



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

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The process performance and metabolic rates of samples of activated sludge dosed with vitamin supplements have been compared. After initial screening, four vitamins and two metals as single supplements and in pairs, were dosed continuously into the mixed liquor of an activated sludge simulation. Toxicity, oxygen demand removal, respiration rates and suspended solids were measured to monitor the effect on process efficiency. It was confirmed experimentally that an industrial wastewater stream did not contain a sufficient supply of micronutrients for efficient biological treatment. This was concluded from the observation that control sludge batches (receiving no supplements) averaged chemical oxygen demand removal efficiency of 58%. Dosing micronutrients into the mixed liquor produced removal efficiencies of up to 69%. Some of the supplements increased the respiration rate of the sludge while some decreased it, indicating a range of stimulatory and inhibitory effects. Complex interactions between micronutrients that were dosed simultaneously were evident. Several positive effects led to the conclusion that micronutrients have the potential to optimise process performance of activated sludge plants treating industrial wastewater. The addition of phosphorus/niacin and molybdenum/lactoflavin removed wastewater components that were toxic to nitrifiers as indicated through toxicity testing, thus protecting downstream nitrification/denitrification treatment processes. *Journal of Industrial Microbiology & Biotechnology* (2000) 24, 267–274.

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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. Direct toxicity assessment 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 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 [9]. Under the name of whole effluent toxicity (WET), DTA has been implemented in the USA where it has been considered successful [7]. 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. Whole effluent toxicity 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 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.

Currently, any individual or company that discharges effluent is bound under Section 7 of the UK Environmental Protection Act (EPA), 1990 [13] 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 [14] '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, ie use-related water quality objectives [27]. 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.

Activated sludge treatment of waste is an aerated oxi-

ation process. It is one of the best established and widespread biological wastewater treatment processes in the developed world for both domestic and industrial wastewaters [3], and 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 microorganisms 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 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 is added via diffusers or by surface agitation. This aerates and maintains the suspension, allowing maximum contact between flocs and 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 (ie 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 0.4–1.0 kg dry weight of sludge is produced per 1.0 kg biological oxygen demand (BOD) removed from the wastewater [2]. 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 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 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 to be maintained such as dissolved oxygen (DO_2) levels, mixing regime, provision of nutrition, 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.

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 [24]. 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 [23]. Environmental factors can affect biodegradation by changing the availability of nutrients and target compounds, thus preventing the growth of microorganisms [6]. Recalcitrance is a problem in industrial wastewater treatment owing to an abundance of xenobiotic compounds that may resist degradation; it can arise from inappropriate conditions, inadequate nutrients and the supply of a substrate that does not activate the appropriate enzymes [8]. The availability of nitrogen and phosphorus can be limiting factors in the degradation of hydrocarbons and supplements (*bioaugmentation*) where concentrations are low can lead to improved wastewater treatment [15]. Bioaugmentation includes the addition of substances such as micronutrients which increase metabolic rates, stimulate cometabolism, or which induce degradative genes [23].

The role of micronutrients in aerobic biological wastewater treatment is not well defined. To achieve sufficient treatment of industrial wastewater, it is often necessary to supply activated sludge with the micronutrients that enable strains capable of degrading 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 and approximates BOD. Enhanced COD removal with no associated increase in BOD removal indicates the 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 [10,11], 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 whether vitamin addition may provide a method for establishing and maintaining 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.

Materials and methods

The screening of potential micronutrients was carried out using activated sludge taken from the return sludge line of an activated sludge pre-treatment plant receiving a chemicals industry effluent that contained a high proportion of organic components (eg toluene, chlorobenzene) and had 1500–2000 mg L^{-1} COD (of which $81 \pm 11\%$ is soluble) and 450–600 mg L^{-1} 5-day BOD (BOD_5) (COD:N:P of 880:20:1; BOD_5 :N:P of 212:20:1; COD: BOD_5 of 4:1). An activated sludge simulation was carried out using the porous pot method for assessing biodegradability of liquid wastes [29,30]. Each pot contained 3 L of activated sludge. The pots were covered to minimise evaporation and insulated to prevent acute temperature changes. Wastewater was supplied at 0.25 L h^{-1} , thus maintaining a 12-h hydraulic retention time (HRT), and nutrients dosed into the mixed liquor as set out in Table 1.

The mean cell retention time (MCRT) was 6 days. Mixing action was provided and DO_2 levels were maintained

Table 1 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

at 1.0–4.0 mg L⁻¹ by aeration from a single sintered glass diffuser placed in the bottom of each pot's conical base. Solids were brushed off the inner walls and the pH, DO₂ and temperature were monitored daily using hand-held probes (Jenway Ltd, Dunmow, Essex, UK, Model 3071 pH and temperature meter; Model 9071 portable DO₂ meter). Mixed liquor suspended solids (MLSS) and influent and BOD₅ and COD were measured three times a week according to Standard Methods [25], and samples of the sludge were analysed respirometrically (CES Ltd, Sittingbourne, Kent, UK, Aerobic Respirometer, Series 17). This model operated in a similar way to the Warburg respirometer [12].

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. CO₂ produced is absorbed in an alkali trap (10 M NaOH, AnalaR, Merck Ltd, Poole, Dorset, UK) 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 electrolysis of an aqueous solution of CuSO₄ (25% w/v, AnalaR, Merck) and the amount of oxygen consumed is directly proportional to the power required to produce replacement oxygen by electrolysis.

After a 24-day acclimation period on 70:30 industrial:domestic mixed feed, the pots were fed 100% industrial wastewater and the influents to all but one pot were supplied with phosphorus (orthophosphoric acid, AnalaR, Merck) and micronutrients as set out in Table 1. The trials proceeded for a further 48 days.

Experiments were performed in a series of tests, each with its own control porous pot. Data were normalised so that each control was reported as 100%, with the experimental pot data reported as a percentage of the concurrent control. After primary data analysis, composite samples of selected effluents were taken over 3 days and tested for toxicity to nitrifying bacteria. Some of the effluents produced were subjected to 2-h toxicity tests, using an Amtox™ (PPM Ltd, Sevenoaks, Kent, UK, Ammonia Toxicity Moni-

tor Version 1.01 F), with 80 ml immobilised nitrifying cultures (PPM Ltd, Wild Type Immobilised Nitrifiers) and a baseline removal limit of 60%. A new culture was used to test each effluent to avoid artefacts arising from acclimation of the bacteria to the effluent. As the Amtox™ uses ammonia removal efficiency of nitrifiers to measure toxicity, the results indicate the potential impact the wastewater may have on a sewage treatment works. Amtox™ has been described in the literature [28]. 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.

Results

The data were used to calculate substrate removal efficiencies of each reactor as removal rates per unit MLSS and percentage removal of the influent oxygen demand. The MLSS in the porous pots and the oxygen uptake rates of the sludge per unit MLSS were also recorded. The MLSS maintained in the treated porous pots varied (Table 2). Treated pots maintained more biomass than the controls, with the exception of the pots receiving supplements of pyridoxine and phosphorus/niacin or a phosphorus/niacin/calcium mix at the lower dose of 0.5 mg L⁻¹. Reducing sludge production is an important cost consideration, and 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). Variation in MLSS must also be taken into account during interpretation of the oxygen uptake rates (Figure 1), as the uptake data were recorded as kg O₂ kg MLSS⁻¹ d⁻¹, and hence do not reflect the oxygen demand of a fixed volume of mixed liquor. 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/1.0 mg L⁻¹ 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 than 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.

The reactor performance was assessed in terms of the removal of BOD₅ 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 BOD₅ by any of the reactors. After 20 days of micronutrient supplements, some scatter was seen in the BOD₅ removal

Table 2 Summary data for all tests

Parameter	Measurement	Mean control	Phosphorus and niacin 1.0 mg L ⁻¹	Phosphorus, calcium and niacin 1.0 mg L ⁻¹	Pyridoxine	Phosphorus and pyridoxine	Phosphorus, molybdenum and lactoflavin	Phosphorus	Phosphorus and niacin 0.5 mg L ⁻¹	Phosphorus, calcium and niacin 0.5 mg L ⁻¹
MLSS (mg L ⁻¹)	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	298.88	715.84	227.27	208.29	478.38	183.10	435.78	198.96	248.17
Respiration rate (kg O ₂ kg MLSS L ⁻¹ d ⁻¹)	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 removal efficiency (%)	Max	78	92	94	52	97	94	95	89	93
	Min	64	88	36	34	91	86	25	38	22
	Mean	72	90	78	38	94	91	75	74	69
	SD	3.87	0.93	14.41	4.31	1.17	1.68	17.64	14.98	21.22
COD removal efficiency (%)	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	3.46	7.27	3.92	4.39	3.38	1.69	9.06	7.60	7.75

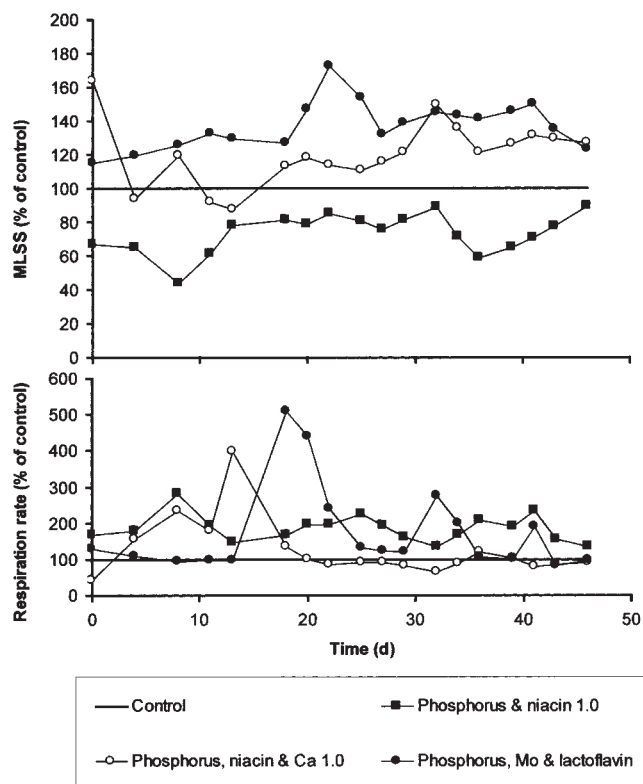


Figure 1 MLSS and oxygen uptake as a percentage of the concurrent control.

efficiencies of the porous pots (Figure 2). The best results in terms of sustained improvements in BOD₅ removal efficiency were observed in the reactors dosed with phosphorus/1.0 mg L⁻¹ niacin (Figure 2) (paired *t*-test, *df* = 26, *t* = 0.43). Slightly less of the influent BOD₅ was removed in porous pot receiving pyridoxine alone than the control (Table 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 BOD₅ removal, but similar mean values and a more vari-

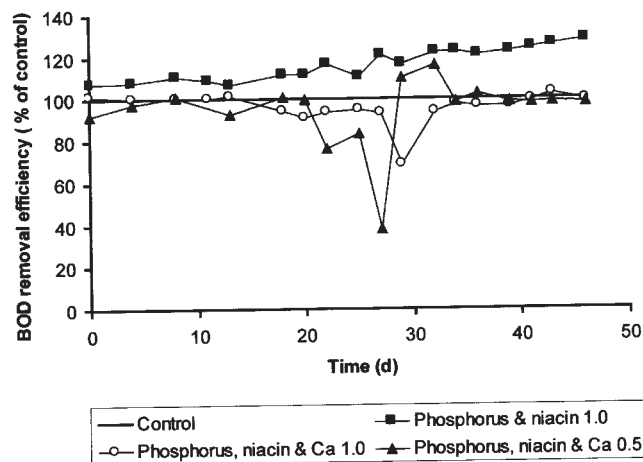


Figure 2 BOD removal efficiencies attained by the reactors, expressed as a percentage of the control.

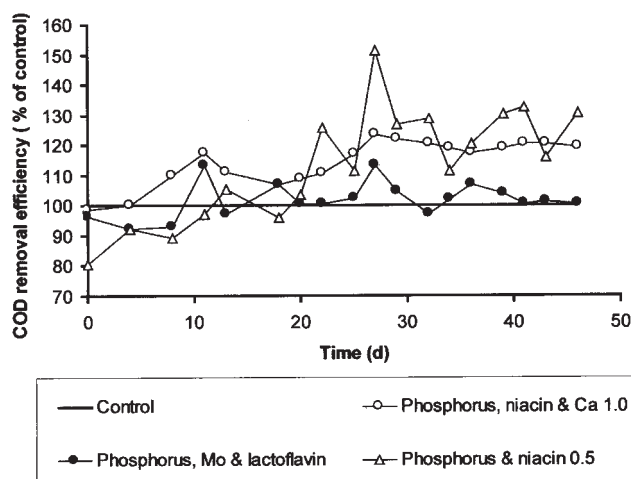


Figure 3 COD removal efficiencies attained by the reactors, expressed as a percentage of the control.

able reactor performance (therefore no significant difference was detected using *t*-test analysis) (Figure 2); these effluents were tested for toxicity to investigate possible relationships between BOD₅ and toxicity removal.

The reactor supplied with phosphorus/niacin/calcium at 1.0 mg L⁻¹ performed significantly better in terms of mean COD removal than the others during the dosing period (paired *t*-test, *df* = 26, *t* = 0.36) (Figure 3); the standard deviation was among the smallest of the values computed for the data sets (Table 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.

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 BOD₅. A certain amount of negative correlation was observed between toxicity reduction and ammonia concentration (Table 3), 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 AmtoxTM bacteria than the control reactor effluent samples (Figure 4). Supplements of molybdenum/lactoflavin and phosphorus/0.5 mg L⁻¹ niacin allowed the efficiency removal of cultures to remain above the 60% removal efficiency baseline throughout the test.

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 (0.0038 kg O₂ kg MLSS⁻¹ d⁻¹ for phosphorus/1.0 mg L⁻¹ niacin to 0.4566 kg O₂ kg MLSS⁻¹ d⁻¹ for phosphorus/0.5 mg L⁻¹ Ca/0.5 mg L⁻¹ niacin dosed sludge) is lower than published values using this model of respirometer (Table 4).

A number of biological factors influence respiration rates

Table 3 Characteristics of the effluents tested for toxicity

Porous pot (in order of decreasing toxicity)	Inhibition (%)	Ammonia (mg L ⁻¹)	COD (mg L ⁻¹)	BOD (mg L ⁻¹)	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 0.5 mg L ⁻¹	1	24.14	737	221	7.86
Phosphorus & niacin 0.5 mg L ⁻¹	-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

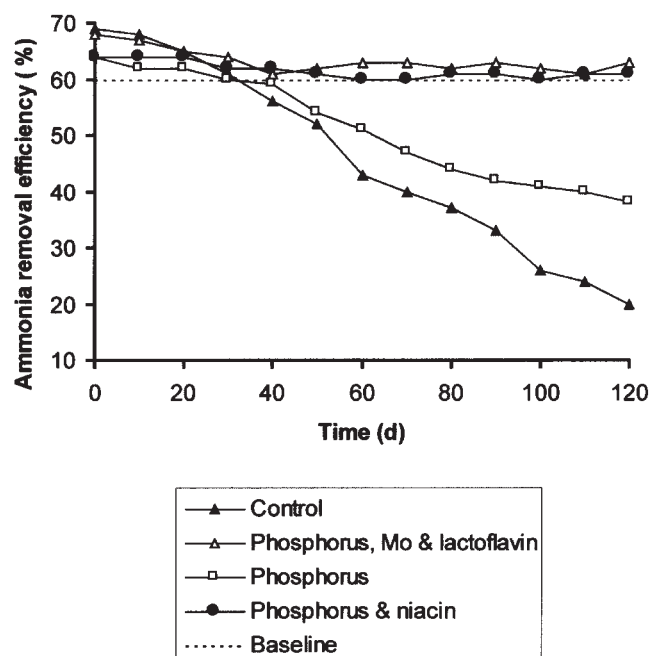


Figure 4 Toxicity of the reactor effluents plotted as ammonia removal efficiency of an immobilised nitrifying culture.

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 [21], long acclimation periods causing low oxygen uptake rates [18]. 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 acclimation of the activated sludge to wastewater and to low levels of nutrients in this wastewater. The high COD:BOD₅ ratio of the wastewater caused the loading rate in terms of BOD₅ load applied per unit MLSS to be low in comparison to the COD load; in addition, the BOD₅: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 4. Clark *et al* [4,5], using the same model respirometer, obtained oxygen uptake rates twenty times higher than those in the current study from activated sludge supplied with domestic wastewater with BOD₅:N:P of 100:17:2 (assuming 90% of available P was removed by chemical precipitation from the original ratio of 100:17:16).

Vitamins are required for the growth of organisms in activated sludge [17]. The ‘vitamin B complex’ is a group of 10 vitamins which have different effects on different microorganisms and should be considered separately. Niacin addition can improve COD removal rates, possibly because it is used in oxidative phosphorylation, the pro-

Table 4 Respiration rates of activated sludge

Respirometer	Process	Waste water	Loading rate (g BOD g SS ⁻¹ d ⁻¹)	Respiration rate (kg O ₂ kg SS ⁻¹ d ⁻¹)
Current study (range of mean rates)			0.16	0.01–0.16
Not known [1]	Aerobic batch digestion	Synthetic	0.31	0.079
Current model [4]	Activated sludge, AlSO ₄ coagulant	Domestic	0.21	0.096
Current model [4]	Activated sludge, polyelectrolyte coagulant	Domestic	0.27	0.24
Current model [5]	Activated sludge, iron (II) sulphate coagulant	Domestic	0.26	0.29
Current model [5]	Activated sludge, iron (III) chloride coagulant	Domestic	0.21	0.206
Not known [19]	Oxidation ditch	Municipal	Not known	0.094
Current model [20]	Activated sludge	Domestic	0.30	0.084

duction of cozymase and as a growth factor in bacterial cells [18,22]. Doses of niacin have been shown to produce greater metabolic activity in industrial sludges [18]. The optimum dose is reported to be 0.1 mg L⁻¹ influent [16,22], 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 [16,18].

Some activated sludges display no requirement for added lactoflavin or pyridoxine [18,22]. Pyridoxine has to be hydrolysed 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 [26]. This accounts for the results here, in which addition of pyridoxine and phosphorus/pyridoxine did not enhance COD removal.

Vitamin shortages can be expected in wastewaters from certain industries [18], 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. 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 degradation of casein hydrolysate, although the effects on COD removal were not reported [18]. In this case, the COD removal efficiencies of the experimental reactors were all improved by micronutrient additions, although this did not correlate directly with removal of toxicity from wastewater. Therefore COD did not equate to the toxicity of wastewaters to nitrifying bacteria, and the operation of unit processes discharging to nitrification systems should focus not only on BOD₅ 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.

Increased COD removal with no associated increase in BOD₅ removal indicates enhanced degradation of recalcitrant COD. This was observed in the pot dosed with phosphorus/0.5 mg L⁻¹ calcium/0.5 mg L⁻¹ niacin. Toxicity of the pot effluents to nitrifying bacteria, as measured by AmtoxTM was removed by addition of phosphorus/0.5 mg L⁻¹ niacin and molybdenum/lactoflavin. The results indicate the potential of the vitamins niacin and lactoflavin, and the trace elements calcium and molybdenum for reducing the toxicity of effluents from biological wastewater treatment processes dealing with recalcitrant organic wastewater.

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 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.

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