%		

3 Your role within the company

Technical staff	27%	Technical management	27%
Strategic management	10%	Researcher	18%
Sales	18%	Other	0%

4 Your interest in Direct Toxicity Assessment (DTA)

Very interested	100%	Slightly interested	0%
Not at all	0%	Don't know	0%

5 Are you concerned about any financial cost to your company of implementing DTA?

Very concerned	37%	Slightly concerned	45%
Not at all	18%	Don't know	0%

If you are concerned, what is the basis for this?

45% Unnecessary extra tests	18% Accept DTA in principle
18% Inappropriate extra tests	10% Lack of control over process inputs
9% Unsure who/what DTA will apply to	

If not, why not?

Cost will be borne in the name of progress.

6 What requirements can you foresee, if any, for:
on-site monitoring capabilities?

45%	Some
36%	None, use testing houses
18%	None, rather use correlating lab tests
27%	Depends on test methods to be used
9%	Only for inlet and outlet monitoring

staff training/recruitment?

73%	Current staff will receive training in new methods
36%	Small amount of recruitment
18%	No recruitment

managerial issues?

9%	Capital outlay
9%	Operating costs
9%	Keeping up to date with technical developments
18%	Overcoming staff objections
36%	Depend on whether DTA becomes a legal tool

7 Would you consider the following to be issues within your company if DTA were to be introduced as:

	Yes	No	Comments
Misapplication of tests	64%	36%	National protocols may not be appropriate to local receiving waters. Fish tests under consideration are not appropriate.
Misinterpretation of tests data	73%	27%	Concerns about technical difficulties. Concern that mixing zones may not be interpreted correctly. National protocols may not be appropriate to specific samples.
Lack of training in personnel	45%	55%	As with many EA issues. Keeping up to date will also be an issue.

8 Are technical concerns regarding the following shared by your company?

	Yes	No
Level of variation in test procedure		
Use of alternatives to *Daphnia* e.g. Amtox™	73%	27%
Use of ecotoxicology data in legal matters		

9 Are current controls adequate for control of discharge consents?

Yes 9% No 73% Depends on case 18%

Do you believe DTA could be used as a tool for pass/fail compliance?

Yes 18 % No 64% Depends on case 18%

Would the introduction of DTA as a trigger for action enhance current environmental practice?

Yes 100% No 0% Depends on case 0%

Do you think that DTA should supersede current tests used to determine discharge consent compliance?

Yes 27% No 55% Depends on case 18%

Would DTA be a worthwhile addition to the range of effluent tests used at present?

Yes 91% No 9% Depends on case 0%

Name_____

Would you like to be sent a copy of the initial results ☐ and/or a copy of the pre-publication paper ☐. Would you be willing to allow a conversation to discuss this topic further? If so, please can you provide a telephone number on which I can contact you to arrange a convenient time and date.

Many thanks for your help.

APPENDIX B

TREATABILITY TEST RESULTS: TRACE ELEMENTS.

TREATABILITY TEST RESULTS: TRACE ELEMENTS.

Table B.1 MLSS (mg/l).

Time (days) (days)	Control (no supplements) 1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	3790	2250	1940	2510	1930	2010	2130	1570	2160	1930
-24	2130	2370	2250	2430	2060	2180	2340	1660	2280	2010
-22	2245	2240	2190	2245	2070	2180	2250	1490	2220	2540
-20	2360	2110	2130	2060	2090	2180	2140	1490	2220	2540
-15	2020	1400	2100	1980	1840	1760	1910	1680	2290	2200
-13	1750	2050	2120	1710	2090	2140	2250	1850	2430	2235
-8	990	1450	2120	1500	2200	2030	2310	2020	2570	2270
-6	1730	2350	1550	2060	1860	2000	2150	1870	2250	2040
-1	1505	2180	1985	2080	1810	1880	2150	1720	1930	1810
0	1280	2010	2420	2100	1770	1770	2150	1510	1740	1700
4	2120	2790	2070	1990	1650	1690	1790	1300	1550	1590
8	1870	2130	2150	2230	1390	1440	1550	1265	1590	1475
11	2305	2245	2140	2120	1110	1460	1610	1230	1630	1360
13	2740	2360	2130	2010	1090	1380	1480	1400	1810	1935
18	1720	2020	2200	1950	1050	1300	1350	1570	1990	2510
20	1420	1750	1850	1680	970	1340	1430	1410	2075	2745
22	1575	1810	1920	1795	960	1220	1250	1250	2160	2980
25	1730	1870	1990	1910	960	1110	1070	1175	1805	2000
27	1765	1990	2115	2045	720	1040	1030	1100	1450	1020
29	1800	2110	2240	2180	840	960	980	1150	1595	1565
32	1800	1940	2860	2690	910	1160	1050	1200	1740	2110
34	1765	1995	2430	2395	900	1200	1070	1210	1730	1685
36	1730	2050	2000	2100	840	1230	1090	1220	1720	1260
39	1695	2235	2055	2140	820	1250	1140	1200	1745	1235
41	1660	2420	2110	2180	840	1270	1190	1180	1770	1210
43	1680	2260	2085	2170	860	1250	1200	1325	1790	1230
46	1700	2100	2060	2160	900	1230	1210	1470	1810	1250
Max	*2740.00*	*2790.00*	*2860.00*	*2690.00*	*1770.00*	*1770.00*	*2150.00*	*1570.00*	*2160.00*	*2980.00*
Mean	*1797.50*	*2115.83*	*2156.94*	*2102.50*	*1032.22*	*1294.44*	*1313.33*	*1286.94*	*1761.11*	*1714.44*
Min	*1420.00*	*1750.00*	*1850.00*	*1680.00*	*720.00*	*960.00*	*980.00*	*1100.00*	*1450.00*	*1020.00*
SD	*324.03*	*246.46*	*228.45*	*220.49*	*288.09*	*202.43*	*308.30*	*133.11*	*177.71*	*565.09*

SD = standard deviation

Table B.2 MLSS as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	59.4	51.2	66.2	104.1	110.4	137.6	122.9
-24	100	111.3	105.6	114.1	105.8	113.6	137.3	121.1
-22	100	99.8	97.6	100.0	105.3	108.7	149.0	170.5
-20	100	89.4	90.3	87.3	104.3	102.4	149.0	170.5
-15	100	69.3	104.0	98.0	95.7	103.8	136.3	131.0
-13	100	117.1	121.1	97.7	102.4	107.7	131.4	120.8
-8	100	146.5	214.1	151.5	92.3	105.0	127.2	112.4
-6	100	135.8	89.6	119.1	107.5	115.6	120.3	109.1
-1	100	144.9	131.9	138.2	103.9	118.8	112.2	105.2
0	100	157.0	189.1	164.1	100.0	121.5	115.2	112.6
4	100	131.6	97.6	93.9	102.4	108.5	119.2	122.3
8	100	113.9	115.0	119.3	103.6	111.5	125.7	116.6
11	100	97.4	92.8	92.0	131.5	145.0	132.5	110.6
13	100	86.1	77.7	73.4	126.6	135.8	129.3	138.2
18	100	117.4	127.9	113.4	123.8	128.6	126.8	159.9
20	100	123.2	130.3	118.3	138.1	147.4	147.2	194.7
22	100	114.9	121.9	114.0	127.1	130.2	172.8	238.4
25	100	108.1	115.0	110.4	115.6	111.5	153.6	170.2
27	100	112.7	119.8	115.9	144.4	143.1	131.8	92.7
29	100	117.2	124.4	121.1	114.3	116.7	138.7	136.1
32	100	107.8	158.9	149.4	127.5	115.4	145.0	175.8
34	100	113.0	137.7	135.7	133.3	118.9	143.0	139.3
36	100	118.5	115.6	121.4	146.4	129.8	141.0	103.3
39	100	131.9	121.2	126.3	152.4	139.0	145.4	102.9
41	100	145.8	127.1	131.3	151.2	141.7	150.0	102.5
43	100	134.5	124.1	129.2	145.3	139.5	135.1	92.8
46	100	123.5	121.2	127.1	136.7	134.4	123.1	85.0
Max	*100.00*	*157.03*	*189.06*	*164.06*	*152.44*	*147.42*	*172.80*	*238.40*
Mean	*100.00*	*119.71*	*123.19*	*119.77*	*128.91*	*128.80*	*137.52*	*133.00*
Min	*100.00*	*86.13*	*77.74*	*73.36*	*102.42*	*108.48*	*119.23*	*85.03*
SD	*0.00*	*16.58*	*23.97*	*20.57*	*16.53*	*12.77*	*14.02*	*40.94*

Table B.3 COD removal rates (kg COD/kg MLSS/d).

Time (days)	Control (no supplements)1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	2.4	4.1	4.7	3.7	1.0	1.0	1.0	2.2	1.5	1.7
-24	4.5	4.0	4.2	3.9	1.8	1.4	1.2	2.1	1.3	1.4
-22	3.5	3.5	3.6	3.5	1.1	0.8	0.6	2.1	1.3	1.0
-20	2.2	2.5	2.5	2.5	1.4	1.2	0.9	2.4	1.5	1.3
-15	1.8	2.7	1.8	1.9	1.4	1.5	1.4	1.8	1.3	1.2
-13	2.4	2.1	2.0	2.5	1.9	1.8	1.7	1.6	1.3	1.5
-8	4.8	3.2	2.2	3.2	1.8	1.9	1.7	1.9	1.3	1.5
-6	3.6	2.6	3.9	3.0	1.6	1.7	1.5	1.9	1.6	1.8
-1	5.0	3.5	3.8	3.6	1.6	1.6	1.4	1.8	1.5	1.6
0	3.8	2.4	2.0	2.3	1.6	1.6	1.3	2.3	1.9	2.2
4	2.7	2.1	2.8	2.9	2.6	2.7	2.5	6.0	4.6	4.8
8	3.2	2.8	2.7	3.0	3.3	3.1	3.0	6.1	4.5	5.4
11	2.7	3.2	3.0	3.4	3.1	2.2	2.2	5.5	4.7	5.8
13	2.2	2.9	3.1	3.3	3.4	2.4	2.5	5.6	4.2	4.2
18	3.6	3.5	3.0	3.4	3.9	3.0	3.0	4.6	3.9	3.3
20	4.4	4.0	3.5	4.0	3.9	2.6	2.6	5.2	3.5	3.1
22	4.0	3.8	3.4	3.9	7.9	6.0	6.0	5.9	3.5	2.8
25	3.6	3.8	3.2	3.8	4.1	3.3	3.5	6.2	4.1	3.9
27	3.5	3.7	3.1	3.7	5.6	3.5	3.7	6.2	5.3	7.7
29	3.5	3.6	3.0	3.5	4.9	3.8	4.0	6.4	4.8	5.1
32	3.6	4.1	2.5	2.9	4.3	3.2	3.8	6.5	4.4	3.9
34	3.6	3.9	2.9	3.2	4.4	3.3	3.8	6.2	4.4	4.9
36	3.6	3.7	3.5	3.5	4.6	3.3	3.7	5.9	4.5	6.6
39	3.6	3.3	3.3	3.4	4.7	3.3	3.6	6.1	4.3	6.8
41	3.5	3.0	3.1	3.2	4.5	3.2	3.4	6.2	4.2	7.0
43	3.5	3.2	3.2	3.3	4.4	3.2	3.3	5.6	4.2	6.8
46	3.5	3.5	3.2	3.3	4.3	3.4	3.3	5.0	4.1	6.6
Max	*4.39*	*4.11*	*3.54*	*4.04*	*7.91*	*6.01*	*5.98*	*6.53*	*5.31*	*7.68*
Mean	*3.45*	*3.36*	*3.01*	*3.33*	*4.19*	*3.17*	*3.29*	*5.64*	*4.18*	*5.04*
Min	*2.19*	*2.06*	*1.98*	*2.31*	*1.62*	*1.59*	*1.33*	*2.30*	*1.92*	*2.22*
SD	*0.50*	*0.55*	*0.37*	*0.40*	*1.30*	*0.89*	*0.96*	*0.97*	*0.71*	*1.64*

Table B.4 COD removal rates as a percentage of the concurrent control.

Time (days)	Control (no supplements)	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	169.0	194.1	150.8	94.0	94.7	66.8	75.8
-24	100	89.3	93.5	87.1	76.2	67.1	59.3	64.2
-22	100	99.5	101.7	99.5	74.4	56.1	60.2	49.4
-20	100	113.2	113.4	115.9	86.6	66.8	64.0	55.4
-15	100	149.0	99.3	104.2	102.9	100.1	71.0	65.4
-13	100	85.4	83.1	102.3	93.9	91.8	82.4	95.4
-8	100	66.6	46.1	65.2	104.9	95.4	66.8	80.9
-6	100	73.3	111.1	83.6	101.0	92.6	84.8	93.2
-1	100	69.6	75.8	72.5	99.7	87.1	82.9	87.1
0	100	63.7	51.6	60.2	98.1	82.0	83.5	96.2
4	100	75.2	100.3	106.5	100.6	93.7	77.4	80.8
8	100	87.8	84.4	92.2	93.5	90.8	74.0	87.6
11	100	119.6	111.9	127.7	70.5	69.4	85.6	104.4
13	100	133.5	141.5	151.3	71.2	73.4	75.1	75.6
18	100	98.4	83.1	94.2	78.3	76.6	84.3	71.6
20	100	90.9	80.4	91.8	66.0	66.1	68.4	59.2
22	100	94.5	84.4	97.0	76.0	75.5	58.2	47.0
25	100	105.6	90.8	106.0	82.2	86.1	66.8	63.7
27	100	106.1	88.4	106.6	63.2	66.1	86.1	124.5
29	100	103.6	86.1	100.7	76.9	80.0	75.5	80.5
32	100	114.3	68.2	80.5	75.1	88.8	67.0	59.4
34	100	108.1	79.8	87.6	74.7	87.1	71.2	78.5
36	100	102.2	96.5	96.6	70.7	80.6	75.8	110.9
39	100	92.4	91.9	94.2	69.0	76.3	71.4	111.6
41	100	84.2	87.2	91.8	70.8	76.0	67.1	112.5
43	100	90.9	89.6	93.1	73.7	76.1	75.0	122.6
46	100	98.8	91.6	94.1	78.2	77.7	81.7	131.8
Max	*100*	*133.52*	*141.50*	*151.31*	*100.58*	*93.70*	*86.06*	*131.80*
Mean	*100*	*98.32*	*89.31*	*98.46*	*77.15*	*79.03*	*74.67*	*89.93*
Min	*100*	*63.68*	*51.60*	*60.21*	*63.22*	*66.11*	*58.25*	*47.01*
SD	*0*	*16.09*	*18.08*	*18.73*	*10.45*	*7.97*	*7.64*	*25.36*

Table B.5 COD removal efficiencies (%).

Time (days)	Control (no supplements)1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	81.5	81.7	81.0	81.4	43.5	42.5	45.4	54.1	49.8	50.4
-24	82.8	82.3	81.8	82.3	50.8	41.0	38.8	54.9	44.7	42.6
-22	79.5	78.9	78.9	79.1	43.2	33.8	26.4	51.9	46.6	43.7
-20	70.4	71.2	72.0	71.2	54.0	48.8	36.9	54.6	52.1	51.5
-15	59.8	61.8	61.8	61.1	52.8	52.0	54.9	50.4	48.7	43.1
-13	65.0	65.0	65.4	65.0	65.0	62.5	64.2	50.0	54.1	57.6
-8	69.6	67.8	68.7	68.7	67.0	64.8	67.1	59.7	50.7	54.3
-6	74.0	73.6	73.6	73.6	55.1	59.9	59.0	56.1	57.2	57.0
-1	77.2	77.8	77.2	77.3	58.9	60.9	60.9	51.1	47.5	46.8
0	51.9	51.9	50.6	51.3	63.0	61.8	62.7	56.3	54.2	61.0
4	59.1	58.5	57.9	59.1	67.9	70.0	69.0	63.7	58.8	63.0
8	59.9	59.9	58.1	65.9	67.5	65.4	68.4	64.2	59.7	65.6
11	59.5	69.4	61.8	69.9	60.2	55.9	60.7	56.7	64.3	65.5
13	58.5	67.3	64.3	64.9	60.6	54.7	60.4	65.1	63.2	68.0
18	60.2	69.6	64.0	64.3	63.2	61.3	62.3	60.7	64.9	69.5
20	60.8	68.1	63.7	66.1	59.5	54.3	58.0	61.3	61.8	70.8
22	61.3	66.6	63.0	67.7	74.5	71.9	73.2	61.7	62.1	69.1
25	60.7	69.2	63.3	71.0	59.6	56.7	57.2	60.4	62.0	65.5
27	60.4	72.2	63.9	74.6	60.8	55.6	57.6	56.7	64.3	65.5
29	60.7	73.7	65.0	74.0	62.2	54.7	58.1	60.9	63.8	66.8
32	61.0	75.1	66.1	73.4	60.7	58.1	62.2	65.1	63.2	68.0
34	60.2	73.6	66.2	71.6	60.0	59.8	62.1	62.9	64.1	68.8
36	59.4	72.0	66.3	69.7	59.4	61.5	62.1	60.7	64.9	69.5
39	58.4	71.2	65.1	69.5	58.6	61.7	62.1	61.1	63.4	70.2
41	57.4	70.4	63.6	69.2	57.2	61.2	61.6	61.3	61.8	70.8
43	58.1	71.1	64.6	69.9	57.2	61.3	60.8	61.5	62.3	70.0
46	58.8	71.8	65.3	70.3	58.1	62.1	60.7	61.7	62.1	69.1
Max	*61.29*	*75.14*	*66.29*	*74.56*	*74.47*	*71.88*	*73.24*	*65.10*	*64.87*	*70.76*
Mean	*59.25*	*68.42*	*62.95*	*67.92*	*61.69*	*60.47*	*62.19*	*61.23*	*62.26*	*67.59*
Min	*51.90*	*51.90*	*50.63*	*51.27*	*57.22*	*54.25*	*57.21*	*56.31*	*54.17*	*60.97*
SD	*2.14*	*5.98*	*3.88*	*5.63*	*4.37*	*5.00*	*4.19*	*2.60*	*2.60*	*2.76*

Table B.6 COD removal efficiencies as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	100.3	99.4	99.9	97.9	104.5	91.9	93.2
-24	100	99.4	98.7	99.4	80.7	76.2	81.5	77.7
-22	100	99.2	99.2	99.5	78.3	61.0	89.7	84.2
-20	100	101.2	102.3	101.2	90.3	68.4	95.4	94.4
-15	100	103.3	103.3	102.1	98.4	103.9	96.8	85.6
-13	100	100.0	100.7	100.0	96.2	98.8	108.2	115.2
-8	100	97.5	98.8	98.8	96.8	100.2	85.0	90.9
-6	100	99.5	99.5	99.5	108.6	107.1	102.0	101.7
-1	100	100.8	100.0	100.2	103.5	103.4	93.0	91.6
0	100	100.0	97.6	98.8	98.1	99.6	96.2	108.3
4	100	99.0	97.9	100.0	103.0	101.6	92.2	98.8
8	100	100.0	97.0	110.0	96.9	101.3	93.0	102.2
11	100	116.5	103.9	117.5	92.8	100.7	113.4	115.5
13	100	115.0	110.0	111.0	90.2	99.7	97.1	104.5
18	100	115.5	106.3	106.8	96.9	98.5	106.9	114.5
20	100	112.0	104.8	108.7	91.1	97.5	100.7	115.3
22	100	108.6	102.9	110.5	96.5	98.3	100.6	112.1
25	100	114.1	104.4	117.1	95.1	96.0	102.6	108.4
27	100	119.6	105.9	123.5	91.3	94.6	113.4	115.5
29	100	121.4	107.1	121.9	87.9	93.4	104.7	109.6
32	100	123.1	108.3	120.4	95.7	102.4	97.1	104.5
34	100	122.2	109.9	118.9	99.6	103.5	101.8	109.3
36	100	121.2	111.5	117.3	103.5	104.6	106.9	114.5
39	100	121.9	111.4	118.9	105.2	106.0	103.8	114.9
41	100	122.7	110.8	120.6	107.0	107.7	100.7	115.3
43	100	122.3	111.2	120.3	107.1	106.2	101.3	113.8
46	100	122.0	111.0	119.5	106.8	104.5	100.6	112.1
Max	*100*	*123.15*	*111.54*	*123.53*	*107.05*	*107.67*	*113.44*	*115.47*
Mean	*100*	*115.40*	*106.22*	*114.53*	*98.04*	*100.90*	*101.84*	*110.51*
Min	*100*	*98.97*	*97.00*	*98.78*	*87.86*	*93.35*	*92.25*	*98.84*
SD	*0*	*8.35*	*4.90*	*7.39*	*6.20*	*4.09*	*5.87*	*5.16*

Table B.7 BOD_5 removal rates (kg BOD_5/kg MLSS/d).

Time (days)	Control (no supplements)1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	0.53	0.87	1.03	0.78	0.90	0.85	0.80	1.08	0.77	0.86
-24	1.12	0.99	1.04	0.96	1.07	1.00	0.92	1.07	0.77	0.87
-22	0.82	0.83	0.87	0.84	0.96	0.92	0.86	1.34	0.88	0.78
-20	0.76	0.87	0.88	0.90	0.76	0.76	0.73	1.48	0.97	0.85
-15	0.36	0.51	0.35	0.34	0.93	0.95	0.88	1.62	1.20	1.24
-13	0.51	0.43	0.41	0.51	0.77	0.76	0.71	0.90	0.68	0.74
-8	0.82	0.55	0.39	0.56	0.72	0.76	0.65	0.92	0.72	0.81
-6	0.47	0.35	0.54	0.42	0.88	0.79	0.74	1.12	0.93	1.02
-1	0.55	0.37	0.42	0.40	0.93	0.86	0.77	1.09	0.97	1.03
0	0.44	0.27	0.23	0.27	0.85	0.95	0.77	1.41	1.22	1.25
4	1.26	0.96	1.29	1.34	0.62	0.67	0.60	2.50	1.86	1.97
8	1.34	1.17	1.16	1.12	0.80	0.83	0.75	2.22	1.74	1.88
11	1.13	1.16	1.22	1.23	1.08	0.86	0.76	2.63	2.01	2.49
13	0.37	0.43	0.47	0.51	1.05	0.90	0.81	2.11	1.67	1.55
18	0.70	0.63	0.55	0.58	1.06	0.97	0.89	1.69	1.30	1.06
20	0.76	0.64	0.58	0.59	1.55	1.20	1.09	1.78	1.21	0.92
22	1.07	0.94	0.88	0.88	1.97	1.60	1.54	2.09	1.21	0.89
25	1.32	1.22	1.15	1.13	1.31	1.27	1.26	2.25	1.46	1.33
27	1.39	1.18	1.10	1.12	1.73	1.37	1.30	2.56	1.93	2.78
29	0.95	0.57	0.47	0.54	1.46	1.50	1.35	2.42	1.74	1.80
32	1.53	1.32	0.89	0.96	1.29	1.25	1.27	2.11	1.46	1.21
34	1.71	1.46	1.21	1.23	1.23	1.23	1.26	2.06	1.43	1.49
36	1.55	1.27	1.31	1.24	1.43	1.20	1.27	2.23	1.57	2.17
39	1.40	1.04	1.13	1.07	1.46	1.06	1.05	2.21	1.52	2.17
41	1.31	0.90	1.01	0.99	1.32	1.13	1.08	2.41	1.60	2.37
43	1.18	0.90	0.94	0.93	1.26	1.10	1.03	2.04	1.51	2.21
46	1.28	1.03	1.04	1.00	1.12	1.18	1.10	1.90	1.54	2.26
Max	*1.71*	*1.46*	*1.31*	*1.34*	*1.97*	*1.60*	*1.54*	*2.63*	*2.01*	*2.78*
Mean	*1.15*	*0.95*	*0.92*	*0.93*	*1.25*	*1.13*	*1.07*	*2.15*	*1.55*	*1.77*
Min	*0.37*	*0.27*	*0.23*	*0.27*	*0.62*	*0.67*	*0.60*	*1.41*	*1.21*	*0.89*
SD	*0.37*	*0.33*	*0.32*	*0.31*	*0.33*	*0.24*	*0.26*	*0.31*	*0.24*	*0.58*

Table B.8 BOD_5 removal rates as a percentage of the concurrent control.

Time (days)	Control (no supplements)	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	165.40	195.36	148.27	94.04	88.74	71.40	79.34
-24	100	88.52	92.76	86.11	92.78	85.64	71.82	81.47
-22	100	101.53	106.87	102.61	96.10	89.78	65.50	58.66
-20	100	114.46	115.60	118.38	99.85	96.19	65.93	57.30
-15	100	143.09	97.79	94.40	102.70	95.32	74.17	76.64
-13	100	85.94	80.31	101.65	99.12	92.89	74.76	81.44
-8	100	66.77	47.04	67.46	105.91	90.55	78.05	88.22
-6	100	75.81	114.93	89.60	89.93	83.66	83.07	91.32
-1	100	68.03	76.37	72.88	92.52	82.69	88.58	94.32
0	100	62.32	53.46	61.60	112.00	90.56	86.29	88.19
4	100	75.99	102.42	106.53	109.12	97.60	74.57	79.04
8	100	87.58	86.98	83.86	104.35	93.31	78.37	84.85
11	100	103.15	107.96	109.23	79.83	70.67	76.30	94.46
13	100	116.10	128.64	137.94	86.26	77.52	79.23	73.23
18	100	89.36	78.18	82.97	91.68	84.08	76.58	62.37
20	100	83.85	76.76	77.01	77.46	70.55	67.75	51.73
22	100	87.95	82.32	82.10	81.19	78.02	57.90	42.42
25	100	92.51	87.39	85.81	96.78	96.13	64.96	59.25
27	100	85.01	79.17	80.40	79.24	74.96	75.39	108.53
29	100	59.87	49.34	56.49	102.44	91.99	71.86	74.27
32	100	86.70	58.29	62.71	97.56	98.89	68.97	57.28
34	100	85.39	70.83	71.86	99.32	102.30	69.60	72.28
36	100	81.56	84.08	79.62	83.66	88.62	70.62	97.49
39	100	73.92	80.40	76.41	72.16	71.93	68.52	97.88
41	100	68.41	77.16	75.52	85.81	82.03	66.26	97.95
43	100	76.14	79.60	79.30	87.91	81.62	73.75	108.35
46	100	80.73	80.93	78.05	105.93	98.80	81.22	119.02
Max	*100.00*	*165.40*	*195.36*	*148.27*	*112.00*	*102.30*	*88.58*	*119.02*
Mean	*100.00*	*89.11*	*88.55*	*87.73*	*93.54*	*87.22*	*73.39*	*80.64*
Min	*100.00*	*59.87*	*47.04*	*56.49*	*72.16*	*70.55*	*57.90*	*42.42*
SD	*0.00*	*13.42*	*18.73*	*19.03*	*12.00*	*10.62*	*6.70*	*21.63*

Table B.9 BOD_5 removal efficiencies (%).

Time (days)	Control (no supplements)1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	92.20	90.53	92.20	90.53	86.84	85.05	85.05	95.90	94.21	93.53
-24	94.52	93.10	92.62	92.86	89.84	88.21	87.40	95.45	94.16	94.16
-22	88.62	89.78	92.38	90.94	86.21	87.25	84.13	86.21	84.13	86.21
-20	88.16	90.21	91.98	91.10	81.51	84.90	80.28	95.30	93.61	93.09
-15	78.96	78.31	80.28	73.07	83.50	82.03	82.62	96.72	97.79	97.08
-13	78.62	79.16	76.49	78.09	83.72	84.97	83.72	96.03	94.31	94.48
-8	87.63	85.70	88.27	89.56	85.11	83.17	80.92	96.48	95.81	95.65
-6	73.62	75.81	75.81	78.54	85.27	82.46	82.46	96.82	96.78	96.45
-1	74.01	72.93	74.55	74.55	85.43	82.10	83.91	96.87	96.29	96.15
0	63.39	62.03	64.07	64.07	42.37	47.45	46.60	96.59	96.04	95.90
4	82.38	82.38	82.38	82.38	33.33	37.25	35.29	96.57	85.86	93.36
8	81.75	81.55	81.75	81.75	35.92	38.83	37.38	91.75	90.37	90.77
11	81.70	82.08	81.89	82.08	38.46	40.38	39.42	93.10	94.14	97.24
13	64.59	64.59	64.59	65.36	37.25	40.68	39.21	89.62	91.80	90.71
18	74.79	78.49	74.79	70.34	36.27	41.17	39.21	92.76	90.03	92.49
20	74.84	77.34	74.84	68.19	48.06	51.42	49.98	91.59	91.31	92.23
22	76.64	77.46	76.91	71.71	59.40	61.29	60.34	92.07	92.11	93.11
25	77.52	77.52	77.93	73.44	40.38	45.18	43.26	91.76	91.57	92.55
27	79.40	76.10	75.33	73.97	39.52	45.23	42.38	92.65	92.07	93.23
29	52.78	37.04	32.41	36.11	38.68	45.28	41.51	92.77	92.46	93.77
32	82.42	77.01	76.34	77.24	37.14	46.19	42.38	91.72	91.72	92.37
34	88.13	85.06	85.93	85.93	35.57	47.11	43.26	91.61	91.16	92.21
36	91.29	88.23	88.74	88.23	39.21	48.03	45.09	92.08	91.68	92.71
39	96.54	94.10	94.10	93.13	39.99	43.99	39.99	91.88	91.55	92.55
41	94.25	93.99	92.43	93.47	35.92	46.59	41.74	93.08	92.51	93.49
43	91.64	93.86	90.53	93.86	34.60	44.21	39.41	91.99	91.64	92.52
46	94.25	93.99	92.43	93.47	31.87	46.14	42.34	91.90	91.90	93.00
Max	*96.54*	*94.10*	*94.10*	*93.86*	*59.40*	*61.29*	*60.34*	*96.59*	*96.04*	*97.24*
Mean	*80.46*	*79.05*	*78.19*	*77.48*	*39.11*	*45.36*	*42.71*	*92.53*	*91.66*	*93.01*
Min	*52.78*	*37.04*	*32.41*	*36.11*	*31.87*	*37.25*	*35.29*	*89.62*	*85.86*	*90.71*
SD	*11.75*	*13.98*	*14.44*	*14.37*	*6.23*	*5.33*	*5.56*	*1.67*	*1.96*	*1.54*

Table B.10 BOD_5 removal efficiencies as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	98.19	100.00	98.19	94.19	92.25	92.25	104.02
-24	100	98.49	97.98	98.24	95.04	93.32	92.46	100.98
-22	100	101.31	104.25	102.61	97.28	98.46	94.94	97.28
-20	100	102.33	104.33	103.33	92.46	96.30	91.07	108.10
-15	100	99.17	101.66	92.53	105.75	103.89	104.63	122.49
-13	100	100.68	97.29	99.32	106.49	108.07	106.49	122.14
-8	100	97.79	100.74	102.21	97.12	94.91	92.34	110.11
-6	100	102.97	102.97	106.69	115.83	112.01	112.01	131.52
-1	100	98.54	100.73	100.73	115.42	110.92	113.38	130.88
0	100	97.86	101.07	101.07	66.83	74.85	73.52	152.37
4	100	100.00	100.00	100.00	40.46	45.22	42.84	117.23
8	100	99.76	100.00	100.00	43.94	47.50	45.72	112.23
11	100	100.46	100.23	100.46	47.08	49.43	48.25	113.96
13	100	100.00	100.00	101.19	57.67	62.98	60.70	138.75
18	100	104.95	100.00	94.06	48.49	55.05	52.43	124.03
20	100	103.33	100.00	91.11	64.21	68.71	66.78	122.37
22	100	101.07	100.36	93.57	77.51	79.97	78.74	120.13
25	100	100.00	100.53	94.74	52.09	58.29	55.81	118.37
27	100	95.84	94.87	93.15	49.77	56.97	53.37	116.68
29	100	70.18	61.40	68.42	73.29	85.80	78.65	175.78
32	100	93.44	92.62	93.72	45.06	56.04	51.42	111.29
34	100	96.52	97.51	97.51	40.36	53.45	49.09	103.95
36	100	96.65	97.21	96.65	42.95	52.61	49.39	100.87
39	100	97.47	97.47	96.46	41.43	45.57	41.43	95.17
41	100	99.72	98.07	99.17	38.11	49.44	44.29	98.76
43	100	102.42	98.79	102.42	37.76	48.25	43.00	100.38
46	100	99.72	98.07	99.17	33.82	48.96	44.92	97.50
Max	*100.00*	*104.95*	*104.33*	*106.69*	*115.83*	*112.01*	*113.38*	*175.78*
Mean	*100.00*	*98.48*	*98.08*	*97.29*	*67.42*	*72.19*	*69.63*	*116.57*
Min	*100.00*	*70.18*	*61.40*	*68.42*	*33.82*	*45.22*	*41.43*	*95.17*
SD	*0.00*	*7.42*	*9.04*	*7.58*	*12.79*	*12.11*	*12.24*	*20.61*

Table B.11 Oxygen uptake rates (kg O_2/kg MLSS/d).

Time (days)	Control (no supplements)1	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Control (no supplements)2	Phosphorus & molybdenum	Molybdenum	Control (no supplements)3	Molybdenum & lactoflavin	Manganese & aluminium
-27	0.1396	0.2378	0.2800	0.1948	0.2270	0.2250	0.2127	0.3048	0.3156	0.1970
-24	0.2571	0.2338	0.1981	0.2309	0.2132	0.2077	0.1939	0.2773	0.2885	0.1868
-22	0.2523	0.2559	0.1590	0.2820	0.2137	0.2092	0.2030	0.1817	0.1794	0.0039
-20	0.2524	0.2462	0.1781	0.3539	0.2132	0.2106	0.2149	0.3035	0.2924	0.1677
-15	0.3094	0.3326	0.1954	0.4166	0.2277	0.2459	0.2270	0.2572	0.2719	0.1897
-13	0.2775	0.1529	0.1753	0.3515	0.2054	0.2070	0.1972	0.2227	0.2453	0.1828
-8	0.3498	0.1111	0.1570	0.2516	0.1971	0.2204	0.1940	0.1940	0.2216	0.1761
-6	0.3235	0.2321	0.2768	0.2927	0.2334	0.2239	0.2086	0.1425	0.2377	0.1805
-1	0.3410	0.1674	0.1881	0.3043	0.2400	0.2384	0.2088	0.1816	0.2758	0.2119
0	0.0117	0.0091	0.0041	0.0048	0.2461	0.2359	0.1946	0.2372	0.3045	0.2347
4	0.1752	0.1930	0.2978	0.2699	0.2240	0.3174	0.3500	0.3108	0.3402	0.2606
8	0.1049	0.2469	0.2670	0.2456	0.2105	0.2690	0.3085	0.3556	0.3301	0.2914
11	0.1478	0.2287	0.2485	0.2633	0.2345	0.2433	0.2769	0.3309	0.3220	0.3160
13	0.0717	0.2153	0.2675	0.2862	0.2092	0.2340	0.2795	0.3024	0.2890	0.2274
18	0.2255	0.2489	0.2763	0.3039	0.2249	0.2546	0.3124	0.0133	0.0677	0.0668
20	0.3448	0.3076	0.3274	0.3455	0.2150	0.2264	0.2756	0.0148	0.0649	0.0611
22	0.3754	0.3171	0.3143	0.3167	0.2251	0.2549	0.3214	0.1218	0.0957	0.0815
25	0.2737	0.2294	0.2645	0.2481	0.2330	0.2870	0.3825	0.1518	0.1986	0.1799
27	0.2016	0.1428	0.2124	0.1855	0.2787	0.2470	0.2895	0.1043	0.1278	0.2437
29	0.2345	0.1848	0.2207	0.1940	0.2114	0.2033	0.1910	0.0769	0.0925	0.1485
32	0.2714	0.2555	0.1886	0.1735	0.2023	0.1767	0.2059	0.0278	0.0766	0.1469
34	0.2806	0.2604	0.2321	0.2499	0.2029	0.1736	0.2050	0.0229	0.0455	0.1346
36	0.2902	0.2651	0.2942	0.3478	0.2157	0.1721	0.2040	0.0005	0.0242	0.1693
39	0.2383	0.1802	0.2160	0.2444	0.2189	0.1705	0.1964	0.2536	0.2524	0.3302
41	0.1842	0.1083	0.1419	0.1448	0.2116	0.1690	0.1894	0.0206	0.0395	0.1944
43	0.2119	0.1596	0.1974	0.1795	0.2013	0.1725	0.1886	0.0028	0.0231	0.1835
46	0.2389	0.2187	0.2542	0.2145	0.1872	0.1761	0.1878	0.0091	0.0865	0.2146
Max	*0.3754*	*0.3171*	*0.3274*	*0.3478*	*0.2787*	*0.3174*	*0.3825*	*0.3556*	*0.3402*	*0.3302*
Mean	*0.2157*	*0.2095*	*0.2347*	*0.2343*	*0.2196*	*0.2213*	*0.2533*	*0.1309*	*0.1545*	*0.1936*
Min	*0.0117*	*0.0091*	*0.0041*	*0.0048*	*0.1872*	*0.1690*	*0.1878*	*0.0005*	*0.0231*	*0.0611*
SD	*0.0914*	*0.0735*	*0.0744*	*0.0819*	*0.0203*	*0.0463*	*0.0643*	*0.1312*	*0.1186*	*0.0798*

Table B.12 Oxygen uptake rates as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & Manganese	Phosphorus, calcium & manganese	Phosphorus, calcium & niacin	Phosphorus & molybdenum	Molybdenum	Molybdenum & lactoflavin	Manganese & aluminium
-27	100	170.377	200.629	139.573	99.091	93.669	103.534	64.620
-24	100	90.918	77.038	89.785	97.441	90.934	104.041	67.355
-22	100	101.402	63.014	111.765	97.893	95.009	98.708	2.166
-20	100	97.513	70.536	140.174	98.818	100.835	96.336	55.250
-15	100	107.497	63.147	134.619	107.961	99.661	105.710	73.740
-13	100	55.091	63.151	126.662	100.778	96.019	110.169	82.093
-8	100	31.778	44.875	71.933	111.795	98.415	114.272	90.818
-6	100	71.734	85.567	90.474	95.933	89.395	166.741	126.614
-1	100	49.091	55.178	89.256	99.310	86.989	151.859	116.694
0	100	77.829	35.261	40.634	95.889	79.088	128.357	98.945
4	100	110.109	169.925	154.006	141.708	156.229	109.466	83.868
8	100	235.420	254.605	234.134	127.837	146.569	92.821	81.951
11	100	154.728	168.119	178.097	103.742	118.101	97.293	95.506
13	100	300.326	373.187	399.287	111.849	133.584	95.587	75.221
18	100	110.360	122.522	134.752	113.229	138.914	510.437	503.615
20	100	89.234	94.967	100.227	105.322	128.194	439.639	413.570
22	100	84.482	83.739	84.362	113.236	142.749	78.587	66.957
25	100	83.826	96.638	90.642	123.173	164.158	130.802	118.511
27	100	70.819	105.355	92.002	88.637	103.899	122.547	233.733
29	100	78.776	94.085	82.726	96.171	90.334	120.227	193.053
32	100	94.125	69.488	63.919	87.357	101.799	275.862	528.917
34	100	92.795	82.695	89.059	85.547	100.992	198.498	587.435
36	100	91.342	101.380	119.855	79.774	94.570	4490.190	31445.204
39	100	75.621	90.653	102.560	77.920	89.727	99.530	130.201
41	100	58.796	77.041	78.590	79.890	89.511	191.566	942.313
43	100	75.329	93.154	84.705	85.697	93.697	815.214	6472.189
46	100	91.553	106.395	89.786	94.053	100.331	954.306	2366.682
Max	*100*	*300.3257*	*373.1874*	*399.2868*	*141.7075*	*164.1577*	*4490.1901*	*31445.2035*
Mean	*100*	*109.7482*	*123.2894*	*123.2967*	*100.6127*	*115.1359*	*497.2738*	*2468.7707*
Min	*100*	*58.7964*	*35.2608*	*40.6338*	*77.9196*	*79.0876*	*78.5872*	*66.9565*
SD	*0*	*62.0761*	*78.7020*	*82.1965*	*18.2272*	*26.2905*	*1029.2883*	*7391.1214*

APPENDIX C

TREATABILITY TEST RESULTS: VITAMINS.

TREATABILITY TEST RESULTS: VITAMINS.

Table C.1 MLSS (mg/l).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	2880	2620	2510	2210	2370	2160	1940	1700	2090
-24	2432	2640	2430	2210	2090	2280	2040	1740	2040
-22	2335	2410	2245	2220	2120	2220	1800	1720	2100
-20	2410	2800	2060	2220	2120	2220	2240	1820	1950
-15	2315	2545	1980	2070	2210	2290	1860	1670	2380
-13	2308	2290	1710	2230	2380	2430	1830	2020	1980
-8	2163	2295	1500	2180	2550	2570	2310	1930	1780
-6	2108	2300	2060	2220	2310	2250	1680	1670	1580
-1	2071	2470	2080	2110	2070	1930	1610	1530	1640
0	2036	2640	2100	2000	1705	1740	2140	1780	1990
4	2126	2680	1990	1580	1340	1550	1760	1780	1810
8	2085	1650	2230	1350	1445	1590	2010	1550	1610
11	2109	2710	2120	1000	1550	1630	2120	1630	1660
13	2164	3260	2010	1010	2110	1810	1570	1310	1190
18	1840	2870	1950	1020	2670	1990	1720	1250	1230
20	1662	2630	1680	930	2650	2075	1230	1190	1040
22	1457	2030	1795	890	2630	2160	1610	1260	1510
25	1443	1775	1910	850	2030	1805	1560	1390	1500
27	1353	1520	2045	790	1430	1450	1580	1530	1530
29	1430	1395	2180	730	1635	1595	2120	1780	1810
32	1392	1270	2690	840	1840	1740	2160	1550	1610
34	1442	1230	2395	860	1615	1730	2200	1630	1660
36	1480	1190	2100	890	1390	1720	2240	1310	1190
39	1490	1300	2140	900	1395	1745	1800	1250	1230
41	1460	1410	2180	910	1400	1770	2240	1190	1040
43	1464	1215	2170	890	1420	1790	2860	1260	1510
46	1418	1020	2160	860	1440	1810	2790	1390	1500
Max	*2164.00*	*3260.00*	*2690.00*	*1580.00*	*2670.00*	*2160.00*	*2860.00*	*1780.00*	*1810.00*
Min	*1353.00*	*1020.00*	*1680.00*	*730.00*	*1340.00*	*1450.00*	*1230.00*	*1190.00*	*1040.00*
Mean	*1636.18*	*1832.65*	*2102.65*	*958.82*	*1764.12*	*1762.35*	*1974.71*	*1426.47*	*1448.82*
SD	*298.88*	*715.84*	*227.27*	*208.29*	*478.38*	*183.10*	*435.78*	*198.96*	*248.17*

SD = standard deviation

Table C.2 MLSS as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	48.250	66.227	114.508	150.955	137.580	115.476	101.190	124.405
-24	100	56.897	114.085	107.282	125.904	137.349	122.156	104.192	122.156
-22	100	58.213	100.000	107.246	142.282	148.993	104.046	99.422	121.387
-20	100	62.500	87.288	106.220	142.282	148.993	137.423	111.656	119.632
-15	100	56.243	98.020	112.500	131.548	136.310	123.179	110.596	157.616
-13	100	50.109	97.714	106.699	128.649	131.351	142.969	157.813	154.688
-8	100	56.181	151.515	99.091	126.238	127.228	151.974	126.974	117.105
-6	100	63.889	119.075	119.355	123.529	120.321	113.514	112.838	106.757
-1	100	65.344	138.206	116.575	120.349	112.209	104.545	99.351	106.494
0	100	66.667	164.063	112.994	112.914	115.232	128.916	107.229	119.880
4	100	65.049	93.868	95.758	103.077	119.231	122.222	123.611	125.694
8	100	43.651	119.251	97.122	114.229	125.692	94.811	73.113	75.943
11	100	61.451	91.974	90.090	126.016	132.520	142.282	109.396	111.409
13	100	77.990	73.358	92.661	150.714	129.286	111.348	92.908	84.397
18	100	81.534	113.372	97.143	170.064	126.752	128.358	93.284	91.791
20	100	78.979	118.310	95.876	187.943	147.163	104.237	100.847	88.136
22	100	84.937	113.968	92.708	210.400	172.800	145.045	113.514	136.036
25	100	80.682	110.405	88.542	172.766	153.617	135.652	120.870	130.435
27	100	75.622	115.864	109.722	130.000	131.818	135.043	130.769	130.769
29	100	81.105	121.111	86.905	142.174	138.696	129.268	108.537	110.366
32	100	88.811	149.444	92.308	153.333	145.000	133.333	95.679	99.383
34	100	71.304	135.694	95.556	133.471	142.975	136.646	101.242	103.106
36	100	58.911	121.387	105.952	113.934	140.984	140.881	82.390	74.843
39	100	64.838	126.254	109.756	116.250	145.417	104.046	72.254	71.098
41	100	70.854	131.325	108.333	118.644	150.000	137.423	73.006	63.804
43	100	77.636	129.167	103.488	107.170	135.094	151.323	66.667	79.894
46	100	89.474	127.059	95.556	97.959	123.129	148.404	73.936	79.787
Max	*100*	*89.47*	*164.06*	*119.35*	*210.40*	*172.80*	*151.97*	*157.81*	*157.62*
Min	*100*	*43.65*	*66.23*	*86.90*	*97.96*	*112.21*	*94.81*	*66.67*	*63.80*
Mean	*100*	*68.04*	*115.85*	*102.22*	*135.29*	*136.14*	*127.57*	*102.34*	*107.67*
Stdev	*0*	*12.54*	*22.73*	*9.21*	*26.28*	*13.35*	*16.30*	*20.69*	*24.91*

Table C.3 COD removal rates (kg COD/kg MLSS/d).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	2.0	3.3	3.7	0.8	1.4	1.5	2.4	2.3	2.5
-24	2.5	2.5	3.9	1.0	1.4	1.3	2.3	2.6	2.5
-22	2.3	2.7	3.5	0.7	1.4	1.3	2.4	2.4	2.5
-20	2.1	2.4	2.5	1.2	1.6	1.5	2.3	2.1	2.3
-15	1.8	2.4	1.9	1.3	1.3	1.3	2.3	2.3	2.4
-13	2.1	2.5	2.5	1.7	1.4	1.3	2.3	2.7	2.6
-8	2.6	2.0	3.2	0.8	1.2	1.3	1.8	2.3	1.9
-6	2.3	1.5	3.0	1.4	1.6	1.6	1.4	1.3	1.8
-1	2.6	2.2	3.6	1.4	1.5	1.5	1.7	1.6	1.0
0	2.4	2.8	2.3	1.4	2.1	1.9	1.4	1.6	1.9
4	3.4	3.4	2.9	2.7	6.0	4.6	3.3	3.0	3.5
8	3.5	5.5	3.0	3.2	5.5	4.5	2.4	2.4	2.4
11	-	3.0	3.4	3.4	4.4	4.7	-	-	-
13	3.5	3.3	3.3	3.7	3.6	4.2	3.7	3.7	3.4
18	3.5	3.6	3.4	3.8	3.0	3.9	3.3	4.0	3.4
20	3.8	3.0	4.0	3.7	3.0	3.5	2.8	3.4	3.9
22	4.7	3.3	3.9	8.2	3.0	3.5	3.4	4.3	4.3
25	4.5	3.5	3.8	4.2	3.6	4.1	5.2	7.6	7.2
27	4.2	3.2	3.7	4.6	4.8	5.3	3.8	5.3	5.5
29	4.7	4.0	3.5	5.1	4.4	4.8	4.7	6.5	5.4
32	4.8	5.0	2.9	4.8	4.2	4.4	5.0	6.4	5.7
34	4.7	5.3	3.2	4.6	4.9	4.4	5.2	5.5	5.4
36	4.2	5.6	3.5	4.3	5.8	4.5	3.8	4.9	4.7
39	4.2	5.2	3.4	4.3	5.7	4.3	3.7	5.8	5.5
41	4.3	4.9	3.2	4.2	5.6	4.2	3.7	5.7	5.5
43	4.3	4.7	3.3	4.3	5.5	4.2	3.7	7.4	8.0
46	4.3	4.5	3.3	4.7	5.4	4.1	4.8	8.0	8.0
Max	*4.82*	*5.60*	*4.04*	*8.19*	*6.03*	*5.31*	*5.25*	*8.04*	*8.01*
Mean	*4.17*	*4.19*	*3.39*	*4.34*	*4.62*	*4.31*	*3.90*	*5.24*	*5.11*
Min	*3.41*	*2.97*	*2.90*	*2.75*	*2.98*	*3.45*	*2.44*	*2.38*	*2.43*
SD	*0.48*	*0.94*	*0.32*	*1.17*	*1.07*	*0.45*	*0.84*	*1.70*	*1.63*

Table C.4 COD removal rates as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	260.0	150.8	81.8	63.0	66.8	75.9	73.9	79.0
-24	100	199.6	87.1	55.9	67.7	59.3	75.4	83.6	81.8
-22	100	178.3	99.5	61.5	67.5	60.2	75.1	76.8	79.6
-20	100	153.0	115.9	91.4	66.8	64.0	70.9	65.3	70.8
-15	100	179.5	104.2	89.3	70.4	71.0	83.9	82.5	87.2
-13	100	215.1	102.3	91.3	85.5	82.4	68.5	79.9	79.6
-8	100	138.2	65.2	46.7	62.2	66.8	59.5	75.8	62.9
-6	100	81.9	83.6	86.3	83.9	84.8	57.7	51.8	73.4
-1	100	116.7	72.5	87.1	81.0	82.9	64.8	60.3	38.5
0	100	144.2	60.2	88.5	91.3	83.5	57.1	63.5	75.9
4	100	161.3	106.5	103.9	101.3	77.4	90.7	81.8	96.1
8	100	260.3	92.2	96.0	90.2	74.0	92.3	90.0	91.8
11	100	196.4	127.7	106.6	79.7	85.6	74.3	72.5	76.9
13	100	149.7	151.3	109.0	64.8	75.1	86.4	85.1	78.8
18	100	139.5	94.2	98.1	65.4	84.3	105.9	131.5	112.1
20	100	153.6	91.8	95.4	57.5	68.4	76.8	94.6	108.2
22	100	138.6	97.0	103.5	50.3	58.2	106.0	135.5	133.1
25	100	135.7	106.0	104.6	58.9	66.8	82.5	119.5	113.7
27	100	142.7	106.6	83.3	77.3	86.1	106.5	150.0	155.7
29	100	142.0	100.7	103.9	69.6	75.5	81.0	111.8	92.4
32	100	136.8	80.5	111.5	63.7	67.0	83.7	106.6	95.5
34	100	186.4	87.6	105.3	78.1	71.2	80.4	85.1	84.4
36	100	247.9	96.6	92.8	97.7	75.8	86.1	110.9	107.2
39	100	202.4	94.2	91.0	94.3	71.4	85.8	136.0	127.7
41	100	168.8	91.8	93.6	91.1	67.1	84.3	131.0	125.7
43	100	136.6	93.1	99.2	99.8	75.0	82.4	163.8	176.5
46	100	101.1	94.1	108.6	108.0	81.7	112.3	190.0	189.3
Max	*100*	*260.33*	*151.31*	*111.47*	*107.96*	*86.06*	*112.29*	*189.98*	*189.31*
Mean	*100*	*163.56*	*98.46*	*99.70*	*79.93*	*74.67*	*87.47*	*114.39*	*113.39*
Min	*100*	*101.12*	*60.21*	*83.29*	*50.26*	*58.25*	*57.14*	*63.46*	*75.94*
SD	*0*	*40.95*	*18.73*	*7.85*	*17.43*	*7.64*	*13.44*	*33.54*	*33.12*

Table C.5 COD removal efficiencies (%).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	61.1	70.4	81.4	40.7	51.5	49.8	61.6	52.5	69.1
-24	64.5	72.2	82.3	30.5	46.7	44.7	64.0	62.1	69.8
-22	62.5	68.7	79.1	28.5	49.9	46.6	66.0	57.5	69.9
-20	64.2	65.5	71.2	52.4	51.9	52.1	66.0	49.3	66.1
-15	58.4	63.9	61.1	53.1	46.6	48.7	57.2	53.8	69.5
-13	62.6	62.2	65.0	63.3	55.0	54.1	70.8	67.2	71.7
-8	65.3	49.8	68.7	31.0	46.9	50.7	48.4	55.3	65.5
-6	65.3	37.1	73.6	56.8	58.1	57.2	56.6	50.2	66.5
-1	63.1	51.0	77.3	59.8	49.9	47.5	57.2	58.7	36.8
0	60.0	61.4	51.3	63.0	58.1	54.2	56.4	52.3	57.8
4	63.9	66.7	59.1	67.5	66.6	58.8	67.0	60.1	66.8
8	63.8	67.6	65.9	63.0	66.2	59.7	65.3	60.5	66.2
11	59.7	66.3	69.9	57.9	56.9	64.3	68.0	65.4	66.2
13	62.6	72.1	64.9	61.2	63.6	63.2	70.7	70.4	66.3
18	62.7	70.2	64.3	60.2	67.5	64.9	68.1	65.3	57.8
20	57.6	68.9	66.1	54.4	66.3	61.8	54.0	51.1	59.6
22	58.6	62.4	67.7	71.5	65.2	62.1	49.9	53.3	47.5
25	59.4	56.8	71.0	55.2	61.5	62.0	68.1	71.6	67.1
27	51.6	53.2	74.6	55.6	56.9	64.3	33.9	46.2	41.9
29	57.7	56.3	74.0	56.2	60.3	63.8	65.5	70.7	70.1
32	58.0	58.9	73.4	62.5	63.6	63.2	61.8	70.2	67.9
34	58.4	60.6	71.6	60.4	65.6	64.1	68.6	70.3	69.7
36	55.8	62.4	69.7	58.4	67.5	64.9	63.3	68.4	67.2
39	55.1	60.7	69.5	58.5	67.0	63.4	58.6	66.7	65.1
41	55.2	59.2	69.2	58.0	66.3	61.8	58.5	67.3	65.8
43	55.3	52.2	69.9	58.8	65.7	62.3	58.4	67.9	66.5
46	55.5	44.2	70.3	60.3	65.2	62.1	58.3	68.5	67.1
Max	*63.89*	*72.11*	*74.56*	*71.47*	*67.54*	*64.87*	*70.66*	*71.63*	*70.05*
Mean	*58.28*	*61.10*	*68.90*	*59.97*	*64.23*	*62.73*	*61.05*	*64.35*	*63.45*
			59.15	*54.44*	*56.94*	*58.79*	*33.88*	*46.17*	*41.89*
			3.92	*4.39*	*3.38*	*1.69*	*9.06*	*7.60*	*7.75*

↑

58.28 % removal

≈ 4.17 removal rate

$\frac{4.17}{58.28} = 1\% = 0.72$

0.72 x 100 = 100 %

7.16 = 100 %

kg COD/kg MLSS/d

= loading

Table C.6 COD removal efficiencies as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	125.4	99.9	93.7	95.1	91.9	87.7	74.7	98.3
-24	100	113.5	99.4	60.0	85.2	81.5	90.7	88.0	98.8
-22	100	103.8	99.5	65.9	96.0	89.7	91.8	80.0	97.3
-20	100	95.7	101.2	97.1	95.1	95.4	89.5	66.9	89.7
-15	100	101.0	102.1	100.5	92.6	96.8	87.3	82.0	105.9
-13	100	107.8	100.0	97.4	110.0	108.2	94.1	89.3	95.2
-8	100	77.7	98.8	46.3	78.6	85.0	73.3	83.8	99.1
-6	100	52.3	99.5	102.9	103.6	102.0	80.1	71.1	94.2
-1	100	76.3	100.2	101.5	97.5	93.0	92.6	95.1	59.5
0	100	96.1	98.8	100.0	103.1	96.2	86.8	80.6	88.9
4	100	104.9	100.0	99.5	104.4	92.2	102.9	92.3	102.6
8	100	113.6	110.0	93.2	103.0	93.0	96.5	89.4	97.8
11	100	120.7	117.5	96.0	100.4	113.4	101.0	97.3	98.4
13	100	116.8	111.0	101.0	97.6	97.1	105.6	105.2	99.0
18	100	113.7	106.8	95.3	111.3	106.9	100.4	96.1	85.1
20	100	121.3	108.7	91.4	108.0	100.7	109.3	103.4	120.6
22	100	117.7	110.5	96.0	105.8	100.6	118.1	125.9	112.4
25	100	109.5	117.1	92.6	101.8	102.6	105.9	111.5	104.4
27	100	107.9	123.5	91.4	100.4	113.4	111.0	151.2	137.2
29	100	115.2	121.9	90.3	98.9	104.7	117.5	126.9	125.7
32	100	121.5	120.4	102.9	97.6	97.1	113.5	128.9	124.6
34	100	132.9	118.9	100.6	104.2	101.8	108.6	111.3	110.3
36	100	146.1	117.3	98.3	111.3	106.9	111.3	120.3	118.3
39	100	131.2	118.9	99.8	109.6	103.8	114.4	130.1	126.9
41	100	119.6	120.6	101.4	108.0	100.7	115.2	132.6	129.6
43	100	106.1	120.3	102.6	106.9	101.3	116.0	135.0	132.1
46	100	90.5	119.5	103.7	105.8	100.6	116.8	137.3	134.6
Max	*100*	*146.05*	*123.53*	*103.73*	*111.30*	*113.44*	*118.06*	*151.23*	*137.23*
Mean	*100*	*115.85*	*114.53*	*97.56*	*104.36*	*101.84*	*108.38*	*115.29*	*113.80*
Min	*100*	*90.48*	*98.78*	*90.32*	*97.63*	*92.25*	*86.84*	*80.58*	*85.10*
SD	*0*	*13.08*	*7.39*	*4.37*	*4.35*	*5.87*	*8.40*	*19.57*	*16.17*

Table C.7 BOD_5 removal rates (kg BOD_5/kg MLSS/d).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	0.8	1.8	0.8	0.8	0.7	0.8	0.6	0.6	0.5
-24	1.0	1.0	1.0	1.0	0.8	0.8	0.5	0.6	0.5
-22	1.0	1.1	0.8	0.9	1.0	0.9	0.5	0.6	0.5
-20	0.9	1.1	0.9	0.7	1.0	1.0	0.5	0.4	0.4
-15	0.9	1.3	0.3	0.8	1.3	1.2	0.6	0.6	0.5
-13	0.8	1.3	0.5	0.8	0.7	0.7	0.6	0.8	0.7
-8	1.3	1.6	0.6	0.7	0.7	0.7	2.2	2.4	1.7
-6	1.1	1.8	0.4	0.7	0.9	0.9	1.0	1.0	1.1
-1	1.0	1.4	0.4	0.8	0.9	1.0	0.9	0.8	0.8
0	1.0	1.2	0.3	0.8	1.3	1.2	1.0	1.0	1.2
4	1.3	1.3	1.3	0.7	2.4	1.9	1.2	1.0	1.3
8	1.2	1.6	1.1	0.9	1.9	1.7	0.9	0.9	0.9
11	1.4	1.7	1.2	1.2	2.1	2.0	0.0	0.0	0.0
13	1.1	1.4	0.5	1.1	1.5	1.7	0.9	0.9	0.8
18	1.1	1.7	0.6	1.0	1.0	1.3	1.2	1.5	1.5
20	1.3	1.7	0.6	1.5	1.0	1.2	1.1	1.5	1.4
22	1.4	1.5	0.9	1.9	1.0	1.2	0.6	1.1	0.8
25	1.6	2.0	1.1	1.4	1.3	1.5	1.6	2.3	1.6
27	1.7	2.4	1.1	1.5	2.0	1.9	2.2	1.2	0.7
29	1.6	2.8	0.5	1.6	1.7	1.7	1.5	2.0	1.5
32	1.6	3.3	1.0	1.3	1.4	1.5	1.2	1.7	1.4
34	1.7	3.0	1.2	1.2	1.6	1.4	1.8	1.8	1.7
36	1.7	2.7	1.2	1.2	2.0	1.6	1.8	2.2	2.1
39	1.7	2.9	1.1	1.2	1.9	1.5	1.8	2.6	2.4
41	1.6	3.2	1.0	1.3	2.1	1.6	1.5	2.1	1.9
43	1.6	3.4	0.9	1.2	1.9	1.5	1.5	2.7	2.8
46	1.5	3.7	1.0	1.2	2.0	1.5	1.7	2.4	2.3
Max	*1.74*	*3.66*	*1.34*	*1.85*	*2.42*	*2.01*	*2.19*	*2.68*	*2.78*
Mean	*1.50*	*2.37*	*0.97*	*1.27*	*1.71*	*1.57*	*1.33*	*1.64*	*1.48*
Min	*1.09*	*1.30*	*0.51*	*0.70*	*0.97*	*1.21*	*0.00*	*0.00*	*0.00*
SD	*0.22*	*0.79*	*0.27*	*0.27*	*0.44*	*0.23*	*0.53*	*0.73*	*0.70*

Table C.8 BOD_5 removal rates as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	208.5	148.3	86.7	66.0	71.4	87.1	97.7	78.6
-24	100	178.7	86.1	90.7	78.9	71.8	84.2	95.5	83.8
-22	100	173.6	102.6	93.2	71.1	65.5	79.8	97.2	81.4
-20	100	160.4	118.4	91.7	70.4	65.9	80.7	69.6	68.5
-15	100	180.3	94.4	90.5	77.9	74.2	99.4	100.6	82.4
-13	100	204.2	101.6	97.6	77.6	74.8	66.8	82.2	78.7
-8	100	183.1	67.5	98.2	79.0	78.0	82.4	91.1	63.7
-6	100	164.1	89.6	81.6	80.9	83.1	72.4	73.3	77.2
-1	100	161.0	72.9	83.7	83.3	88.6	69.0	61.1	60.1
0	100	160.7	61.6	95.6	88.6	86.3	64.6	66.3	78.5
4	100	166.0	106.5	113.6	97.0	74.6	87.8	70.2	91.5
8	100	252.9	83.9	107.1	86.8	78.4	97.2	96.2	94.7
11	100	177.1	109.2	111.0	81.1	76.3	-	-	-
13	100	136.7	137.9	105.1	69.4	79.2	86.2	77.8	73.5
18	100	136.8	83.0	97.4	59.6	76.6	105.4	136.4	132.9
20	100	141.5	77.0	93.9	54.2	67.7	70.6	91.8	89.7
22	100	137.1	82.1	94.2	48.7	57.9	62.0	128.1	90.3
25	100	137.1	85.8	107.6	59.7	65.0	95.1	135.0	90.6
27	100	159.7	80.4	87.8	78.7	75.4	127.0	67.0	43.3
29	100	143.8	56.5	112.3	71.9	71.9	80.9	106.0	81.0
32	100	132.8	62.7	104.2	66.9	69.0	80.3	115.2	95.8
34	100	167.3	71.9	99.0	76.7	69.6	78.0	78.8	75.3
36	100	205.0	79.6	87.3	89.7	70.6	80.1	97.2	92.8
39	100	188.7	76.4	84.3	88.0	68.5	76.4	107.5	99.4
41	100	175.2	75.5	94.8	86.6	66.3	75.3	102.1	94.9
43	100	162.3	79.3	96.6	94.8	73.7	73.0	126.9	131.8
46	100	143.1	78.1	110.9	104.7	81.2	100.6	145.1	138.3
Max	*100.00*	*252.95*	*148.27*	*113.64*	*104.72*	*88.58*	*127.02*	*145.08*	*138.26*
Mean	*100.00*	*168.06*	*87.73*	*96.91*	*77.35*	*73.39*	*80.08*	*93.19*	*84.02*
Min	*100.00*	*132.79*	*56.49*	*81.64*	*48.70*	*57.90*	*0.00*	*0.00*	*0.00*
SD	*0.00*	*27.68*	*21.77*	*9.29*	*13.12*	*6.92*	*21.34*	*29.68*	*26.86*

Table C.9 BOD_5 removal efficiencies (%).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	90.2	94.7	90.5	86.2	95.6	94.2	82.5	81.1	80.2
-24	90.4	96.5	92.9	87.4	94.8	94.2	78.1	77.7	78.1
-22	87.1	98.6	90.9	86.2	87.3	84.1	75.2	78.0	76.6
-20	88.6	93.1	91.1	79.4	95.4	93.6	86.8	60.7	74.0
-15	88.9	94.9	73.1	85.0	99.1	97.8	94.9	91.8	91.8
-13	89.7	94.2	78.1	87.2	95.9	94.3	89.9	89.9	92.2
-8	91.2	94.5	89.6	82.9	96.3	95.8	96.5	95.8	95.4
-6	88.2	94.2	78.5	83.1	96.8	96.8	95.6	95.6	94.2
-1	86.9	92.2	74.6	83.3	97.1	96.3	89.4	87.5	84.3
0	76.9	93.0	64.1	45.8	96.6	96.0	93.8	80.4	87.9
4	78.3	90.8	82.4	36.3	96.6	85.9	94.6	75.2	92.6
8	76.8	90.9	81.7	37.4	91.0	90.4	93.8	88.3	93.1
11	74.1	90.6	82.1	38.5	95.2	94.1	-	-	-
13	73.1	91.8	65.4	36.3	93.8	91.8	92.6	84.6	81.3
18	70.4	91.1	70.3	34.3	94.1	90.0	66.3	66.2	67.0
20	72.2	89.6	68.2	43.3	93.3	91.3	66.7	66.7	66.3
22	68.3	89.4	71.7	51.9	94.3	92.1	25.4	43.9	28.1
25	68.4	89.7	73.4	38.5	94.6	91.6	62.7	64.6	42.7
27	68.6	90.2	74.0	38.1	94.8	92.1	75.0	38.3	21.6
29	64.1	89.8	36.1	37.7	94.8	92.5	69.8	71.5	65.5
32	67.8	89.6	77.2	35.7	94.1	91.7	56.5	72.2	64.8
34	74.6	89.5	85.9	33.6	93.8	91.2	86.9	85.0	81.2
36	76.2	89.3	88.2	36.3	94.1	91.7	87.5	89.1	86.6
39	76.3	89.1	93.1	37.0	94.0	91.5	81.7	82.5	79.2
41	74.8	88.9	93.5	36.9	95.6	92.5	81.2	81.5	77.2
43	73.8	88.7	93.9	34.6	93.4	91.6	82.6	84.0	79.2
46	73.2	88.5	93.5	33.8	94.3	91.9	82.7	82.8	77.7
Max	*78.25*	*91.84*	*93.86*	*51.86*	*96.57*	*94.14*	*94.56*	*89.14*	*93.09*
Mean	*72.41*	*89.86*	*78.27*	*37.64*	*94.22*	*91.41*	*75.37*	*73.53*	*69.01*
Min	*64.13*	*88.48*	*36.11*	*33.65*	*90.96*	*85.86*	*25.44*	*38.28*	*21.60*
SD	*3.87*	*0.93*	*14.41*	*4.31*	*1.17*	*1.68*	*17.64*	*14.98*	*21.22*

Table C.10 BOD_5 removal efficiencies as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	100.6	98.2	99.3	99.6	98.2	100.6	98.9	97.8
-24	100	101.7	98.2	97.3	99.3	98.6	101.2	100.6	101.2
-22	100	101.1	102.6	100.0	101.2	97.6	97.5	101.2	99.4
-20	100	100.3	103.3	97.4	100.1	98.2	101.8	71.3	86.8
-15	100	101.4	92.5	101.8	102.5	101.1	103.4	100.0	100.0
-13	100	102.3	99.3	104.1	99.8	98.2	91.8	91.8	94.1
-8	100	102.9	102.2	97.3	99.8	99.3	101.5	100.7	100.4
-6	100	104.8	106.7	97.4	100.0	100.0	100.5	100.5	99.0
-1	100	105.2	100.7	97.5	100.3	99.4	98.6	96.5	92.9
0	100	107.1	101.1	108.0	100.0	99.4	98.1	84.2	92.0
4	100	108.0	100.0	108.8	100.0	88.9	99.7	79.2	97.7
8	100	110.4	100.0	104.1	99.1	98.5	101.6	95.6	100.8
11	100	108.8	100.5	100.0	102.2	101.1	-	-	-
13	100	106.6	101.2	97.4	104.7	102.4	105.3	96.2	92.4
18	100	111.5	94.1	94.6	101.4	97.1	100.0	99.7	101.0
20	100	111.8	91.1	90.0	101.9	99.7	100.5	100.5	100.0
22	100	116.5	93.6	87.3	102.5	100.0	69.0	119.0	76.2
25	100	110.6	94.7	95.2	103.1	99.8	122.1	126.0	83.1
27	100	120.8	93.2	96.4	102.3	99.4	132.4	67.6	38.1
29	100	116.7	68.4	97.6	102.2	99.7	117.4	120.3	110.1
32	100	117.9	93.7	96.2	102.6	100.0	108.9	139.3	125.0
34	100	119.3	97.5	94.6	102.4	99.5	105.3	103.0	98.5
36	100	120.8	96.6	92.5	102.2	99.6	103.5	105.5	102.4
39	100	122.4	96.5	92.5	102.3	99.6	101.9	102.8	98.8
41	100	124.1	99.2	102.7	102.8	99.4	102.9	103.3	97.9
43	100	126.0	102.4	100.0	101.6	99.6	102.9	104.6	98.6
46	100	128.1	99.2	106.0	102.6	100.0	104.6	104.8	98.3
Max	*100.00*	*128.08*	*106.69*	*108.82*	*104.67*	*102.43*	*132.40*	*139.29*	*125.00*
Mean	*100.00*	*111.39*	*97.29*	*98.37*	*101.42*	*99.05*	*102.81*	*100.51*	*95.48*
Min	*100.00*	*100.26*	*68.42*	*87.30*	*99.15*	*88.91*	*69.05*	*67.60*	*38.14*
SD	*0.00*	*8.66*	*6.90*	*5.11*	*1.41*	*2.30*	*10.74*	*15.18*	*14.54*

Table C.11 Oxygen uptake rates (kg O_2/kg MLSS/d).

Time (days)	Mean control (no supplements)	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	0.1653	0.0060	0.1948	0.2111	0.2113	0.3156	0.1472	0.2086	0.2106
-24	0.1819	0.0032	0.2309	0.2113	0.2352	0.2885	0.1502	0.2060	0.2155
-22	0.1636	0.0051	0.2820	0.2117	0.1807	0.1794	0.1574	0.2153	0.2130
-20	0.1942	0.0058	0.3539	0.2131	0.2308	0.2924	0.1400	0.2668	0.2171
-15	0.1980	0.0055	0.4166	0.2158	0.2167	0.2719	0.2175	0.2489	0.1975
-13	0.1782	0.0050	0.3515	0.2049	0.1969	0.2453	0.1770	0.1776	0.2068
-8	0.1827	0.0059	0.2516	0.2116	0.1797	0.2216	0.1872	0.2285	0.1715
-6	0.1836	0.0067	0.2927	0.2080	0.1428	0.2377	0.1984	0.1815	0.2155
-1	0.1919	0.0059	0.3043	0.2190	0.1857	0.2758	0.2349	0.2062	0.2331
0	0.1579	0.0053	0.0048	0.2000	0.2575	0.3045	0.1947	0.2158	0.2097
4	0.1982	0.0056	0.2699	0.3678	0.3683	0.3402	0.2714	0.2922	0.2041
8	0.1839	0.0096	0.2456	0.3580	0.3793	0.3301	0.2866	0.3257	0.2928
11	-	0.0052	0.2633	0.3695	0.3536	0.3220	-	-	-
13	0.1806	0.0038	0.2862	0.2532	0.2770	0.2890	0.2688	0.2555	0.2430
18	0.1313	0.0053	0.3039	0.2460	0.0675	0.0677	0.2382	0.3058	0.3206
20	0.1623	0.0068	0.3455	0.2320	0.0680	0.0649	0.2286	0.3025	0.3570
22	0.2127	0.0089	0.3167	0.2282	0.1284	0.0957	0.3104	0.2061	0.4150
25	0.1891	0.0103	0.2481	0.2240	0.1700	0.1986	0.1966	0.3079	0.3454
27	0.1844	0.0112	0.1855	0.2235	0.2215	0.1278	0.2926	0.1554	0.4565
29	0.1670	0.0123	0.1940	0.2230	0.1855	0.0925	0.1956	0.2843	0.3207
32	0.1690	0.0125	0.1735	0.1820	0.1259	0.0766	0.2358	0.3460	0.3080
34	0.1635	0.0140	0.2499	0.1824	0.1609	0.0455	0.1909	0.2111	0.3027
36	0.1448	0.0151	0.3478	0.1806	0.1760	0.0242	0.1306	0.1734	0.2531
39	0.1600	0.0144	0.2444	0.1787	0.3387	0.2524	0.1041	0.2150	0.2587
41	0.1157	0.0129	0.1448	0.1767	0.1940	0.0395	0.0988	0.1804	0.2618
43	0.1136	0.0077	0.1795	0.1847	0.1840	0.0231	0.0855	0.2149	0.3595
46	0.1146	0.0183	0.2145	0.1954	0.1712	0.0865	0.0919	0.2151	0.3424
Max	*0.2127*	*0.0183*	*0.3478*	*0.3695*	*0.3793*	*0.3402*	*0.3104*	*0.3460*	*0.4565*
Mean	*0.1619*	*0.0102*	*0.2478*	*0.2356*	*0.2100*	*0.1457*	*0.2016*	*0.2495*	*0.3151*
Min	*0.1136*	*0.0038*	*0.1448*	*0.1767*	*0.0675*	*0.0231*	*0.0855*	*0.1554*	*0.2041*
SD	*0.0305*	*0.0041*	*0.0604*	*0.0666*	*0.0994*	*0.1160*	*0.0778*	*0.0600*	*0.0651*

Table C.12 Oxygen uptake rates as a percentage of the concurrent control.

Time (days)	Control	Phosphorus & niacin 1.0	Phosphorus, niacin & Ca 1.0	Pyridoxine	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5
-27	100	358.9480	139.5730	92.9748	69.3231	103.5335	95.9230	135.9264	137.2656
-24	100	222.0968	89.7846	99.0857	84.8105	104.0406	93.5751	128.3584	134.2634
-22	100	296.6978	111.7647	99.0763	99.4717	98.7075	93.4144	127.7635	126.4135
-20	100	340.0000	140.1744	99.9917	76.0473	96.3355	69.9767	133.2973	108.4646
-15	100	258.8153	134.6190	94.7590	84.2590	105.7104	112.3700	128.6042	102.0541
-13	100	200.3450	126.6621	99.7636	88.4099	110.1686	96.7512	97.0661	113.0077
-8	100	212.7021	71.9329	107.3569	92.6298	114.2720	110.1743	134.4517	100.9338
-6	100	217.6688	90.4742	89.1250	100.1798	166.7410	92.0175	84.1845	99.9591
-1	100	189.8884	89.2562	91.2456	102.2593	151.8593	121.1133	106.3212	120.1821
0	100	166.2195	40.6338	81.2821	108.5421	128.3568	66.7892	74.0193	71.9095
4	100	179.1877	154.0056	164.2179	118.5190	109.4656	97.5876	105.0691	73.4119
8	100	279.7098	234.1335	170.1090	106.6736	92.8209	116.9025	132.8487	119.4026
11	100	192.5211	178.0971	157.5779	106.8608	97.2927	-	-	-
13	100	145.8461	399.2868	121.0169	91.6142	95.5872	84.8050	80.6040	76.6551
18	100	165.5430	134.7516	109.4046	508.6986	510.4371	125.5331	161.1136	168.9517
20	100	195.8982	100.2270	107.9070	460.3047	439.6395	97.8106	129.4389	152.7692
22	100	196.5277	84.3624	101.3593	105.4377	78.5872	92.1573	61.2017	123.2295
25	100	224.3046	90.6420	96.1373	111.9650	130.8018	69.6702	109.0890	122.3809
27	100	193.4985	92.0016	80.2170	212.4166	122.5470	88.1781	46.8374	137.6010
29	100	162.3757	82.7256	105.4730	241.1609	120.2268	64.1885	93.3028	105.2509
32	100	134.9941	63.9187	89.9718	453.2648	275.8621	70.5260	103.5015	92.1234
34	100	168.0480	89.0586	89.8549	702.0742	198.4976	63.0266	69.7057	99.9434
36	100	206.6580	119.8554	83.7322	760.1806	104.3748	62.0859	82.4460	120.3184
39	100	190.5763	102.5602	81.6375	133.5605	99.5305	127.1681	262.5384	315.9459
41	100	235.5895	78.5901	83.5436	940.6680	191.5661	63.0609	115.1312	167.0585
43	100	154.2726	84.7054	91.7880	648.9161	81.5214	58.1468	146.2291	244.6517
46	100	133.8614	89.7857	104.3580	188.8542	95.4306	73.9357	173.1027	275.4566
Max	*100*	*279.7098*	*399.2868*	*170.1090*	*940.6680*	*510.4371*	*127.1681*	*262.5384*	*315.9459*
Min	*100*	*133.8614*	*63.9187*	*80.2170*	*91.6142*	*78.5872*	*58.1468*	*46.8374*	*73.4119*
Mean	*100*	*185.8478*	*128.1593*	*108.1356*	*346.5394*	*167.3052*	*84.6739*	*117.0100*	*149.6969*
SD	*0*	*37.7053*	*82.0142*	*28.9639*	*278.0499*	*127.1544*	*23.1393*	*52.4589*	*70.9207*

Table C.13 Percentage ammonia removal by Amtox™ cultures during toxicity testing.

Time (minutes)	Control sample 1	Control sample 2	Phosphorus & pyridoxine	Phosphorus, Mo and lactoflavin	Phosphorus	Phosphorus & niacin 0.5	Phosphorus, niacin & Ca 0.5	Baseline
0	65	69	66	68	64	64	66	60
10	65	68	65	67	62	64	66	60
20	66	65	65	65	62	64	67	60
30	60	61	65	64	60	62	65	60
40	57	56	58	61	59	62	62	60
50	53	52	58	62	54	61	60	60
60	45	43	54	63	51	60	60	60
70	40	40	45	63	47	60	59	60
80	38	37	43	62	44	61	60	60
90	35	33	41	63	42	61	61	60
100	29	26	40	62	41	60	61	60
110	24	24	39	61	40	61	60	60
120	19	20	39	63	38	61	59	60

APPENDIX D

PILOT-SCALE TRIAL RESULTS: NIACIN.

PILOT-SCALE TRIAL RESULTS: NIACIN.

Table D.1 MLSS (mg/l) and SSVI (g/ml).

	MLSS		SSVI	
Time (days)	Control	Test	Control	Test
0	2562	2447	-	-
7	320	386	250	207
8	473	440	169	181
11	652	552	123	145
13	400	1250	64	55
15	394	477	203	168
18	800	1030	66	74
20	668	776	120	103
22	644	1036	124	77
25	648	1040	102	82
27	788	972	-	-
29	576	1058	139	76
36	448	1312	179	61
41	708	1120	113	71
43	1180	1788	68	45
46	1500	2472	-	-
48	1808	3200	47	44
50	1808	3200	44	44
55	1772	2848	56	56
57	980	2472	92	58
63	1268	1340	79	180
Max	*2562.00*	*3200.00*	*250.00*	*207.00*
Mean	*971.29*	*1486.48*	*113.22*	*95.94*
Min	*320.00*	*386.00*	*44.00*	*44.00*
SD	*603.44*	*913.41*	*57.41*	*54.37*
RSD	*62.13*	*61.45*	*50.70*	*56.67*

SD = standard deviation

RSD = relative standard deviation (%)

Table D.2 Oxygen uptake data.

	Time (days)	Control	Test
Uptake per respirometry cell per 12h (kg)	**0**	7.1430	5.8102
	7	4.8939	7.6636
	14	7.3720	11.5790
	21	3.5403	10.4130
	28	8.9339	13.1200
	35	9.4129	13.3280
	42	13.5570	14.1820
	49	13.0780	15.4940
	56	15.4100	12.8700
Uptake per respirometry cell (kg/d)	**0**	14.2860	11.6204
	7	9.7878	15.3272
	14	14.7440	23.1580
	21	7.0806	20.8260
	28	17.8678	26.2400
	35	18.8258	26.6560
	42	27.1140	28.3640
	49	26.1560	30.9880
	56	30.8200	25.7400
Uptake per litre (kg/d)	**0**	285.7200	232.4080
	7	195.7560	306.5440
	14	294.8800	463.1600
	21	141.6120	416.5200
	28	357.3560	524.8000
	35	376.5160	533.1200
	42	542.2800	567.2800
	49	523.1200	619.7600
	56	616.4000	514.8000
Uptake per unit MLSS (kg/kg MLSS/d)	**0**	0.6041	0.5282
	7	0.4968	0.6427
	14	0.4579	0.4471
	21	0.2459	0.3937
	28	0.7977	0.4000
	35	0.3191	0.2982
	42	0.2999	0.1773
	49	0.5338	0.2507
	56	0.4861	0.3842
	Max	*0.7977*	*0.6427*
	Mean	*0.4712*	*0.3913*
	Min	*0.2459*	*0.1773*
	SD	*0.1705*	*0.1412*
	RSD	*36.1727*	*36.0719*

Table D.3 Pilot plant performance determinant data (mg/l).

Time (days)	Feed BOD_5	Control effluent BOD_5	Test effluent BOD_5	Feed COD	Control effluent COD	Test effluent COD	Feed NH_4	Control effluent NH_4	Test effluent NH_4	Feed SS	Control effluent SS	Test effluent SS	Feed Tot-P	Control effluent Tot-P	Test effluent Tot-P
0	1010	881	807	1880	1100	938	19.7	23.2	28.9	345	136	120	-	-	-
7	1100	732	577	2290	1580	1450	20.5	25.1	26.4	140	144	156	-	-	-
8	-	-	-	-	-	-	18.7	26.9	14.5	-	-	-	-	-	-
11	-	-	-	1420	1520	1410	57.2	36.7	33.4	-	-	-	30.4	4.87	3.88
15	-	-	-				28.3	24.6	23.8	-	-	-	24.1	3.13	5.66
18	-	-	-	1380	1130	1080	8.26	22.6	24.7	-	-	-	29.5	5.44	4.03
20	-	-	-	1370	888	918	28.7	26	24.8	314	132	120	25.5	13.3	7.53
22	620	428	353	1560	1230	1250	26.2	15	54.3	108	272	308	33.32	13.5	12.8
25	-	-	-	1640	1810	1170	53.3	24.1	31.9	32	132	128	72.1	24.1	22.9
27	-	-	-	1600	1760	1640	36.4	25.5	38.1	61	94	118	-	-	-
29	911	525	400	1620	1430	1270	31.7	34.9	31	50	164	108	-	-	-
36	885	325	410	2060	1210	1110	-	-	-	-	-	-	-	-	-
41	-	-	-	-	-	-	41.6	23.9	22.2	-	-	-	24.4	15.8	16.7
43	953	175	275	1790	848	776	38.9	45.6	22.8	600	141	148	45.1	12.3	6.43
46	-	-	-	1800	777	833	26	35.3	34.3	-	-	-	-	-	-
48	-	-	-	1700	1060	1110	8.15	33.3	39.3	-	-	-	-	-	-
50	1100	322	402	2110	1310	1300	39.1	44.5	18.9	314	80	212	67.9	28.2	19.4
53	278	890	419	2070	2260	1890	26.5	31.1	35	108	122	80	19.6	22.6	15.9
55	376	283	122	1460	1070	1155	36.3	35.3	45.2	287	96	98	-	-	-
57	984	337	665	1910	1250	1110	31.9	57.9	39.1	61	334	148	-	-	-
63	840	202	863	1780	1210	1140	10.7	28.3	35.5	176	484	192	25.7	27	26.2
Max	*1100.00*	*890.00*	*863.00*	*2290.00*	*2260.00*	*1890.00*	*57.20*	*57.90*	*54.30*	*600.00*	*484.00*	*308.00*	*72.10*	*28.20*	*26.20*
Mean	*823.36*	*463.64*	*481.18*	*1746.67*	*1302.39*	*1197.22*	*29.41*	*30.99*	*31.21*	*199.69*	*179.31*	*148.92*	*36.15*	*15.48*	*12.86*
Min	*278.00*	*175.00*	*122.00*	*1370.00*	*777.00*	*776.00*	*8.15*	*15.00*	*14.50*	*32.00*	*80.00*	*80.00*	*19.60*	*3.13*	*3.88*
SD	*279.49*	*259.37*	*224.65*	*269.14*	*374.76*	*276.68*	*13.28*	*9.87*	*9.46*	*164.58*	*116.20*	*60.05*	*18.02*	*8.97*	*7.91*
RSD	*33.95*	*55.94*	*46.69*	*15.41*	*28.77*	*23.11*	*45.18*	*31.84*	*30.32*	*82.42*	*64.80*	*40.33*	*49.84*	*57.95*	*61.52*

Table D.4 Pilot plant performance determinant removal efficiencies (%).

	BOD		COD		Ammonia		SS		Total Phosphorus	
Time (days)	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
0	12.8	20.1	41.5	50.1	-17.8	-46.7	60.6	65.2	-	-
7	33.5	47.5	31.0	36.7	-22.4	-28.8	-2.9	-11.4	-	-
8	-	-	-	-	-43.9	22.5	-	-	-	-
11	-	-	-7.0	0.7	35.8	41.6	-	-	84.0	87.2
15	-	-	-	-	13.1	15.9	-	-	87.0	76.5
18	-	-	18.1	21.7	-173.6	-199.0	-	-	81.6	86.3
20	-	-	35.2	33.0	9.4	13.6	58.0	61.8	47.8	70.5
22	31.0	43.1	21.2	19.9	42.7	-107.3	-151.9	-185.2	59.5	61.6
25	-	-	-10.4	28.7	54.8	40.2	-312.5	-300.0	66.6	68.2
27	-	-	-10.0	-2.5	29.9	-4.7	-54.1	-93.4	-	-
29	42.4	56.1	11.7	21.6	-10.1	2.2	-228.0	-116.0	-	-
36	63.3	53.7	41.3	46.1	-	-	-	-	-	-
41	-	-	-	-	42.5	46.6	-	-	35.2	31.6
43	81.6	71.1	52.6	56.6	-17.2	41.4	76.5	75.3	72.7	85.7
46	-	-	56.8	53.7	-35.8	-31.9	-	-	-	-
48	-	-	37.6	34.7	-308.6	-382.2	-	-	-	-
50	70.7	63.5	37.9	38.4	-13.8	51.7	74.5	32.5	58.5	71.4
53	-220.1	-50.7	-9.2	8.7	-17.4	-32.1	-13.0	25.9	-15.3	18.9
55	24.7	67.6	26.7	20.9	2.8	-24.5	66.6	65.9	-	-
57	65.8	32.4	34.6	41.9	-81.5	-22.6	-447.5	-142.6	-	-
63	76.0	-2.7	32.0	36.0	-164.5	-231.8	-175.0	-9.1	-5.1	-1.9
Max	81.64	71.14	56.83	56.65	54.78	51.66	76.50	75.33	87.01	87.24
Mean	25.59	36.51	24.54	30.38	-33.77	-41.80	-80.67	-40.86	52.05	59.64
Min	-220.14	-50.72	-10.37	-2.50	-308.59	-382.21	-447.54	-300.00	-15.31	-1.95
SD	84.66	36.29	21.46	17.07	88.93	110.74	169.56	117.31	34.56	30.03
RSD	330.81	99.40	87.46	56.17	-263.34	-264.97	-210.19	-287.11	66.41	50.35

Table D.5 Pilot plant performance determinant removal rates (kg/kg MLSS/d).

	BOD		COD		Ammonia		SS		Total Phosphorus	
Time (days) (d)	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
0	0.003	0.002	0.018	0.004	0.000	0.000	0.005	0.000	-	-
7	0.069	0.029	0.133	0.024	-0.001	0.000	-0.001	-0.002	-	-
8	-	-	-	-	-0.001	0.002	-	-	-	-
11	-	-	-0.009	0.010	0.002	0.000	-	-	0.004	0.000
15	-	-	-	-	0.001	0.000	-	-	0.003	0.000
18	-	-	0.019	0.004	-0.001	0.000	-	-	0.002	0.000
20	-	-	0.043	-0.003	0.000	0.000	0.016	0.001	0.001	0.001
22	0.018	0.007	0.031	-0.002	0.001	-0.004	-0.015	-0.003	0.002	
25	-	-	-0.016	0.059	0.003	-0.001	-0.009	0.000	0.004	0.000
27	-	-	-0.012	0.009	0.001	-0.001	-0.003	-0.002	-	-
29	0.040	0.013	0.020	0.017	0.000	0.000	-0.012	0.006	-	-
36	0.075	-0.011	0.114	0.013	-	-	-	-	-	-
41	-	-	-	-	0.002	0.000	-	-	0.001	
43	0.040	-0.005	0.048	0.004	0.000	0.001	0.023	0.000	0.002	0.000
46	-	-	0.041	-0.002	0.000	0.000	-	-	-	-
48	-	-	0.021	-0.002	-0.001	0.000	-	-	-	-
50	0.026	-0.003	0.027	0.000	0.000	0.001	0.008	-0.004	0.001	0.000
53	-	-	-	-	-	-	-	-	-	-
55	0.003	0.006	0.013	-0.003	0.000	0.000	0.007	0.000	-	-
57	0.040	-0.020	0.040	0.009	-0.002	0.001	-0.017	0.011	-	-
63	0.030	-0.031	0.027	0.003	-0.001	0.000	-0.015	0.014	0.000	0.000
Max	*0.075*	*0.029*	*0.133*	*0.059*	*0.003*	*0.002*	*0.023*	*0.014*	*0.004*	*0.001*
Mean	*0.034*	*-0.001*	*0.033*	*0.009*	*0.000*	*0.000*	*-0.001*	*0.002*	*0.002*	*0.000*
Min	*0.003*	*-0.031*	*-0.016*	*-0.003*	*-0.002*	*-0.004*	*-0.017*	*-0.004*	*0.000*	*0.000*
SD	*0.024*	*0.017*	*0.039*	*0.015*	*0.001*	*0.001*	*0.013*	*0.006*	*0.001*	*0.000*
RSD	*70.379*	*-1199.129*	*119.026*	*177.387*	*1687.538*	*-2308.223*	*-1292.627*	*332.644*	*71.315*	*185.879*

APPENDIX E

INTERVIEWING AIDE-MÉMOIRE AND DATA.

INTERVIEWING *AIDE-MÉMOIRE* AND DATA.

E.1 *Aide-mémoire* for DTA interviews.

Opening question: Do you foresee any problems for your company in adapting to DTA?

As the interviewee speaks, note: Technical Financial Managerial

Option list presented to interviewees:

1. Refuse to accept highly toxic wastewater.
2. Charge for discharges to sewer according to toxicity.
3. Implement pre-treatment regulations.
4. Segregate component wastewater streams.
5. Use product substitution (manufacturers) to reduce by-product/wastewater toxicity.
6. Optimise existing treatment processes.
7. Include GAC or activated sludge and biofilms.
8. Use online toxicity monitors and pass the responsibility of toxicity reduction back to the polluter.
9. Use wastewater minimisation.
10. Start/increase recycling of toxic wastewater components.
11. Use nitrification/denitrification treatment processes* to remove ammonia.
12. Use more pre-treatment upstream of sewage treatment works.
13. Use more biological processes.

Have you heard of micronutrient addition (MNA)?

Have you considered it in the past?

Would you consider its use in the future?

Why? / Why not?

E.2 **(Table E.1) Raw data from interview notes.**

Nature of business	Do you foresee problems for industry in adapting to DTA?	Which of the list of options are available to you?	Which options would you prefer to take?	Have you heard of MNA ?	Have you considered using MNA?	Do you see MNA as an option ?	Why? / Why not?
Oil refinery	Have already adapted to WET. Some cost involved but no more than increasing chemical standards.	4, 5, 6, 7, 9, 10, 11, 13	5 4, 9, 10 7, 13	Yes	Yes	Yes	Have used successfully for 7 years. Low / no capital investment Gravity fed dosing means no operating costs either. No change to sludge production in AS. Useful in all biological processes.
Regulation	Nothing insurmountable.	For water companies: 1, 2, 3, 5, 6, 7, 9, 10	N/A	No	N/A	Yes	Biological processes are the most adaptable and sustainable. Any way to optimise them is good.
Consultancy	No	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13	7 – GAC is last resort	Yes	Yes	Yes	Nutritional requirements are well known quantities and basic physiological requirements of WWT processes. Fundamental operating conditions – the obvious choice for optimisation.
Waste disposal	Possible addition to technical costs.	1, 6, 7	6 7	No	No	Yes	Would accept contractor's advice to use MNA.

Nature of business	Do you foresee problems for industry in adapting to DTA?	Which of the list of options are available to you?	Which options would you prefer to take?	Have you heard of MNA?	Have you considered using MNA?	Do you see MNA as an option?	Why? / Why not?
Water company	Technical problems in tracing sources of toxicity	1, 2, 3, 6, 7, 8, 11, 13	6 11, 12 2	Yes	Yes	Yes	Use where imbalances are a problem. Cheaper and simpler than activated carbon.
Water company	Minor problems financially.	1, 2, 3, 6, 7, 8, 11, 12, 13	Whichever is most apt.	Yes	Yes	Maybe	No need for domestic waste. For enhanced COD / toxicity removal the usual options (e.g. longer sludge age) are more likely. MNA is an ongoing cost, but cheaper than activated carbon.
Dairy processing	Yes – but not sure what.	6, 7, 9, 10, 11, 13	9, 11, 7	Yes	Yes	In the future.	If plant need optimising in the future, then it will be considered in more detail.
Chemical manufacture	Some, depending on the actions of water companies. Also some re. cost of increasing lab work.	4, 5, 6, 7, 9, 10, 11, 12, 13	5, 9 10, 12	Yes	Yes	Yes	Often use MNA where WW is not balanced. Prefer MNA to GAC (also used in some cases) as it is simpler, cheaper and easier to tailor to specific WW.
Consultancy	Can imagine small companies struggling.	All possible for someone.	Depends on case.	Yes	Yes	Yes – in some cases.	Useful for chemical, food and paper WW. Small capital costs make it easy to incorporate.

Nature of business	Do you foresee problems for industry in adapting to DTA?	Which of the list of options are available to you?	Which options would you prefer to take?	Have you heard of MNA?	Have you considered using MNA?	Do you see MNA as an option?	Why? / Why not?
Research & contracting	No.	4 – 13	4, 5 6, 12 7	Yes	Yes	Yes – in certain cases.	Removing the toxin at source is the first choice, but if you have to treat toxins then MNA is one of the options to consider.
Chemical manufacture	No. Most developments are based on established tests. 'Progress' always leads to cost; DTA is no different.	4, 5, 6, 7, 9, 10, 11, 12, 13	4, 5, 9, 10 6, 7, 11, 13	Yes	Yes	Yes	Prefer to adapt existing processes once rather than pay inflated discharge costs. Separate WW streams are relatively easy to treat, so would use own PTP.
Water company	Some changes, not really problems.	2, 3, 6, 7, 8, 11, 13	Ask traders to do 4, 12, then 6, 7, 11, 13	Yes	No	Yes	DTA is a new principle and needs new ideas. Never needed MNA for sewage, maybe WW with large trade input will need MNA in the future. It will have to be cost effective to be a real option, but the fact that MNA = simple dose into existing WWTWs is an advantage.
Chemical manufacture	Not really. Initial costs of increasing monitoring may be high.	4, 5, 6, 7, 9, 10, 12	4, 5, 9 6, 12	Yes	No	Yes	Best option = prevent toxicity, 2nd = minimisation, 3rd = biotreatment. Very keen to optimise own PTP. Open to any new ideas, the more proven the better.