CHAPTER IV

THE EFFECTS OF OPERATIONAL CONDITIONS ON EBPR PERFORMANCE

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Abstract

Excessive nitrogen and phosphorus can cause eutrophication in water bodies and needs to be reduced in most wastewaters before discharge to receiving waters. The excess biological phosphorus removal (EBPR) process has been shown to be an economical and environmentally compatible method of reducing phosphorus from wastewaters. Despite the complexity of EBPR mechanisms, appropriately designed and operated treatment plants can easily achieve phosphorus (P) removal as long as EBPR-available organic substrates such as short chain volatile fatty acids (VFAs) and an anaerobic-aerobic reactor configuration are provided. Some factors and operating conditions, however, adversely affect the performance of EBPR plants. For this paper, the effects of aerobic recycle and SRT on system performance were investigated using modified UCT configuration systems operated at 20 (System 1) and 5^{o}C (System 2), respectively. The results showed that electron acceptor loadings of 999 and 552 mgO_2/d to the respective anoxic reactors reduced P removal performance by 12 and 29 mg/L in Systems 1 and 2, respectively. The reason for the difference in the magnitude of the reduced performances was that the denitrifying bacteria of System 1 used internally stored PHA for denitrification, while the denitrifying bacteria of System 2 used externally available acetate. The study also implied that the best P removal can be achieved with a total SRT value of at least 16 days at 5^{o}C. The washout biomass solids retention time (SRT) of the EBPR functions was determined to be 3.5 and 1.8 days at 5 and 10 ^{o}C, respectively. These values are significantly lower than the value predicted by Mamais and Jenkins (1992). The reason for this difference is the presence of highly enriched poly-P organisms (55% and 39% P as VSS at 5 and 10 ^{o}C, respectively) in this study. The high

poly-P reserves were not completely depleted even at the washout SRT (6.0% and 2.1 % P as VSS remained at 5 and 10^{o}C, respectively). It was demonstrated that the reason for washout was not the exhaustion of poly-P reserves but was related to the shutdown of glycogen metabolism at cold temperatures. It was concluded that the most rate-limiting step of EBPR is glycogen metabolism under all temperature conditions. It also was concluded that glycogen metabolism is an integral part of the EBPR process as proposed by Mino and coworkers (Mino *et al.* 1987).

Keywords: Excess biological phosphorus removal, activated sludge, denitrification, UCT configuration, aerobic recycle, PHA, glycogen, poly-P, SRT, washout SRT, nitrate.

INTRODUCTION

Several biochemical models have been developed to explain the mechanisms of excess biological phosphorus removal (EBPR) processes using activated sludge. Even though the most widely accepted current models (Comeau and Wentzel, 1986; Mino, 1987; Pereira *et al.*, 1996) agree on the aerobic EBPR metabolic pathways, some differences exist when the anaerobic EBPR processes are considered. The differences are mainly related to the means of producing reducing power for PHA synthesis. It is apparent that EBPR biochemistry is complex and no exact model exists yet, but despite this complexity, wastewater treatment plants can achieve phosphorus removal as long as wastewater containing organic substrate in the form of short-chain volatile fatty acids (SCVFAs) is introduced to the anaerobic zone of the treatment plant, and the mixed liquor is alternated between anaerobic and aerobic conditions. The purpose of the alternation is to favor the growth of phosphorus accumulating organisms (PAOs). PAOs can store inorganic phosphorus as intracellular poly-phosphate (poly-P) to a much greater extent than is needed for growth metabolism, and can use the energy stored in the poly-P

bonds to polymerize and store poly-hydroxyalkanoates (PHAs) from SCVFAs. In wastewater treatment practice, carbon and nitrogen also can be removed along with the phosphorus, and the combined processes are refer to as a biological nutrient removal (BNR) system. Several flow configurations have been used for BNR, and some of them, such as the UCT, were specifically designed to protect the anaerobic zone from the introduction of oxidized nitrogen forms, and recycled dissolved oxygen.

It is known that the best EBPR performance is obtained when influent containing volatile fatty acids (VFAs) is initially introduced into a strictly anaerobic zone. Hence, an oxygen and/or oxidized nitrogen free environment is a major prerequisite for a successful EBPR process (Barnard, 1985; Jenkins and Tandoi, 1991; Randall et al. 1992). In addition, complete VFA (e.g. acetate) uptake in the anaerobic stage is a key parameter affecting EBPR performance. The effects of different types of VFAs on EBPR performance have been investigated by Abu-gararah and Randall (1991), and Hood and Randall (1999). It was found that acetate is the best substrate among other short chain fatty acids such as butryate, valerate, their isoforms and propionate in terms of yielding the highest phosphorus removal per unit COD of VFA. However, even when the nutritional prerequisites are strictly provided, the success of the EBPR process also strongly depends on other factors such as pH and temperature. Additionally, wastewater and operating conditions such as the influent COD/TP ratio, the amount of electron acceptor entering the anaerobic stage, and the biomass solids retention time (SRT) can influence the efficiency of EBPR.

Temperature is one of the key parameters that affects the performance of the EBPR process (Erdal and Randall, 2002b). Two major effects are exerted by temperature: It influences the rates of enzymatically catalyzed reactions, and affects the rate of diffusion of substrate into the cells (Grady *et al*., 1999). Substantial research efforts have been made to more fully define temperature effects on the kinetics and performance of EBPR systems during the last two decades. Early researchers (Sell, 1981; Kang *et al*., 1982; Ekama, *et al*., 1984; Siebrietz, 1984; Barnard *et al*., 1985) reported that EBPR efficiency was greater at lower temperatures than at higher temperatures. McClintock *et al.* (1991)

determined wash-out values of an EBPR system at 20 and 10^{o}C, and reported that EBPR functions would "wash-out" of activated sludge systems before other heterotrophic functions. Mamais and Jenkins (1992) demonstrated the wash-out of EBPR processes at several combinations of temperature and SRT. In recent studies, John and Stephenson (1996); Brdjanovic, *et al.* (1997 and 1998); Choi (1998); Beatons *et al*. (1999) and other researchers have shown that EBPR reaction rates become slower with decreasing temperature, as is typical of biochemical reactions. Thus, although decreasing temperature appears to slow down EBPR reaction rates, a substantial body of evidence indicates that many EBPR systems perform more efficiently as the temperature decreases.

In a very recent study Erdal and Randall (2002b) showed that EBPR performance is considerably better at 5^{o}C than at 20^{o}C. It was concluded that reduced competition for substrate in the non-oxic zones results in an increased population of PAOs and greater EBPR efficiency at steady state, given that the SRT of the system is above the critical washout SRT for the prevailing temperature. Nearly non-existent nitrification renders the anoxic zone nitrate free and further increases the anaerobic time. In spite of the reduced rate of acetate uptake at 5^{o}C, an increased detention time provided complete acetate utilization through the non-oxic stages. This had a significant contribution to increased P removal performance at 5^{o}C. The results also showed that the proliferation of PAOs was due to cellular and biochemical adaptive mechanisms that provide them with a growth advantage over the non-PAO heterotrophs typical of BNR activated sludge biomass.

It is well known that NO_3-N and other electron acceptors (e.g. O_2 and NO_2-N) entering the anaerobic stage will adversely affect EBPR process performance. This is because, in the presence of nitrate, denitrifying bacteria utilize VFAs and electron flow is diverted from PHA production to NO_3^- reduction (Bond and Rees, 1999). This reduces the amount of VFAs available for PAOs and poly-P metabolism. It was proposed by Tetrault et al. (1986) that the concentration of oxidized nitrogen should be less than 10 mgN/L in recycled flow to achieve good EBPR performance. In actuality, the amount of electron acceptor that can be tolerated is a function of the total amount of VFA that becomes available in the anaerobic zone relative to the amount of P that must be removed. Even

though this concept was investigated to some extent under warm temperatures (Kuba *et al*., 1996), no study has been performed to fully investigate the effects of NO_3-N at cold temperatures. It should be remembered that even though cold temperatures may partially benefit EBPR by reducing nitrification and, therefore, reducing the recycle of nitrates, they also may have a negative impact by increasing the amount of O_2 recycled in addition to slowing down the EBPR kinetic rates. In this study, the combined effects of nitrate and oxygen on EBPR performance were investigated using two-identical UCT pilot plants operated with steady state conditions at 20 and 5^oC, respectively.

Another important parameter affecting EBPR system performance is SRT. The solids retention time (SRT) is a unified design parameter that is related to the steady state specific growth rate of the biomass in a bioreactor system. It determines electron acceptor requirement and the excess biomass production rate (Grady *et al.,* 1999). "Minimum SRT", another design parameter, defines a critical SRT point, below which no growth of biomass can occur. The washout SRT value is selected considering the growth of the slowest growing organisms desired in the system, usually nitrifiers, and sludge flocculation (Grady *et al*., 1999). Because biochemical kinetic rates become slower at cold temperatures, the desired organisms will need more time to grow; i.e. their required retention time in the system will need to become larger. Failure to increase the SRT as temperature decreases may result in partial or complete lost of the desired biological treatment function(s), e.g., nitrification or EBPR. In their study, McClintock *et al.* (1992) completely lost EBPR when the SRT was reduced from 15 to 5 days at 10^oC. Mamais and Jenkins (1992) showed that EBPR functions can be lost from EBPR activated sludges at several combinations of SRT and temperature, implying that there is an EBPR washout SRT for any given temperature that is higher than heterotrophic washout.

Matsuo (1994) showed that a short anaerobic SRT (0.9 days) in an EBPR system resulted in an apparent decrease in P removal efficiency due to the growth of non phosphate accumulating organisms that competed with the PAOs for substrate in the anaerobic zone. Beatons *et al.* (1999) used the same SRT value of 10 days over an entire temperature range from 5 to 20^oC, and observed lower P removal efficiency at the colder

temperatures. It is likely that they observed partial washout of the EBPR functions because the 10 day SRT was too low for the cold mixed liquor temperatures. In this respect, a relatively high SRT would seem to be a promising parameter to enhance the performance of biological treatment processes, particularly if they are seasonally operated at low temperatures. Brdjanovic *et al.* (1998) arbitrarily increased the SRT values to 32 days at 5^{o}C. This high SRT value resulted in increased endogenous respiration, and decreased O_2 transfer to the mixed liquor (Grady *et al.*, 1999). The effect of excessive aeration was later found to reduce EBPR efficiency due to depleting energy reserves (e.g. PHA) (Brdjanovic *et al.*, 1999). Temperature studies without consideration of SRT may not result in optimum EBPR system performance and may result in confusion.

METHODS and MATERIALS

Experimental Design

1. The effect of nitrate recycle

The purpose of this research was to determine in what extent EBPR system performance is affected by the electron acceptors that enter the anoxic stage of the UCT process. The system performances with NO_3-N recycle were determined previously and the results were reported by Erdal and Randall (2002b). In their study two UCT systems were operated with and without aerobic recycle. Performance data, including the measurement of internal storage products were collected both at steady state and shortly after nitrate recycle was stopped. It was initially thought that it would be useful to compare system 2 performance with system 1 when both were receiving the same amount of NO_3-N recycle. However, mass balance calculations of nitrate and O_2 entering the anoxic zones revealed that there was a negligible difference between the two systems as initially operated. Mass balance calculations were performed for all of the organic components

utilized by and generated by the biomass. The organic carbon mass balance calculations were performed using the conversion factors given in Table 1.

Table 1. Carbon and COD equivalents of acetate, PHB&PHV and glycogen

	Molecular formula	MW mg/mmol	Carbon mmolC/mg	COD mgCOD/mg
Acetate	CH_3COO^-	59	29.5	0.950
HB	$C_4H_6O_2$	86	21.5	1.674
HV	$C_5H_8O_2$	100	20	1.920
Glycogen	$C_6H_{10}O_5$	162	27	1.185

2. The effect of SRT under cold temperature conditions

The system operated at 20^{o}C (System 1) showed that a 10 day SRT is enough to achieve complete utilization of acetate in the anaerobic stage thereby promoting very good P removal. It also was shown that when the SRT was increased to 17 days, the P removal performance of System 1 did not change. On the other hand, maintaining a larger SRT was necessary for better P removal at 5^{o}C. An SRT value of 18 days was thought to be large enough to accomplish complete acetate uptake through the non-oxic stages. Previous operating experience showed that higher SRT values (20 days or more) did not enhance P removal performance. This may suggest that the same or better P removal can be achieved with lower SRT values. To evaluate this, the SRT values of the system were decreased from 18 days to very low values, and this decrease continued up until the wash-out point was determined. The same experiment was performed with System 2, which was operated at 10^{o}C and 12 days SRT.

Experimental Setup

Two identical laboratory-scale UCT systems with two anaerobic, two anoxic and three aerobic reactors in series, were set up in two different constant temperature rooms set at 20^{o}C (System 1) and 5^{o}C (System 2), and operated at 10 and 18 day SRTs, respectively. Both systems were fed acetate and supplemental yeast extract as the organic carbon source (450-500 mg/L COD). The complete composition of the synthetic wastewater is given in Erdal and Randall (2002b).

The flow schematic of the lab scale UCT systems also is given in Erdal and Randall (2002b). The pH of the system influent was kept within a narrow range (7.2-7.6) to minimize pH effects. To avoid the formation of P precipitates in the feed, the delivery of P to the first anaerobic reactor was made separately using a small pump, whereas the rest of the feed chemicals, both organic and inorganic, were delivered to the same reactor using another peristaltic pump. The combined influent was defined as the combination of both flows entering the first anaerobic reactor.

The seed for both systems were obtained from the Roanoke, Virginia, wastewater treatment plant, which achieves partial biological P removal. Sludge wasted from System 1 was added to the aerobic section of System 2 on a daily basis for about one week during startup. By this method, the acclimation period was shortened and a similarity in microbial community was obtained. System 1 was operated at steady state for more than a year and steady state data was collected (Erdal and Randall, 2002b). Following steady state data collection of system 1 at 20^{o}C, the aerobic recycle line was removed. However, System 2 (5^{o}C) was operated without an aerobic recycle line for about 6 months after which the aerobic recycle line was placed into operation. The flow rates of both systems during the study are given in Table 2. Steady state data collected included MLSS, MLVSS, COD, acetate, NH_4^+-N, NO_2-N, NO_3-N, soluble PO_4, total P, and the cellular internal components, PHB, PHV and glycogen. The samples were collection at the locations summarized in Table 5.

Table 2. Internal and external flow rates and nominal detention times of the systems.

	System 1 at 20^{o}C	System 2 at 5^{o}C
Synthetic feed pump rate	31.9 L/day	29.1 L/day
Phosphorus feed pump rate	2.4 L/day	1.6 L/day
Anoxic recycle flow rate	42.1 L/day	39.8 L/day
Aerobic recycle flow rate	38.2 L/day	38.6 L/day
Return activated sludge flow rate	40.4 L/day	39.3 L/day
Anaerobic detention time	2.8 hr	3.1 hr
Anoxic detention time	2.8 hr	3.1 hr
Aerobic detention time	7.3 hr	8.1 hr

Table 3. The sampling parameters and their locations

Parameter	Sample Location									
	CI	AN1	AN2	AX1	AX2	AE1	AE2	AE3	Effluent	RAS Line
Acetate	x	x	X	x	x	x	x	x	x	x
Ammonia N	x	x	X	x	x	x	x	x	x	x
COD	x	x	X	x	x	x	x	x	x	x
DO	x					x	x	x		
Glycogen		x	X	x	x	x	x	x		x
MLVSS, MLSS		x	X	x	x	x	x	x	x	x
Nitrite, nitrate N	x	x	X	x	x	x	x	x	x	x
OUR						x	x	x		
pH	x		X		x	x	x	x		x
PHB, PHV		x	X	x	x	x	x	x	x	x
Soluble P	x	x	X	x	x	x	x	x	x	x
TKN	x								x	x
Total P		x	X	x	x	x	x	x		x

CI: Combined influent
AN: Anaerobic reactor
AX: Anoxic reactor
AE: Aerobic reactor
RAS: Return activated sludge

Analytical Methods

Mixed liquor solids (MLSS, MLVSS) and COD measurements were analyzed according to APHA (1995) as defined in sections 2540D, 2540E, and 5220C, respectively. Anions (acetate, PO4-P, NO_2-N and NO_3-N) were analyzed by a Dionex 2010I ion chromatograph with an IONPAC AS40A-SC column. Cations including NH_4-N were analyzed by Dionex 120 ion chromatography furnished with electrochemical conductivity detector.

PHB and PHV analysis

The mixed liquor samples from designated reactors were placed into 15 mL plastic centrifuge tubes. They were immediately centrifuged at 10,000 rpm for about 10 minutes and clear supernatant was poured off. The tubes were immersed into liquid nitrogen for quick freezing. Frozen samples were then lyophilized, and 25 to 50 mg homogeneous samples were weighed out into a 5 mL high pressure Wheaton "V-vial". A minimum of 8 external PHA standards (0 to 20 mg) were prepared. Two mL of methanol-sulfuric acid-benzoic acid solution was added to each vial. Benzoic acid served as an internal standard. Benzoic acid solution was prepared freshly by solubilizing 50 mg benzoic acid into 100 mL of 3% sulfuric acid in methanol solution (v/v). Before the vials were tightly sealed, 2 mL of chloroform was added to each vial. The vials were then incubated in a drying oven at 100^{o}C for 3.5 hours. Following digestion, the vials were cooled down to room temperature, and 1 mL of distilled water was added into each vial. The vials were shaken about 10 minutes to separate methanol and chloroform layers. A sufficient volume of the chloroform phase (1 mL) was transferred into GC autosampler vials by Pasteur pipettes. The samples were injected automatically to a GC with a Reoplex 400 Chromosorb GAW column and a FID detector. The oven, injector and detector temperatures were set to 130, 160 and 200^{o}C, respectively.

Glycogen analysis

Glycogen measurements were performed according to a modification of the method outlined by Gerhardt *et al.* (1995). Glycogen is resistant to hydrolysis in alkali environments, but is readily soluble in water and acid, and insoluble in ethanol (ASM Manual, 1981). Lyophilized solid samples were weighed into screw-capped centrifuge tubes, and 1 to 2 mL of 30% wt/vol KOH was added depending on the quantity of the solids. The samples were digested in a 100ºC drying oven for 3 hours to break down the cells and to solubilize the glycogen homopolymer. Following digestion, the samples were taken out and left to cool down to room temperature. Then, to each tube 3mL of water and 8 mL of ice-cold ethanol was added to precipitate the glycogen. The opaque solution formed after addition of ethanol was centrifuged for 15 min at 10 000*g*. The pellet was washed with 60% (vol/vol) ice-cold ethanol. The remaining precipitate was dried at 60ºC. To breakdown and solubilize glycogen, dried solids were further digested at 100ºC in 3 mL of 6N HCl for 1 hour. Measurement of soluble glucose was done by the Phenol method (Gerhardt *et al*., 1995). The straw yellow color developed during the phenol test was used to determine the concentration of the glucose in the samples. The absorbance of each sample along with the standards was determined using a Spec 20 spectrometer set at a wavelength of 490 nm. The suggested enzymatic method was not used because of serious impracticalities. The measured glycogen was then reported as mg glucose per mg of dry solids.

RESULTS AND DISCUSSION

The effects of aerobic recycle on EBPR performance:

The steady state solids concentrations at 5°C are summarized in Table 4. The MLVSS concentrations with and without aerobic recycle were very similar, but the amount of acetate removed from solution in the anaerobic zone decreased by more than (6%) when aerobic recycle was implemented as shown in Figure 1. The first anoxic section of the system received average acetate concentrations of 121 and 97 mg/L with and without aerobic recycle, respectively. The phosphorus removal efficiency was reduced even more when aerobic recycle was implemented, as shown in Figure 2. Phosphorus removals (ΔP) were averaged 98.9±6.3 and 71.8±3.3 mg/L without and with aerobic recycle, respectively. Phosphorus mass balance calculations (Figure 3) showed that the net P uptake was 3246 and 1956 mg/day, respectively, when the system was operated without and with aerobic recycle, a 40% reduction.

Table 4. Steady-state biomass solids concentrations throughout System 2

	without aerobic recycle		with aerobic recycle	
	MLSS mg/L	MLVSS mg/L	MLSS mg/L	MLVSS mg/L
Anaerobic 1	4448±399	2779±173	4682±205	2550±52
Anaerobic 2	4395±413	2781±196	4645±254	2537±61
Anoxic 1	7456±720	4518±186	8512±419	4492±119
Anoxic 2	7410±678	4543±220	8497±423	4581±142
Aerobic 1	7727±650	4557±237	8667±480	4562±206
Aerobic 2	7878±688	4537±203	8763±504	4539±124
Aerobic 3	7972±699	4506±207	8806±467	4526±183
Effluent	44±19	25±10	61±11	32±5
RAS	14350±2007	8271±581	17158±495	8840±471
	n=8, ±: standard deviation		n=4, ±: standard deviation	

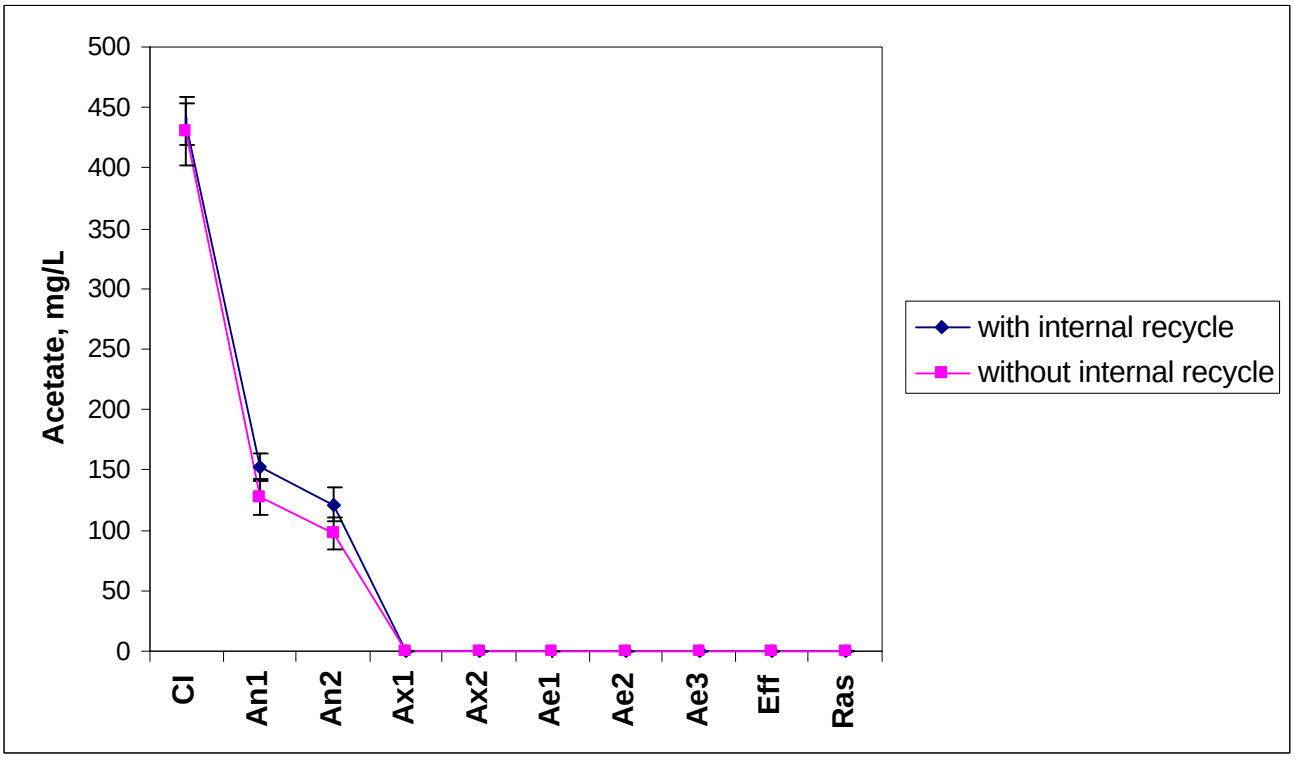

Figure 1. Acetate utilization under both operating conditions at 5^{o}C (error bars represents the standard deviations of the mean values; N=4 with internal recycle and N=9 without internal recycle.

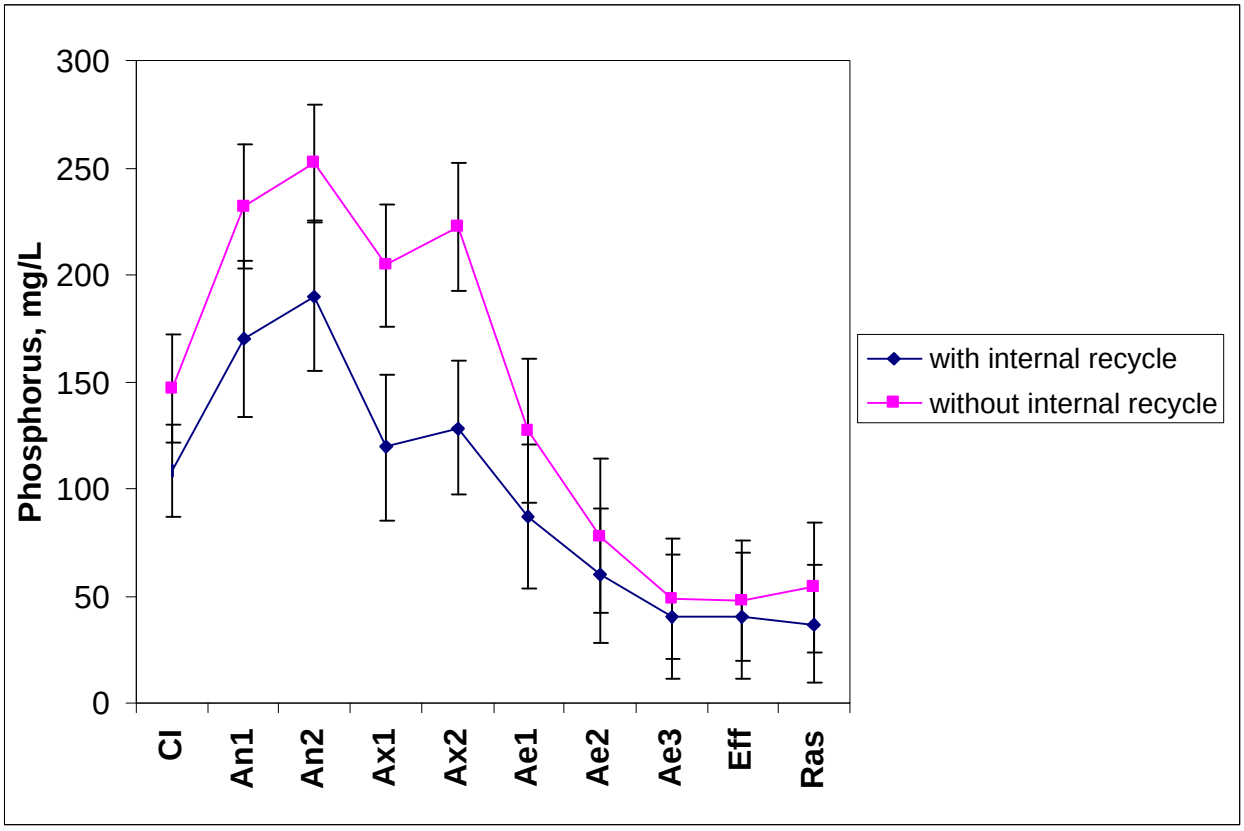

Figure 2. Phosphorus release and uptake with and without aerobic recycle at 5^{o}C (error bars represents the standard deviations of the mean values, N=4 with internal recycle and N=9 without internal recycle.

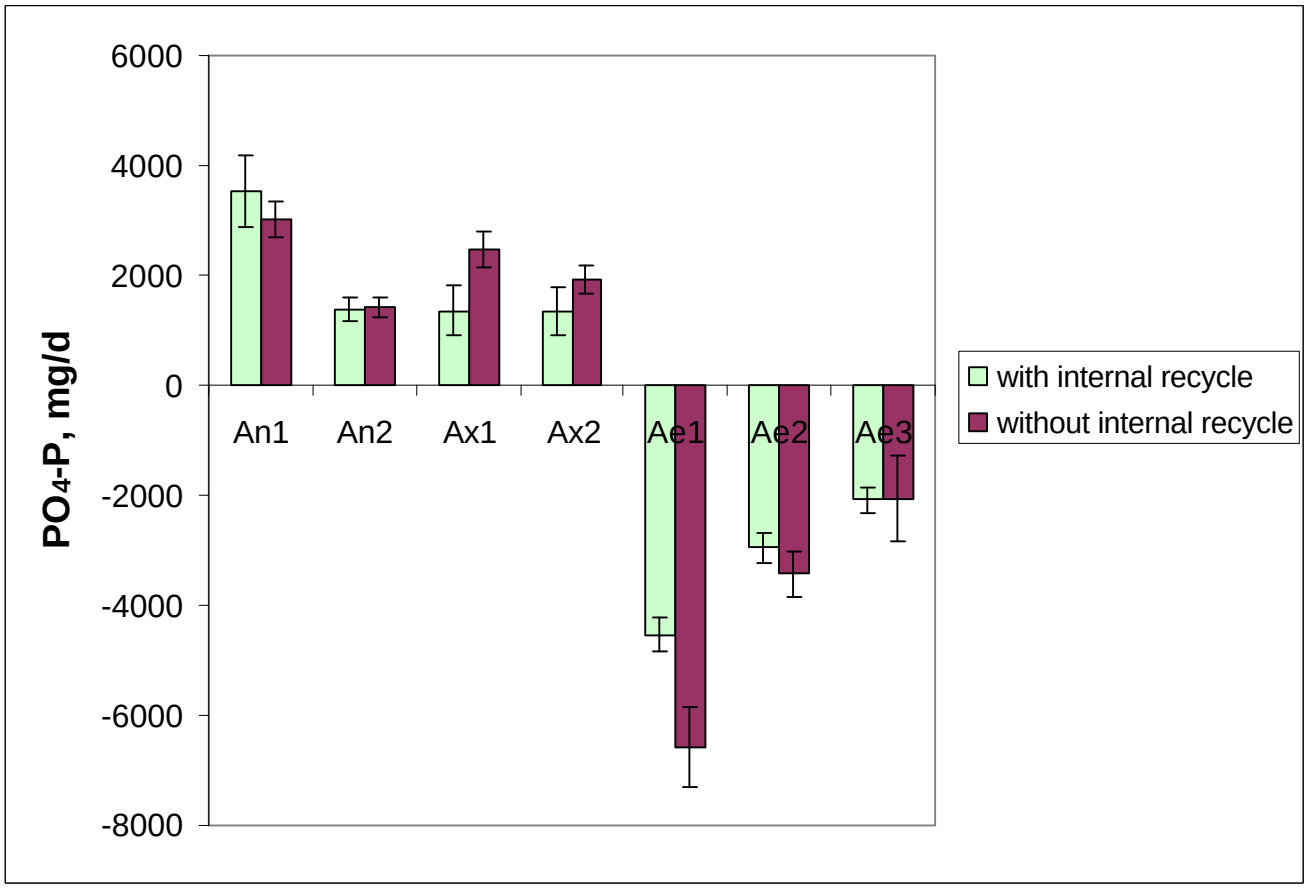

Figure 3. Mass balance of phosphorus at 5^{o}C (error bars represents the standard deviations of the mean values, N=4 with internal recycle and N=9 without internal recycle).

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Mass balances of PHA and glycogen are given in Figures 4 and 5, respectively. To better compare the system under both recycle conditions, mass balances of the organic compounds were calculated on a carbon basis (Figure 6). As the figure shows, the system produced a considerably smaller amount of PHA (72 mmol C/d) when the aerobic recycle line of the system was implemented. The averaged nitrate and oxygen concentrations in the aerobic recycle line of System 2 were 1.6 and 10.1 mg/L, respectively. This increased the mass of electron acceptor flux to the anoxic zone by 552 mg/d as O_2, which should have been how much acetate, as COD, was consumed in the anoxic stage by the introduced electron acceptors. This amount should have consumed only 20 C-mmol/d of acetate based upon conversion factors listed in Table 1. In other words, if all the remaining acetate was utilized to synthesize PHA, only a 20 mmol C difference should have been observed in PHA formation when the system was operated with and without aerobic recycle. However, 80 mmolC was consumed when aerobic recycle was implemented. The reason for the observed differences might be interpreted as the presence of non-poly P bacteria competing with the PAOs for acetate. However, a previous study (Erdal 2002 c) showed that PAOs were dominate in the 5^oC mixed liquor. Therefore, only a small fraction of the bacterial community could have been non-poly P organisms.

Careful examination of the glycogen mass balance data (Figure 5) revealed that the net utilization of glycogen in the first anoxic reactor was significantly decreased when aerobic recycle was introduced to the system. The mass balance of phosphorus also showed that the net P release was significantly reduced in the anoxic stage when the aerobic recycle was introduced. Both mass balances taken together showed that as P uptake was taking place in the anoxic section, glycogen was being replenished.

Comparison of the carbon mass balances of the organic compounds (Figure 6) strongly suggests that glycogen metabolism is a key factor for EBPR. The extent of its involvement, however, may change depending upon operating conditions.

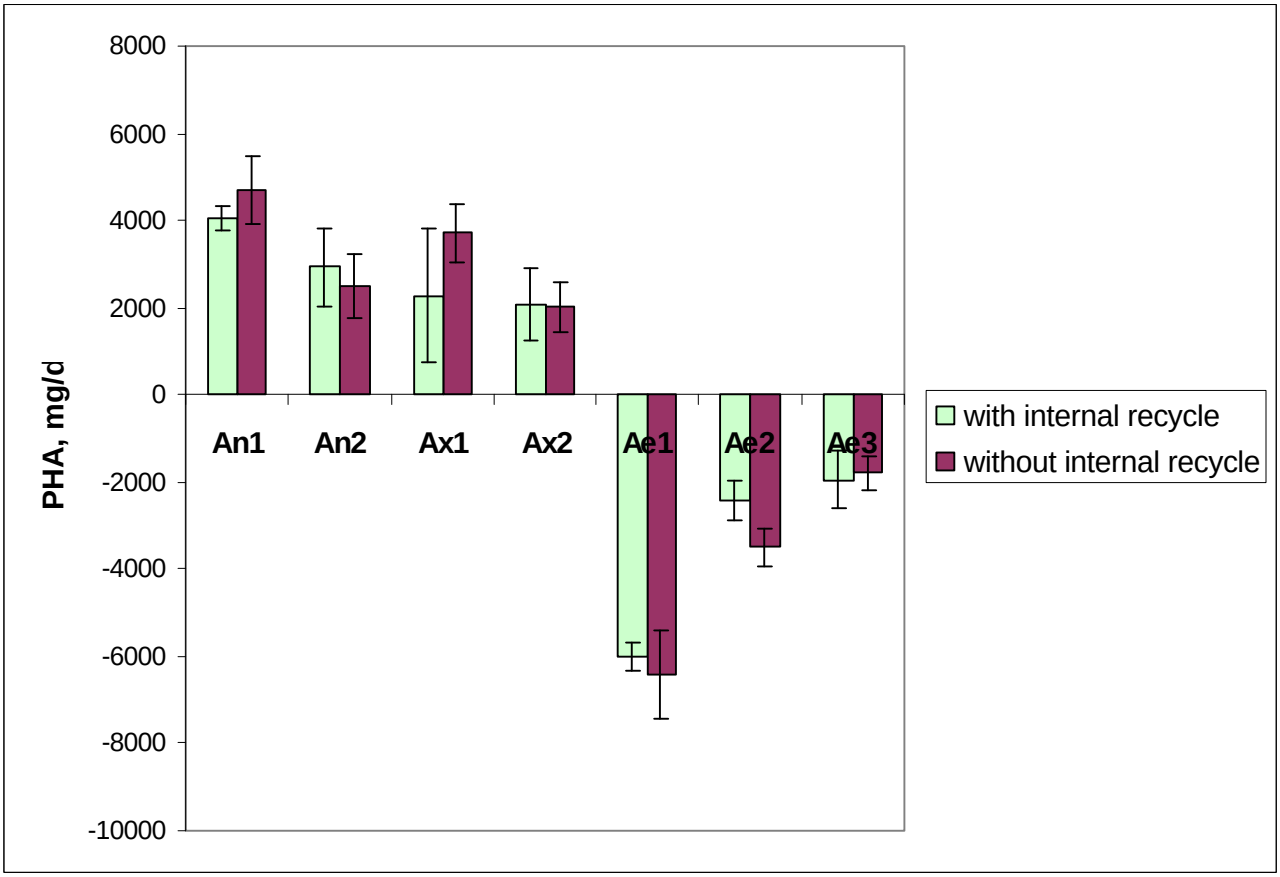

Figure 4. Mass balance of PHA at 5^{o}C with and without aerobic recycle (error bars represents the standard deviations of the mean values, N=4 with internal recycle and N=9 without internal recycle).

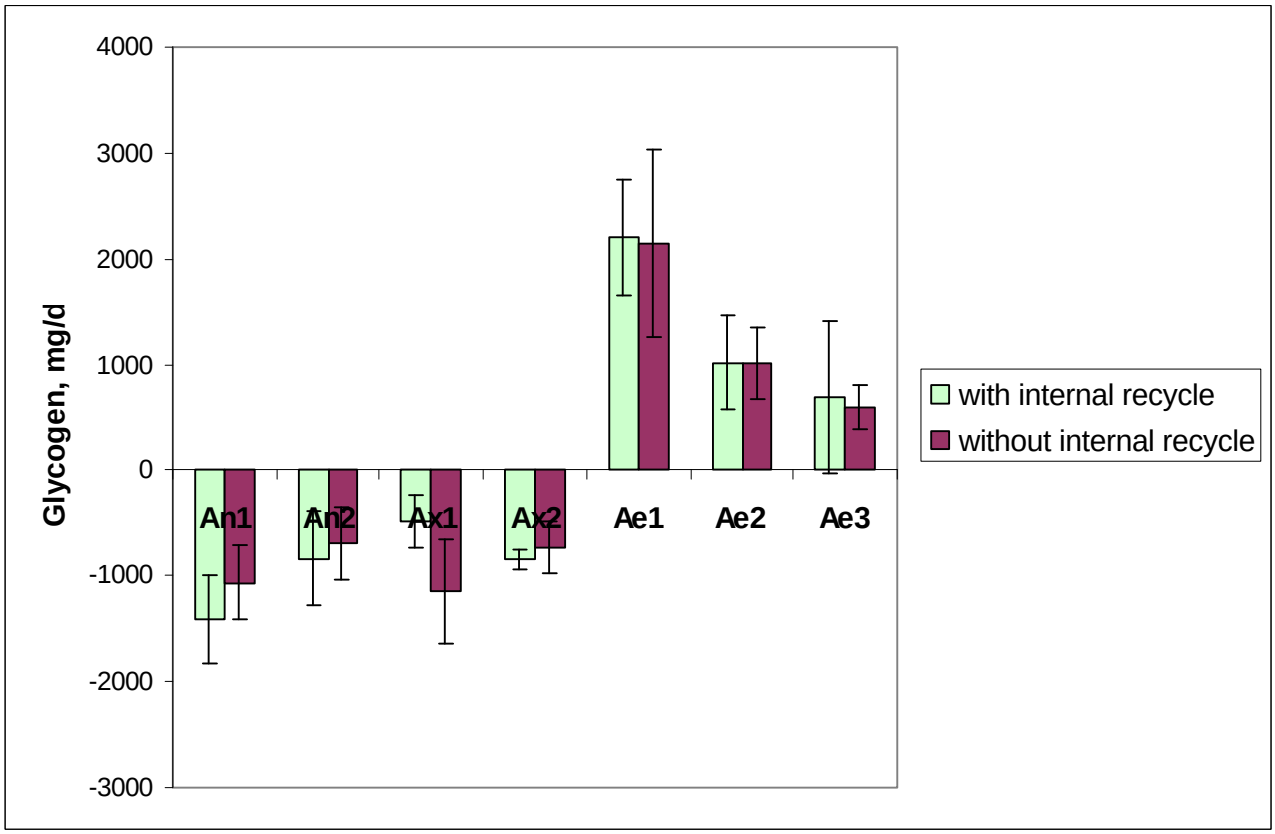

Figure 5. Mass balance of glycogen at 5^{o}C with and without aerobic recycle (error bars represents the standard deviations of the mean values, N=4 with internal recycle and N=9 without internal recycle).

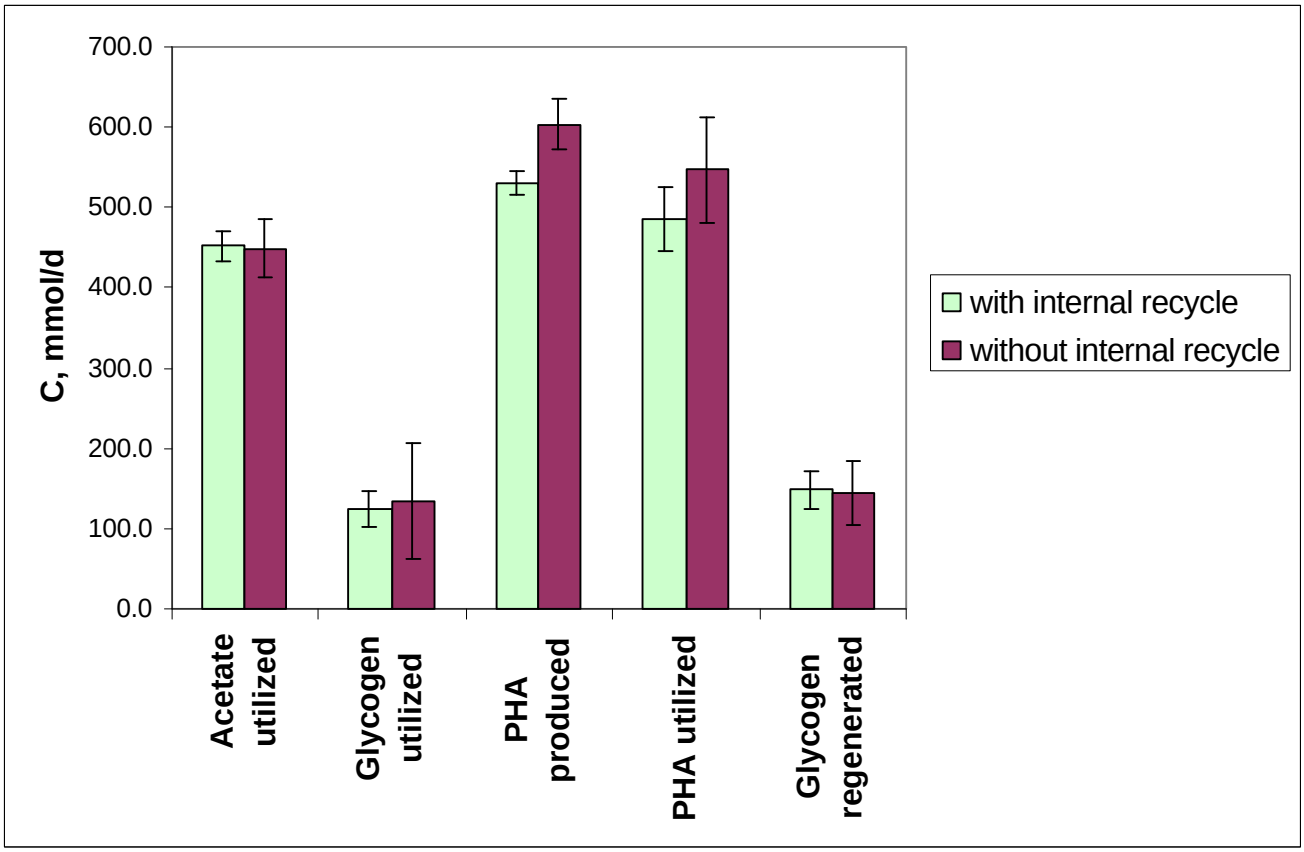

Figure 6. Carbon mass balance of acetate, glycogen and PHA at 5^{o}C. (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=9 without internal recycle).

The effects of aerobic recycle at 20°C:

The effects of aerobic recycle on P and acetate removal performances of EBPR processes at 20°C are given in Figures 7 and 8. Even though the acetate utilizations are nearly identical with and without operation of the aerobic recycle, more P (36 mg/L vs 24 mg/L) was removed when there was no aerobic recycle. The fundamental reason for the reduced P removal was the entry of substantial amounts of nitrate and oxygen (999 mg O_2/d equivalent) to the anoxic zone. Unlike the 5°C operation, no acetate was available in the anoxic zone for denitrification purposes. However, the PHA data revealed that the

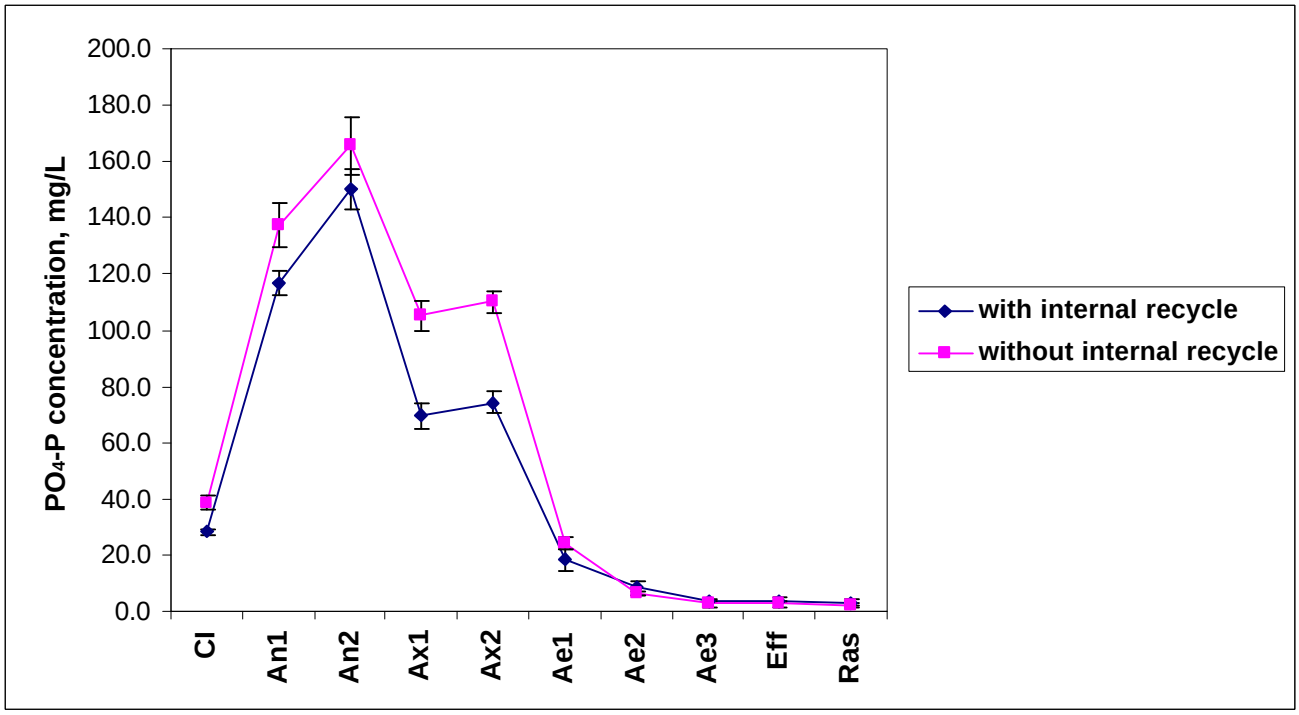

Figure 7. The effect of aerobic recycle on P removal in System 1 at 20°C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

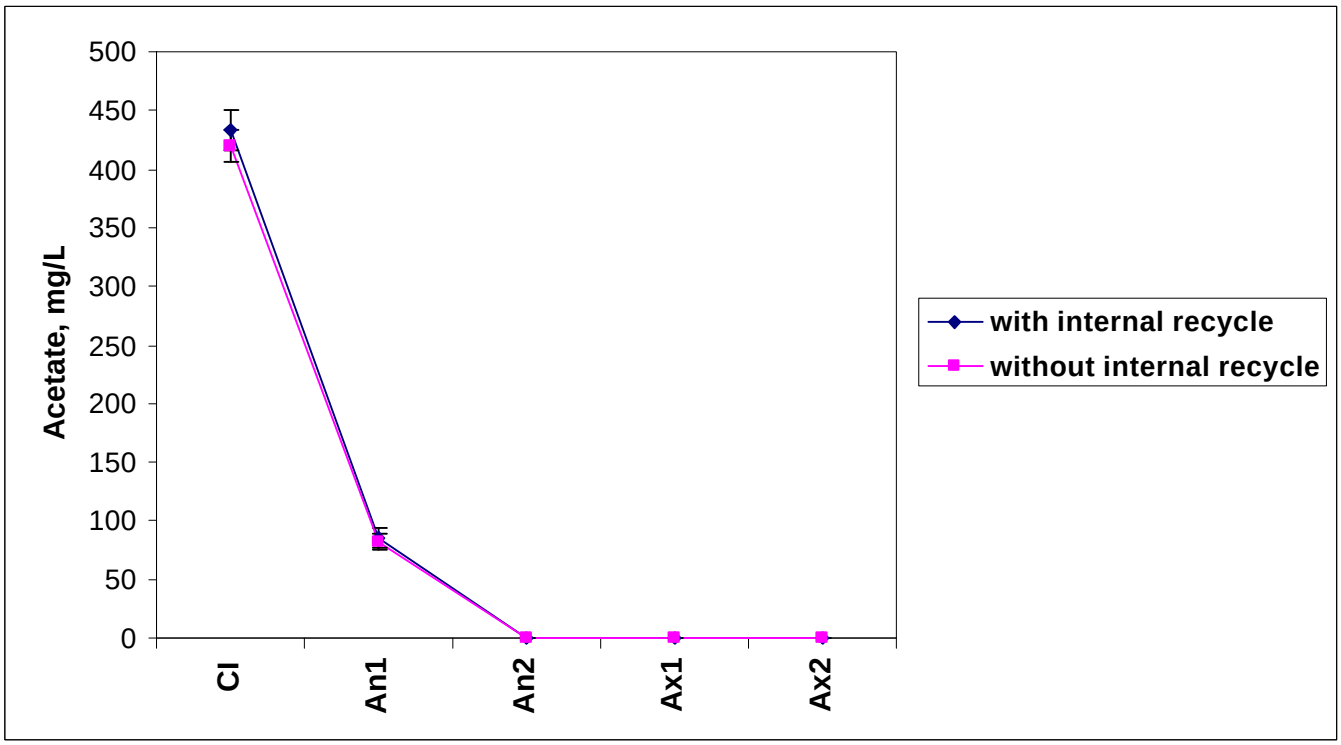

Figure 8. Acetate utilizations through the non-oxic stages of System 1 at 20^{o}C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

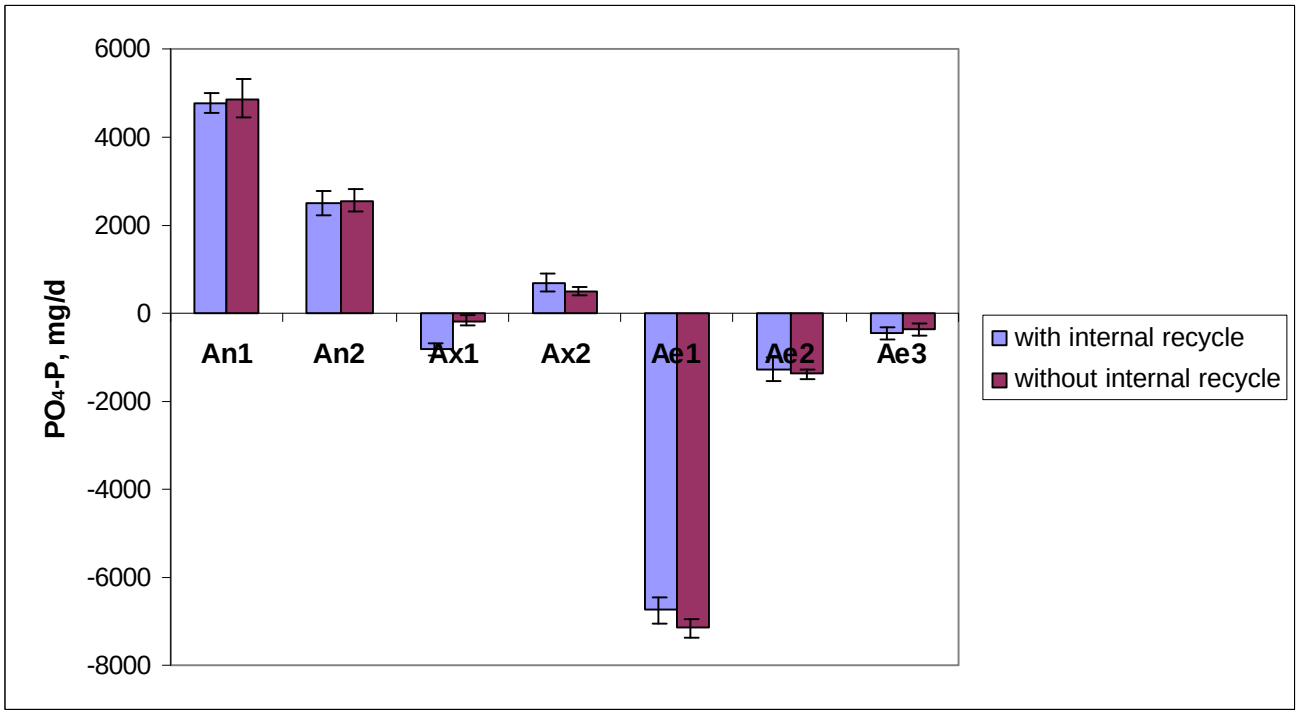

Figure 9. Mass balance of PO_4-P in System 1 at 20^{o}C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

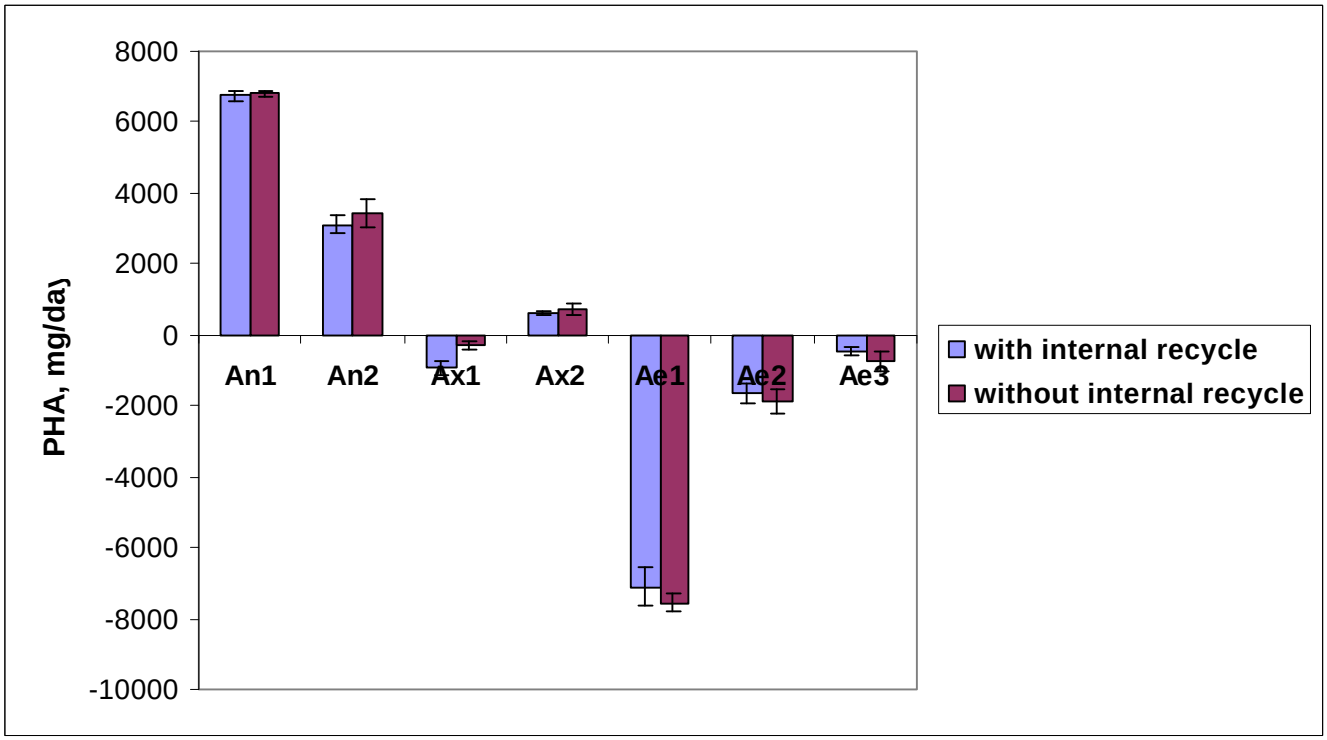

Figure 10. Mass balance of PHA in System 1 at 20^{o}C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

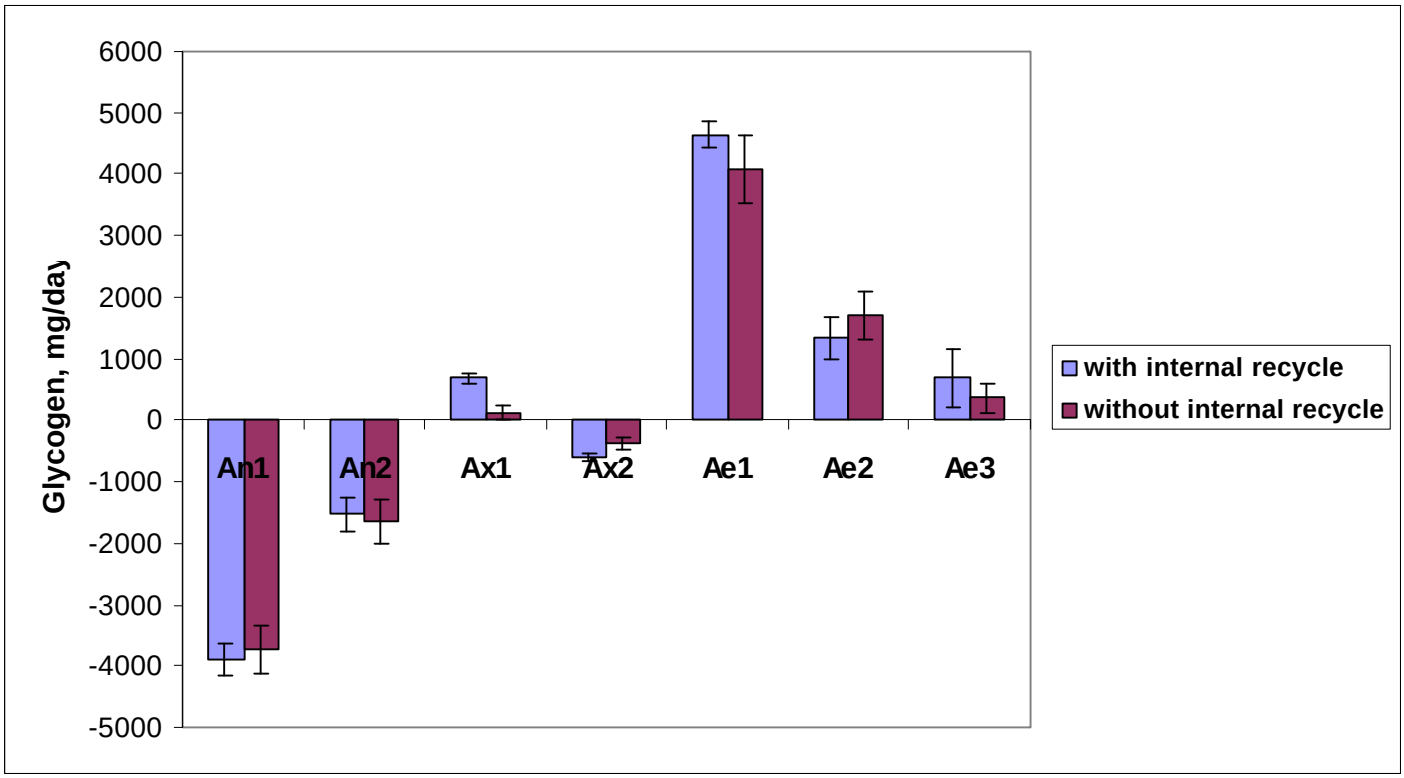

Figure 11. Mass balance of glycogen in system 1 at 20^{o}C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

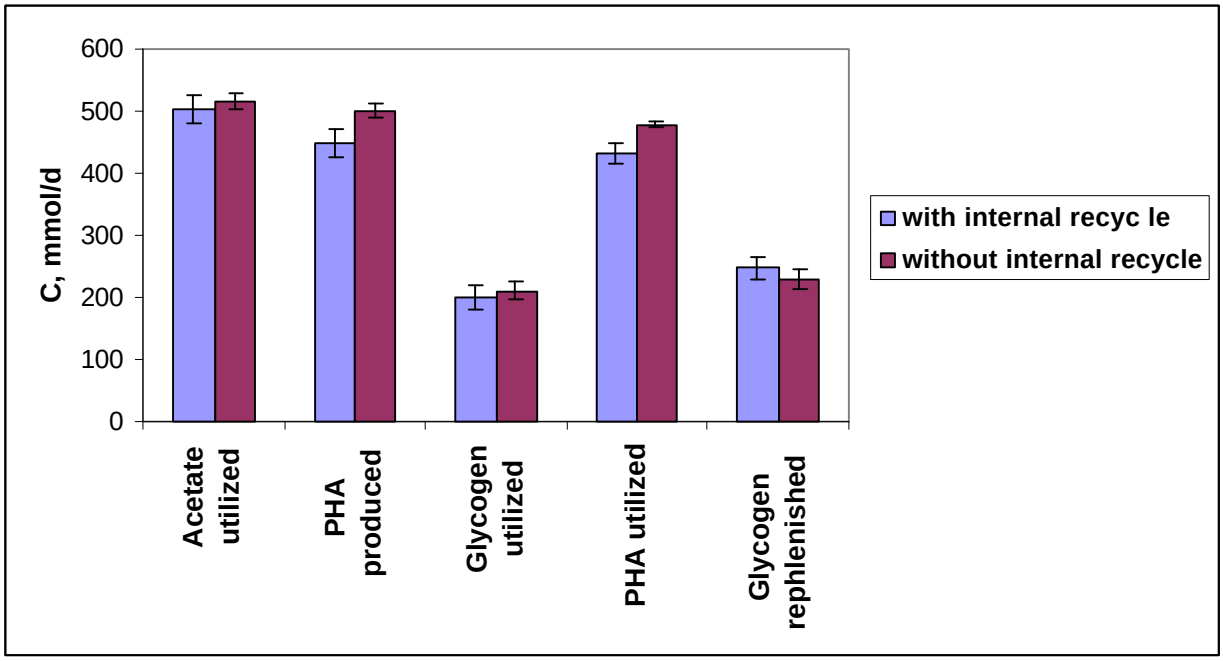

Figure 12. Carbon mass balance of Acetate, PHA and glycogen in System 1 at 20^{o}C (error bar represents the standard deviations of the mean values, N=4 with internal recycle and N=7 without internal recycle).

denitrifying bacteria had the ability to utilize PHA under anoxic conditions (Figure 10). It was concluded that less PHA (53 Cmmol/d) was available to the PAOs for growth and maintenance, poly-P storage and glycogen replenishment. It was also concluded that PHA can be used as the organic substrate for denitrification in the absence of external substrate. Mixed liquor solids concentrations with and without aerobic recycle cases are given in table 5.

Table 5. Steady state biomass solids concentrations throughout System 1 at 20^{o}C.

	with internal recycle		without internal recycle	
	MLSS	MLVSS	MLSS	MLVSS
Anaerobic 1	3202±119	2287±69	2930±80	2112±38
Anaerobic 2	3191±139	2283±71	2896±79	2107±49
Anoxic 1	4591±107	3231±54	4542±71	3246±27
Anoxic 2	4560±76	3197±86	4509±55	3238±44
Aerobic 1	4702±91	3212±113	4653±57	3255±94
Aerobic 2	4762±99	3208±104	4737±101	3303±67
Aerobic 3	4839±91	3240±84	4789±97	3281±43
Effluent	45±9	29±12	36±5	24±7
RAS	7594±167	5197±125	7578±124	5297±59
	n=4, ±: standard deviation		n=7, ±: standard deviation	

System Performance at 10^{o}C:

The same UCT process used previously at 20^{o}C was operated at 10^{o}C without aerobic recycle at steady state for more than 3 months. The steady state data (Figure 13-19) showed that the system removed an average P concentration of 71 mg/L. Similar to 5^{o}C operation, P release continued throughout the anoxic stages at 10^{o}C. The comparison of C mass balance of PHA and glycogen revealed that PHA production and glycogen uptake showed similar trends in the non-oxic stages at 10 and 5^{o}C, whereas this trend was not observed in the non-oxic stages at 20^{o}C. The sludge P content of 39% as VSS indicated that the system operated at 10^{o}C had a large poly-P population. The electron microscopy micrographs detected indivudual cells with very large poly-P granules in the aerobic stage of this system (Erdal and Randall 2002 c). On the other hand, another distinct population with no poly-P accumulation (non-poly-P bacteria) was detected in one out of 5 grids. However, the extent of non poly-P presence at 10^{o}C was relatively low compared to that observed at 20^{o}C. The presence of fewer Poly-P bacteria at 10^{o}C operation was one of the reasons why 5^{o}C operation (99 mg P/L) out competed 10^{o}C operation (71 mgP/L) with respect to P removal performance. The other reason for the reduced P removal performance at 10^{o}C was related to an increased amount of NO_3-N recycled to the anoxic stage with the return activated sludge (RAS). The anoxic stage at 10^{o}C received 332 mgO_2/day more electron acceptor compared to that received at 5^{o}C. Total PHA production through the non-oxic stages was 12905 mg/day at 5^{o}C whereas it was 11792 mg/day at 10^{o}C.

The stoichiometry of EBPR processes have been reported found to be insensitive to temperature (Brdjanovic et al. 1997). However this study showed that (Figure 17 and 18) the EBPR stoichimetry is sensitive to temperature. The observed stoichometry between anaerobic PHA production and glycogen utilization was 4.47, 3.28 and 2.37 for 5, 10 and 20^{o}C, respectively. Aerobic stoichiometry was also affected by temperature and the aerobic glycogen rephlenished per PHA utilized was 0.26, 0.37 and 0.49 for 5, 10 and 20^{o}C, respectively. It is obvious that glycogen metabolism is adversely affected by cold temperatures. Reduced demand for glycogen at cold temperatures, however, resulted in

better EBPR performance. Enzyme assay tests performed by Z. K. Erdal et al. (2002) showed that the activity of phospho-frukto-kinease enzyme, one of the key enzymes of glycolysis, is very slow at 5^{o}C. It was concluded that cold temperatures change EBPR metabolisms in such a way that EBPR bacteria store less glycogen due to glycolysis kinetic limitation. Thus, more substrate as PHA was available for poly-P metabolism in the aerobic stage. The need for maintanence energy requirement is also low at cold temperatures (Grady et al. 1999). These factors caused the PAOs to accumulate more poly-P than they would at higher temperatures. The P content (P_x) of EBPR sludges under different temperatures showed increased poly-P storage ability of the EBPR biomass as temperature decreased.

In summary, it was concluded that three factors cause EBPR performance to improve as temperature decreases, even though kinetic rates are slower. These are:

- The selective pressure of colder temperatures results in a population shift which greatly reduces the non poly-P bacterial population, and the PAOs progressively dominate the overall population. The small amount of non-poly-P population observed at 10^{o}C suggests that 10^{o}C is low enough to enhance the PAO fraction of the population in EBPR systems. PAOs are even more dominant at 5^{o}C.
- Cold temperatures exert strong pressure on the metabolic pathways of EBPR processes. Due to the slow down of glycolylosis as temperature decreases, EBPR bacteria accumulate less glycogen even when PHA is not limited. Therefore most of the energy is stored as poly-P and the amount stored as glycogen decreases as temperature decreases.
- Reduced nitrate entry to the anoxic stage at 5^{o}C minimized the acetate consumption for denitrification purpose whereas reduced nitrate mass at 20^{o}C minimized the PHA utilization for denitrification, resulting in a greater amount of energy available for utilization by poly-P metabolism.

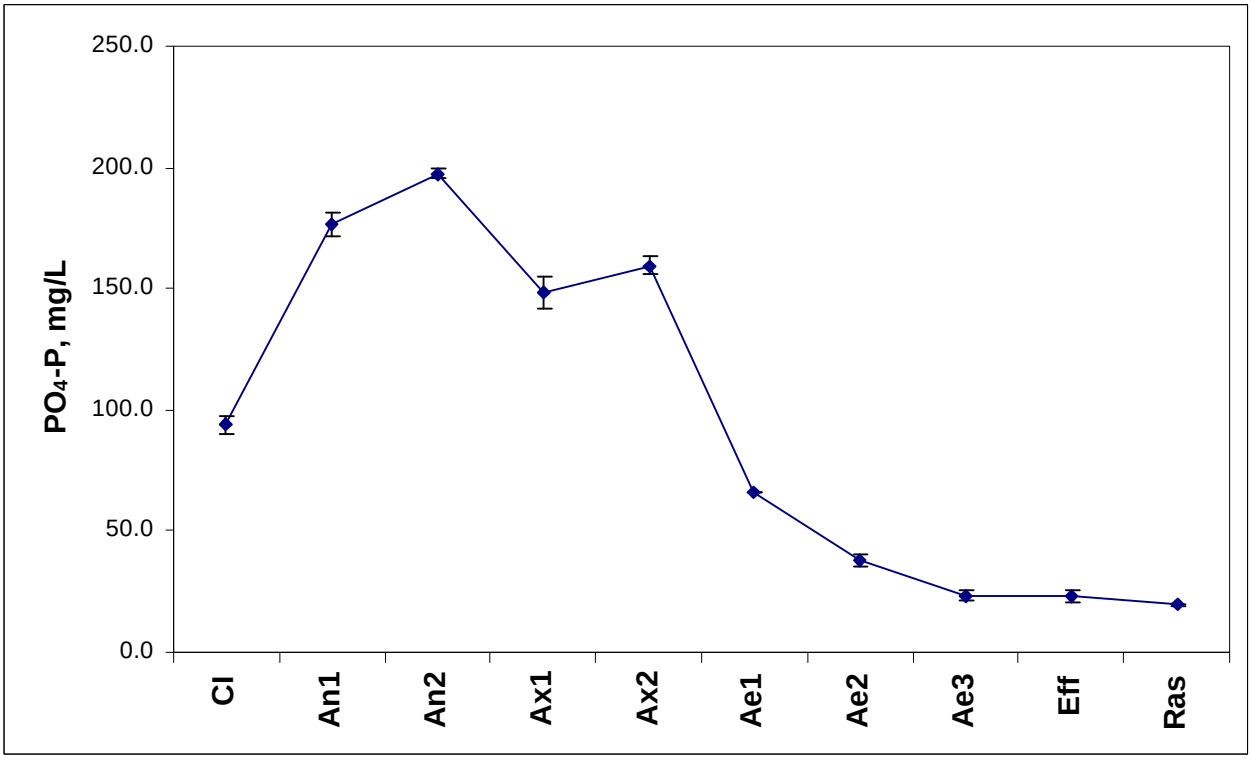

Figure 13. Soluble P concentration through the System 1 reactors at 10^{o}C.

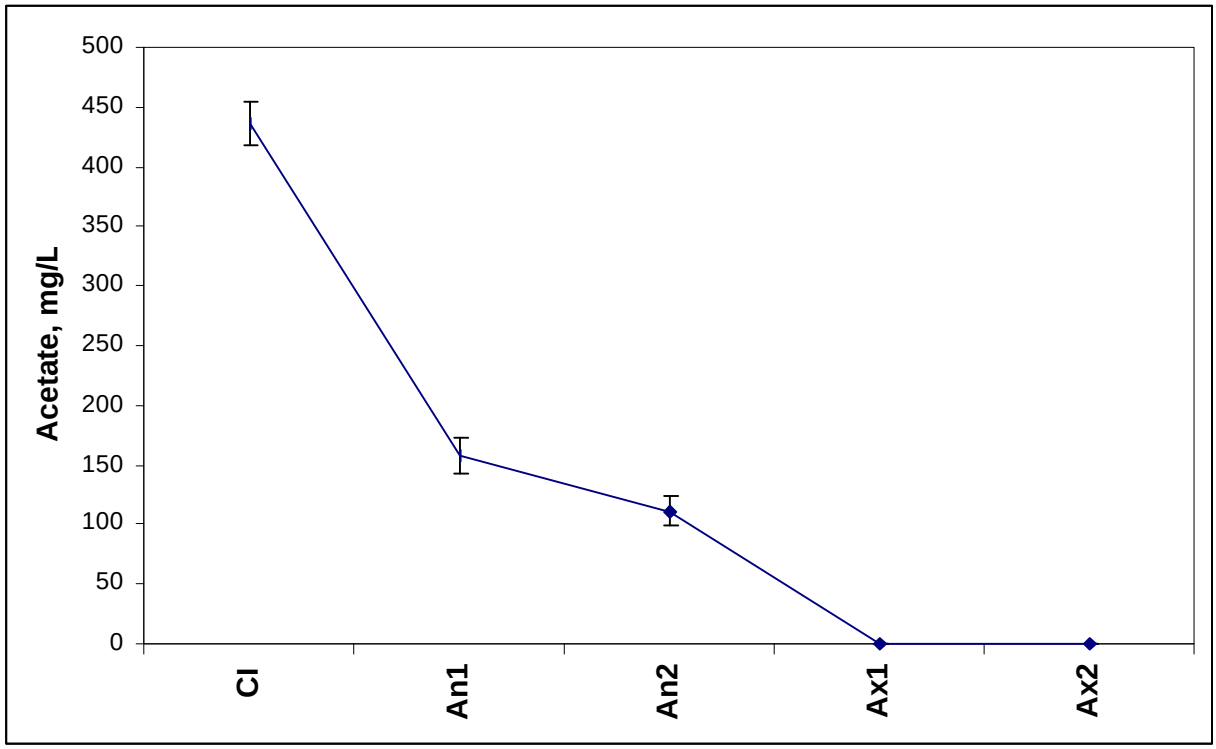

Figure 14. Acetate concentrations through the System 1 reactors at 10^{o}C.

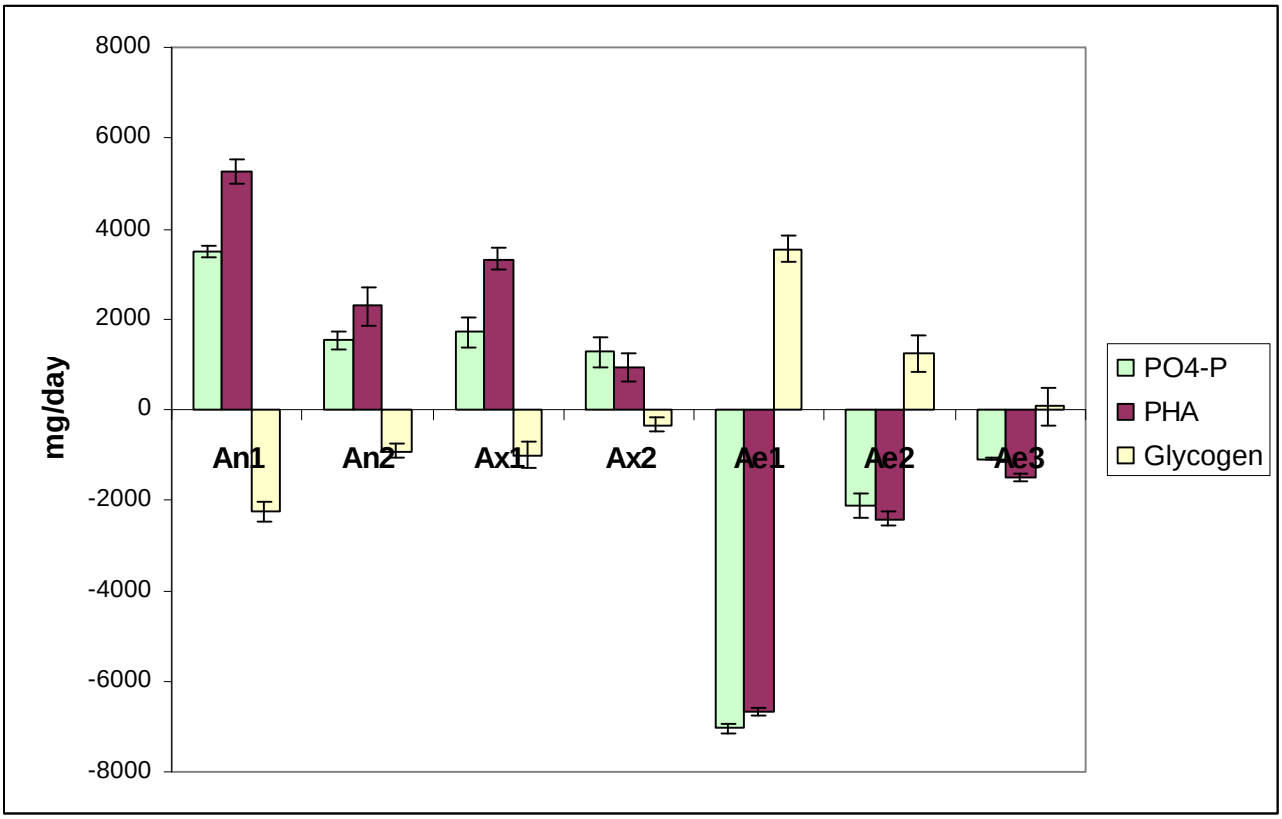

Figure 15. Mass balance of PO_4-P, PHA and glycogen through the System 1 reactors at 10^oC.

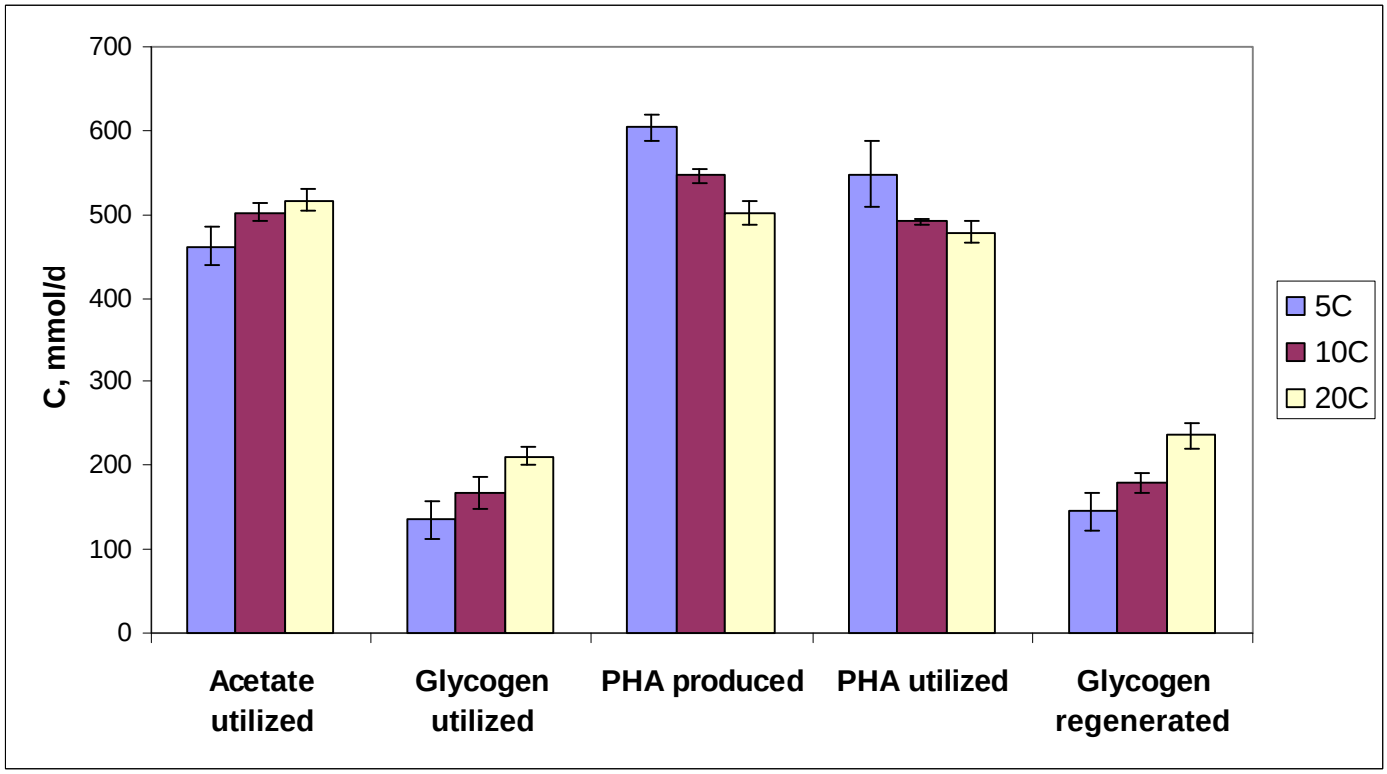

Figure 16. Comparison of C mass balance of acetate, PHA and glycogen for 5,10 and 20^{o}C in the UCT systems operated without internal recycle.

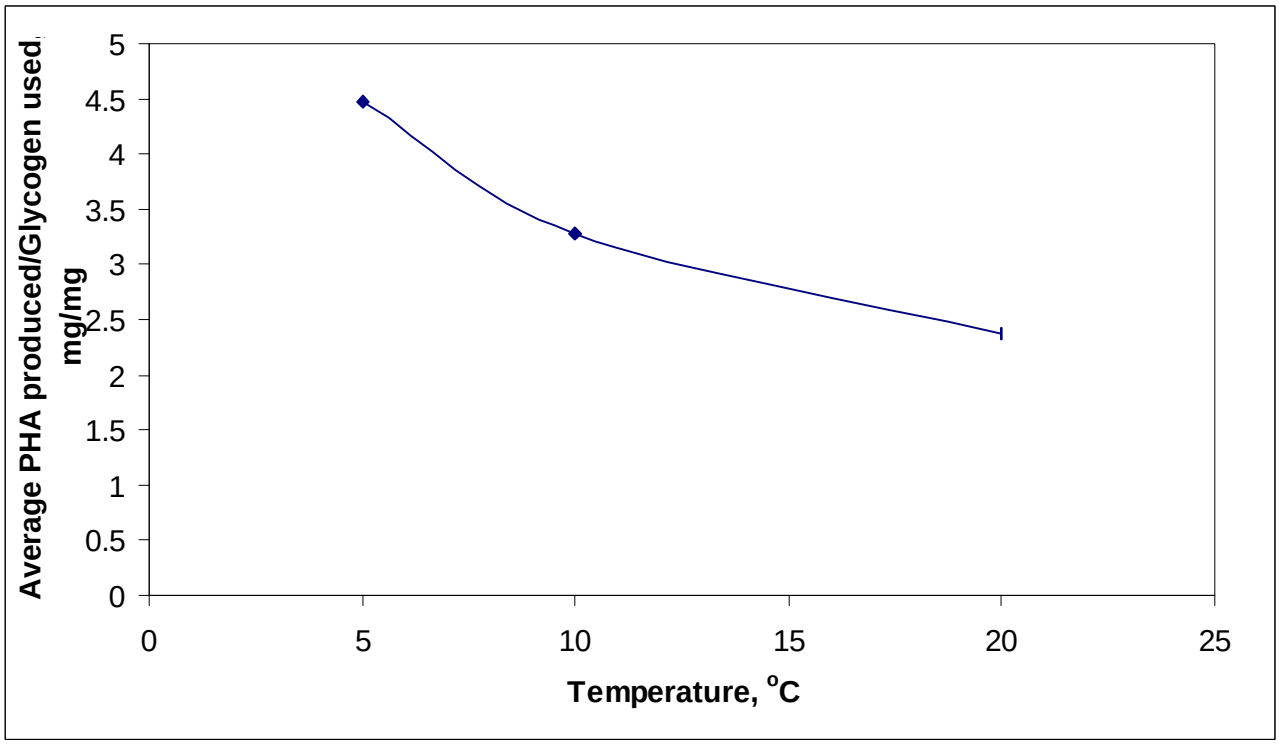

Figure 17. The observed stoichiometry between anaerobic PHA produced and glycogen utilized at 5,10 and 20°C in UCT systems operated without internal recycle.

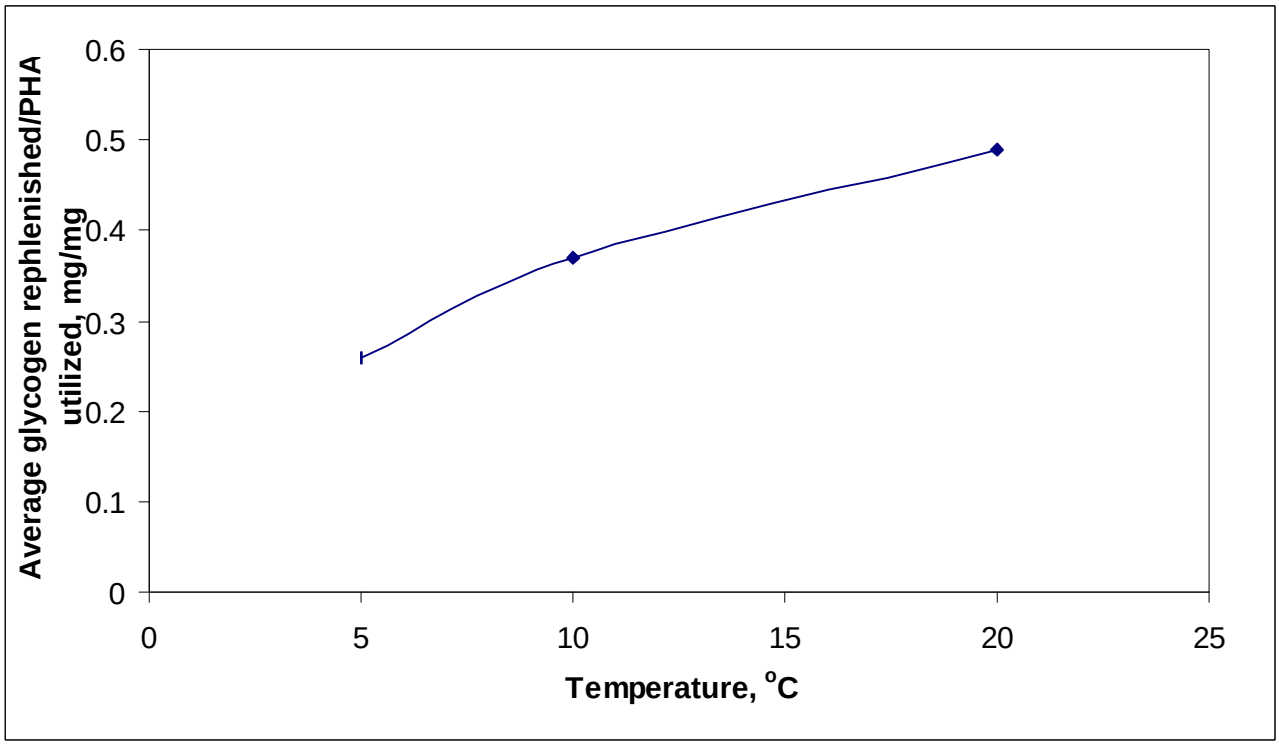

Figure 18. Observed stoichiometry between aerobic glycogen replenished and PHA utilized at 5,10 and 20^{o}C in UCT systems operated without internal recycle.

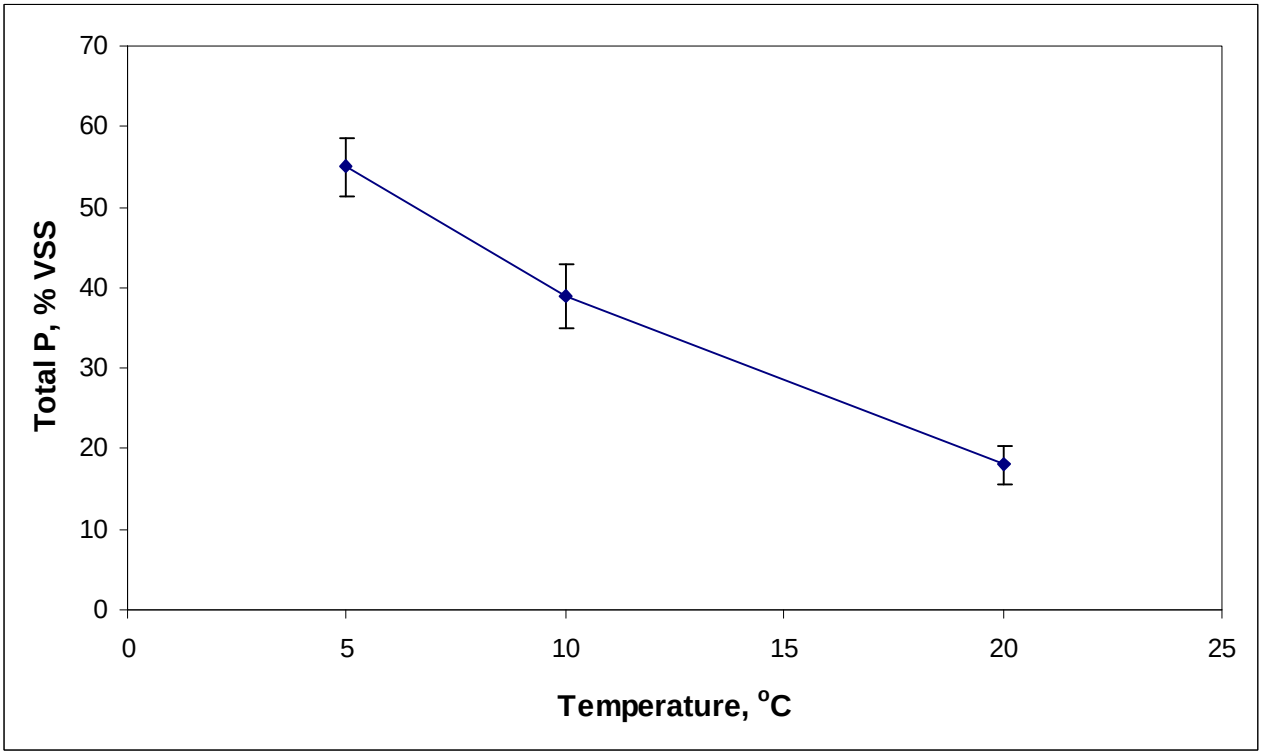

Figure 19. P content (P_x) of sludge for different temperatures in UCT system operated without internal recycle.

The effects of SRT

The effects of SRT on EBPR performance and on internal storage products are illustrated by Figures 20 through 23. The changes in the biomass solids concentrations are summarized in Table 1. In this study system performance was not affected by SRT over the range of 18 to 16.4 days. However, EBPR performance was linearly reduced over the SRT range of 15 to 11 days, but the EBPR system was still removing a considerable amount of P when operated at a total SRT of 5 days. Then, EBPR performance was completely lost and wash-out of the biomass occurred at a total SRT of 3.5 days. Glycogen utilization and regeneration were dramatically reduced at the 5 day-SRT (424 and 488 mg/d, respectively), and no utilization and regeneration was observed at the SRT of 3.5 days. PHA production and utilization and P release and uptake patterns were still observed even at wash-out SRT. The Poly-P content of the bacterial cells was not depleted completely at wash-out (6% P as VSS) and they released approximately 2000 mg/d P in the non-oxic reactors. However, PHA storage was not enough to take up the additional P entering the system. This suggests that glycogen is not the sole reducing power source. In a related study, Z. K. Erdal (2002) showed that the key TCA cycle enzymes (isocitrate dehydrogenase and malate dehydrogenase) were active at 5^{o}C. This possibly suggests that even though PAOs may use the TCA cycle or other mechanisms (e.g. lipid degradation) for reducing power, the results indicate that glycogen metabolism is obligatory for successful EBPR operation. In a series of batch tests, glycogen metabolism was found to be very slow at 5^{o}C (U. Erdal 2002 c). It appears that glycogen metabolism kinetically limited EBPR performance.

Mamais and Jenkins (1991) showed that EBPR can be washed out at any temperature give the appropriate SRT combination. A very good linear relationship between aerobic SRT and temperature was reported over the temperature range of 10 to 20^{o}C. This relationship is given in Figure 26.

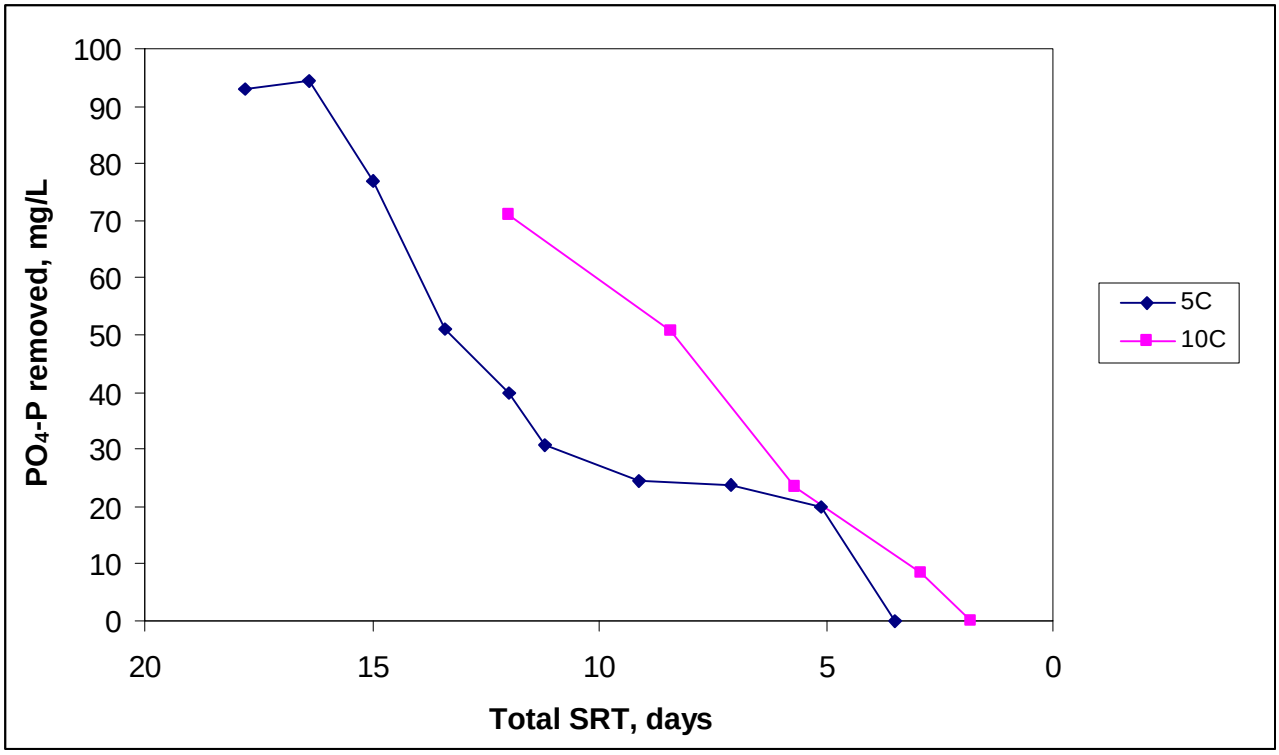

Figure 20. P removal as a function of SRT in UCT Systems at 10 and 5°C.

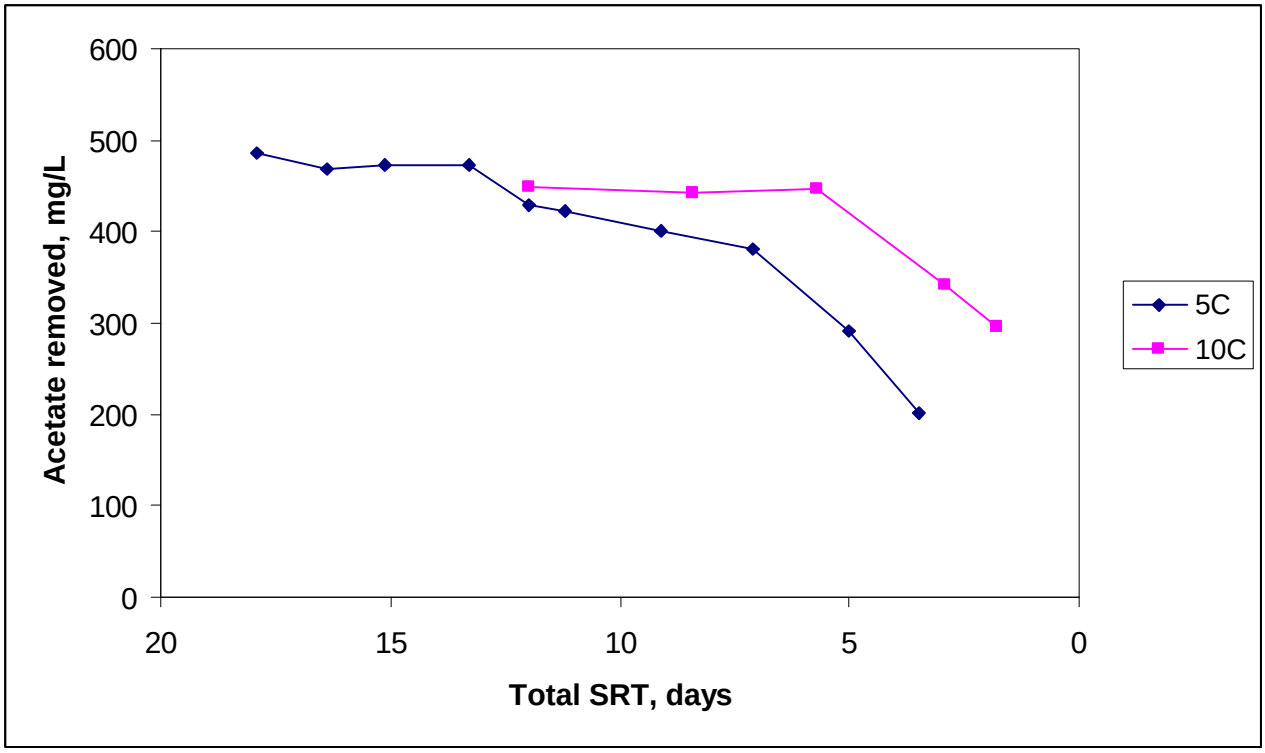

Figure 21. Acetate removal through the non-oxic stages at 10 and 5^{o}C as a function of SRT.

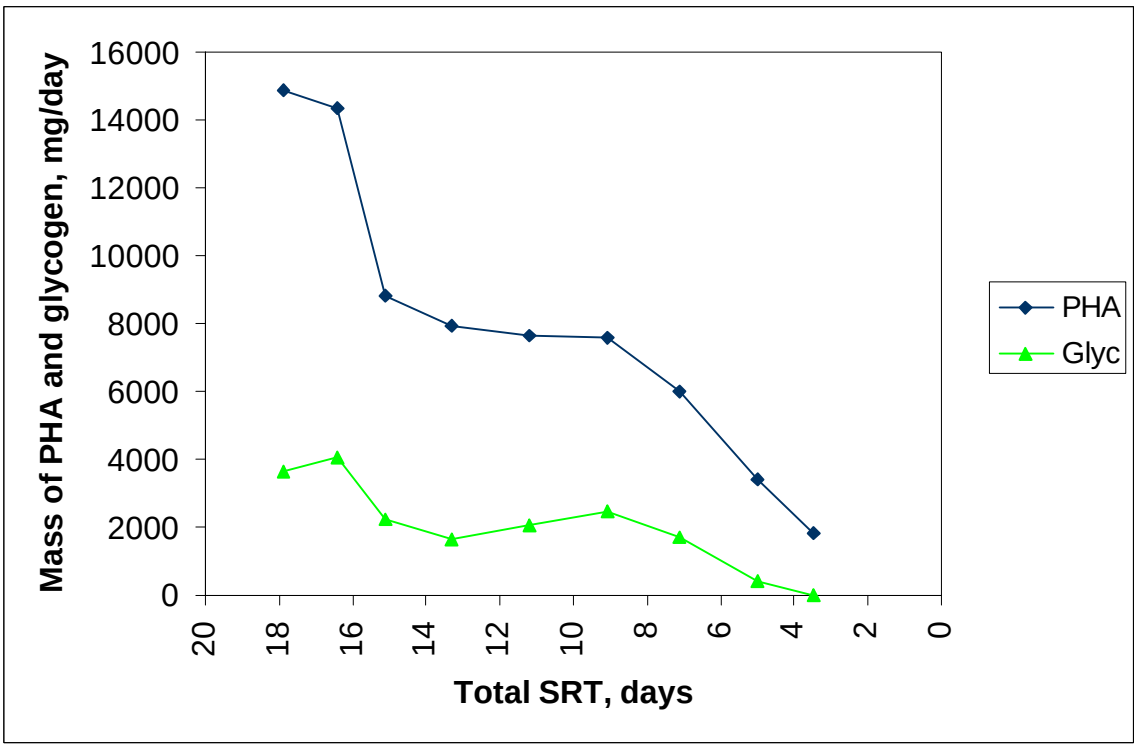

Figure 22. PHA production and glycogen utilization through the non-oxic stages of a UCT system operated without internal recycle at 5°C.

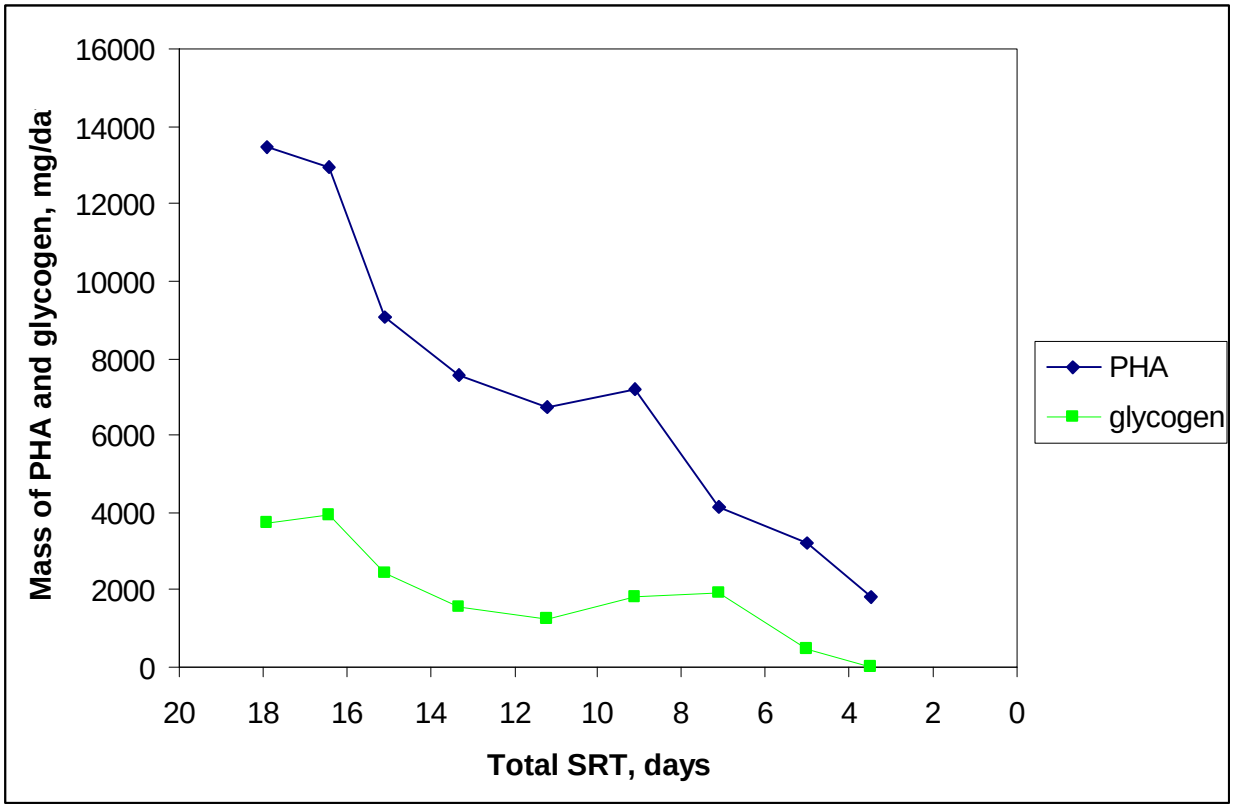

Figure 23. PHA utilization and glycogen replenishment through the aerobic stage in a UCT system operated without internal recycle at 5^{o}C.

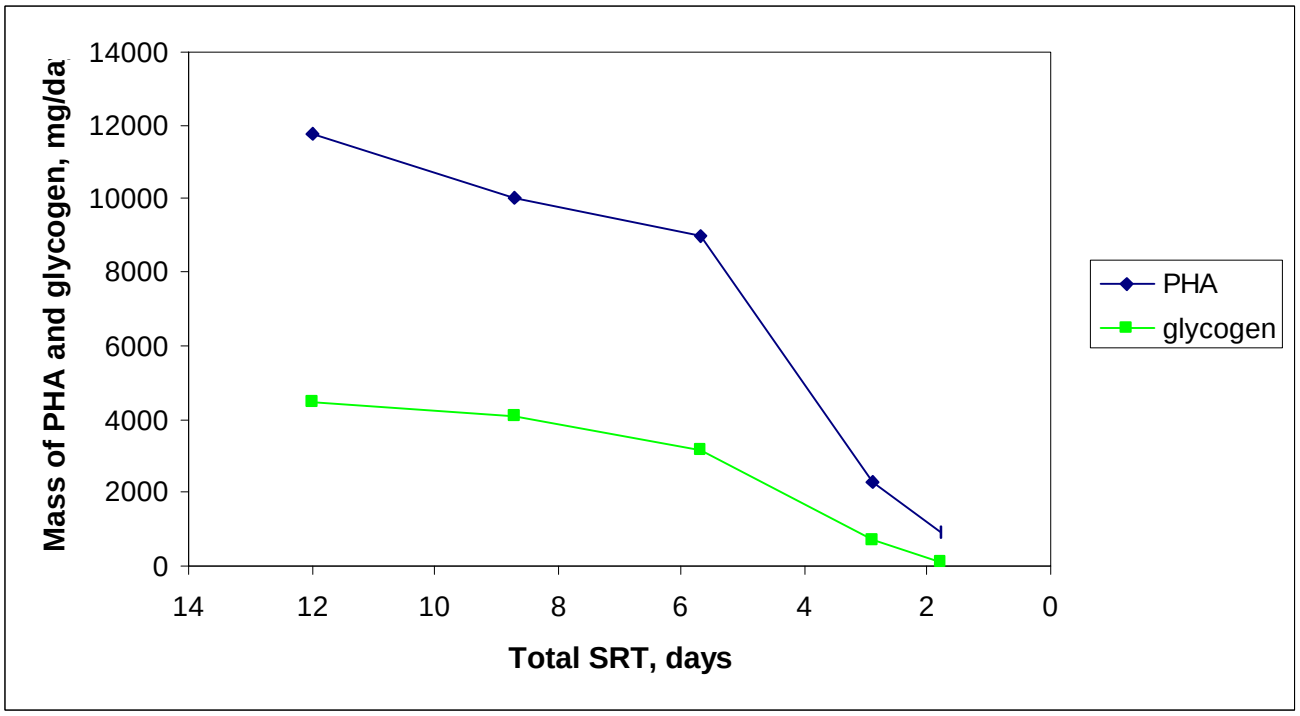

Figure 24. PHA production and glycogen utilization through the non-oxic stages of a UCT system operated without internal recycle at 10^{o}C.

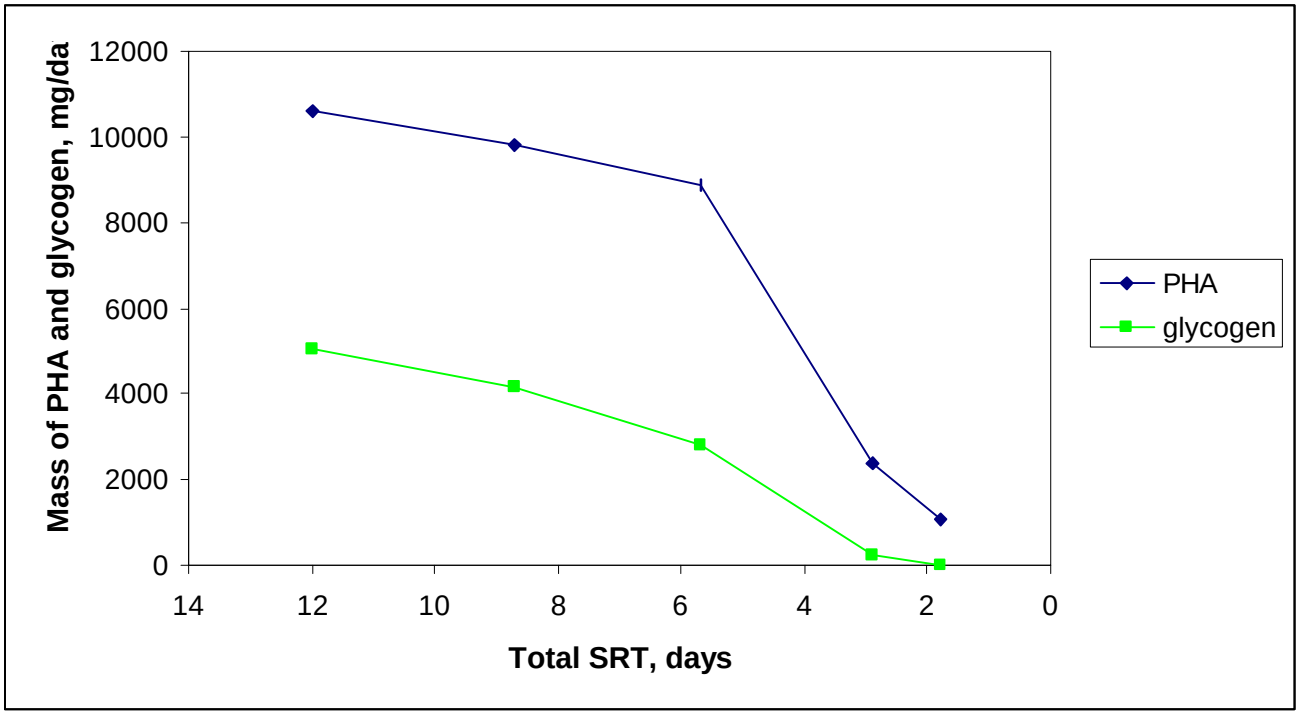

Figure 25. PHA utilization and glycogen replenishment through the aerobic stage in a UCT system operated without internal recycle at 10^{o}C.

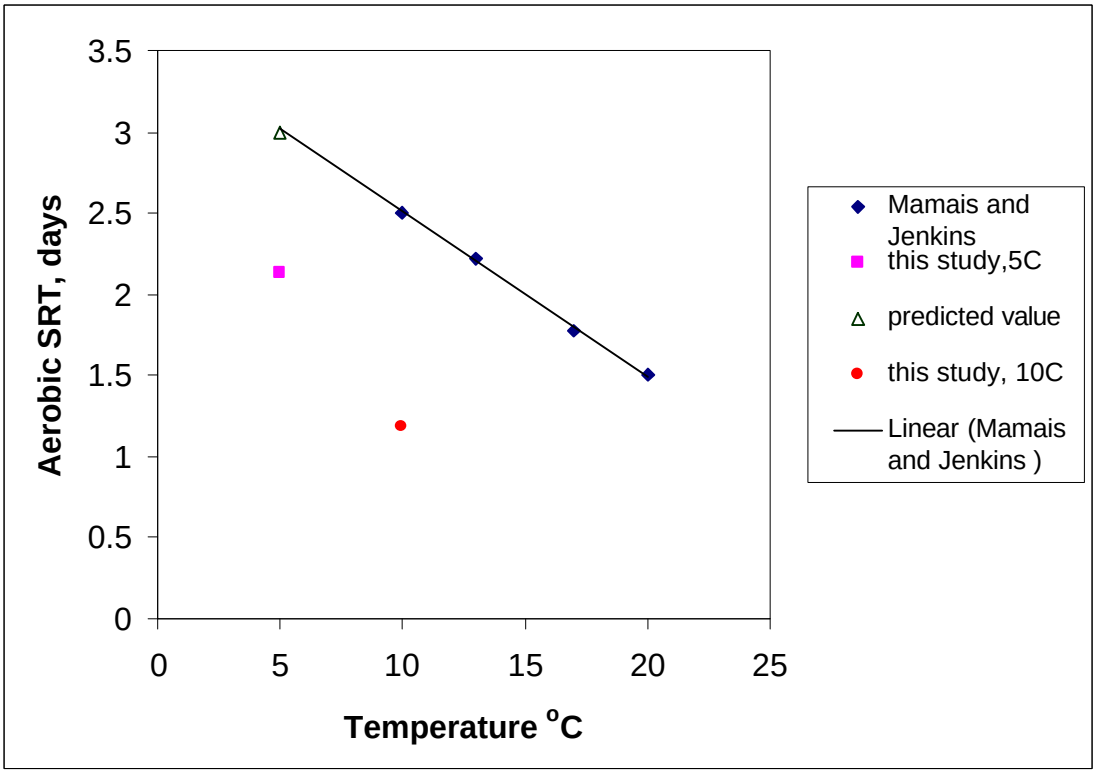

Figure 26. The relationship between washout SRT and temperature given by Mamais and Jenkins (1992).

The extension of Mamais and Jenkins linear line (predicted value in the graph) shows that EBPR would wash out at an aerobic SRT of 3 days at 5^{o}C. However, the aerobic washout SRT in this study was 2.1 days (61% of the total SRT), i.e., somewhat smaller than their prediction. Similarly, the aerobic washout SRT (1.2 days) was much lower than their predictions (2.55 days) at 10^{o}C. Perhaps the difference should not be surprising because the P concentration in their study was limiting at only 8.5 mg/L, and the P content of their sludge was only 6.5 % of the VSS at 20^{o}C. In this study, COD was limiting and a much higher sludge P content was obtained (18, 39 and 55% of VSS at 20,10 and 5^{o}C, respectively). In their study, poly-P was depleted before glycogen was sdepleted, and it caused washout to occur sooner. In this study, washout in cold temperatures was not linked to exhaustion of poly-P but was related to the loss of glycogen metabolism.

CONCLUSIONS

- The performance of EBPR systems is strongly affected by the amount of electron acceptor recycled to the anoxic reactor. The results showed that a moderate amount of electron acceptor entering to the anoxic stage reduced the EBPR performance by d
- Even though bacterial cells contained poly-P and showed similar release and uptake pattern at washout SRT, no additional P was taken up in the absence of glycogen involvement. Near washout SRT, very low solids concentration and slowing kinetics resulted in no replenishment of glycogen in the aerobic stage, and this resulted in washout of EBPR. If the cells had been able to utilize glycogen, it is hypothesized that washout of the sludge would not have occurred until the poly-P pool of the cells were completely depleted.
- Both anaerobic and aerobic stoichiometry of EBPR process are sensitive to temperature.

- A much lower wash-out SRT value was observed at 10^{o}C than that reported by Mamais and Jenkins (1992). This difference can be explained because P limiting conditions were applied during their study, but not in this study. Thus much lower sludge P content was achieved (8 %) by them relative to this study. More likely poly-P became limiting before glycogen in their study, and a relatively higher washout SRT was observed.
- Glycogen metabolism is a key parameter and seems to be the most rate-limiting step in EBPR processes under cold temperatures.
- The PHA production in the absence of glycogen indicates that PAOs may use different pathways to generate reducing power. This concept needs further investigation.
- Operating UCT plants without aerobic recycle seems to be a promising means for improving P removal under cold temperatures. Wastewater treatment plants with no strict effluent N regulation may use this modification to improve P removal in winter.
- SRT values of 16 days or more must be provided to achieve optimum P removal at 5^{o}C.

REFERENCES

Abu-Ghararah, Z.H. and Randall, C.W. (1991) The effect of organic compounds on biological phosphorus removal. *Wat.Sci.Tech.*, **23**, 585-594.

APHA (1985). Standard Methods for the Examination of Water and Wastewater. 16th Edition, American Public Health Association, Washington D.C.

Barker, P.S. and Dold, P.L. (1996). Denitrification behaviour in biological excess phosphorus removal activated sludge systems. *Wat. Res.* **30** (4) 769-780.

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.

Beatons, D., Vanrolleghem, P.A., vanLoosdtrecht, M.C.M. and Hosten, L.H. (1999) Temperature effects in bio-P removal. *Wat. Sci. Tech.* **39** (1) 215-225.

Brdjanovic, D., Slamet, A., van Loodsdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J. and Heijnen, J.J. (1998) Impact of excessive aeration on biological phosphorus removal from wastewater. ***Wat. Res***. **32** (10) 200-208.

Brdjanovic, D., Slamet, A., van Loodsdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J. and Heijnen, J.J. (1998) Influence of Temperature on BPR: Process and Molecular Ecological Studies. *Wat. Res*. **32** (4) 1035-1048.

Brdjanovic, D., van Loodsdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J. and Heijnen, J.J. (1997) Temperature effects on physiology of biological phosphorus removal. *Journal of Environmental Engineering* **123** (2) 144-153

Cech, J. S. and Hartman, P. (1990) Glucose induced break down of enhanced biological phosphate removal. *Environ. Tech*. **11** 651-656.

Cech, J.S. and Hartman P. (1993). Competition between polyphoshate and polysaccharide accumulating bacteria in enhanced biological phosphorus removal systems. *Water Res.* **27** 1219-1225.

Choi, E., Rhu, D., Yun, Z. and Lee, E (1998). Temperature effects on biological nutrient removal system with municipal wastewater. *Wat.Sci. Tech*. **37** (9), 219-226.

Comeau, Y., Hall, K.J., Hancock, R.E.W. and Oldham, W.K. (1986) Biochemical Model for enhanced biological phosphorus removal. *Wat. Res*. **20** (12) 1511-1521.

Comeau, Y., Oldham, W.K. and Hall, K.J. (1987) Dynamics of carbon reserves in biological dephosphatation of wastewater. *Proc. of IAWPRC Specialized Conference*, **28-30** Sept. 1987. Rome. Italy. 39-55.

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

Daigger, G.T., Randall, C.W., Waltrip, G.D. and Romm, E.D. (1987). Factors affecting biological phosphorus removal for the VIP process, a high-rate University of Cape Town type process. *Proceedings of an IAWPRC specialized conference held in Rome, Italy Ramadori Ed*.

Ekama, G., Marais, G. and Siebritz, I. (1984). Biological Excess Phosphorus Removal, in Theory, Design and Operation of Nutrient Removal Activated Sludge Processes, Water Research Commission, Pretoria, South Africa.

Erdal, U.G. and Randall, C.W. (2002 c) The effects of temperature on system performance and bacterial community structure in EBPR systems. PhD dissertation. Virginia Polytechnic Institute and State University.

Erdal, Z.K., Randall C.W. and Gregory E.M. (2002). An investigation of biochemistry of biological phosphorus removal systems. PhD dissertation. Virginia Polytechnic Institute and State University.

Filipe, C.D.M., Daigger, G.T., and Grady, C. P. L. Jr (2001 a). Effects of pH on the rates of aerobic metabolism of phosphate-accumulating and glycogen-accumulating organisms. *Water Environment Research*. **73** (2), 213-221.

Filipe, C.D.M., Daigger, G.T., and Grady, C. P. L. Jr (2001 b). pH as a key factor in the competition between glycogen-accumulating organisms and phosphate-accumulating organisms. *Water Environment Research*. **73** (2), 223-232.

Gerhardt, P., Murray, R.G.E., Wood, W.A. and Krieg, N.R. (1994) Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington D.C.

Grady, C.P.L.Jr., Daigger, G. T., and Lim, H.C. (1999). *Biological Wastewater Treatment 2nd edition*. Marcel Dekker , Inc.Newyork.

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.

Hood, C.R. and Randall, A.A. (2001) A biochemical hypothesis explaining the response of enhenced biological phosphorus removal biomass to organic substrates. *Wat. Res.* **35**(11) 2758-2766.

Kisoglu, Z., Erdal, U.G., and Randall, C.W. (2000). The effect of COD/TP ratio on intracellular storage materials, system performance and kinetic parameters in a BNR system. WEFTEC 2000 Anaheim CA.

Liu, W., Mino, T., Nakamura, K. and Matsuo, T. (1996) Glycogen accumulating population and its anaerobic substrate uptake in anaerobic-aerobic activated sludge without biological phosphorus removal. *Wat. Res.* **30** (1) 75-82.

Liu, W., Nakamura, K., Matsuo, T. and Mino, T. (1997) Internal energy-based competition between polyphosphate-and glycogen-accumulating bacteria in biological phosphorus removal reactors-effect of P/C feeding rato. *Wat. Res.* **31** (6) 1430-1438.

Mamais, D. and Jenkins, D. (1992). The Effects of MCRT and Temperature on Enhanced Biological Phosphorus Removal, *Wat.Sci. and Tech.*, **26**, (5-6), 955-965.

McClintock, S., Randall, C.W. and Pattarkine, V. (1992). The effects of temperature and mean cell residence time on enhanced biological phosphorus removal. Environmental Engineering, the Proceedings of the 1991 Specialty Conference on Environmental Engineering, ASCE. 319-324.

Mino, T., Arun, V., Tsuzuki, Y. and Matsuo, T. (1987) Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. *Proc. of IAWPRC Specialized Conference*, 28-30 Sept. 1987. Rome. Italy. 27-38.

Mino, T., van Loodsdrecht, M.C.M. and Heijnen, J.J. (1998) Microbiology and biochemistry of the enhanced biological phosphate removal process. *Wat. Res.* **32** (11)3193-3207.

Oldham, W.K. and Dew, H.P. (1979). Cold temperature operation of the Bardenpho process. *Proc., 14th Can. Symp. On Water Pollution Res.*, Toronto, Canada.

Pereira, H., Lemos, P.C., Reis, M.A.M., Crespo, J.P.S.G., Carrondo, M.J.T. and Santos, H. (1996) Model for carbon metabolism in biological phophorus removal processes based on *in vivo* ^{13}C-NMR labeling experiments. *Wat. Res.* **30**(9) 2128-2138.

Randall, C.W., Barnard, J.L. and Stensel, H.D. (1992). Design and retrofit of wastewater treatment plant for biological nutrient removal. Water Quality Management Library, Lancaster Pensylvania USA.

Sell, R. (1981). Low Temperature Biological Phosphorus Removal, Presented at the 54th Annual Conference of the Water Pollution Control Federation, Detroit, Michigan. Air Products and Chemicals, Inc. Allentown, PA, USA.

Seviour, R.J. and Blackall, L.L. (1999) *The Microbiology of Activated Sludge*. Kluwer Academic Publisher Dordrecht, Netherlands.

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M. and Heijnen, J.J. (1994). Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. *Biotechnology and Bioengineering*. **43**, 461-470.

Wentzel, M.C., Lotter, R.H., Loewenthal, R.E. and Marais, G.V.R. (1986). Metabolic behaviour of Acinetobacter spp. in enhanced biological phosphorus removal-a biochemical model. *Wat.SA* **12**, 209-224.