Pergamon

Wat. Sci. Tech. Vol. 39, No. 1, pp. 215–225, 1999.

0273–1223/99 $19·00 + 0·00

PII: S0273-1223(98)00787-2

TEMPERATURE EFFECTS IN BIO-P REMOVAL

D. Baetens*, P. A. Vanrolleghem**,
M. C. M. van Loosdrecht*** and L. H. Hosten*

* *Laboratory for Technical Chemistry and Environmental Engineering, University of Gent, Technologiepark 9, 9052 Gent (Zwijnaarde), Belgium*
** *BIOMATH Department, University of Gent, Coupure Links 653, 9000 Gent, Belgium*
*** *Department of Biochemical Engineering, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands*

ABSTRACT

This paper discusses the temperature influence in biological phosphorus removal through literature review and experimental evidence. An SBR (Sequencing Batch Reactor) was operated in an anaerobic-aerobic sequence to cultivate an enriched biological phosphorus removing sludge. The impact of long term temperature changes on the stoichiometry and kinetics of the different processes involved was studied at 20, 15, 10 and 5°C. At 5°C breakthrough of acetate to the aerobic period occurred. It was shown that the stoichiometry of the anaerobic processes was insensitive to long-term temperature changes, whereas the kinetics of the aerobic and anaerobic processes were clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates. It was shown that a prediction of the temperature effect on a full scale biological nutrient removal plant is not a straight forward case because of the different influence of temperature on the subprocesses. All these influences should be accounted for. © 1999 IAWQ Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

Biological nutrient removal; kinetics; phosphorus removal; stoichiometry; temperature effect.

INTRODUCTION

Biological phosphorus removal from waste water is preferred above chemical phosphate precipitation because of its lower environmental impact. A conventional waste water treatment plant can easily be converted into a biological phosphorus removal plant by providing an anaerobic tank ahead of the aerobic reactor.

Waste water treatment plants can be subjected to wide temperature ranges giving a strong need to determine possible influences of temperature variations on the process. Recently several investigators have been interested in this temperature influence on Enhanced Biological Phosphorus Removal processes (EBPR) leading to a number of papers, unfortunately not always with comparable results. These differences are often

due to non-comparable experimental set ups. Laboratory as well as full scale results are available, with temperatures ranging from as low as 3°C up to temperatures as high as 45°C.

Most studies can be categorised into those looking at the ***efficiency*** of EBPR processes under varying temperatures (often dealing with full scale waste water treatment plants) and/or publications which focus on the ***kinetics*** of the process (laboratory scale studies primarily). Recently attention is also being paid to the effects of temperature on the ***stoichiometry*** of the EBPR process.

Efficiency

Especially when looking at EBPR efficiency at different temperatures, results from literature are revealing contrasting observations. Where at first it would seem logical to find higher efficiencies at elevated temperatures (20-37°C) (Yeoman *et al.*, 1988; McClintock *et al.*, 1993; Converti *et al.*, 1995; Jones *et al.*, 1996 and Scheer, 1994), different authors report on improved efficiencies at lower temperatures (5-15°C) (Sell *et al.*, 1981; Kang *et al.*, 1985; Krichten *et al.*, 1985; Barnard *et al.*, 1985; Vinconneau *et al.*, 1985 and Florentz *et al.*, 1987). According to Helmer and Kunst (1997) a drop in temperature from 15°C to 10 and then to 5°C had no significant influence on the efficiency of the EBPR capacity. From full-scale SBR results (Marklund and Morling, 1994) a sharp decrease in EBPR efficiency was observed when the waste water temperature dropped below 4.5-5°C.

Kinetics

When the kinetics of the EBPR process are being studied, more consistency is observed in the literature. In the temperature range from 5°C to around 30°C, increased P-release and/or P-uptake rates with increased temperatures are being reported by Shapiro *et al.* (1967), Boughton *et al.* (1971), Spatzierer *et al.* (1985), Mamais and Jenkins (1992) and Brdjanovic *et al.* (1997). Helmer and Kunst (1997), however, reported a higher specific P-uptake at 5°C than at 10°C.

Declining phosphate release and uptake rates were observed at temperatures of 35°C and higher, with a significant inhibition at 42.5°C while no phosphate release or uptake was observed at 45°C, indicating that at this temperature the phosphate removing bacteria were probably dead (Jones and Stephenson, 1996).

Stoichiometry

A recent study showed that the stoichiometry of the anaerobic process is insensitive towards temperature changes whereas some effects on the aerobic stoichiometry was observed (Brdjanovic *et al.*, 1997).

The objective of this paper is to indicate possible causes for the sometimes conflicting observations of temperature influence in biological phosphorus removal waste water treatment plants. Operational parameters that may be influencing the observations are considered and discussed.

In practice biological phosphorus removal is usually integrated with biological nitrogen removal in waste water treatment processes to comply with the actual discharge regulations. The temperature influence in both bio-P and bio-N removal can be different and these differences might influence the overall impact of the temperature influence on the biological phosphorus removal process.

METHODS

Apparatus

The study was carried out in a laboratory fermentor (Biostat) filled with 8 l of mixed liquor. The reactor was operated as a Sequencing Batch (SBR) with a cycle length of 6 hours, notably 15 minutes filling (anaerobic), 1.5 hours anaerobic phase, 3.5 hours aerobic phase, a maximum 10 minutes anoxic in between phase, a minimum 33 minutes settlement phase and a 17 minutes effluent withdrawal period. At the end of the

aerobic period 200 ml of mixed liquor was removed to obtain a sludge age of 10 days. 3.8 litres of supernatant was removed at the end of the cycle and 4 litre of medium was fed at the beginning of the cycle. The hydraulic retention time thus was 12 hours. When performing experiments, the filling of the batch reactor was always performed within 3 minutes. In a later stage the reactor configuration was modified to allow a filling period of only 3 minutes in all cases. The anaerobic period was then prolonged with 12 minutes.

The SBR consisted of a Pyrex vessel with an internal diameter of 20 cm and a total height of 40 cm. A constant stirrer speed of 350 rpm was maintained except for the settlement and effluent purge period.

A time controller was used to obtain the settings for the different phases. Nitrogen gas was bubbled through the reactor during the anaerobic phase when the oxygen concentration was raised above 0.05 mg O_2/l. When performing experiments, nitrogen was continuously bubbled through the reactor during the anaerobic period. During the aerobic phase the dissolved oxygen (DO) concentration was automatically controlled to obtain a constant value around 2 mg/l. The band width was set at 0.2 mg/l, however, a practical band width up to 1 mg/l usually occurred due to overshoot in both directions. Gas flows were controlled with massflow controllers (Bronkhorst). The flow rates, however, were fixed at a given value for nitrogen and air and can, during the course of the cycle, only be changed manually. The experiments are performed at controlled temperature and pH (7.5 ± 0.1). The pH was maintained by dosing Na_2CO_3 or HCl (both $\pm$ 1N). An external heating/cooling device allows for operating at different, but constant temperatures. The SBR is equipped with an internal coil.

Medium

A non sterilised medium was used containing 0.85g NaAc.$3H_2O$ (400 mg COD/l), 65.81 mg KH_2PO_4 (15 mg P/l), 90 mg $MgSO_4.7H_2O$, 14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N/l), 1 mg yeast extract and 0.3 ml nutrient solution per litre.

The nutrient solution contained per litre: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$ and 10 g EDTA. Only reagent grade products were used. The mixture was adopted from Smolders *et al.* (1995).

Analytical methods

Orthophosphate analyses, using the ascorbic acid method, were performed with a colorimetric autoanalyser (TrAAcs 800) with a measurement range between 0.5 and 5 mg P/l. Nitrate measurements, using the hydrazine reduction method, and ammonia measurements, using the Berthelot reaction, were also performed on TrAAcs, with a measurement range between 0.2 and 2 mg N/l and 0.5 to 10 mg N/l respectively. Potassium measurements were conducted on a flame photometer. Colorimetric COD analyses were performed according to the Standard Methods using Hach tubes and a Nanocolor colorimeter. Acetate analysis was performed on a Gas Chromatograph (GC) equiped with an FID. For polyhydroxybutyrate (PHB) analysis, lyophilised biomass was subjected to a propylation reaction. The organic phase was analysed by gas chromatography. For MLSS measurements 20 ml mixed liquor was filtered on a Whatman glass microfibre filter (GF/C) in a crucible. Before filtration the crucibles were dried at 550°C and weighed. After filtration the crucibles were first dried at 105°C followed by weighing of the recipient. For VSS determination the crucibles were then heated at 550°C followed by weighing again. The polyphosphate concentration can be approximated by the difference between MLSS and VSS. Polyphosphate can also be obtained by performing a total phosphate analysis. Therefore, lyophilised biomass was subjected to a destruction reaction in an H_2SO_4-HNO_3-mixture (1:1). Organic phosphates were thereby converted to orthophosphate and measured as such. Glycogen measurements were not performed. The initial anaerobic concentration was estimated as 2.5% of the MLSS content. The anaerobic glycogen profile was calculated based on the stoichiometric ratio between acetate-uptake and glycogen derived by Smolders *et al.* (1995). Aerobically the glycogen was supposed to be replenished linearly. The active biomass concentration was calculated as the difference between MLSS and the sum of PHB, glycogen and polyphosphate.

Operation of the Sequencing Batch Reactor

On an average basis, the unit was sampled twice a week to verify the stability of the phosphorus removal capacity. For this purpose, samples were taken at the end of the previous cycle in the settlement phase, at the end of the feeding phase, at halftime and at the end of the anaerobic phase, and at halftime and at the end of the aerobic phase. Samples were then analysed at least for orthophosphate, acetate, nitrate, ammonia and MLSS.

Initially the SBR was operated at 20°C and an SRT of 10 days. Once steady state operation was achieved, experiments were performed followed by switching the temperature to 15°C. The SBR operated at this temperature until steady state again was achieved. This procedure was repeated for 10 and 5°C. The concentration profiles for orthophosphate were used to define steady state. About 2 to 3 sludge ages were neccesary to obtain steady state after switching the operational conditions. An SRT of 10 days was only sufficient for complete phosphorus removal at 20, 15 and 10°C. At 5°C incomplete P-removal occurred and acetate broke through to the aerobic phase. No attempts were made to correct for this situation. Short-term temperature shock experiments were performed at the lowest temperature.

When experiments were performed to elucidate the kinetics of the various reactions, in total 30 samples were taken during the anaerobic and aerobic phase of one cycle. Samples were taken every five minutes, gradually enlarging the sampling interval to 20 minutes towards the end of both anaerobic and aerobic phases when limited changes were expected. Samples were analysed for orthophosphate, acetate, nitrate, ammonia and PHB. At five moments samples were taken for MLSS and VSS measurement.

RESULTS AND DISCUSSION

Performance of the EBPR activity in the SBR

The SBR was initially inoculated with sludge originating from a lab scale Phoredox installation. After some start-up difficulties (Baetens and Hosten, 1996) the SBR has exhibited good phosphorus removal capacity over a two and a half year period. Anaerobically orthophosphate is released, whereas acetate and glycogen are consumed with concurrent formation of PHB (and PHV not taken into account here). Aerobically, phosphate is stored internally by the bacteria using PHB as carbon source, while glycogen is replenished. Potassium follows the same profile as orthophospate, according to the formula $K_{1/3}Mg_{1/3}PO_3$ (Comeau *et al.*, 1987). At ambient temperatures the MLSS concentration became fairly stable with an average value of 3.5 g/l. An average VSS/MLSS ratio of 70% was obtained. At 20, 15 and 10°C complete phosphorus removal was achieved over long periods. Maximum anaerobic phosphorus concentrations of 140 mg P/l were recorded. For this temperature range acetate was always fully consumed, whereas at 5°C acetate broke through to the aerobic phase. Nitrification was observed at 20, 15 and 10°C, whereas at 5°C nitrifiers were washed out. Nitrate was never observed anaerobically. It seemed that during the 3 minutes filling period all nitrate present from the preceding aerobic period, was already consumed.

Qualitative interpretation

In Figure 1 (a-d) the patterns of the relevant parameters are shown, recorded at different steady state temperatures.

From the figures it can be seen that complete anaerobic acetate consumption occurred at temperatures 20, 15 and 10°C, whereas breakthrough to the aerobic phase occurred at 5°C. At this lowest temperature, acetate is aerobically consumed with concomitant PHB production. During aerobic acetate consumption, the phosphate concentration remained constant, a finding in contrast to other authors (Brdjanovic *et al.*, 1998c) who observe a net P-release and PHB-formation when acetate breaks through to the aerobic stage.

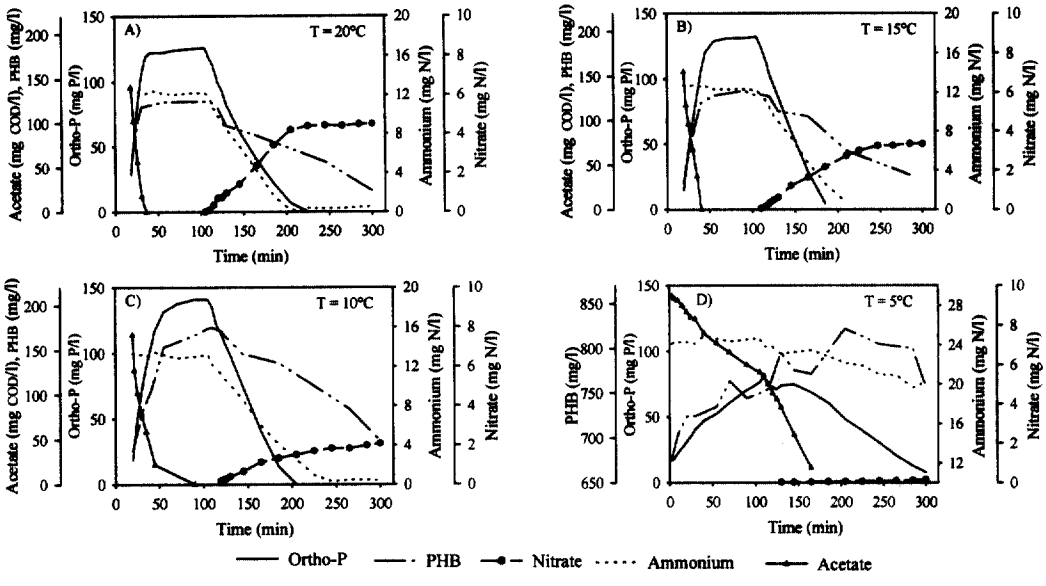

Figure 1. Patterns of relevant parameters recorded at different temperatures (Active biomass concentrations: 20°C: 2.61 g/l; 15°C: 2.06 g/l; 10°C: 2.57 g/l; 5°C: 1.84 g/l) Graph D: use axis of graph C for acetate concentration.

From the graphs it can be seen that with decreasing temperature, the PHB concentration slightly increased. After switching the operating temperature to 5°C, however, the PHB concentration in the cells increased drastically and reached a final maximum value of 30% based on MLSS (50% based on VSS). It can be assumed this high value is the maximum storage capacity of the cells. Note that the PHB measurements at 5°C show a much higher scatter than at the other temperatures. No validated explanation for this observation is found, except maybe that homogenisation of the sludge after freeze-drying is more difficult at high PHB concentrations because of the plastic character of the samples.

Whether P-release occurs or phosphorus remains constant during aerobic acetate consumption, the fact is that part of the aerobic period is lost for P-uptake. From Figure 1d it can be seen that increasing the aerobic retention time significantly might result in a total P-removal at 5°C as well. The results obtained by Brdjanovic *et al.* (1998b) showed that complete P-removal could be obtained at 5°C, provided the SRT was increased from 16 to 32 days. PHB concentration then dropped to normal values again and complete anaerobic acetate consumption was achieved.

Quantative interpretation: stoichiometry and kinetics of the anaerobic and aerobic phase

The anaerobic and aerobic stoichiometry and kinetics (i.e. maximum specific reaction rates) were calculated for each temperature and presented in Table 1. Calculation of the rates mentioned in Table 1 has been performed using raw data. Initial maximum rates are used. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates by fitting equation 1 to the data. In Table 2 the temperature coefficient θ is presented for the anaerobic and aerobic reaction rates observed.

$$r_T = r_{20} \exp^{(\theta.(T-20))} \quad (1)$$

From the values in Table 1, it can be concluded that temperature has little or no impact on anaerobic stoichiometry. On the kinetics, however, in both the aerobic and anaerobic phases, temperature has a strong influence.

Table 1. Stoichiometric and kinetic parameters for the anaerobic and aerobic phases of the EBPR process

		20°C	15°C	10°C (a)	10°C (b)	5°C
Anaerobic period						
Max. spec. Acetate uptake rate	g COD/g active BM/h	0.243	0.193	0.171	0.113	0.058
Max. spec. P-release rate	g P/g active BM/h	0.137	0.122	0.105	0.069	0.033
Stoichiometry	g P/g COD	*0.58±0.04*	*0.63±0.01*	*0.61±0.06*	*0.61*	*0.56*
Max. spec. PHB production	g PHB/g active BM/h	0.149	0.108	0.094	0.067	0.036
Stoichiometry	g PHB/g COD	*0.64*	*0.56*	*0.54±0.08*	*0.60*	*0.59*
Aerobic period						
Max spec. P-uptake rate	g P/g active BM/h	0.0373	0.0532	0.0411	0.0397	0.027
Max spec. PHB consump. rate	g PHB/g active BM/h	0.0256	0.0222	0.0138	0.0089	n.a.
Max spec. total NH_4-uptake rate	g N/g active BM/h	0.0035	0.0034	0.0028	0.0025	0.0002
Max spec. tot. NO_3-prod rate	mg N/g active BM/h	1.056	0.889	0.548	0.306	0

(a)-(b) Time gap of 4 months in between measurements.

Table 2. Temperature coefficient θ ($°C^{-1}$) and standard error for the anaerobic and aerobic reaction rates

Anaerobic phase	θ ± stand. error	Aerobic phase	θ ± stand. error
Max spec. Acetate uptake rate	0.0764 ±0.011	Max spec. P-uptake rate (5-15°C)	0.0648 ± 0.003
Max spec. P-release rate	0.0683 ± 0.015	Max spec. PHB cons. rate	0.0742 ± 0.028
Max spec. PHB production rate	0.0816 ± 0.007	Max specific NO_3-prod rate	0.0842 ± 0.033

Whereas nearly all rates show a declining trend with decreasing temperature over the complete temperature range, the aerobic specific P-uptake rate seems to attain a maximum value between 20 and 15°C. Brdjanovic *et al.* (1997) observed a maximum for the phosphate release rate.

From the results obtained from experiments performed at 10°C with a considerable acclimation time in between, it can be seen that the observed rates differ, but the stoichiometry remains constant. This observation indicates a potential difficulty when one tries to compare rates recorded by different authors. However, stoichiometry appears not to be affected. For the calculation of the temperature coefficient θ the results from 10b have been used because of the longer acclimisation time applied for the experiment.

The anaerobic P-release/HAc uptake ratio reported by Brdjanovic *et al.* (1997) is lower than the value reported here. This is due to the higher pH applied in our experiments and may also be caused by not accounting for endogenous P-release here. The stoichiometric PHB/HAc ratio is lower than reported by Brdjanovic *et al.* (1997) since here PHV production is not yet taken into account.

For the calculation of the NH_4-uptake NO_3-production rates the total active biomass was considered. Due to the small fraction of nitrifiers and denitrifiers present the relative influence of a decreasing amount of these organisms will have an important influence on the rate calculation. For the calculation of the other rates, this problem is less important because of the much higher fraction of Poly-P organisms. The values mentioned in Table 1 for NH_4-uptake NO_3-production rates should thus be regarded with some scepticism.

According to the classification of the temperature coefficients in ASM2 and the values obtained in this study, BPR has a low to medium degree of temperature dependency. The temperature coefficient observed for nitrification, however, is much lower than typical values mentioned in literature ($\theta = 0.113°C^{-1}$)

Generally speaking the experimental results obtained using the enriched culture are consistent with the results obtained by Brdjanovic *et al.* (1997, 1998b) who used a similar experimental setup. However from the introduction in this paper it shows that in practice many conflicting results are obtained. In the following section of this paper possible causes which can explain the results are indicated.

Biomass acclimation to temperature

Whereas short term temperature changes are supposed only to influence the kinetics and eventually the stoichiometry, long term temperature changes might also influence the biomass population. Hence, a distinction should be made between experimental results on pure cultures of bio-P organisms, or results obtained with enriched and mixed cultures. In the literature no results were found for temperature effects on pure cultures.

Enriched cultures

In two recent papers Brdjanovic *et al.* (1997, 1998a) studied short term as well as long term temperature effects on stoichiometry and kinetics of the anaerobic and aerobic phases of the biological phosphorus removal process. The process was operated in such a way to enrich the biomass with P-removing organisms. From their results it can be concluded that the anaerobic temperature coefficients (θ) obtained from long and short term tests were similar and consistent with results observed by Jones and Stephenson (1996), with θ being approximately 0.077 C^{-1}. In contrast to this observation under anaerobic conditions, different temperature coefficients for the aerobic phase were obtained depending on either long or short term tests. This observation was attributed by the authors to a probable population shift. Their conclusion is supported by molecular ecological techniques showing a distinct population shift in this enrichment culture with temperature.

Jones and Stephenson (1996) studied the temperature dependency of the EBPR process at different temperatures performing batch tests using sludge that had first been acclimatised to different temperatures. For anaerobic P-release, and even more apparent for aerobic P-uptake, θ values generally decreased with increasing temperatures. According to these results the authors describe the EBPR process to be more sensitive to temperature changes at lower temperatures. A population shift is not discussed, but could be an alternative explanation for their observations.

Sell *et al.* (1981) and Krichten *et al.* (1985) explained the enhanced EBPR efficiency at lower temperatures by stating that the EBPR bacteria were psychrophilic. Above 10°C the non-EBPR mesophilic bacteria would then compete for substrates with the psychrophiles resulting in less phosphate removal at higher temperatures. Helmer and Kunst (1997) also attribute their results to an accumulation of cold tolerant P-removing bacteria.

Mixed Cultures

When using mixed cultures, as in full-scale studies, population shifts are of even greater importance. When operating at constant MCRTs, nitrification can easily be suppressed at lower temperatures, resulting in washout of nitrifiers and, henceforth, denitrifiers. Furthermore, lower temperatures might lead to incomplete anaerobic substrate uptake, resulting in a breakthrough of substrate to the aerobic stage. This will result in an increase of heterotrophic bacteria.

Temperature influence on active biomass concentration

Mathematical models that include the presence of EBPR in activated sludge processes, such as the Activated Sludge Model No2 (ASM2) (Henze *et al.*, 1995), University of Capetown Activated Sludge Model (UCTPHO) (Dold *et al.*, 1994) or the metabolic model of the EBPR (Smolders *et al.*, 1995) take into account the active biomass instead of the MLSS measurement. The active biomass can be calculated from the MLSS measurement obtained through standard procedures by subtracting the PHB, poly-P and glycogen content. While the active biomass remains constant under anaerobiosis (no growth and negligible decay), the MLSS value will decrease due to loss of poly-P and its comparatively higher molecular weight than stored acetate. Aerobically, the active biomass will increase due to growth and the poly-P content will increase whereas the PHB concentration will decrease. An overall increase in active biomass as well as MLSS should be observed. At decreasing temperatures less orthophosphate will be taken up and thus less poly-P will be formed. The ratio active biomass/ MLSS will thus be a function of temperature. This phenomena will result in different specific P-release and P-uptake rates when MLSS instead of active biomass is used as a biomass count. The observed higher specific P-uptake rate at 5°C (Helmer and Kunst, 1997), might be explained by this MLSS based calculation.

Temperature influence on observed yield and SRT

With increasing temperature the ATP requirement for maintenance increases (Brdjanovic *et al.*, 1997) causing a decrease of the substrate available for net biomass growth. Decay processes increase with increasing temperature as well. Both phenomena result in less biomass production for the same amount of substrate used, causing a decrease in the net observed yield and thus a decrease in phosphorus removal capacity.

Working with full-scale continuous flow waste water treatment plants, the biomass concentration in the reaction basins is maintained constant through adjustment of the sludge wasted. When temperature decreases sludge production increases and more sludge needs to be purged to maintain this constant biomass concentration. With decreasing temperature a decrease in solids retention time (SRT) and thus an increase in the observed yield occurs.

Within the temperature range 13.5 to 20°C, it was demonstrated that EBPR functions efficiently and independently of SRT for aerobic SRTs above 2.1 days. At lower SRT values EBPR capability may be lost at an aerobic SRT that depends on temperature. Higher temperatures allow efficient EBPR to be maintained at lower SRT values (Marnais and Jenkins, 1992). The authors observed washout of P-removing organisms at an aerobic SRT of 2.1 days at a temperature of 13.5°C while washout only occurred at an aerobic SRT of 1.5 days for a temperature of 20°C. Their results are in good agreement with observations made by McClintock *et al.* (1993) and Shao *et al.* (1991) who observed washout for an aerobic SRT of 2.5 days at 10°C and 1.45 days at 23°C respectively.

Temperature influence on nitrification and denitrification

A prerequisite for biological phosphorus removal to occur, is the existence of a true anaerobic zone, preceding an anoxic or aerobic stage. A carbon source should be available for the micro-organisms in this true anaerobic zone. Poly-P organisms will store this carbon source as polyhydroxyalkanoates for later use in the anoxic or aerobic stage. Nitrate entering the anaerobic zone will cause denitrification, depleting part of the carbon source present. Several process configurations have thus been modified to avoid breakthrough of nitrate in the anaerobic stage as much as possible (e.g. Modified UCT).

Whereas the influence of temperature on the bio-P process still remains partially unresolved, the influence of temperature on nitrification is well described and reveals a high correlation between temperature and nitrification (θ=0.113 C^{-1}). So, when temperature decreases, less ammonium might be converted to nitrate, causing less nitrate to enter the anaerobic stage. With decreasing temperature, more carbon will thus be available for poly-P bacteria. An increase in the fraction of poly-P bacteria will occur due to washout of the nitrifiers and denitrifiers.

For denitrification with acetic acid, 2.16 g C-HAc/g $N\text{-}NO_3$ are consumed (Henze *et al.*, 1996). When denitrification occurs together with phosphorus removal, a higher acetic acid uptake rate will be observed. With decreasing temperature denitrification will disappear because of washout of nitrifiers and thus denitrifiers. The resulting decrease in acetic acid uptake rate will then not only be caused by the influence on the bio-P process, but also because of the lack of nitrate production, and thus the lack of denitrification.

Temperature influence on simultaneous precipitation reactions

Ever since the discovery of biological phosphorus removal, researchers also focused on the possible contribution of simultaneous phosphorus precipitation reactions. Orthophosphates are known to form stable complexes with many cations. In most waste waters primarily calcium and magnesium are of importance but also sodium is often taken into account. When cation concentrations are high enough precipitation should be considered. Moreover the temperature influence on the precipitation will influence the observed overall temperature effects on the biological phosphorus removal process. Unfortunately the temperature effect on CaP-precipitation seems to be a complicated process with no straightforward answers considering the overall effect. The difficulty is that the probability of CaP-precipitation decreases with decreasing temperature but if the solubility product is once exceeded the formation of hydroxyapatite is quicker at lower temperatures (Maurer and Boller, 1999).

Temperature influence on anaerobic volatile fatty acid (VFA) availability

When the anaerobic uptake of acetate is incomplete at lower temperatures, leading to a breakthrough of acetate to the aerobic phase, an increase of heterotrophic bacteria will occur. However, it should also be taken into account that for real waste water treatment plants the available carbon source first has to be fermented to short-chain-fatty acids. This process can take place in a prefermentor, but also occurs naturally in slow-flowing outfall sewers or in pumping stations and force mains. Generally, it was found that without this incidental fermentation or prefermentation, it is very difficult to produce sufficient VFAs in the anaerobic zone of an EBPR plant when winter mixed liquor temperatures are below 17°C. Below this point the additional secondary release of phosphates in the anaerobic zone is more than the uptake possible through the production of VFA by increasing the anaerobic retention time (Randall *et al.*, 1992). Low temperature will thus cause less hydrolysis and fermentation, causing less PHB to be formed anaerobically, resulting in less energy available for later aerobic phosphate uptake.

Table 3. The effect of a decreased temperature in Bio-P removal

Level	Action	Resulting effect	Effect
Organism	• Lower rates	• Kinetic limitation	-
	• Lower decay resulting in higher observed yield	• Less limitation due to increased storage capacity	+
Population	• Acclimation leading to population shift within PAO's	• Changed Arrhenius coefficients, changed kinetics	+/-
	• Less nitrification, less nitrate	• More substrate for PAO's, more storage capacity	+
	• Less fermentation	• Less substrate for PAO's, less storage capacity	-
Physical-chemical	• Precipitation	• Probability of precipitation decreases	-
		• If solubility product is exceeded, precipitate formation quicker	+

Temperature influence in Bio-P removal: an overview

In Table 3 an overview is given of the expected temperature effects on the different processes occurring in a biological nutrient removal plant. In this table an important terminology, namely the storage capacity, is

introduced. This storage capacity is not the capacity of one single organism, but the overall storage capacity given by the product of the individual storage capacity and the amount of organisms.

CONCLUSIONS

This paper discusses the temperature influence on biological phosphorus removal through literature review and experimental evidence. From this review it becomes obvious that temperature has an important influence on bio-P processes, especially due to the storage processes involved. As such, MLSS measurements are not a good representation of the active biomass. Temperature changes can also cause a shift in composition. When comparing results between different researchers, attention should be focussed on differences between several operational conditions and all rates should be expressed on the basis of active phosphorus removal biomass.

An SBR (Sequencing Batch Reactor) was operated in an anaerobic-aerobic sequence to cultivate an enriched biological phosphorus removing sludge. The impact of long term temperature changes on the stoichiometry and kinetics of the different processes involved was studied at 20, 15, 10 and 5°C. At 5°C breakthrough of acetate to the aerobic period occurred.

It was shown that the stoichiometry of the anaerobic processes was insensitive to long term temperature changes, whereas the kinetics of the aerobic and anaerobic processes were clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates.

It was shown that a prediction of the temperature effect on a full scale biological nutrient removal plant is not a straightforward case because of the different influence of temperature on the subprocesses. All these influences should be accounted for.

ACKNOWLEDGEMENT

Financial support provided by the Fund for Scientific Research (F.W.O.) and the University of Gent Research Fund (BOZF) is greatly acknowledged.

REFERENCES

Baetens, D. and Hosten, L. H. (1996). Biological phosphorus removal using SBR technology: phenomenological aspects during start up period. *Preprint of First IAWQ Specialized Conference on Sequencing Batch Reactor Technology*, Munich, 357-363.

Barnard, J. L., Stevens, G. M. and Leslie, P. J. (1985). Design strategies for nutrient removal plant. *Wat. Sci. Tech.*, **17**(11/12), 233-242.

Boughton, W. H., Gottfried, R. J., Sinclair, N. A. and Yall, I. (1971). Metabolic factors affecting enhanced phosphorus uptake by activated sludge. *Appl. Microbiology*, **22**(4), 571-577.

Brdjanovic, D., van Loosdrecht, M. C. M., Hooijmans, C. M., Alaerts, G. J. and Heijnen, J. J. (1997). Temperature effects on physiology of biological phosphorus removal. *J. Env. Eng.*, 144-153.

Brdjanovic, D., Slamet, A., van Loosdrecht, M. C. M, Hooijmans, C. M., Alaerts, G. J. and Heijnen, J. J. (1998a). Impact of excessive aeration on biological phosphorus removal from wastewater. *Wat. Res.*, **32**(1), 200-208.

Brdjanovic, D., Logemann, S., van Loosdrecht, M. C. M., Hooijmans, C. M., Alaerts, G. J. and Heijnen, J. J. (1998b). Influence of temperature on biological phosphorus removal: process and molecular ecological studies. *Wat. Res.*, **32**(4), 1035-1048.

Brdjanovic, D., van Loosdrecht, M. C. M., Hooijmans, C. M., Alaerts, G. J. and Heijnen, J. J. (1998c). Minimal aerobic sludge retention time in biological phosphorus removal systems. (Submitted).

Comeau, Y., Rabionwitz, B., Hall, K. J. and Oldham, K. (1987). Phosphate release and uptake in enhanced biological phosphorus removal from wastewater. *Journal WPCF*, **59**(7), 707-715.

Converti, A., Rovatti, M. and del Borghi, M. (1995). Biological removal of phosphorus from wastewaters by alternating aerobic and anaerobic conditions. *Wat. Res.*, **29**(1), 263-267.

Dold, P. L., Wentzel, M. C., Billing, A. E. and Marais, G. v. R. (1994). Activated sludge systems simulation programs. *Rep. To Water Res. Commission*, South Africa.

Florentz, M., Caille, D., Bourdon, F. and Sibony, J. (1987). Biological phosphorus removal in France. *Wat. Sci. Tech.*, **19**(4), 1171-1173.

Helmer, C. and Kunst, S. (1997). Low temperature effects on phosphorus release and uptake by microorganisms in EBPR plants. *Wat. Sci. Tech.*, **37**(4-5), 531-539.

Henze, M., Gujer, W., Mino, T., Matsuo, T. Wentzel, M. C. and Marais, G. v. R. (1995). *Activated Sludge Model No 2.* IAWQ Scientific and Technical Report No. 3, IAWQ, London, UK.

Henze, M., Harremoës, P., La Cour Jansen, J. and Arvin, E. (1996). *Wastewater Treatment. Biological and Chemical Processes.* Springer.

Jones, M. and Stephenson, T. (1996). The effect of temperature on enhanced biological phosphate removal. *Env. Techn.*, **17**, 965-976.

Kang, S. J., Horvatin, P. J. and Brisco, L. (1985). Full-scale biological phosphorus removal using A/O process in a cold climate. *Proc. Int. Conf. Mgmt. Strategies for Phosphorus in the Environment,* Selper Ltd, UK.

Krichten, D. J., Hong, S. N. and Tracy, K. D. (1985). Applied biological phosphorus removal technology for municipal wastewater by the A/O process. *Proc. Int. Conf. Mgmt. Strategies for Phosphorus in the Environment,* Selper Ltd, UK.

Mamais, D. and Jenkins, D. (1992). The effects of MCRT and temperature on enhanced biological phosphorus removal. *Wat. Sci. Tech.*, **26**(5/6), 955-965.

Marklund, S. and Morling, S. (1994). Biological phosphorus removal at temperatures from 3 to 10°C - a full scale study of a sequencing batch reactor unit. *Can. J. Civ. Eng.*, **21**, 81-88.

Maurer, M. and Boller M. (1999). Modelling of phosphorus precipitation in wastewater treatment plants with enhanced biological phosphorus removal. *Wat. Sci. Tech.*, **39**(1), 147-163 (this issue).

McClintock, S. A., Randall, C. W. and Pattarkine, V. M. (1993). Effects of temperature and mean cell residence time on biological nutrient removal processes. *Wat. Env. Res.*, **65**(5), 110-118.

Randall, C. W., Barnard, J. L. and Stensel, H. D. (1992). *Design and retrofit of wastewater treatment plants for biological nutrient removal.* Water Quality Management Library, **5**, 85-95.

Shao, Y. J., Abkian, V., Crosse, J., Horenstein, B. and Jenkins, D. (1991). The effect of MCRT on biological phosphorus removal. *California Annual WPCF Conference*, Pasadenea, CA.

Shapiro, J., Levin, G. V. and Humberto, Z. G. (1965). Metabolic uptake of phosphorus by wastewater organisms. *J. Water Pollution Control Fed.*, **37**(6), 800-824.

Scheer, H. (1994). Vermehrte biologische Phosphorelimination - Bemessung und Modellierung in Theorie und Praxis. *Veröffenlichungen des institutes für siedlungswasserwirtschaft und abfalltechnik der Universität Hannover*, **88**.

Sell, R. L., Krichten, D. J., Noichl, O. J. and Hartzog, D. G. (1981). Low temperature biological phosphorus removal. *Proc., 54th WPCF Conf.*

Smolders, G. J. F., van der Meij, J., van Loosdrecht, M. C. M. and Heijnen, J. J. (1995). A structured metabolic model for anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process. *Biotechnol. Bioeng.*, **47**, 277-278.

Spatzierer, G., Ludwig, C. and Matsche, N. (1985). Biological phosphorus removal in combination with simultaneous precipitation. *Wat. Sci. Tech.*, **17**(11/12), 163-176.

Vinconneau, J. C., Hascoet, M. C. and Florentz, M. (1985). The first application of biological phosphorus removal in France. *Proc. Int. Conf. Mgmt. Strategies for Phosphorus in the Environment,* Selper Ltd, UK.

Yeoman, S., Hunter, M., Stephenson, T., Lester, J. N. and Perry, R. (1988). An assessment of excess biological phosphorus removal during activated sludge treatment. *Envir. Technol. Letters*, **9**, 637-646.