Enhanced Biological Phosphorus Removal: Competition and symbiosis between SRBs and PAOs on lactate/acetate feed

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ABSTRACT

Sulphate reduction in activated sludge cultivated with a mixture of lactate and acetate as carbon source was investigated in a sequencing batch reactor. The system was initially operated to cultivate phosphorus removing bacteria using acetate as sole carbon source. Sulphate reduction was initially not observed when lactate was added as feed component, whereas the typical anaerobic/aerobic phosphorus profile was recorded. When anaerobic and aerobic cycle lengths were adjusted to allow growth of sulphate reducing bacteria, sulphate reduction was observed concurrent with complete phosphorus removal. Bulking and complete deterioration of the enhanced biological phosphorus removal was observed after several months when operating problems gave the sulphate reducing population opportunity to become dominant. Complete recovery of phosphorus removal activity was obtained after only 25 days when the operating conditions were switched back to the initial conditions. Bulking even disappeared after only 2 days. Combined sulphate reduction and phosphorus removal can be possible, provided bulking conditions of the sludge and thus wash out of the EBPR active biomass can be pre-vented.

KEYWORDS

Acetate feed, Enhanced Biological Phosphorus Removal (EBPR), Filamentous bulking, Lactate feed, Sequencing Batch Reactor (SBR), Sulphate reduction, Sulphate reducing bacteria

INTRODUCTION

With the discovery of Enhanced Biological Phosphorus Removal (EBPR) (Barnard, 1975), many wastewater treatment plants have been built with the aim to reduce phosphorus in the effluent by means of a biological rather than a chemical process. The EBPR process is supposed to achieve highest removal ratios when Short Chain Fatty Acids (SCFAs), with as key component acetate, are present in the influent (Fuhs and Chen, 1975; Potgieter and Evans, 1983; Malnou, 1984; Ekama *et al.*, 1984; Arvin and Kristensen, 1985 and Comeau *et al.*, 1987). Reports on the influence of non-SCFAs on the EBPR efficiency are less consistent. Using glucose or glucose/acetate as carbon sources caused in certain cases the proliferation of so called G-bacteria with a complete deterioration of EBPR activity (Cech and Hartman, 1990, 1993), whereas other authors report symbiosis between lactic acid producing bacteria and polyphosphate accumulating bacteria (PAOs) with excellent EBPR performance (Jeon and Park, 2000). Beginning of the 90'ies it was reported that anaerobic sulphate reduction caused the proliferation of filamentous bacteria with as a consequence possible hindrance of the EBPR activity (Yamamoto-Ikemoto *et al.* 1991, 1994). Wanner *et al.* (1987), however, provided evidence that inducing EBPR by enhancing growth of PAOs could suppress filamentous bulking in anaerobic-aerobic systems, even if only limited poly-P accumulation occurred.

In this article evidence is gathered for deterioration of EBPR activity due to induced anaerobic sulphate reduction activity when a sequencing batch reactor (SBR) is fed with a synthetic wastewater containing lactate and acetate as carbon sources. Operating conditions obviously determine the proliferation of a filamentous population with complete washout of the PAO population.

MATERIALS and METHODS

The study was carried out in a laboratory fermentor (Biostat, B. Braun, Melsinger) with a maximum volume of 11ℓ filled with 8ℓ of mixed liquor. To achieve EBPR the reactor was operated as a SBR with a cycle length of 6 hours: filling (15 minutes) (anaerobic), anaerobic phase (1.5 hours), aerobic phase (3 hours and 15 minutes), anaerobic in-between phase (maximum 10 minutes), during which nitrogen gas is flushed through the mixed liquor to prevent remaining oxygen entering the anaerobic period of the following cycle, settling phase (minimum 33 minutes) and an effluent purge (17 minutes). At the end of the aerobic phase 200 ml of mixed liquor is removed to obtain a sludge age of 10 days. Four litres of supernatant were removed at the end of the cycle and 4 litres of medium were fed at the beginning of the cycle, yielding a hydraulic retention time of 12 hours. Using lactate/acetate mixtures, the length of the aerobic phase was increased by 2 hours in order to obtain complete phosphorus removal. To promote growth of SRBs, the length of the anaerobic phase was increased to 2 hours and 45 minutes and the length of the aerobic phase was shortened to 2 hours and 15 minutes. The feed compositions and phase lengths during the different research periods are presented in Table 1.

Table 1 Feed compositions and phase lengths during the different research periods

	Acetate [g COD/ℓ]	**Lactate [g COD/ℓ]**	**Ortho-P [mg P/ℓ]**	**Anaerobic phase length**	**Aerobic phase length**	**Duration [days]**
1st research period	400	-	15	1h30min	3h15min	136
2nd research period	800	-	15	1h30min	3h15min	22
3rd research period	800	-	45	1h30min	3h15min	10
4th research period*	800	-	45	1h30min	5h15min	4
5th research period	400	400	45	1h30min	5h15min	24
6th research period	400	400	15	2h45min	2h15min	116
7th research period	400	-	15	1h30min	3h15min	…..

* All other nutrients were doubled in concentration

The unit was sampled twice a week to verify the phosphorus removal capacity. For this purpose, samples were taken at least at the end of the previous cycle in the settling phase, at the end of the feeding phase, at the end of the anaerobic phase and at the end of the aerobic phase. All samples were then analysed at least for ortho-P, nitrate and MLSS. COD, PHA and sulphate were analysed less frequently. When experiments were performed to elucidate the kinetics of the different reactions, at least 30 samples were taken during the anaerobic and aerobic phase of one cycle. Samples were then analysed for ortho-P, COD, nitrate, sulphate and PHA.

RESULTS and DISCUSSION

The SBR was initially operated to enhance biological phosphorus removal with acetate as the sole carbon source (400 mg COD/ℓ) and 15 mg P/ℓ ortho-phosphate. The initial cycle lengths, i.e. an anaerobic phase of 1 hour and 30 minutes and an aerobic phase of 3 hours and 15 minutes, were used for low acetate concentration. It was observed that full phosphorus removal could still be achieved using a higher acetate concentration, only by increasing the length of the aerobic phase with 2 hours. No acetate breakthrough to the aerobic phase occurred. Higher phosphorus and PHA concentrations were reached at the end of the anaerobic phase compared to the first research period.

Table 1 Ortho-P release to COD uptake ratios, PHA formation to COD uptake and PHV to PHB ratios

Period	No of days elapsed	Carbon source in feed [mg COD/ℓ]	Ortho-P in feed [mg P/ℓ]	Ortho-P release to COD uptake [mg P/mg COD]	(PHB+PHV) formation to COD uptake [mg PHA/mg COD]	PHV/PHB ratio [-]	P-removal ratio [%]
1st	36	547 (acetate)	12.998	0.142	0.334 + 0.063 = 0.397	0.188	100
1st	43	319 (acetate)	12.880	0.209	0.365 + 0.089 = 0.454	0.244	84
1st	80	220 (acetate)	12.760	0.665	1.020 + 0.304 = 1.324	0.298	64
2nd	18	573 (acetate)	13.371	0.744	2.463 + 0.868 = 3.331*	0.353	100
3rd	5	751 (acetate)	39.581	0.404	0.771 + 0.241 = 1.012	0.312	72
4th	1	851 (acetate)	42.251	0.376	0.837 + 0.236 = 1.073	0.282	100
5th	16	lactate/acetate	38.50	n.m.	n.m.	1.019	47
6th	14	785 lactate/acetate	10.07	0.209	n.m.	1.17	100
6th	15	lactate/acetate	low (~ 15)	n.m.	n.m.	0.990	0

* See further, this value is too high. Most probably the COD measurement was not correct. n.m.: not measured

From Table 2 it can be observed that the higher the acetate concentration in the feed, the higher the observed phosphorus release and PHB formation during the anaerobic phase. When increased concentration of carbon sources are used, the PHA-formation to carbon utilisation ratio increases. The PHV/PHB ratio remains nearly constant. During the 5th research period a mixture of acetate and lactate was used as carbon source. Only after as few as 6 days, complete phosphorus removal could no longer be obtained. However, an anaerobic/aerobic phosphorus profile was still recorded, whereas still no sulphate profile was observed. It could be deduced that using a 50/50w% lactate/acetate mixture as carbon source had almost no effect on the maximum anaerobic phosphorus concentration compared to acetate alone. However, the PHV/PHB ratio increased from about 0.3 for pure acetate feed to about 1 for the mixture of acetate and lactate (Table 2).

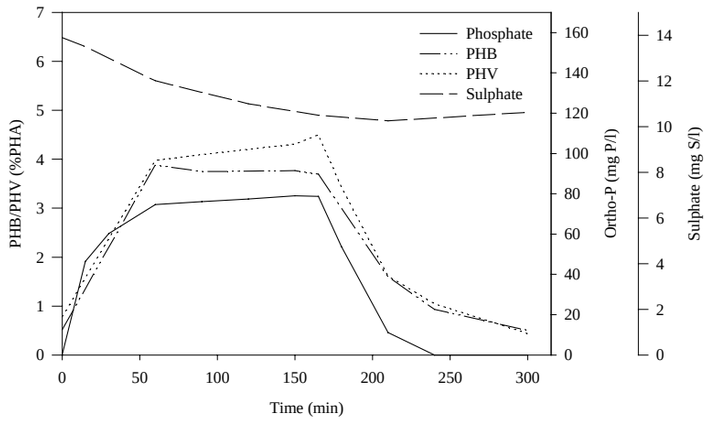

Figure 1 PHB/PHV and ortho-P profiles for lactate/acetate feed (50/50) after 13 days of acclimation to the 6th research period.

Increasing the length of the anaerobic phase to 2 hours and 45 minutes and shortening the aerobic phase to 2 hours and 15 minutes, anaerobic sulphate reduction was clearly observed (Figure 1). From the experimental results it was obvious that most of the carbon source was already used during the feeding phase (not shown). In this phase of the process, the sulphate reduction is only minor compared to the reduction in the true anaerobic phase. Sufficient carbon source thus remains for PAOs. These results indicate that sulphate reduction and phosphorus removal occur simultaneously.

One month after the operating and feeding conditions were changed to enhance sulphate reduction, the typical phosphorus profile and complete phosphorus removal were still present. However, the sludge showed very bad settling characteristics, i.e. the SVI changed from 10 ml/g to more than 500 ml/g. Due to growth of biomass on the membrane of the pH electrode, a pH value of 8.8 occurred for a period of at maximum 2 weeks. After this period, the biomass did not settle at all and biomass was washed out with the effluent. As a result phosphorus was no longer removed.

Finally, the feeding of lactate was ceased to enhance phosphorus removal again. It was observed that only after as few as 99 cycles, i.e. 25 days (3 sludge ages), complete phosphorus removal capacity was regained. The sulphate reduction capacity of the sludge completely disappeared after only two days and at the same moment the sludge's settling characteristics improved drastically.

During the second start-up period of the EBPR process, the biomass was observed microscopically. Starting from a sludge with nearly only filamentous bacteria a bacterial population was created with clear inclusions of intracellular storage polymers. Initially the sludge had very poor settling characteristics with a SVI of over 500 ml/g. The population consisted of filamentous bacteria belonging to *Beggiatoa* spp. It was observed that the biomass immediately responded to the changed operating conditions and growth started of two clearly different microbial populations. At the moment the SBR had fully restored its phosphorus removal capacity, the population containing storage polymers dominated, however, the growth of the second population continued and possible symbiosis between both occurred.

Although the experimental evidence gained provides proof that SRBs and PAOs can co-exist under defined conditions, it was also shown that this co-habitation is vulnerable and dominant growth of filamentous bacteria as *Beggiatoa* spp. can completely suppress the EBPR activity. Although Eikelboom (2000) indicated four filamentous species known to store elemental sulphur granules inside their cells, i.e. *Thiotrix* spp., *Beggiatoa* spp., Type 021N and Type 0914, they stated that *Beggiatoa* spp. is rather uncommon in activated sludge plants. In 1994, Yamamoto-Ikemoto *et al.* proposed a model for the possible ecological interactions among SRBs, PAOs, denitrifying bacteria and filamentous sulphur bacteria based on own experimental evidence and general microbial knowledge (Yamamoto-Ikemoto *et al*., 1991, 1994, 1996) (Figure 2). Denitrifying conditions suppress the growth of sulphate reducing bacteria because of the competition for the available carbon source. In the absence of denitrifiers, the authors observed sulphate reduction in wastewater treatment.

According to Yamamoto-Ikemoto *et al*. (1991), deterioration of EBPR activity was linked with high sulphate reduction producing high concentration of sulphide. The high sulphide concentration induced growth of filamentous bacteria, i.e. *Beggiatoa* spp. Consequently, the longer the sulphate reduction activity, the higher the phosphate concentration observed in the effluent. Whereas sulphide was never observed in our system, except for the times when nitrogen bubbling was deliberately switched off, growth of filamentous bacteria was clearly observed. Fast uptake of sulphide by the microbial population is a possible explanation for the sulphide profile to be absent.

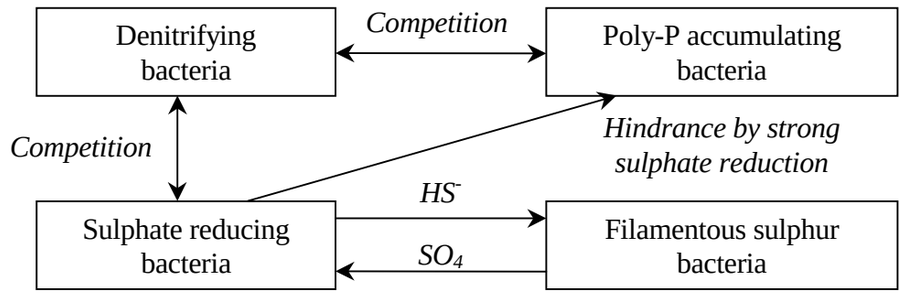

Figure 2 Model of ecological interactions among denitrifying bacteria, PAOs, SRBs and filamentous sulphur bacteria in activated sludge (after Yamamoto-Ikemoto et al., 1994)

CONCLUSIONS

Simultaneous occurrence of anaerobic sulphate reduction and phosphorus release was possible, but proved to be a process vulnerable to fast deterioration of EBPR activity due to changed operating conditions. Growth of filamentous bacteria, with *Beggiatoa* spp. as dominant organism, was always observed concurrent with sulphate reduction, even at low levels. It is expected that the higher the sulphate reduction, the more dominant the growth of these filamentous bacteria will become, with fast deterioration of EBPR.
It was observed that PAO activity was suppressed, but could very quickly restore its acetate uptake and overall P-removal capacity. Regrowth of PAOs allowed complete recovery of EBPR activity within 3 sludge ages.

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