Investigation into a biosupplement for possible reduction of activated sludge production in a system with excess biological phosphorus removal

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Abstract

The availability and use of so-called "biosupplements" (or biological "catalysts") in wastewater treatment systems has increased significantly in recent years. The effectiveness of adding either live cultures of micro-organisms produced commercially (or enzyme products from such cultures) to systems which naturally develop mixed and complex populations of many different micro-organisms (e.g. activated sludge) may be questioned. Comparatively few studies have been published in which the commercially-produced cultures have been tested under controlled conditions. This study was aimed at conducting a controlled study of one "liquid live micro-organism" (LLMO) product in a nitrification-denitrification biological enhanced phosphorus removal (NDBEPR) activated sludge system. The product was marketed by the supplier for its ability to reduce sludge production in activated sludge systems. Using two parallel pilot-scale NDNEPR activated sludge systems operated in parallel under identical conditions, one with and one without the addition of the LLMO product, this study found no evidence to support the supplier's claim for the product. Two experiments were conducted: one in which the LLMO product was dosed in relatively large amounts without prior aerobic activation, and one in which activation was carried out aerobically for 24 to 36 h, as specified by the supplier. For both experiments, satisfactory mass balances for the systems could be shown, but no statistical difference in sludge production was observed, despite dosing at least ten times more product than that specified by the supplier for typical full-scale applications. Performance of the test and control systems was also virtually identical in all other respects.

Introduction

Biocatalysts (or so-called biosupplements) have been marketed and used in wastewater treatment processes for a number of years with varying degrees of success. According to Koe and Ang (1992), results from the commercial application of such biocatalysts, particularly in wastewater treatment systems facing operational problems, have tended to be positive. However, laboratory research investigations have often contradicted these results and many researchers have concluded that no significant improvement in process performance can be achieved with biosupplementation (Kunst, 1989; Koe and Ang, 1992).

On the simplest level of wastewater treatment processes, some manufacturers claim that bio-enhancement technology can improve the performance of oxidation ponds (Ascough, 1997). Other products use either enzymes or live bacterial cultures to accelerate degradation of fats, greases, proteins and carbohydrates (including cellulose) in structures such as septic tanks, pipes, greases traps, and sumps (Jager et al., 1989; Mylie, 1990; Beams and Burns, 1991). Koe and Ang (1992) investigated a biocatalyst which had reportedly improved performance of full-scale anaerobic digesters. They identified the bacterial flora present in the biocatalyst and found that, with the exception of certain strict aerobes, all were facultative anaerobes (typically acid-forming fermenters) which were shown to exist as part of the natural bacterial flora in a normal anaerobic digester without biocatalyst addition. Since no significant shift in bacterial population was detected in either the control or augmented laboratory reactors, it was concluded that the commercial biocatalyst is unlikely to improve the performance of the digestion process (Koe and Ang, 1992).

In activated sludge systems, there have been commercially-oriented reports of bacterial cultures or mixtures of cultures which could be useful in improving biodegradation of specific target effluents. For example, particular attention may be directed to situations where consistent nitrification is the objective, but has proved difficult to achieve in cold climates. The addition of pure cultures of nitrifying organisms was reported to significantly improve nitrification within 10 d (Anonymous, 1991). Similarly, Glancer et al. (1994) cited the use of denitrifying organisms *Moraxella* and *Corynebacterium* for improving denitrification on a full-scale biological nutrient removal activated sludge plant in Salzburg (Austria). Glancer et al. (1994) also cited the use of a mixed culture containing the yeast *Trichosporon*, which produces increased amounts of the enzyme peroxidase, for accelerated degradation of complex refractive molecules such as lignins, and mixed cultures of *Pseudomonas*, *Flavobacterium* and *Bacillus* spp. for improved biodegradation of arylsulphonic acids (used as auxiliaries in tanning, paper and textile industries). However, a criticism of the work of Glancer et al. (1994) may be that the use of suitable controls was not reported. Potentially, this may be a common error in research where a number of variables could influence the observed result. Vansever et al. (1997) drew attention to the need for controlled experiments when investigating the potential effects of supplements to activated sludge systems. Since it is rare to have identical but separate systems in parallel for test and control purposes at full scale, such experiments are usually conducted on small-scale systems. The latter allow statistical analysis of the data obtained. From their experiments with a nutritive supplement containing grain supplements, ferrous sulphate and alumino

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silicates as principal constituents (by mass) along with magnesium sulphate, calcium phosphate and manganese sulphate, Vansever et al. (1997) concluded that improved sludge settleability, lower effluent COD and stimulated nitrification resulted from the addition of the supplement to laboratory-scale aerobic activated sludge systems with synthetic wastewater (based on powdered milk) or industrial wastewater as influent. Since both types of influent were balanced in mineral nutrients, Vansever et al. (1997) found it unlikely that the effect of the supplement would be primarily due to the addition of N, P or trace elements. Instead, they drew attention to the large part of the COD in the supplement being in particulate form which could constitute a nucleus for aggregation, thereby improving flocculation. Vansever et al. (1997) did not draw specific attention to the high content of ferrous ions in the supplement. In an aerobic system, rapid oxidation of the ferrous ions to ferric form may be expected (Singer, 1972). The chemical coagulation properties of ferric ions are very well known in water and wastewater treatment systems, and the doses used by Vansever et al. (1997) amounted to approximately 4 mg Fe/ℓ, which would be significant as a simultaneous precipitant in an activated sludge system (e.g. Yeoman et al., 1988; De Haas et al., 1996).

The aim of this paper is to present the results of an eight-week trial in which a commercial biosupplement was tested at a pilot plant in a biological excess P removal (BEPR) activated sludge system. This particular biosupplement was selected since it was claimed by the supplier to be useful in reducing sludge production in activated sludge systems (Becker, 1997; Dorfling 1998). BEPR activated sludge systems (i.e. incorporating an anaerobic reactor) are known to produce greater quantities of both volatile and total suspended solids (VSS and TSS) than conventional activated sludge systems, on a comparative basis (Wentzel et al., 1990).

Materials and methods

Pilot plants

Two identical units (R1 and R2) were set up in the three-stage Phoredox configuration and operated at 20 (±1) °C, with a sludge age of 10 d. The reactor configuration was as follows: anaerobic (AN) reactor (8 ℓ); anoxic (AX) reactor (4 ℓ); first aerobic (AE1) reactor (10 ℓ); second aerobic (AE2) reactor (10 ℓ); clarifier (2.5 ℓ); target influent flow rate (Q_i) = 36 ℓ/d ; s-recycle ratio from clarifier to AN reactor = 1:1; a-recycle ratio from AE2 to AX reactor = 3:1. The influent consisted of settled sewage pumped from the balancing tank at Darvill Wastewater Works (WWW) in Pietermaritzburg (South Africa). Batches of sewage (160 ℓ) were prepared every second day, stored at 4°C and served as common feed to both Unit R1 and Unit R2. Each sewage batch was augmented with the following constituents: sodium acetate (100 mg/ℓ as COD); magnesium chloride (84 mg Mg/ g COD as acetate added). In some cases, sodium bicarbonate was added for alkalinity supplementation (100 mg/ℓ as $CaCO_3$) in order to maintain a median pH in the test units in the range 6.9 to 7.4. In Experiment 2 only (see below), phosphate was added to the influent (common to both units) at a concentration of 10 mgP/ℓ (as K_2HPO_4).

The reactor and clarifier interior surfaces were brushed every day and the appropriate volume of mixed liquor (3.2 ℓ) was wasted daily from the AE2 reactor to maintain a sludge age of 10 d. Oxygen uptake rates (OUR) were measured in the AE2 reactors by means of OUR meters (Randall et al., 1991). The air supply to both aerobic reactors was controlled so that the air pumps switched on at a dissolved oxygen (DO) concentration of 3.0 mg/ℓ and switched off at a DO of 5.0 mg/ℓ. Approximately once a fortnight, the mixed liquor of both units was passed through a sieve (kitchen type, with a mesh size of ca. 0.5 mm) to remove lumps from the mixed liquor which otherwise may have caused blockages of the pipework in the system (dia. <5 mm in some places). In some cases, whitish lumps were caught on the sieve and identified mainly as dense colonies of the protozoan *Vorticella*; in other cases, blackish lumps were found to contain larvae of pyschodid flies. The mixed liquor was always saved in such cases and returned after the reactors had been washed. Pump tubing lines were cleaned daily by means of squeezing or brushing.

Figure 1 shows a layout of the pilot plants. Pilot plant Unit R1 served as the test system (dosed with biosupplement), while Unit R2 served as the control.

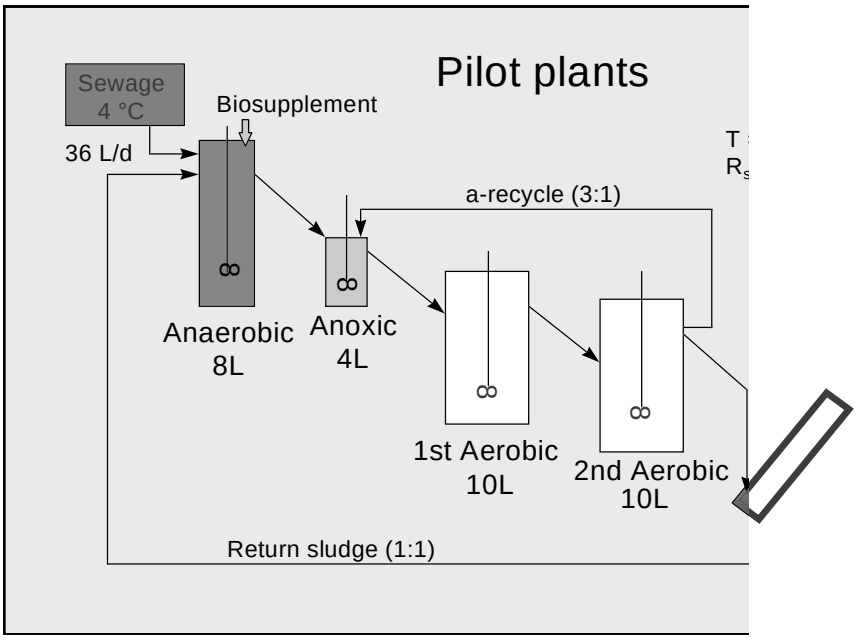

Figure 1
Layout of three-stage Phoredox pilot plants

Biosupplement

The product tested in this study was LLMO-S1 supplied by Amitek Environmental Engineering (Becker, 1997; Dorfling, 1998). LLMO denotes "live liquid micro-organisms" (Becker, 1997). This product is described by the supplier as consisting of "bacteria specially selected for their ability to produce extracellular enzymes". According to the supplier, these extracellular enzymes serve to solubilise colloidal and particulate material in sewage/activated sludge systems and hence to reduce sludge production. It is claimed by the supplier that many bacterial species produce extracellular enzymes in appreciable quantities while growing and reproducing in the presence of soluble substrate; it is claimed that bacterial starvation induces greater production of the enzymes required. The starved bacteria are then activated by means of the addition of nutrients, and aeration for 24 to 36 h at room temperature (Becker, 1997). The active bacteria are then added to the wastewater treatment system, where the effects of the enzyme may be exerted.

Experiments using biosupplement

Two experiments were conducted using the AMITEK LLMO-S1 product.

In **Experiment 1**, the product was added to Unit R1 in large excess of the required dose. In this experiment, the LLMO-S1 bacterial product was exposed to nutrients during the 24 h "activation" stage but in the **absence of aeration**. Hence, for Experiment 1, the protocol used was:

- 400 mℓ LLMO-S1 (bacterial product) and 100 mℓ "activator" (nutrient solution), both supplied by *Amitek* (Johannesburg) were initially added to 2 ℓ of tap water in a 3 ℓ glass reactor termed the activation tank.
- The mixture was gently stirred for 24 h at a temperature of 26 (±2)°C without aeration.
- After 24 h, 400 mℓ of the "activated/ unaerated" mixture was dosed to Unit R1 (anaerobic reactor). Dosing took place over 23 h by means of a slow feed system using a Gilson peristaltic pump fitted with tubing of suitable diameter.
- Every second day, the activation tank was refilled with 640 mℓ tap water, 128 mℓ LLMO-S1 and 32 mℓ "activator", while 400 mℓ of the mixture was withdrawn every day and dosed into the Unit R1 (anaerobic reactor).
- This procedure amounted to dosing 400 mℓ/d of "activated/ unaerated" product consisting of 64 mℓ/d LLMO-S1 and 16 mℓ/d "activator" (nutrient) solutions, and the balance tap water. It was estimated (Becker, 1997), that this represented an excess of approximately 1400-fold of the required dose of LLMO-S1 product for full-scale operating plants, based on the normal method of activation with aeration.

In **Experiment 2,** the normal procedure was followed for the activation of the LLMO-S1 product under aerobic conditions (i.e. **with aeration**) in the presence nutrients (i.e. "activator" solution). Due to the relatively small scale of the pilot plants (Fig. 1), for this experiment it was decided to dose ten times the amount of LLMO-S1 compared to that required for full-scale operating plants (Becker, 1997). In this manner, a more practical daily dose volume (3.0 mℓ/d) was applicable to the pilot plants. Moreover, it was anticipated that the higher dose would exaggerate any effect which the product might have on sludge production in the pilot plants, and hence allow an assessment to be made of whether or not a full-scale trial at more realistic doses should be attempted. The daily protocol for this experiment was as follows:

- Two 1 ℓ glass beakers served as activation vessels. These were "activated" on alternate days, so that on any given day, one served as the source of activated mixture for dosing to the pilot plant, while the other was undergoing activation for 24 to 36 h.
- To activate each batch, 75 mℓ LLMO-S1 and 35 mℓ "activator" solution were added to 390 mℓ of tap water, and the mixture was aerated for between 24 h and 36 h. Aeration was carried out at room temperature (20 ± 2°C) using an aquarium air pump via a small ceramic diffuser stone.
- After 24 h aeration, 1.5 mℓ of the activated mixture was withdrawn and dosed at once to the anaerobic reactor of Unit R1. After a further 8 to 12 h aeration (i.e. total 32 to 36 h aeration), a second aliquot (1.5 mℓ) of the mixture was withdrawn and dosed to the same reactor of Unit R1. On weekends, 3.0 mℓ of activated mixture was dosed in the same manner, but only once per day (i.e. after 24 h activation).
- After dosing the required aliquot(s) from an "activated" batch, the remainder of the batch was discarded and a new activation cycle commenced the following day, as described above.

Measurements

OUR was measured automatically, as described by Randall et al. (1991). Flow rates were recorded daily and the pump speeds adjusted accordingly to achieve average flows of 1.5 ℓ/h (± 0.05) as far as possible.

All chemical and physical parameters measured were in accordance with *Standard Methods* (1985). The only exceptions were: dilute sludge volume index (DSVI), which was measured according to Ekama and Marais (1984); and COD, which was measured both by the open reflux method in *Standard Methods* (1985) and a microwave digestion method, followed by automated potentiometric titration (Slatter and Alborough, 1990). It was found that the open reflux method gave poor recoveries (56 to 66%) of sodium acetate COD, both from pure solutions and in admixture with settled sewage, possibly due to the reflux condenser length (50 cm) being inadequate. Better recoveries (90 to 105%) were obtained by the microwave method which uses closed reflux in teflon pressure vessels (De Haas, 1998). In summary, the parameters routinely measured were:

- Chemical oxygen demand (COD) (influent, effluents)
- Total Kjeldahl Nitrogen (TKN) (influent, effluents)
- Soluble reactive phosphate (SRP)
- Total phosphate (total P) (effluents and filtered AN, AX, AE1, AE2 reactors)
- Soluble ammonia (influent and effluents)
- Soluble nitrate (effluents, filtered AN, AX, AE1, AE2 reactors)
- Mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) (mixed liquors)
- DSVI (mixed liquors)
- pH (in AN, AE1, AE2 reactors)
- Oxygen uptake rate (OUR) (AE2 reactor)

A limited number of samples of batches of "activated" biosupplement mixture were analysed for COD, TKN and total P.

TABLE 1
SUMMARY RESULTS FOR EXPERIMENT 1.
BIOSUPPLEMENT (LLMO-S1) WAS DOSED TO PILOT PLANT UNIT R1; UNIT R2 WAS THE CONTROL.
INF = INFLUENT; EFF = EFFLUENT; *f* = FILTERED; AN = ANAEROBIC; AX = ANOXIC; AE1 = FIRST AEROBIC; AE2 = SECOND AEROBIC REACTOR; N = NO. OF OBSERVATIONS; SD = SAMPLE STANDARD DEVIATION; MIN. = MINIMUM; MAX. = MAXIMUM. ALL RESULTS IN mg/ℓ, UNLESS OTHERWISE INDICATED.

Determinand	n R1	Mean R1	Min. R1	Max. R1	SD R1	n R2	Mean R2	Min. R2	Max. R2	SD R2
FLOW, ℓ/d *	46	**35.8**	33.8	37.4	0.7	47	**36.2**	33.8	37.7	0.7
COD, INF	47	**366**	191	544	78	47	**366**	191	544	78
COD, EFF	47	**49**	28	88	11	47	**47**	26	70	9
TKN, INF	47	**27.1**	20.0	37.8	4.2	47	**27.1**	20	37.8	4.2
TKN, EFF	47	**3.3**	2.0	7.27	1.43	47	**3.4**	2.0	7.58	1.54
SRP, EFF	46	**1.60**	0.12	7.30	1.80	46	**0.86**	<0.1	5.65	1.18
NH_3-N, EFF	46	**0.53**	<0.5	3.28	0.62	46	**0.38**	<0.5	2.64	0.43
NO_3-N, EFF	46	**8.11**	3.15	13.70	2.56	46	**7.22**	2.87	13.00	2.47
f NO_3-N, AN	18	**<0.5**	<0.5	0.5	-	18	**<0.5**	<0.5	<0.5	-
f NO_3-N, AX	18	**4.55**	3.04	6.52	0.98	18	**3.89**	2.29	6.09	1.06
f NO_3-N, AE1	18	**7.90**	4.59	12.10	2.05	18	**7.11**	4.15	11.70	2.03
f NO_3-N, AE2	18	**8.24**	4.72	13.10	2.23	18	**7.52**	4.10	12.60	2.20
MLSS, mg/ℓ	46	**1316**	983	1660	161	46	**1283**	1003	1682	165
VSS, mg/ℓ	46	**923**	660	1188	123	46	**910**	642	1208	125
DSVI, mℓ/g	45	**304**	74	791	224	45	**273**	77	624	172
OUR, mg/(ℓ·h)	58	**11.07**	7.86	14.78	1.73	57	**11.77**	7.86	15.86	1.96
Temp., °C	58	**19.4**	18.5	20.8	0.4	57	**19.5**	18.5	21.8	0.5
pH, AN	40	**7.24 #**	6.96	7.47	-	40	**7.19 #**	7.00	7.51	-
pH, AE1	40	**7.31 #**	7.04	7.56	-	40	**7.28 #**	7.06	7.51	-
pH, AE2	40	**7.48 #**	7.08	7.69	-	40	**7.48 #**	7.21	7.63	-

# denotes *median* in place of mean.
* Flow to R1 was corrected for volume of biosupplement mixture dosed (i.e. 0.4 ℓ/d subtracted).

Results and discussion

Experiment 1

Experiment 1 was conducted over an eight-week period from 8 April to 12 June 1997. During the preceding period extending over six months, the pilot plants had been operated using the same influent composition, although small differences may have arisen due to variations in the source settled sewage from Darvill WWW.

The results for Experiment 1 are given in Table 1. The nitrogen mass balances for this experiment were acceptable: 110% for Unit R1 and 108% for Unit R2. The COD mass balances for this experiment were 72% and 74% for Unit R1 and Unit R2 respectively. The COD mass balances were <100%. This could be partly due to the fact that OUR was only measured in the second aerobic reactor, and the same OUR was assumed to apply to the first aerobic reactor for the purposes of the mass balance calculation. The UCTPHO model of Wentzel et al. (1992) was used to estimate the OUR in the first aerobic reactor. The model predicted that the OUR in the first aerobic reactor to be approximately 50% higher than that in the second aerobic reactor. Applying this correction to the mass balance calculations improved the result to between 83% (Unit R1) and 85% (Unit R2). The failure to achieve complete mass balance further suggests that true steady-state was probably not achieved during this experimental period. The VSS concentrations showed an overall increasing trend through the experiment (Fig. 2). This may have been due to a gradual increase in the influent COD from the Darvill WWW settled sewage, which typically occurs at the transition from the wet season (summer, ending April) to the dry season (winter, commencing May/June) at this works. The observed trend in influent COD (Fig. 2) shows that this was the case.

Despite the indication from mass balance data that steady-state conditions had not been completely achieved, it is permissible to compare the results for sludge production (VSS and TSS) since the two units were operated in parallel under identical conditions. From the results in Table 1, it can be seen that the performance of the two pilot plants (Unit R1 and R2) was virtually identical with respect to all determinands. The MLSS and VSS of Unit R1 was slightly greater than that of Unit R2, but the differences were small, namely 33 mg/ℓ MLSS and 13 mg/ℓ VSS, respectively. This represented <3% of the mean MLSS and VSS. The differences between the mean MLSS and VSS in the two units were not statistically significant at the 95% confidence level when tested using the "Student t" method. Nevertheless, it is possible that the slightly greater COD load imposed by the biosupplement mixture dosed to Unit R1 may have contributed to the slightly greater VSS and MLSS production in that unit.

The COD of the activated LLMO-S1 biosupplement mixture was measured and found to be approximately 455 mg/ℓ. This represents an additional COD load of approximately 182 mg/d. Based on an assumption of 13% unbiodegradable particulate COD and an unbiodegradable soluble COD fraction of 25% (high

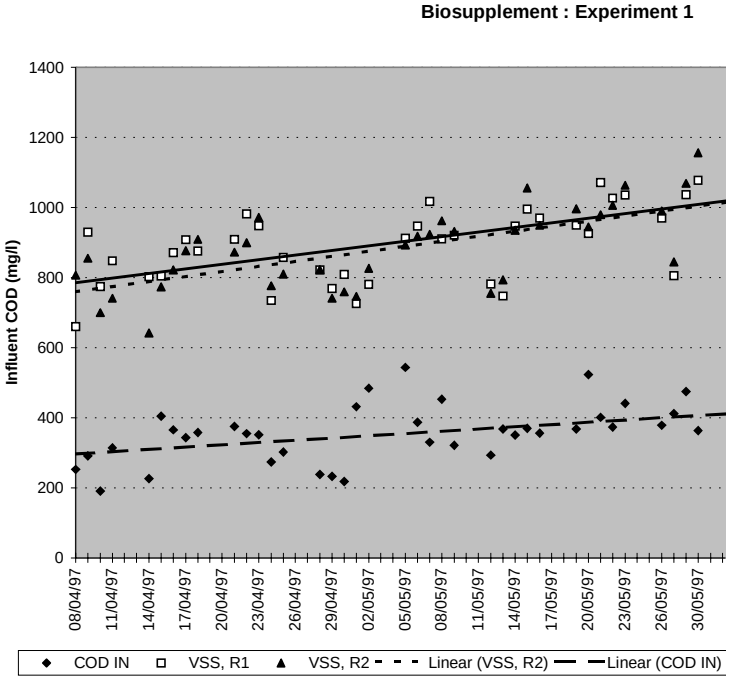

Figure 2
Time plots of influent COD and second aerobic reactor VSS concentrations in the pilot plants during Experiment 1

due to the apparent presence of molasses in the "activator" solution), and using the steady state calculations described by WRC (1984), it may be estimated that this additional COD load would contribute approximately 12 mg/ℓ additional VSS to a system with a 10 d sludge age and a process volume of 32 ℓ. The observed difference in mean VSS between the two units was 13 mg/ℓ (Table 1), which correlates well.

Similar to the additional COD load, the biosupplement mixture appeared to contribute slightly to the phosphate and nitrogen loads of Unit R1, judging from increases of approximately 0.8 mgP/ℓ and 0.9 mgN/ℓ in the mean effluent SRP and nitrate concentrations respectively (Table 1).

The DSVI in both Unit R1 and Unit R2 was high during Experiment 1 (Table 1). This indicated that a sludge with a tendency to bulk emerged in both units. The problem was slightly worse in Unit R1 than Unit R2, judging from the DSVI data. However, sludge carryover into the effluent did not occur in either unit since the clarifiers continued to function satisfactorily at the relatively low MLSS concentrations which prevailed in the systems.

In summary, Experiment 1 showed that the biosupplement at high doses (developed without aeration during activation) had no beneficial effect on the operation of the test pilot plant, relative to the control. The observed differences were either too small to be statistically significant, or could be attributed to the additional carbon or nutrient load posed by the biosupplement-activator mixture.

Experiment 2

Three weeks prior to the commencement of Experiment 2, both pilot plant units had been seeded with activated sludge (16 ℓ per unit) from the aerobic area of the Darvill WWW full-scale plant. Experiment 2 was conducted over a ten-week period from 13 January 1998 to 27 March 1998. The dose of biosupplement LLMO-S1 was much smaller than that used in Experiment 1, but still ten times greater than that expected for full-scale applications (see **Materials and Methods**). The activation step for the biosupplement was, however, conducted to the supplier's specifications, under aerobic conditions for 24 h to 36 h.

TABLE 2
SUMMARY RESULTS FOR SELECTED DETERMINANDS FOR PHASE 1 (13/1/98 TO 10/2/98) OF EXPERIMENT 2

Determinand	Mean R1	SD R1	Mean R2	SD R2
Influent COD (mg/ℓ)	**329**	98	**329**	98
OUR (mg/ℓ·h)	**10.97**	1.46	**11.10**	1.24
MLSS (mg/ℓ)	**1400**	68	**1408**	102
VSS (mg/ℓ)	**970**	50	**971**	56

OUR: Oxygen uptake rate
SD: Standard deviation

Phase 1 of Experiment 2 extended over the first month (13 January to 10 February 1998). During this period, the mixed liquor total P content showed an increase, due to the development of the biological excess P removal capacity of the systems, in response to the relatively high acetate content of the feed. This phenomenon is well described for modified activated sludge systems (Wentzel et al., 1988). The increased P content of the mixed liquor would have contributed toward the small increase in MLSS noted for Phase 1 of this experimental period (Fig. 3). However, by the end of this period, the MLSS and VSS concentrations were tending toward stability. No differences were noted in the trends for MLSS and VSS concentrations between Unit R1 and Unit R2 for Phase 1 of Experiment 2 (Fig. 3). The mean concentrations and standard deviations for this period are given in Table 2. Similarly, no significant difference in OUR was noted for this period (Table 2).

The biosupplement LLMO-S1 had been dosed throughout Phase 1 of Experiment 2, as described under **Materials and Methods.** However, in order to rule out any bias which may have

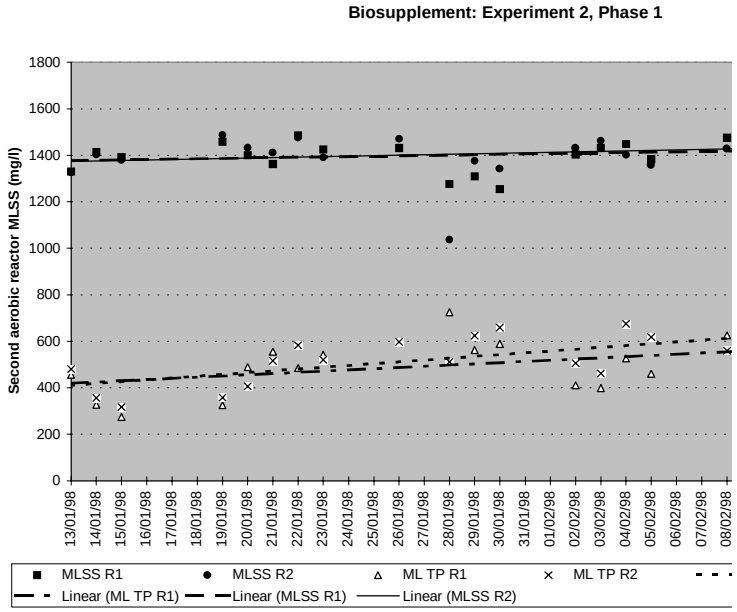

Figure 3
MLSS and mixed liquor total P concentrations in the second aerobic reactor of pilot plant units during the first month (Phase 1) of Experiment 2

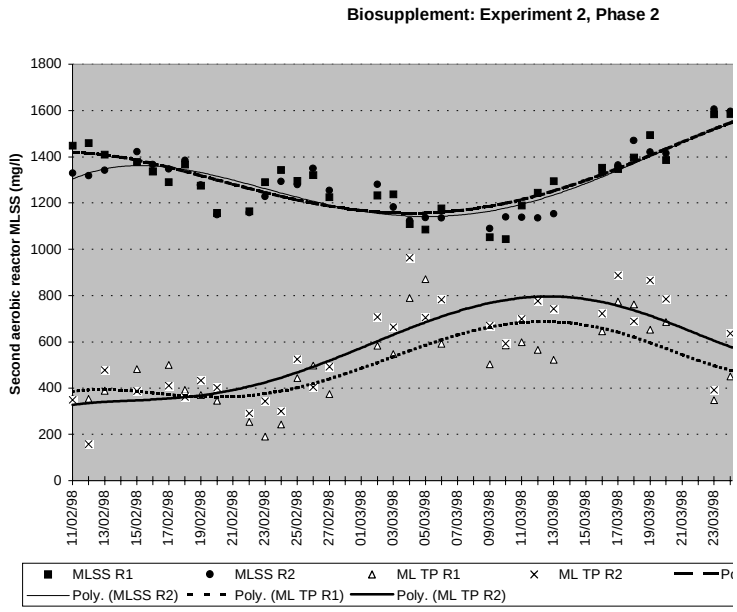

Figure 4
MLSS and mixed liquor total P concentrations in the second aerobic reactor of pilot plant units over a six-week period (Phase 2) in Experiment 2. 'Poly' implies polynomial trend-fitting function.

developed in the pilot plant units as a result of the seeding and mixed liquor development, at the end of Phase 1 the mixed liquors from the two units (R1 and R2) were pooled, mixed and equally re-divided between the two. The ensuing six-week period constituted Phase 2 of Experiment 2.

Phase 2 of Experiment 2 extended over the six weeks from 11 February until 27 March 1998. Throughout this period, Unit R1 received biosupplement addition, as described under **Materials and Methods.**

Fairly stable concentrations of MLSS and VSS were observed in the pilot plants during this period (Figs. 4 and 5). The most probable sources of variation in solids concentrations in the units were changes in the influent COD concentration (Fig. 5) and composition (not determined), arising from changes in the source sewage reaching Darvill WWW. During summer (e.g. the month of February 1998 - see Fig. 5), this works often receives a significant ingress of rain water and groundwater due to problems in the city's sewer reticulation system, which results in dilution of the influent COD. Figures 4 and 5 show that VSS and MLSS concentrations followed a trend in response to the trend in influent COD concentration, but delayed by approximately 10 d (one sludge age), as would be expected. The other factor influencing MLSS concentrations would have been bio-P removal. Although the bio-P removal processes respond relatively quickly to changes in influent composition (Wentzel et al., 1990), the total P content of the mixed liquor will also be subject to sludge wasting from the system; that is, a delayed response to changes in influent COD may also be expected for the MLSS due to this effect (Figs. 4 and 5).

Comparing the relative responses of Units R1 (with biosupplement) and R2 (without biosupplement) in Figs. 4 and 5, it may be seen that the two systems behaved almost identically. This was expected since the systems had a common sewage source and were fed at closely similar flow rates. These results imply that the biosupplement had no observable effect on sludge production in the test system.

TABLE 3
SUMMARY RESULTS FOR EXPERIMENT 2 (PHASE 2).
BIOSUPPLEMENT (LLMO-S1) WAS DOSED TO PILOT PLANT UNIT R1; UNIT R2 WAS THE CONTROL.
INF = INFLUENT; EFF = EFFLUENT; *f* = FILTERED; AN = ANAEROBIC; AX = ANOXIC; AE1 = FIRST AEROBIC; AE2 = SECOND AEROBIC REACTOR; N = NO. OF OBSERVATIONS; SD = SAMPLE STANDARD DEVIATION; MIN. = MINIMUM; MAX. = MAXIMUM. SRP = SOLUBLE REACTIVE (ORTHO)PHOSPHATE. ALL RESULTS IN mg/ℓ, UNLESS OTHERWISE INDICATED.

Determinand	n R1	Mean R1	Min. R1	Max. R1	SD R1	n R2	Mean R2	Min. R2	Max. R2	SD R2
FLOW, ℓ/d	42	**35.6**	15.6*	40.1	3.3	43	**35.7**	15.6*	39.1	3.3
COD, INF	32	**292**	160	408	66	32	**292**	160	408	66
COD, EFF	32	**38**	10	69	11	32	**38**	10	72	14
TKN, INF	30	**21.5**	8.8	35.0	5.9	30	**21.5**	8.8	35.0	5.9
TKN, EFF	32	**2.2**	<3.0	5.8	0.8	32	**2.8**	<3.0	3.5	0.4
TP, INF	31	**14.90**	11.73	20.57	2.10	31	**14.90**	11.73	20.57	2.10
TP, EFF	32	**3.37**	1.23	10.76	2.29	32	**2.98**	0.99	15.39	2.94
SRP, EFF	32	**2.91**	0.73	9.79	2.29	32	**2.49**	0.49	13.42	2.74
NH_3-N, EFF	32	**1.06**	<0.5	3.27	0.82	32	**0.61**	<0.5	2.46	0.51
NO_3-N, EFF	32	**5.48**	3.44	9.05	1.43	32	**5.10**	3.07	8.29	1.33
f NO_3-N, AN	12	**<0.5**	<0.5	<0.5	-	12	**0.28**	<0.5	0.6	-
f NO_3-N, AX	12	**3.29**	1.95	5.19	0.88	12	**2.88**	1.33	5.10	0.87
f NO_3-N, AE1	12	**5.46**	3.55	8.29	1.52	12	**5.00**	2.86	7.62	1.27
f NO_3-N, AE2	12	**5.75**	3.51	8.80	1.65	12	**5.17**	2.84	7.82	1.45
f SRP, AN	12	**40.11**	28.05	52.20	7.39	12	**41.82**	27.14	52.80	7.99
f SRP, AX	12	**15.78**	10.30	20.94	3.21	12	**15.77**	9.69	22.10	3.88
f SRP, AE1	12	**7.59**	4.11	12.40	2.11	12	**6.82**	3.67	9.46	1.73
f SRP, AE2	12	**2.92**	1.08	7.73	1.77	12	**2.14**	0.77	6.32	1.49
MLSS, mg/ℓ	34	**1313**	1044	1620	145	34	**1301**	1090	1606	140
VSS, mg/ℓ	34	**841**	659	1061	100	34	**829**	662	1065	106
DSVI, mℓ/g	33	**102**	80	138	17	33	**112**	90	166	17
OUR, mg/(ℓ·h)	41	**8.86**	6.24	11.02	1.6	41	**9.69**	5.79	12.77	2.07
Temp., °C	41	**20.1**	19.5	20.6	0.3	41	**20.2**	19.1	21.0	0.4
pH, AN	31	**6.91 #**	6.8	7.21	-	31	**6.91 #**	6.75	7.19	-
pH, AE1	31	**7.16 #**	7.03	7.56	-	31	**7.09 #**	6.97	7.6	-
pH, AE2	31	**7.4 #**	7.12	7.86	-	31	**7.35 #**	7.2	7.88	-

# denotes *median* in place of mean.
* Low flow to both units on 21/2/98 due to power failure.

A comprehensive set of results for Phase 2 of Experiment 2 is given in Table 3. The nitrogen mass balances for this period were 101% for Unit R1 and 100% for Unit R2. Using the data in Table 3, the COD mass balances were 80% and 84% for Unit R1 and Unit R2 respectively. When applying an estimate for the OUR in the first aerobic reactor (not measured) obtained using the UCTPHO model (Wentzel et al., 1992), the COD mass balances improved to 90% and 95% respectively for Units R1 and R2. This suggests that steady state conditions were closely approached during Phase 2 of Experiment 2. This is also reflected in the phosphorus mass balances, namely 99% and 98% for Unit R1 and Unit R2 respectively.

Despite the relatively good mass balances, variation in the influent COD did occur and may be expected to reflect in the performance of both systems. The closely similar results for the two units allows comparisons to be drawn between them.

From Table 3 it can be seen that the mean MLSS and VSS of Unit R1 were slightly higher than those of Unit R2 for Experiment 2 (Phase 2) as a whole. However, the difference in the means between the units amounted to only 12 mg/ℓ (for both MLSS and VSS). Using the "Student t" method, it was found that this difference was not statistically significant at a 95% confidence level.

The biosupplement mixture dosed to Unit R1 was analysed several times for COD, TKN and total P. The results are given in Table 4. Based on these results and the daily dose of 3.0 mℓ/d of biosupplement mixture to Unit R1, it can be calculated that the additional COD load to this unit was a mere 1.26 mg/d. Against a mean COD load of ca. 10 400 mg/d from the influent sewage, the COD load from the biosupplement may be considered to be negligible (0.01%). Similarly, the additional N and P loads amounted to 0.4 mgN/d and 0.3 mgP/d respectively, which are also negligible amounts (0.05%), compared to the mean N and P loads from the influent sewage.

It is worth noting that the mean effluent total P (and SRP) as well as nitrate concentrations from Unit R1 were slightly higher (ca. 0.5 mgP/ℓ or 0.5 mgN/ℓ) than those for Unit R2 (Table 3). Again, these differences were probably not statistically significant since they fall within the 95% confidence interval of the means. Nevertheless the possibility cannot be ruled out that the biosupplement resulted in minor changes in the activated sludge microbial ecology of Unit R1, which in turn, may have resulted

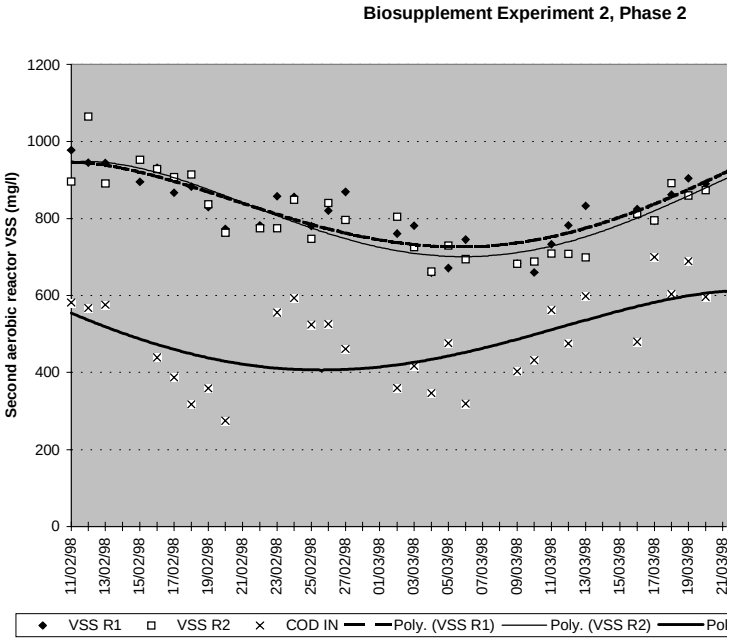

Figure 5
MLVSS and influent COD concentrations in the second aerobic reactor of pilot plant units over a six-week period (Phase 2) in Experiment 2. 'Poly' implies polynomial trend-fitting function.

TABLE 4
RESULTS OF ANALYSIS FOR BIOSUPPLEMENT MIXTURE DOSED TO UNIT R1 DURING EXPERIMENT 2.

Determinand	n	Mean	SD
COD, mg/ℓ	8	420	89
TKN, mgN/ℓ	8	137	40
TP	8	93	11

n: no. of observations
SD: Standard deviation

in weakened denitrification and biological P removal potentials, compared to the control (Unit R2).

Settling properties of the activated sludge mixed liquor, as measured by the DSVI parameter, were very similar in the two units. Judging from the DSVI data (Table 3), settling was marginally better in Unit R1. This difference was statistically significant when the 95% confidence interval of the mean was taken into account. However, the difference in mean DSVI was only 10 mℓ/g, which is small from secondary settling tank design considerations. It should be borne in mind that the biosupplement dose used in this experiment was ten times higher than that expected to be used at full scale (see **Materials and Methods**). Hence, it is unlikely that a significant improvement in settling would result from the application of the biosupplement at full scale at this Works.

The mean OUR was marginally lower (0.8 mg/ℓ·h) in Unit R1 than Unit R2 (Table 3). This difference was marginally significant when taking the 95% confidence limits into account (namely, 0.5 and 0.6 mg/ℓ·h for Unit R1 and Unit R2 respectively). Again, considering the relative magnitude of the biosupplement dose (see above), this difference is unlikely to be significant at full scale.

Conclusions

The addition of biosupplement LLMO-S1 (supplied by Amitek) had no significant effect on the performance of a pilot-scale activated sludge system operated for nitrification-denitrification and biological enhanced phosphorus removal (NDBEPR). The dose of biosupplement tested was at least ten times greater than that projected to be necessary for full-scale applications. The biosupplement is marketed on the basis of its expected propensity to reduce sludge production. No reduction in sludge production was found for the test unit over experiment periods of six to ten weeks. Most of the differences in relative mean performance of the test and control systems were minor, and not statistically significant when variance in the data was taken into account. Minor changes in sludge settleability and OUR were found for the system receiving biosupplement. However, these differences were of marginal statistical significance and unlikely to be significant in full-scale applications at practicable lower biosupplement doses.

Neither unaerated (unactivated) biosupplement in relatively large amounts, nor aerated (activated) biosupplement had any significant effect on the performance of the NDBEPR systems tested here. This implies that neither the seed bacteria in the biosupplement product, nor the enzymes they produce upon activation (Becker, 1997), are likely to exert any major influence over the microbial ecology and resultant performance of such activated sludge systems.

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