Laboratory-scale investigation of biological phosphate removal from municipal wastewater

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Abstract

Biological phosphate removal in the wastewater treatment processes is an acknowledged phenomenon having gained worldwide support and can either be implemented independently or in synergy with chemical precipitation. The objective of this study was to evaluate the effect of biomass concentration on phosphate uptake. Procedures entailed exposing varying biomass concentrations to anaerobic environments for 2 h followed by aeration for 5 h to achieve phosphate uptake. Orthophosphate concentrations were determined after anaerobic and aerobic incubation using a Merck SQ118 spectrophotometer. There was a noticeable change in phosphate removal with changes in biomass concentration. Optimal phosphate removal was achieved at biomass concentration of 1 900 mg·ℓ^{-1}. The findings of this investigation suggest a direct relationship between biomass concentration and phosphate removal capacity.

Nomenclature

BPR	=	biological phosphorus removal
BNR	=	biological nutrient removal
COD	=	chemical oxygen demand
DO	=	dissolved oxygen
DWTP	=	Darvill wastewater treatment plant
EBPR	=	excess biological phosphorus removal
ML	=	mixed liquor
MLOSS	=	mixed liquor organic suspended solids
MLSS	=	mixed liquor suspended solids
P	=	phosphorus
Poly-P	=	poly-phosphate
SQ	=	spectroquant
UCT	=	University of Cape Town

Introduction

Due to rapid industrialisation there has been an increase in the amount of effluent being disposed to natural water bodies. Major contaminants found in wastewater include biodegradable, volatile and recalcitrant organic compounds, toxic metals, suspended solids, plant nutrients (nitrogen and phosphorus), microbial pathogens and parasites (Bitton, 1994). The discharge of nitrogen (as nitrate) and phosphorus (as phosphates) to inland rivers, lakes and dams causes massive growth of algae and plants due to the "fertiliser type" effect of the phosphate and nitrate (Steyn et al., 1975). This process is called **eutrophication** and disturbs the natural balance that exists in the water body. Phosphate is a more limiting factor than nitrate in eutrophication, because some bacteria and algae are able to fix atmospheric nitrogen and convert it to the more oxidisable states of nitrates and nitrites for growth (Wentzel, 1990). Therefore, reducing phosphorus concentrations to the lowest possible level is vital to the maintenance of unpolluted water supplies.

Biological phosphorus removal (BPR) is preferred by wastewater operators to chemical phosphorus removal since it lowers process costs and reduces the problem of mineralisation. Biological phosphorus removal techniques are based on the principle that, given optimal conditions, some heterotrophic bacteria in the activated sludge biomass are able to remove solubilised phosphates by accumulating them intracellularly in the form of polyphosphates.

During biological wastewater treatment it is the active biomass that is responsible for nutrient removal. Mixed liquor organic suspended solids (MLOSS) is composed of four components viz., heterotrophic active biomass; endogenous residue; inert material and autotrophic active biomass (Ubisi et al., 1997).

The original UCT model (Dold et al., 1980; Van Haandel et al., 1981) did not consider active heterotrophic or autotrophic biomass to be present in the municipal wastewater in South Africa. However, investigations in Europe have indicated that municipal wastewater can contain a significant heterotrophic active biomass fraction (Henze, 1989), up to 20% of the total COD (Kappeler and Gujer, 1992). It is the heterotrophic active biomass that is responsible for the uptake of phosphates in the form of polyphosphates. Some researchers maintain that the active fraction of bacteria in activated sludge flocs amounts to only 1 to 3% of the total bacterial population whereas the other 97 to 99% can be referred to as inactive (Hanel, 1988).

The concentration of suspended solids in the aeration tank, commonly referred to as mixed liquor suspended solids (MLSS), is a crude measure of the biomass within the aeration tank. Normal MLSS concentrations range from 1 500 to 3 500 mg·ℓ^{-1} for conventional activated sludge units, rising to 8 000 mg·ℓ^{-1} for high-rate systems (Gray, 1989). In theory, the higher the MLSS concentration in the aeration tank the greater the efficiency of the process as there is a greater biomass concentration to utilise available COD or nutrients (Gray, 1989). Decreasing excess biological phosphorus removal (EBPR) efficiencies were always synonymous with decreasing poly-P bacterial counts (Cech et al., 1991, Cech and Hartman, 1993). Biomass in an enhanced phosphate removal process is capable of accumulating phosphorus in excess of 3%; in some cases sludge phosphorus contents of up to 18% have been obtained with artificial, tailored substrates (Appeldoorn et al., 1992).

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In this study, MLSS biomass concentrations were manipulated to determine the effect on P-uptake capacities. Previous researchers (Streichan et al., 1990; Bosch, 1992) reported that phosphate uptake was related to an increase in biomass concentration. Momba and Cloete (1996) reported that high initial cell concentrations of *Acinetobacter junii* (a poly-P organism) removed more phosphate than low cell concentrations and therefore phosphate uptake was directly related to biomass concentration and high nutrient availability. Bosch (1992) stipulated that biomass was a more significant factor than the type of organism/s present with reference to BPR. The aim of this study was to investigate the relationship between biomass concentration and phosphate uptake.

Materials and methods

Sampling

Mixed liquor samples were obtained from a full-scale modified Johannesburg 5-stage BNR system namely Darvill Wastewater Treatment Plant (DWTP). System received raw influent which was composed of 70% industrial and 30% domestic effluent. Samples were collected from the aerobic zone of the BNR system in sterile 1ℓ Schott bottles containing glass beads (diameter of 3 mm) and shaken manually to enhance the breakup of flocs. Sludge was stored at 4°C during transit (which was approximately 1 h) and processed immediately upon return to the laboratory.

Culture media for phosphate uptake studies

Mixed liquor samples (5 ℓ) from the anaerobic zone were allowed to settle for 2 h and centrifuged at 3480 *X g* for 20 min using a Beckman J6-MC centrifuge. Supernatant was filtered using Whatman No.1 filter paper to remove biomass and other suspended solids. The salts, sodium acetate (5 g·ℓ^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g·ℓ^{-1}) and KNO_3 (0.18 g·ℓ^{-1}), were added to the filtrate. Filtrate was diluted with deionised water to render a final phosphate concentration of 38 mg·ℓ^{-1} and pH adjusted to 7 with 2.0 M HCl before autoclaving at 121°C for 15 min (Bosch and Cloete, 1993).

Biomass studies

Initial MLSS of the activated sludge from the aerobic zone was determined using the method number 2540 D (*Standard Methods*, 1989). MLSS was then adjusted to the desired concentration by either dilution or settling. Adjusted biomass concentrations were placed in separate 2 ℓ Erlenmeyer flasks and subjected to anaerobic conditions by bubbling nitrogen gas through the sludge for a period of 10 to 15 min. Sludge was agitated for the duration of anaerobic incubation (2 h). Once settled, supernatant was discarded and the mixed liquor centrifuged (3480 *X g* for 20 min). Supernatant was discarded and 1 ℓ of ML media, with predetermined phosphate concentrations, was added. Sludge was aerated continuously at dissolved oxygen (DO) concentrations of 4.0 mg·ℓ^{-1} for 5 h. Ten mℓ of supernatant was extracted after every hour of aeration to conduct orthophosphate analyses.

Orthophosphate analysis

Mixed liquor media supernatant was filtered through a 0.22 µm filter (millipore) to extract cells. Filtrate was analysed and phosphate content determined spectrophotometrically using the SQ118 (Merck) and test kit P(VM) 14842.

Table 1
Effect of Altering Biomass Concentration on Phosphate Removal

MLSS (mg·ℓ^{-1})	Initial ortho-P conc. (mg·ℓ^{-1})	Final ortho-P conc. (mg·ℓ^{-1})	Total ortho-P uptake (mg·ℓ^{-1})
1 155	38	35.8	2.2
1 900	38	22.0	16.0
2 330	38	22.9	15.1
3 060	38	22.1	15.9
*3 830	38	19.8	18.2
5 000	38	17.6	20.4
5 990	38	15.4	22.6
7 400	38	14.6	23.4
11 200	38	3.9	34.1

* Initial MLSS value of Darvill Wastewater Works.

Results and discussion

Findings of this investigation suggest that increasing sludge biomass concentrations affect P-removal. The patterns of phosphate uptake capacity demonstrated by different MLSS concentrations are summarised in Table 1. Initial MLSS concentrations of sludge obtained were 3 830 mg·ℓ^{-1}. Orthophosphate concentrations of the ML medium were adjusted to 38 mg·ℓ^{-1} due to the fact that when edible oil wastes from surrounding industries enter the particular activated sludge system, phosphate concentrations of the influent, normally in the region of 5 to 6 mg·ℓ^{-1}, increase to 35 to 40 mg·ℓ^{-1}. This increase is due to the high quantity of phosphates present in oil effluents (Pechey, 1997). Final orthophosphate concentrations were measured after aerating sludge for 5 h in order to determine total phosphate uptake.

Concentrations of phosphorus accumulated at varying MLSS concentrations are shown in Table 1. Low quantities of phosphate were accumulated i.e. 2.2 mgP·ℓ^{-1} at MLSS concentrations of 1 155 mg·ℓ^{-1} compared to phosphate accumulated at higher MLSS concentrations. An increase in phosphate uptake was noted when biomass concentrations were increased from 1 155 to 1 900 mg·ℓ^{-1} i.e. from 2.2 to 16.0 mgP·ℓ^{-1}. This increase in phosphate uptake was expected due to the fact that increase in MLSS concentrations caused a concurrent increase in the poly-P population in the sludge. At MLSS concentrations of 2 330 and 3 060 mg·ℓ^{-1}, phosphate uptake rate reduced slightly from 16.0 mgP·ℓ^{-1} (accumulated by MLSS concentration of 1 900 mg·ℓ^{-1}) to 15.1 and 15.9 mgP·ℓ^{-1} respectively. Schon et al. (1993) reported that activated sludge which aerobically accumulated phosphorus in an enhanced uptake system released phosphate when an oxygen deficiency occurred. A concomitant increase in phosphorus accumulation was noted as MLSS concentrations increased (Table 1).

MLSS concentrations of 1 900 mg·ℓ^{-1} accumulated a maximum of 16.0 mgP·ℓ^{-1} of phosphate from the medium. Phosphate accumulation (mg·ℓ^{-1}) over a 5 h period is shown in Fig. 1. During the 1st hour of aeration 5 mgP·ℓ^{-1} was accumulated by the biomass. During the 2nd hour of aeration phosphate uptake rate was noted to be increasing. At the end of the 3rd hour of aeration the phosphate uptake rate decreased. This could possibly be due to the malfunctioning of the aeration source and therefore lowering the dissolved oxygen concentration. During the last 2 h of aeration the phosphate uptake rate increased steadily. Phosphate analyses were conducted

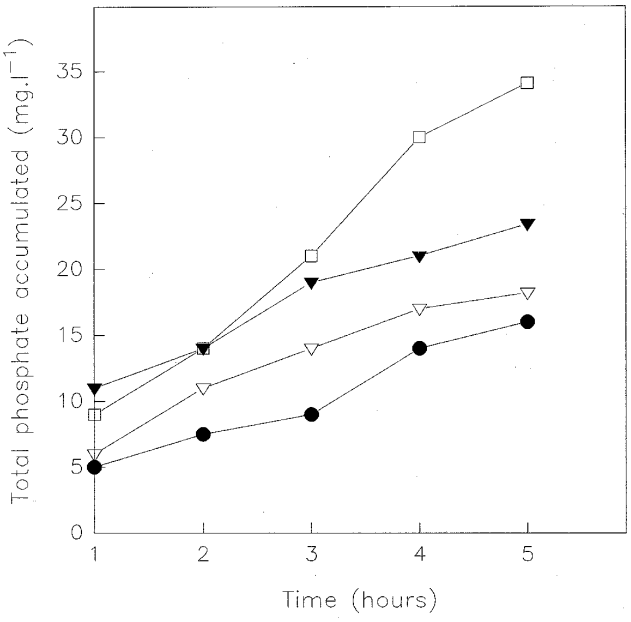

Figure 1
Quantity phosphorus accumulated (mg·ℓ$^{-1}$) at various time intervals by sludge biomass at MLSS concentrations of 1 900 mg·ℓ$^{-1}$(•), 3 830 mg·ℓ$^{-1}$(▽), 7 400 mg·ℓ$^{-1}$(▼) and 11 200 mg·ℓ$^{-1}$(□)

on an hourly basis. Phosphate uptake capacity at this biomass concentration was higher than the uptake capacity of biomass concentrations 2 330 mg·ℓ$^{-1}$ and 3 060 mg·ℓ$^{-1}$. A possible explanation could be that the small floc size allowed maximum exposure of the poly-P organisms in the biomass to the soluble phosphate in the medium, therefore facilitating optimal phosphate removal to take place.

Phosphate uptake studies were also conducted on the initial MLSS concentration of the aerobic sludge obtained (3 830 mg·ℓ$^{-1}$) (Fig.1). It was essential to conduct experiments on initial MLSS concentrations to assist in the understanding of phosphate uptake capacity regarding the varying biomass concentrations. During the entire aeration period phosphate uptake rate increased gradually, with most of the phosphate accumulated within the first 2 h of aeration (11 mgP·ℓ$^{-1}$). Sludge biomass accumulated 18.2 mgP·ℓ$^{-1}$ in total at the end of the 5 h aeration period. Comparing phosphate accumulated by sludge biomass at MLSS concentration of 1 900 mg·ℓ$^{-1}$ (i.e. 16.0 mgP·ℓ$^{-1}$) to Darvill Wastewater Treatment Plant's MLSS concentration, it is clear that the lower MLSS concentration proved to accumulate phosphate more effectively. A possible explanation could be that aeration caused the flocs of the less concentrated sludge to disperse and therefore allowed maximum exposure of poly-P organisms to the phosphorus in the medium. At MLSS concentrations of 5 000 and 5 990 mg·ℓ$^{-1}$, respectively, sludge biomass was capable of accumulating 20.4 and 22.6 mg·ℓ$^{-1}$ phosphate from the medium (Table 1). This implies that increasing biomass concentrations do not necessarily increase the phosphate uptake capacity to any great extent.

Increasing biomass concentrations to 7 400 mg·ℓ$^{-1}$ resulted in the accumulation of 23.4 mg·ℓ$^{-1}$ phosphate from the medium (Fig. 1). Sludge biomass accumulated most of the phosphate during the first 3 h of aeration (19 mgP·ℓ$^{-1}$) with the highest amount of phosphate being accumulated during the 1st hour of aeration. During the last 2 h of aeration the phosphate uptake rate slowed down. An increase of 5.2 mg·ℓ$^{-1}$ in the phosphate uptake capacity was noted (at MLSS concentration of 7 400 mg·ℓ$^{-1}$) when compared to the phosphate uptake capacity of the initial biomass concentration of 3 830 mg·ℓ$^{-1}$. This suggests that doubling biomass concentration increased the phosphate uptake capacity only minimally by 5.2 mg·ℓ$^{-1}$.

Tripling of the biomass concentration to 11 200 mg·ℓ$^{-1}$ accumulated 34.1 mg·ℓ$^{-1}$ of phosphate from the medium. Phosphate uptake increased progressively with time and is shown in Fig.1. Highest amount of phosphate was accumulated during the 1st hour of aeration whereas during the rest of the aeration period the uptake rate increased steadily. Some reasons for low phosphate uptake capacities for high MLSS values could be due to the limited availability of oxygen during aeration (Hawkes, 1983). Total phosphate accumulation obtained during this experiment was much lower than what was expected, as it was hypothesised that doubling up of sludge biomass concentration should double phosphate uptake capacity of the sludge.

Due to the fact that oxygen levels in the flocs are diffusion-limited, therefore decreasing the number of aerobic bacteria as the floc size increased, could be a possible reason why high MLSS values did not accumulate much phosphate (Hanel, 1988). One of the main factors that led to the increase of the floc size could have been the biomass concentration. The more concentrated the sludge the larger the flocs (Hanel, 1988). Although high MLSS concentrations did not accumulate high amounts of phosphate, the overall uptake rate increased gradually with increase in biomass concentration.

Conclusions

Increasing biomass concentrations increases phosphate uptake capacity. This was attributed to an increase in the nutrient utilisation rate of the poly-P organisms. High nutrient utilisation rates stimulate the multiplication rate of poly-P organisms. The more viable poly-P organisms present in the sludge the higher the phosphate removal capacity of that sludge. Although P-removal capacities of highly concentrated sludges did not double, the sludges still showed the tendency to remove phosphate by accumulating it intracellularly. Incapability of the sludges to double their P-removal capacities could be attributed to the fact that oxygen levels in the flocs are diffusion-limited and high substrate utilisation rates decrease the phosphate uptake capacity of the sludge i.e. the food:micro-organisms ratio becomes limiting. Optimal phosphate removal was obtained with biomass concentration of 1 900 mg·ℓ$^{-1}$.

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