INTRODUCTION

The effects of temperature on the efficiency and the kinetics of excess biological phosphorus removal (EBPR) systems have been under investigation for the past two decades, but with contradictory results. Early researchers (Sell, 1981; Ekama, *et al.* 1984; Barnard *et al.* 1985) reported that EBPR efficiency was greater at lower temperatures than at higher temperatures over the range from 5 to 24 ^{o}C. The first contradictory finding was reported by McClintock *et al.* (1991). They showed that EBPR functions would "wash-out" of activated sludge systems before other heterotrophic functions at a temperature of 10 ^{o}C and a sludge retention time (SRT) of 5 days, whereas wash-out did not occur at 10 ^{o}C when the SRT was 15 days. Then, Mamais and Jenkins (1992) showed that there is a wash-out SRT for all temperatures over the range of 10 to 30^{o}C. This introduced the paradox that, even though EPBR system performance becomes more efficient at lower temperatures, if the SRT-temperature combination is below a critical value, EBPR ceases before other heterotrophic functions wash-out. More recently, John and Stephenson (1996); Brdjanovic *et al.* (1997 and 1998); Choi et al. (1998); Beatons *et al.* (1999) have shown that EBPR biochemical reaction rates become slower with decreasing temperature, as is typical of biochemical reactions, if the microbial population is unchanged. Thus, although temperature appears to affect EBPR reaction rates as expected, a substantial body of evidence including full-scale experience indicates that many EBPR systems perform more efficiently as the temperature decreases. The research presented in this thesis was designed to investigate this apparent contradiction.

It is evident that NO_3-N and other electron acceptors (e.g. O_2 and NO_2-N) entering the anoxic stage will adversely affect the EBPR system performance. Since, in the presence of nitrate, denitrifying bacteria utilize volatile fatty acids (VFAs) and electron flow is diverted from polyhydroxyalkanoate (PHA) production to NO_3^- reduction (Bond and Rees, 1999). Thus it reduces the amount of VFAs available for phosphate accumulating organisms (PAOs) and poly-P metabolism. It was proposed that the concentration of oxidized nitrogen should be less than 10 mgN/L in recycled flow in order to achieve good EBPR performance (Tetroult *et al.*, 1986). Even though this concept was investigated to

some extent under warm temperatures (Kuba *et al.*, 1996), no study has been done to see NO_3-N effect under cold temperatures. It should be remembered that even though cold temperatures can be advantageous for EBPR due to reducing nitrate entry (lower nitrification), it has a negative impact due to recycling more dissolved oxygen (DO) (greater saturation of O_2 at cold temperatures). It causes oxidation of readily available substrate in the anoxic zone instead of being stored as PHA. In this study, the combined effects of nitrate and oxygen were investigated in two-identical UCT pilot plants operated at 20 and 5^{o}C.

Mamais and Jenkins 1992 showed that there is a critical SRT for all temperatures, below which EBPR would not function, which is consistent with the predicted impact of temperature on chemical/biochemical reactions in accordance with the Arrhenius relationship. However, the SRT-temperature relationship was unknown to the researchers who concluded that PAOs are psychrophilic relative to typical activated sludge heterotrophic populations and assumed this would explain why they become more efficient at lower temperatures. Due to the selective pressure exerted by the cold temperature, microorganisms with growth characteristics that most closely fit the environmental conditions become dominant (Morris and Clarke, 1981). It is known that the diverse bacterial consortium typical of biological wastewater treatment systems in temperate climates consist mainly of psychrophilic, pyschrotrophic and mesophilic bacteria. Due to their different optimum temperature growth ranges, a distinct group may be dominant over a temperature range below or above which they will "wash-out" or be quantitatively insignificant. This phenomenon is referred to as population shift (Morris and Clarke, 1981), but it requires time-dependent acclimation of the culture to the environmental conditions before it occurs.

In summary, this research was designed to develop evidence that could be used to resolve the EBPR temperature paradox. The experimental approach was designed to investigate the following issues:

1. The importance of acclimation to the experimental temperature;

2. Differences between system responses to short-term and long-term temperature exposures;
3. Psychrophilic nature of PAOs, which would give them a competitive advantage over other groups of bacteria at low temperatures;
4. Washout phenomenon that results from the critical SRT-temperature combination imposed on the system, and its relation to the reaction rates that become too slow to support cell growth;
5. The nature of the limiting reaction that leads to the washout of EBPR functions;
6. Transport processes and cellular membrane fluidity as maintained by homeo-viscous adaptation;
7. Population shift from PAOs to GAOs as it may lead to a complete or partial loss of EBPR performance.

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CHAPTER I
LITERATURE REVIEW

Ufuk G. Erdal and Clifford W. Randall

Introduction

Biological nutrient removal (BNR) is an effective and economical way to remove phosphorus along with nitrogen and organic materials from wastewater, and it has been the subject of a lot of research in recent years. During the past two decades, the mechanisms of excess biological phosphorus removal (EBPR) have been investigated by numerous researchers, and different biochemical models have been developed, e.g., by Wentzel *et al.* (1986), Mino *et al. (1987)* and Smolders *et al (*1994), to explain the mechanisms of EBPR. In addition, new models and new mechanisms have been developed in light of these models (Pereira *et al.*, 1996 and Louie, *et al.* 2000). Basically, EBPR systems utilize alteration of anaerobic and aerobic cycles to favor the growth of phosphorus accumulating organisms (PAOs). PAOs can store inorganic phosphorus as intracellular poly-phosphate to a greater extent than is needed for growth metabolism. They do this as an energy-storing mechanism. In anaerobic zones, organic substrates (short chain fatty acids (VFAs)) are taken into the cells, and stored in the form of poly-hydroxy-alkanoates (PHAs). Degradation of poly-P bonds provides energy to drive these reactions, and orthophosphorus is simultaneously released to the medium as Poly-P is de-polymerized. Then, in the aerobic stage the PAOs grow, produce excess energy, and take up orthophosphates to store the energy, using the stored PHA as the energy and carbon source for growth (Mino *et al.*, 1998). In addition to the released phosphorus, some of the phosphorus initially present in the wastewater is removed, resulting in net removal when sludge is wasted from the system. The overall process is known as excess biological phosphorus removal (EBPR). Reducing equivalents (NADH) needed for ATP production are produced in the anaerobic zone as PHA is broken down. NADH is generated through either tricarboxylic acid cycle (TCA) or other glyoxylate cycles. Although current EBPR

models mostly agreed about the aerobic reactions of the EBPR process, there are significant disagreements about the reactions that occur under anaerobic conditions.

Biochemical Models of EBPR Metabolism

The major EBPR models, and significant additions to them, are given below in chronological order of their development, with brief explanations:

Comeau-Wentzel Model (1986):

PHA is a reduced energy source and its synthesis therefore requires reducing powers. Partial oxidation of acetyl CoA through the TCA cycle produces the reducing power. The combination of acetate (acetyl CoA) and reducing power produce PHA. The proposed stoichiometry of the EBPR process is given by Comeau-Wentzel in Figure 1.

Mino Model (Mino et al., 1987):

The lack of evidence for the operation of the TCA cycle under anaerobic conditions and the observation of significant changes in intracellular carbohydrate (glycogen) content motivated the development of the Mino model (1987). In this model, reducing power is generated by the degradation of intracellularly stored glycogen (carbohydrate) via the Embden-Meyerhoff Parnas (EMP) pathway. The stoichiometry of the proposed model is also given in Figure 1.

Modified Mino Model (Wentzel, 1991):

The only major change from the Mino model is that the modified Mino model postulates the Entner-Doudoroff (ED) pathway for degradation of intracellular glycogen instead of the EMP pathway. This modification was proposed by Wentzel (1991) based upon the

results of a single study in which the apparent use of the ED pathway by an Acinetobacter was observed.

Pereira et al. (1996):

Pereira and coworkers (1996) showed that a small portion of labeled acetate was released as CO_2 during an anaerobic batch test experiment. Therefore, their ^{13}C NMR results suggest that at least part of the TCA cycle is still operable under anaerobic conditions, and that some fraction of the reducing power needed is generated through the TCA cycle. The complete pathway of the model is illustrated in Figure 2.

Maurer et al. (1997):

Maurer *et al.* (1997) used a solid state NMR to track carbon flow in EBPR sludge fed with domestic sewage. Although no suggestion was made about the operation of the TCA cycle under anaerobic conditions, they suggested that the ED pathway was used during glycogen breakdown.

Louie et al. (2000):

More recently, Louie *et al.* (2000) suggested that the glyoxylate pathway is active under anaerobic conditions to provide reducing equivalents and to maintain stable NAD^+/NADH balance.

It is obvious that all of the biochemical pathways of EBPR are not completely defined as yet. In addition to unknown biochemical mechanisms, the effects of other factors that affect the performance of EBPR processes are incompletely understood, notably temperature. Conflicting reports concerning the effects of temperature upon EBPR processes have repeatedly appeared in the research literature over the last two decades.

The diverse bacterial consortium responsible for the EBPR processes in biological wastewater treatment systems consist of psychrophilic, pyschrotrophic and mesophilic heterotrophic bacteria. Because they have different optimum growth temperatures, the temperature of the wastewater-microbial mixture (mixed liquor) strongly influences the population composition of the consortium. Temperature is also a key parameter that affects the performance of the microbial consortium. 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; and Barnard *et al.*, 1985) reported that EBPR efficiency was greater at lower temperatures than at higher temperatures. Temperatures investigated ranged from 5 to 24^{o}C. McClintock *et al.* (1991) measured the performance of an EBPR system at 20, 15 and 10^{o}C and reported that EBPR functions would "wash-out" of activated sludge systems at 10^{o}C before heterotrophic COD removal functions. Mamais and Jenkins (1992) showed the early wash-out of EBPR functions 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 temperature appears to affect EBPR reaction rates in a normal manner, a substantial body of evidence indicates that many EBPR systems perform more efficiently as the temperature decreases. Helmer and Kunst (1997), have speculated that PAOs are more psychrophilic than competing heterotrophs, and this gives them a competitive advantage at low temperatures, resulting in a population shift towards PAOs and greater P removal efficiency in spite of the decreasing reaction rates. Similar observations supporting better EBPR removal at cold temperature were made by Panswad *et al.* (2000). Although the results of temperature effects on EBPR kinetic rates are similar, research findings show considerable disagreement about the performance of the EBPR systems under different temperature conditions.

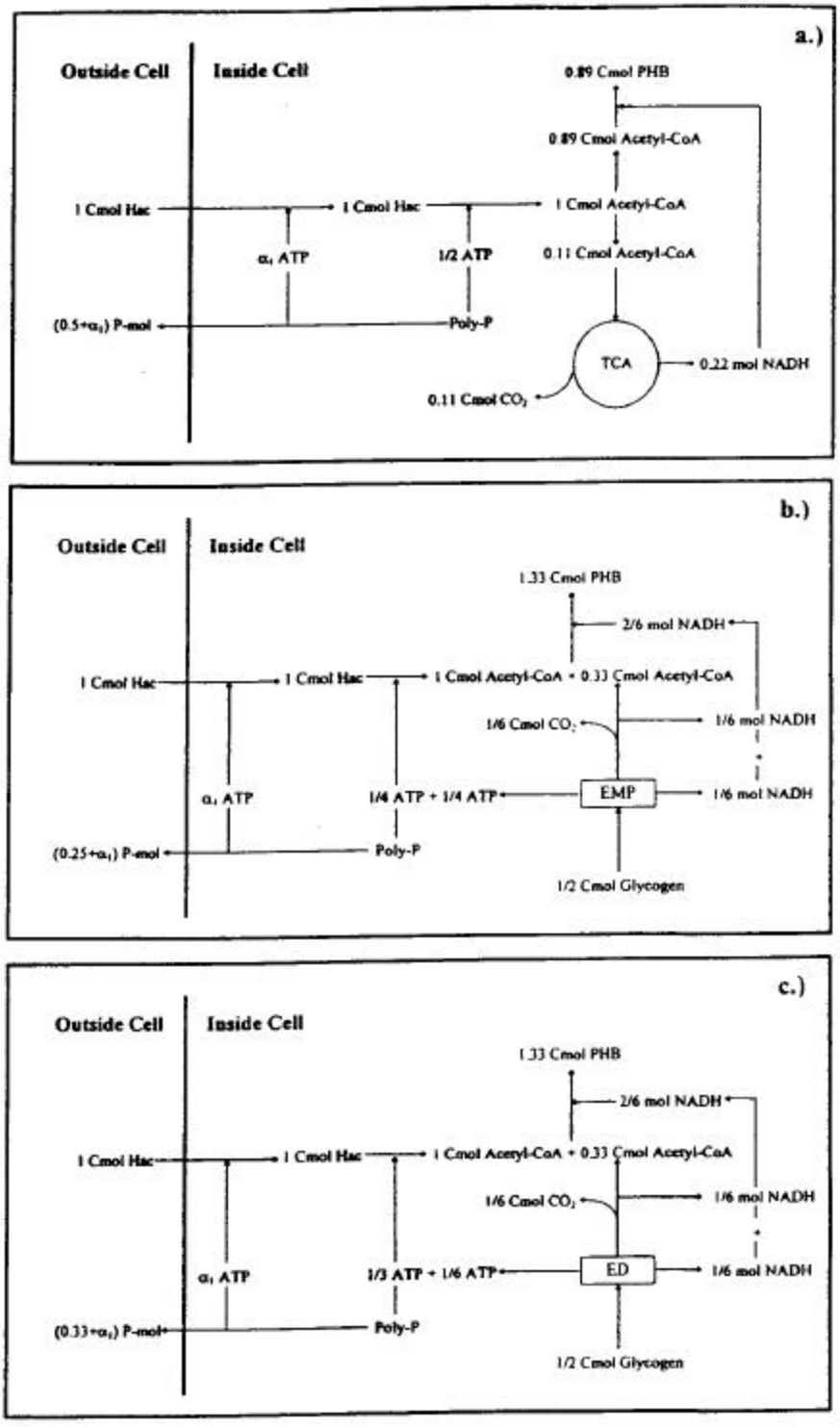

Figure 1. Comeau-Wentzel, Mino and Modified Mino models

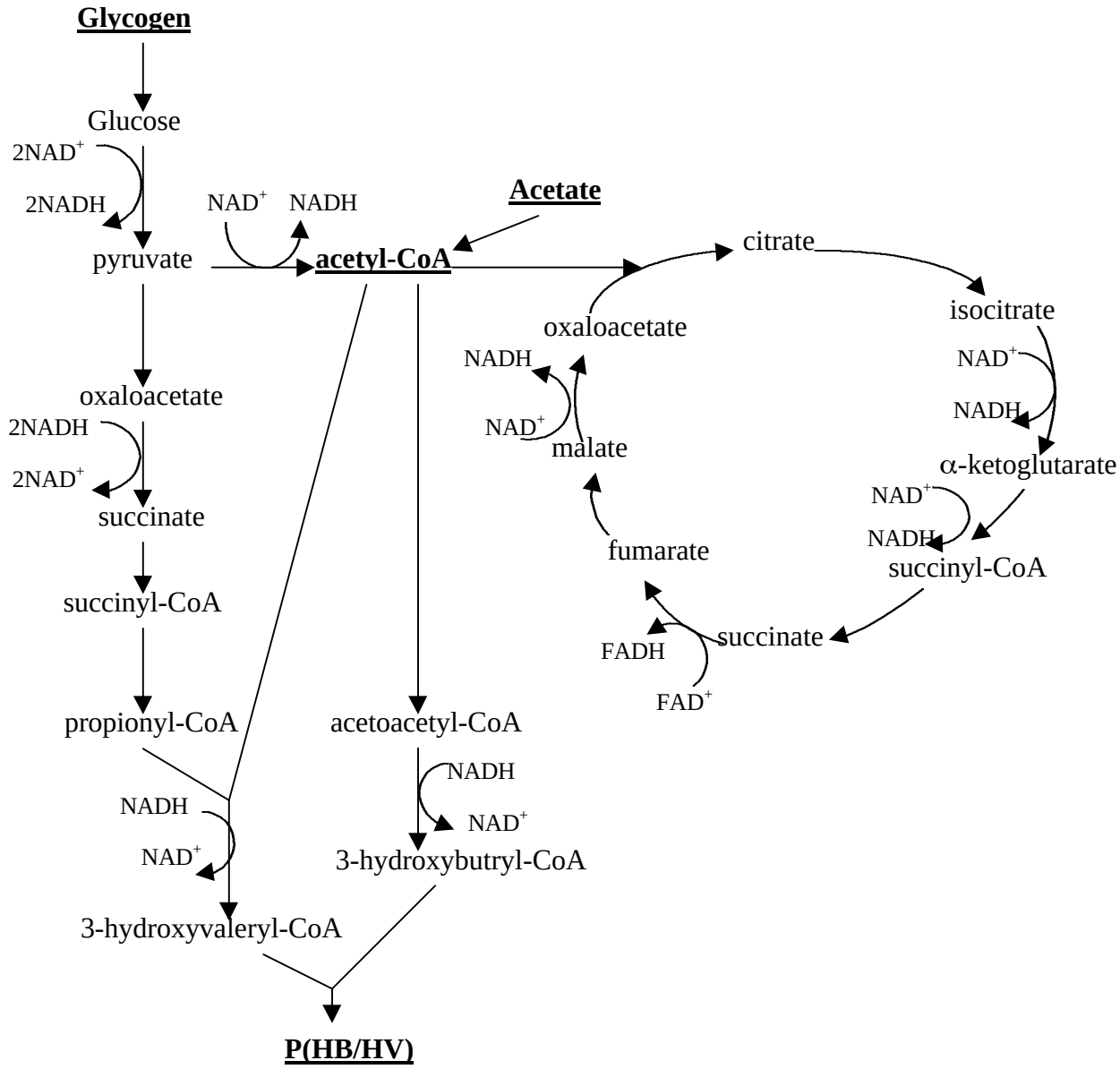

Figure 2. EBPR model proposed by Pereira *et al.* (1996).

An Analysis of the Temperature Related EBPR Research

Several factors may contribute to the seemingly contradictory results of temperature on EBPR performance and reaction rates. The results potentially could be explained through the use of different substrates, different system configurations, the use of different analytical techniques, the application of different operational conditions (SRT, different anaerobic and aerobic contact time etc.) (Brdjanovic *et al.*, 1998), plus acclimated versus non-acclimated systems. Some studies have focused on the short-term (non-acclimated) effects of temperature on EBPR kinetics and performance while others have focused on long term (acclimated) temperature effects on EBPR system performance. Therefore, it is difficult to directly compare the results of the studies.

The results of the EBPR temperature performance studies in the literature can be divided into two broad groups:

a. Lower EBPR efficiency as temperature decreased.
b. No change or better EBPR performance as temperature decreased.

a. Lower EBPR efficiency as temperature decreased:

Shapiro *et al.* (1967) found that the anaerobic P-release rate decreased significantly when temperature was reduced by 10^{o}C. They reported the temperature coefficient (Q_{10}) to be 2.1-2.6 in the temperature range of 10-30^{0}C.

Hashimoto and Furukawa (1984) investigated anaerobic P release in activated sludge over the temperature range of 12 to 28^{o}C. They reported that P-release increased by a factor of 2.4 as temperature increased from 20-28^{o}C, and they determined an activation energy (E_a) value of 68.7 kJ/mol for P- release within the temperature range of 12-28^{o}C.

Jones *et al.* (1987) found 75 percent more P-release and 30 percent more uptake at 29^{o}C that those observed at 24^{o}C.

Boughton *et al.* (1971) investigated the uptake of labeled ^{32}P through aerobic batch studies over a wide temperature range (5-45^{o}C). The temperature optima for P-uptake were reported as 24-37^{o}C. At two extreme temperatures, 10^{o}C and 45^{o}C, P-uptakes were 50% of the P-uptake observed within the temperature optima.

Fuhs and Chen (1975) found that P uptake in aerobic conditions was significantly inhibited at 10^{o}C compared to 37^{o}C. Optimum growth temperature for *Acinetobacter iwoffi* was determined to be 20 to 24^{o}C because the maximum aerobic P uptake was observed within this range.

Spatzierer *et al.* (1985) investigated biological phosphorus removal in combination with simultaneous precipitation in three different full-scale BNR treatment plants located in Austria. They reported that biological P-removal was reduced under winter conditions with temperatures below 12^{o}C.

The impact of temperature on EBPR efficiency was investigated in a modified Bardenpho type process in Canada (Vassos *et al.*, 1987). P-removal efficiency was reduced when the temperature was below 15^{o}C and further decreases below this temperature significantly reduced plant phosphorus removal performance.

McClintock and Randall (1991) simultaneously operated side-by-side a lab scale Virginia Initiative Plant (VIP) configuration system and a conventional activated sludge (CAS) system using a feed of domestic wastewater supplemented with acetic acid to compare temperature effects on acclimated performance. EBPR was maintained for all temperatures investigated (20, 15, and 10^{o}C) as long as a 15 day MCRT was maintained However, the EBPR functions were completely lost when the MCRT of the VIP system was decreased to 5 days., even though COD removal remained at the same efficiency.

Mamais and Jenkins (1992) investigated the effects of SRT and temperature combinations on EBPR using continuous flow bench scale activated sludge systems treating wastewater supplemented with 50 mg/L acetate over ranges of SRT and temperature of 2-4 days and 13.5-20^{o}C, respectively. The kinetic rates of the EBPR processes also were investigated during batch test experiments performed at 10 to 37^{o}C. With the inclusion of the McClintock data at 10^{o}C, it was stated that EBPR functioned efficiently and independently of SRT as long as SRTs were selected above 2.9 days for the temperature range studied. At lower SRT values EBPR capabilities might be lost at an SRT value that depends on temperature. The optimum temperature for aerobic P-removal was reported to be between 28-33^{o}C and Q_{10} was calculated as 1.5-1.7 through the batch test experiments performed at 10-30^{o}C.

Marklund (1993) investigated low temperature effects on the performance of a full scale BNR plant located beyond the Arctic circle in Sweden. The results of acclimated temperature studies using the SBR mode of operation showed that EBPR was maintained over the temperature range of 3-8^{o}C. However, the system did not meet the effluent limits of 0.5 mg P/L and 15 mgBOD7/L except at 8^{o}C.

Jones and Stephenson (1996) suggested that the optimum temperature was 30^{o}C for anaerobic release and aerobic uptake of phosphate. EBPR was also observed at two extreme temperatures, 5 and 40^{o}C, but the efficiency of EBPR was reduced significantly. Activation energies were determined within 33-35kJ/mol and 39.5-41 kJ/mol for anaerobic P-release and aerobic P-uptake.

Brdjanovic *et al.* (1997), using a lab scale SBR, determined the short-term effects of temperature on EBPR performance and kinetics at 5, 10, 20 and 30^{o}C. Sludge that had been acclimated to 20^{o}C was used for the entire kinetic studies. The optimum temperature for anaerobic P release and acetate uptake was found to be 20^{o}C. However a continuous increase in aerobic P-uptake was obtained for temperature values up to 30^{o}C. The overall anaerobic and aerobic temperature coefficients were reported to be 1.078 and 1.057,

respectively. The stoichiometry of EBPR was found to be insensitive to temperature changes.

Choi *et al.* (1998) used a lab scale modified UCT process fed with weak sewage with an average soluble COD of 100 mg/L, and investigated the BNR efficiency of the system over the temperature range of 20 to 5^{o}C. It was reported that the denitrification rate at 5^{o}C was roughly 10 times lower than at 10^{o}C. A rapid decrease was observed in P removal efficiency as temperature decreased from 20 to 5^{o}C. However recovery of P-removal at 5^{o}C was observed during continued operation.

Beatons *et al.* (1999) investigated the temperature effects on EBPR kinetics and performance in a SBR type process operated at temperatures of 20, 15, 10 and 5^{o}C and at a constant SRT of 10 days. It was reported that the aerobic P-release was maximum over the temperature range of 15 –20^{o}C and all other reaction rates increased as temperature increased. Acetate breakthrough to the aerobic phase was reported at 5^{o}C because of incomplete P removal.

Krishna and Van Loosdrecht (1999) investigated the effect of temperature on storage polymers in a lab scale SBR unit fed with acetate. The specific acetate uptake rate was found to increase from 0.22 (C-mol/C-mol h) at 15^{o}C to 0.43 at 35^{o}C. However, the specific PHB formation rate decreased and the highest PHB formation and consumption was reported at 15^{o}C, the lowest temperature used in the investigation.

b. Unchanged or better P removal efficiency at cold temperatures:

Oldham and Dew (1979) investigated cold temperature effects on the system performance of a bench scale Bardenpho process. Over the temperature range of 18 to 6^{o}C their results showed that EBPR efficiency was not affected by cold temperature and 90% P removal was achieved at 6^{o}C.

Sell *et al.* (1981) investigated low temperature (5, 10, and 15^{o}C) effects using an A/O process and found that EBPR performance was not lost even when the temperature decreased down to 5^{o}C. Moreover, EBPR efficiency was 40% greater at 5^{o}C than at 15^{o}C. It was postulated that the EBPR bacteria are psychrophilic and work efficiently below 10^{o}C. They reported that temperatures above 10^{o}C resulted in a population shift from phosphate accumulating psychrophilic organisms to mesophilic non-phosphate accumulating organisms.

Krichten *et al.* (1983) obtained results similar to those reported by Sell *et al.* (1981). Increased P-removal was observed in an A/0 process at 5^{o}C compared to 10^{o}C and 15^{o}C.

Siebrietz (1984) ran numerous studies at 24 and 14^{o}C, and obtained superior EBPR performance at 14 ^{o}C.

Barnard *et al.* (1985) reported as high as 90 % P-removal in a wastewater treatment plant in Canada operating at 9^{o}C.

Kang *et al.* (1985) operated a full scale A/O process in Pontiac, Michigan. Wastewater temperatures as low as 10^{o}C did not affect P-removal efficiency. The lowest effluent soluble and total P concentrations of 0.3 and 0.4 mg/L, respectively, were observed at 11^{o}C, compared to 0.7 and 0.8 mg/L TP observed at 16 and 17^{o}C, respectively.

Van Groenestijin and Deinema (1985) showed that the P content of a pure culture of *Acinetobacter* was maximum at 5^{o}C (10%) and minimum at 35^{o}C (1.4%).

Daigger *et al.* (1988) achieved very good P-removal in a VIP process at 13^{o}C. However, temperatures as low as 5^{o}C did not change the system performance and an effluent P concentration of 1 mg/L was still reached, but more contact time was provided at the lower temperature.

Marklund and Morling (1994), using an SBR, showed that EBPR was not lost even at temperatures as low as 3 – 8^{o}C. However, a significant increase in effluent P concentration was reported when the temperatures were below 5^{o}C. On the other hand, biofloculation was enhanced at the lower temperatures.

Converti *et al.* (1995) used a modified A/O process to determine temperature effects on EBPR kinetics and performance at 5, 15, 25, 30 and 35^{o}C. The results showed that P removal efficiency varied between 60 to 62.5% over the temperature range studied. It was stated that the time necessary to achieve the desired level of P removal was strongly increased as the temperature decreased.

Jonsson *et al.* (1996) operated a full scale UCT plant in Helsinborg, Sweden between June, 1993 and July, 1994. The concentration of soluble phosphorus in the plant effluent was lower than 0.3 mg/L even at temperatures below 10^{o}C as long as enough VFA potential was present.

Helmer and Kunst (1997) used a lab scale Johannesburg process treating domestic wastewater with supplemental peptone to show that a drop in temperature to 10^{o}C and then 5^{o}C had no significant effect on the efficiency of EBPR in spite of reduced P release observed at 5^{o}C. The dominant organisms at 5^{o}C were identified as facultative anaerobic microorganisms, which showed the best ability to store poly-P under cold temperatures. The temperature coefficients were reported as 1.20 and 1.28 for anaerobic P-release and aerobic P-uptake, respectively.

Brdjanovic *et al.* (1998) extended their previous studies to include long term temperature effects on EBPR performance, kinetic rates and molecular effects. It was found that EBPR efficiency was low at 10^{o}C with an 8-day SRT, but complete removal was achieved when the SRT was increased to 16 days. The P removal efficiency was still excellent at 5^{o}C when the SRT value was increased to 32 days. While only a very slight deviation from the short term temperature coefficient under anaerobic condition was observed (1.078 vs. 1.085), the temperature coefficients for oxygen uptake and PHA

consumption were significantly changed following temperature acclimation. Electron microscopy and dry denaturing gradient get electrophoresis (DDGGE) techniques showed the existence of a population shift when the temperature dropped to 5^0C.

In an as yet unpublished study, Panswad *et al.* (1999) investigated the long-term effect of temperature on EBPR using an A/0 process and feeding synthetic wastewater. The experiments were performed at 5, 15, 25, 35, and 40^{o}C, and showed that P-removal efficiency was not adversely affected by temperatures below 25^{o}C. Instead, P removal efficiency was reduced by 28% and 39% at 35^{o}C and 40^{o}C, respectively. Complete P-removal was achieved for three temperatures studied (5, 15 and 25^{o}C). Interestingly, nearly no P-release was reported at 40^{o}C although 61% P- removal was observed.

In order for readers to make an easy comparison among the temperature studies, a summary table is presented as Table 1. The major factors leading to the contradictory results, and possible explanations for them are presented in the discussion section.

Table1. Summary of temperature effects on EBPR performance in lab and full scale applications.

Study	Temperature studied, ^{o}C	Temperature acclimation	Process type	Substrate source	SRT day	Influent COD mg/L	Influent P mg/L	P removal
Spatzierer et.al. 1985	varying	seasonal	full scale AS	domestic sewage	?	?	?	30% reduction below 12^{o}C.
Barnard et.al. 1985	varying	seasonal	full scale Bardenpho	domestic sewage	?	?	?	very good as low as 8^{o}C.
McClintock et.al. 1990	20,15 and 10	provided	lab scale UCT	domestic and additional Ac	15 and 5	~ 250	~ 19	no P removal at 5^{o}C with 5day-SRT
Mamais and Jenkins 1992	13.5-20	provided	bench scale AS	domestic ww additional Ac	4 to 2	430	8.5	lost at 13.5^{o}C with SRT below 2.9 days.
Marklund 1993	3, 8	seasonal	full scale SBR	domestic sewage	?	88-167	3.1-9.6	80% P removal
Converti et.al. 1995	5,15,25,30,35	only at 20	modified A/O chemostat	synthetic ww mainly HAc	N/A		36	60-64% P removal in given Temperature range
Jonsson et.al. 1996	10 to19	seasonal	full scale UCT	domestic sewage	?	420	5.7	as low as 0.3 mgP/L in effluent at 10^{o}C.
Brdjanovic et.al. 1997	5,10,20 and 30	only at 20	SBR	synthetic ww with NaAc	8	400 250 at 5^{o}C	15	No P removal at 5^{o}C 4 mgP/L in effluent at 10^{o}C
Brdjanovic et.al. 1998	5,10,20 and 30	provided	SBR	synthetic ww with NaAc	8,16,32	400	15	complete P removal at 10^{o}Cand16 days complete P removas at 16 and 32 days at 5^{o}C
Helmer and Kunst, 1997	5 to 20	provided	full-scale Johannesburg	domestic ww plus peptone	12	0.3gCOD/SS.d	18	no change in EBPR removal
Choi et. al. 1997	5 to 20	only at 20	lab-scale BNR	domestic sewage	10	220	4	decreased P removal at 12^{o}C and below.
Beatons et.al. 1999	5,10,15 and 20	provided	SBR	synthetic ww with NaAc	10	400	15	incomplete P removal at 5^{o}C.
Panswad et.al. 1999	5,15,25,35,40	provided	SBR	with NaAc and nutrient broth	12	300	15	100% P removal at 5,15 and 25^{o}C 72% at 35^{o}C and 61% at 40^{o}C

DISCUSSION

A review of the literature provides some answers but suggests that several questions still need to be answered before the effects of temperature on EBPR system performance will be fully understood. It seems fairly clear that EBPR kinetic reaction rates decrease as temperature decreases, as is common for other biochemical rates. It also seems clear that EBPR functions will 'wash-out' before other aerobic heterotrophic functions will wash-out as temperature and SRT decrease. On the other hand, the reported evidence is very contradictory regarding changes in EBPR system performance as temperature decreases. Does the efficiency of EBPR performance improve as the temperature decreases below 20^{o}C? There is a lot of evidence both for and against. If EBPR performance improves as temperature decreases and the PAOs are psychrophilic, why do the EBPR functions wash-out before other heterotrophic functions? Are PAOs less able to maintain their membranes in a fluid state relative to other aerobic heterotrophs, or do one or more of the enzymes essential to EBPR become non-functional at low temperatures? The temperature effects questions would be easier to resolve if the biochemical pathways of EBPR were more fully understood, but the existing biochemical models do not agree on several points and the current biochemical evidence is inconclusive.

The subsequent discussion will seek to assess the extent to which the existing literature answers the following questions:

- Do major shifts in the EBPR bacterial populations occur as the consortium acclimates to changes in the mixed liquor temperature?
- Is temperature acclimation required to sustain EBPR performance?
- What are the relative effects of P-limiting vs. COD-limiting conditions on EBPR performance?
- Is the P-removal efficiency of EBPR systems determined by the competition between PAOs and GAOs, and how are the two populations affected by temperature?
- Are EBPR bacteria able to maintain their cellular membranes in a fluid-like state, or do the membranes gel and become rigid at cold temperatures?

- What type(s) of transport mechanism(s) are utilized in the anaerobic zone to transport VFAs into the cells and to maintain the proton motive force?
- Does the type(s) of VFA available influence the temperature effect?
- How important is the metabolism of glycogen to EBPR, and how is glycogen metabolism affected by low temperatures?
- What are the effects of SRT changes on temperature influenced EBPR performance?
- What is the role of electron acceptors such as NO_3-N and DO in the performance of EBPR at low temperatures?

a. Does the EBPR Bacterial Population Shift as the Mixed Liquor Temperature Changes?

Resolution of this question requires that the EBPR bacteria in the activated sludge be accurately identified. Early efforts to indentify the bacterial types responsible for EBPR concluded that *Acinetobacter* species were the dominant type, and the early models were developed based on the biochemistry of *Acinetobacter* species (Buncan, 1983; Kerdachi and Healey, 1987; Lotter 1985; and Wentzel *et al.* 1988 a). However, later studies produced results that contradicted this by showing that several other P-removing genera were present in EBPR systems, and were frequently more dominant, and provided evidence that a key identification technique used in the early studies contained an error (Kavanaugh, 1991; Kavanaugh and Randall, 1992). When methods other than cultivation were used for the enumeration of bacteria from EBPR sludges, the results also showed that *Acinetobacter* are less prominent than other genera (Seviour and Blackall, 1999). In a recent study, Mudaly *et al.* (2000) identified the predominant bacterial species in an enriched culture fed with acetate. The results showed that the four largest groups in the bacterial community were as the follows: ♌-Proteobacteria (22%); ♋-Protobacteria (19%) and ♑-Proteobacteria (17%) and, *Acinetobacter* (11%). Further, there has not, as yet, been a successful demonstration that a pure culture of any bacteria can achieve sustained EBPR.

It is well known that temperature may exert a pressure on the selection of the dominant organisms in activated sludge systems, resulting in population shifts (Grady *et al.* 1999). The induced shifts may significantly influence the performance of the biological process. Even though temperature selection effects have been suggested for EBPR systems,, very few studies have been performed to investigate the effects of temperature changes on EBPR bacterial communities. Only two studies have been done to show temperature population relationships in EBPR systems: Helmer and Kunst (1997) and Brdjanovic *et al.* (1998). Helmer and Kunst (1997) used colony identification techniques and homogenized EBPR sludge spread on agar plates for their investigation. Numerical methods were used during identification of the isolates. While *Acinetobacter* sp. or *Moraxella* sp. were the largest group of microorganisms (20%) at 20 and 15^{o}C, the sludge contained only 5% of these organisms at 5^{o}C. The gram positive *Staphylococcus* group made up the largest group in the 5^{o}C activated sludge and nearly all isolated strains were capable of storing poly-P at 5^{o}C. Improved P removal was observed at the lower temperature and was attributed to a population shift that selected facultative anaerobic organisms with high P accumulating capacity. This concept was further investigated by Brdjanovic *et al.* (1998). The dry gel denaturation technique (DDGDE) and electron microscopy were used to track the potential population shifts. Comparison of the DDGDE bands showed that the EBPR sludges contained a diverse group of bacteria, and their relative fractions changed as temperature changed. Electron microscopy study captured at least 6-7 different types of bacterial cells at 20^{o}C. However, no visual comparisons were reported. Unlike the previous study, they could not identify the bacterial species in their EBPR sludge because DDGDE is just a qualitative method and cannot provide any taxonomic or quantitative information. More micro-ecological studies are needed to determine EBPR population shifts with temperature.

b. How does SRT influence the performance of biological treatment systems?

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). Washout or minimum SRT, another design parameter, is a critical point below which no growth of biomass occurs. 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 resident time in the system will become larger. Failure to increase the SRT as temperature decreases may result in partial or complete loss 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^{o}C. 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.

Harada and Matsumoto (1981) stated that both SRT and temperature cause shifts in the predominant bacterial population, resulting in changes in the overall growth activities of activated sludge. It was also proposed that higher respiratory activities and higher protein to carbohydrate cellular content resulted from shorter SRTs and higher acclimation temperatures. Matsuo (1994) showed that a short anaerobic SRT (0.9days) in an EBPR system resulted in an apparent decrease in P removal efficiency due to 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 the entire temperature range from 5 to 20^{o}C, 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 caused endogenous respiration to increase and O_2 transfer requirements to decrease. The effect of excessive aeration, (e.g. high SRT and high endogenous respiration) was later found to reduce EBPR efficiency (Brdjanovic *et al.*, 1999). Temperature studies without consideration of SRT may not result in optimum EBPR system performance and may result in confusion.

c. Is temperature acclimation required to sustain EBPR performance?

It is evident that bacterial communities need time to adapt to environmental conditions such as pH, temperature, substrate type, etc., before the population mix stabilizes (Brocks, 1999). This period is usually referred to as the acclimation period, and before its completion the growth rate of the bacterial consortium is inconsistent because the population mixture has not reached steady state conditions. Benedict and Carlson (1993) investigated the effect of temperature acclimation in a conventional activated sludge system. Activated sludge that had been acclimated to 15-19^{o}C was then exposed to 4, 19 and 32^{o}C., Adaptation periods of 14 and 52 days were required for the temperatures of 4 and 32^{o}C, respectively, based upon endogenous respiration rate measurements. It is commonly accepted that three times the design SRT value is enough to approximate the acclimation period needed to reach steady state (McClintock *et al.* 1991). However, most of the researchers that investigated the effects of temperature on the biochemical reaction rates of EBPR populations purposely did not permit acclimation to occur during their investigations, except at the reference temperature(s). In most of the studies, temperature acclimation was carried out for a single temperature (usually 20^{o}C) (Brdjanovic *et al.*, 1997; Choi *et al.*, 1998) or a couple of intermediate temperatures (Mamais and Jenkins, 1992; Stephenson and Jones, 1997). The acclimated sludge was then exposed to temperatures other than the acclimation temperatures during the batch test experiments to study short-term temperature effects.

Short-term effects can be important when biological system performance under shock temperature stress is of interest. John and Stephenson (1996) investigated EBPR performance and kinetics over a broad temperature range (5 to 45^{o}C). The microbial consortiums were acclimated at 15 and 25^{o}C. Then batch test studies were performed at 10 and 5^{o}C using the sludge acclimated to 15^{o}C to determine system performance and kinetic rates. Based upon their batch test studies using non-acclimated sludge, it was concluded that EBPR was partially lost at cold temperatures. However, their results are valid only for short-term effects on EBPR performance and kinetic rates, and not for acclimated consortiums. Brdjanovic *et al.* (1997 and 1998) performed back to back investigations of both short and long-term temperature effects using sequencing batch reactor (SBR) EBPR systems. Although the worst EBPR performance was reported at 5^{o}C after both short and long-term temperature acclimation, they reported a very large difference in the aerobic P uptake rate at 5^{o}C for the short and the long-term temperature exposures, with a much greater efficiency following the long-term acclimation period. Choi *et al.* (1998) investigated short term effects on EBPR kinetics and performance. They observed that EBPR was completely lost at 5^{o}C when a sludge acclimated at 20^{o}C was exposed to 5^{o}C for a short period. However, continuous operation at the same temperature for several days resulted in a significant recovery of P removal. It appears that acclimation of the microbial population during the last two studies resulted in very different results when compared to a non-acclimated population exposed to the same temperature. While it is useful information to know the effects of sudden temperature shocks on EBPR system performance, the more common temperature condition in full-scale treatment systems is gradual temperature change, which permits seasonal acclimation. It seems very likely that a major reason for the apparently contradictory EBPR temperature effects reported in the literature is because some researchers used acclimated cultures for the temperatures investigated and some did not.

d. Is it a simple competition between PAOs and GAOs?

The morphological characteristics of PAOs were first described by Fuhs and Chen (1975) based on microscopic observations of PAOs in enriched activated sludge cultures. Subsequent studies showed that the PAOs were non-motile rods or cocci, usually in clusters, and they contained Neisser positive granules in the cells (Mino *et al.,* 1998). Although it was previously thought that the important EBPR bacteria are gram negative, later studies have shown that some PAOs are gram positive (Wagner *et al.,* 1994; and Liu *et al*., 1995). Experiments have suggested that EBPR activated sludge contains two groups of bacteria that use different internal energy sources to co-exist and compete for substrate during the anaerobic stage (Liu *et al.,* 1997). The PAOs use energy stored in Poly P bonds for the uptake of acetate and synthesis into poly-hydroxy-butyrate (PHB) for cellular storage. It is unknown how they obtain the necessary reducing power for the synthesis reaction. It has been postulated by Comeau *et al.* (1986) and Wentzel *et al.* (1987) that it is accomplished by incomplete operation of the TCA cycle, but by Mino *et al.* (1987) by degradation of intracellular glycogen through glycolysis. Recently, Perreira *et al.* (1996) have proposed that it is obtained through a combination of both partial use of the TCA cycle and glycolysis. Glycogen accumulating organisms (GAOs) are the competing group of bacteria, and they that use glycogen as an the energy source for acetate uptake and storage, and obtain the needed reducing power through glycolysis. GAOs have similar features to PAOs except they do not accumulate excess phosphorus and they tend produce Poly-hydroxy-valerate (PHV) from acetate. Consequently, "they lack EBPR ability." (Liu *et al*., 1997). A high sludge P content is a good indicator that PAOs are dominant in EBPR activated sludge (Liu *et al.*,1997). PAO enriched EBPR sludges with P content of up to 37 and 43 % as VSS have been reported by Wentzel *et al.* (1987) and Copp and Dold (2001), respectively.

It is possible to have a lot of organic storage in the anaerobic stage without P release and with no excess P uptake in the aerobic stage. Inhibition of EBPR processes has been reported in several studies due to the presence of non Poly-P bacteria, called G bacteria by Cech and Hartman (1993), or GAOs by Mino *et al.* (1994); Liu *et al.* (1995), and

Filipe *et al*. (2001). Activated sludge dominated by GAOs will have low P and high glycogen content as indicators of their dominancy. It appears that under some conditions there are bacteria that can compete successfully with Poly P organisms and actually inhibit EBPR. This type of inhibition has been linked to long sludge residence time (Fukase *et al.* 1984), high ratio of anaerobic/aerobic hydraulic detention time (Matsuo, 1994), presence of glucose in the influent feed (Cech and Hartman 1993; Mino *et al.* 1997), and a low P/C ratio in the feed (Liu *et al.* 1994). However, there is no evidence in the literature that SRT and HRT changes contribute to the proliferation of GAOs.

Although glucose is the main substrate and energy source for GAO growth, several studies have reported very good EBPR even when the influent contained significant amounts of glucose (Fukase *et al.*, 1982, Matsuo, 1994). In low P/C (2/100) conditions, Liu *et al.* (1997) found that low P in the influent limited Poly-P formation in the aerobic stage and thereby provided less energy for acetate uptake. Under these conditions, poly-P becomes a limited energy source for the cells while glycogen is not limited. Continuous operation at low influent P may select a population where the GAOs are dominant.

The effect of solution pH on PAO and GAO competition was investigated in a recent study (Filipe *et al.*, 2001). It was reported that a pH value of less than 7.25 yields reduced P removal efficiency. Based upon increased glycogen values, decreased P removal efficiency could be attributed to proliferation of GAOs induced by low pH conditions.

The literature results suggest that the efficiency of EBPR is determined by competition between PAOs and GAOs, and the relative populations are influenced by several factors. However, the effects of temperature on this competition have never been investigated.

e. Do the cellular membranes of EBPR bacteria become gel-like and rigid at cold temperatures?

Most microorganisms must accommodate to a variety of changing conditions and stresses in their surrounding environment to survive and grow. Adjustment to fluctuations in temperature is possibly the most common form of adaptation because of the impact of temperature on the biochemical reaction rates of the cells. Typically, cell wall membrane fluidity changes with temperature, generally decreasing as temperature drops and increasing as it rises. It is of vital importance for cells to keep their membrane fluid-like otherwise many cell functions including solute transport are not operable. Most prokaryotic organisms, but not all, are able to compensate for temperature changes by altering the lipid composition of their membranes, thereby regulating membrane fluidity. This ability is called 'homeoviscous adaptation' because the main goal of such regulation is to keep the membrane viscosity approximately the same despite the changes in temperature (Becker *et al.*1996). Although the mechanism of homeoviscous adaptation is not fully understood for most organisms and living cells, an appreciable amount of effort has been made to determine such mechanisms for *E.coli* and some food related bacteria. Okuyama *et al.* (1986) demonstrated the preservation of membrane fluidity at 10 and 0^{o}C in the pyschrophilic bacteria, *Vibrio* Strain ABE-1. Fluidity was maintained in the cell wall by developing an extremely high content of hexadecanoic acid (16:1, i.e., 16 carbons with one double bond) in the membrane phospholipids. Fodor *et al.* (1997) investigated the lipid compositions of two symbiotic photosynthetic bacteria, *Xenorhabdus nematophilus* and *Photorhadbus liminescens*, at 28 and 18^{o}C. Lipid fatty acid composition from primary and secondary cultures of both bacterial species grown at 18^{o}C were more ordered (i.e. less fluid) than those grown at 28^{o}C. It suggested that, unlike the *Vibrio* strain, these particular bacterial species were unable to perform homeoviscous adaptation. In the light of the literature information, it is clear that this unique ability, when possessed, can provide a very substantial advantage to bacteria over their competitors when temperature varies, especially when it is low. It is also clear that some bacteria cannot make such adjustments. Reduced EBPR performance, especially washout, under cold temperature conditions may be related to the inability of EBPR bacteria to

perform homeoviscous adaptation. However, no study has been performed to investigate this mechanism in EBPR activated sludges.

f. Does NO_3-N entering the anoxic zone affect EBPR performance?

Autotrophic organisms are very slow growing organisms and nitrification rates significantly decrease at cold temperatures (Metcalf & Eddy, 1991; Randall *et al.,* 1992; Grady *et al.,* 1999). Nearly non-existent nitrification at low temperatures frequently results in a nitrate free anoxic zone, which further increases the anaerobic retention time. The near complete uptake of volatile fatty acids (VFAs) in the anaerobic stage as one of the essential requirements for optimum P removal was proposed by Randall (1991). In spite of the reduced rate of acetate uptake at 5^oC, an increased detention time may provide complete acetate utilization through the non-oxic stages of a typical BNR configuration. This condition may significantly enhanced EBPR performance at 5^oC. Kuba *et al.* (1996) investigated the effect of the nitrate-N loading to the anoxic stage on EBPR performance. They reported reduced P removal efficiency at high nitrate loadings. Their investigation, however, did not include temperature effects. It should be noted that even though cold temperatures tend to reduce the nitrate loading to the anoxic zone, they tend to increase the DO loading to the zone if the nitrate recycle is maintained. The full impacts of nitrate recycle on EBPR performance at different temperatures needs to be investigated.

g. Does the type(s) of VFA available for EBPR influence the temperature effects?

Full-scale studies have demonstrated that short-chain volatile fatty acids (SCVFAs) are essential for EBPR during the treatment of municipal wastewaters (Barnard, 1985; Randall *et al.* 1992). The most comprehensive study on the effects of different SCVFAs on EBPR performance was performed by Abu-ghararrah and Randall (1991) using municipal sewage supplemented with SCVFAs. Similar investigations using synthetic

wastewater were performed by Mino *et al.* (1993) and Hood and Randall (2001). The studies agreed that acetate is the best carbon source of the SCVFAs, i.e., isovalerate, valerate, isobutyrate, butyrate and propionate, and of the simple sugars such as glucose, for efficient EBPR. The SCVFAs in the preceding sentence are listed in order of their EBPR efficiencies.

Most domestic wastewaters are initially SCVFA deficient, but SCVFAs are provided through fermentation reactions (Randall *et al.,* 1992). Therefore, in full-scale plants treating municipal wastewaters, the more complete the fermentation of the wastewater before or in the anaerobic stage, the more efficient the EBPR. Cold temperatures strongly reduce the rates of fermentation (Grady *et al.,* 1999). Despite the potential limitation of fermentation processes, full-scale studies performed by Barnard *et al.(*1985); Kang *et al.* (1985) and Daigger *et al.* (1988) showed that EBPR performance was not reduced by low temperatures and sometimes even better P removal was reported at the lower temperatures.

Acetate was the sole carbon source in the majority of the studies (e.g. Brdjanovic *et al.*, 1997 and 1998; Choi *et al.,* 1998; Johns and Stephenson, 1996; and Panswad *et al.,* 1999) that reported decreasing EBPR efficiencies as temperature decreased. Clearly, in these studies fermentation was not needed and the efficiencies were not affected by changes in the fermentation rates. Randall and Hood (2001) have questioned whether EBPR might be more robust and more efficient when fed mixtures of SCVFAs rather than a single SCVFA, but it seems clear that substrate cannot be a factor that influences temperature effects on EBPR performance when the systems are fed only acetate as a primary organic carbon source..

h. P-limiting vs. COD-limiting conditions in EBPR system.

The effects and importance of the COD/TP ratio on EBPR performance was initially presented and discussed by Randall in Randall *et al.* (1992). Since then, the COD/TP

ratio has been investigated by several researchers to better understand the effects of P limiting vs COD limiting conditions on EBPR system performance (Schuler and Jenkins, 1996; Liu *et al*., 1996; Punrattanasin, 1997; Punrattanasin and Randall, 1998; Kisoglu *et al*., 2000). The results of all of the studies agree that the highest % P in the mixed liquor will occur when the COD/P ratio is low, i.e., under COD limiting conditions. Paradoxically, the results also agree that the minimum effluent P concentrations will be obtained when EBPR systems are operating under P limiting conditions, i.e., when the %P in the EBPR sludge is relatively low. Randall *et al.* (1992) established a relationship between the influent COD/P ratio and the effluent P concentration, and suggested that a COD/P ratio greater than 40:1 was usually needed to obtain an effluent P concentration less than 1.0 m/L because the typical P concentration in USA municipal wastewaters is less than 10 mg/L, and may be as low as 3.5 mg/L, USA wastewaters are usually P limited rather than COD limited. However, the PAOs actually utilize SCVFAs rather than all of the COD, and some wastewaters may be SCVFA deficient even though they have relatively high COD/P ratios in the influent. Therefore no consensus exists when results of COD limiting and P limiting conditions are compared.

From a practical viewpoint, when a system has non detectable P in the effluent, it does not mean that the PAOs are utilizing their full capacity for P removal, it simply means that the available P was exhausted before all of the available COD was utilized. Brdjanovic *et al*. (1998), and Panswad *et al*. (2000) investigated P removal and temperature relationships under P-limited conditions (COD/P ratios were 26 and 20, respectively). They both reported 100% P removal efficiency over the temperature range of 5 to 25^{o}C. Because P was limiting the EBPR performance over the entire temperature range, it was not possible to compare the actual EBPR capacities at the different temperatures, and the temperature effect results were inconclusive. A general rule of thumb is that EBPR capacity comparisons cannot be made unless the effluents contain at least 1 mg/L P.

i. What type of transport mechanisms are active?

The mechanism of SCVFAs transport into PAO cells has never been demonstrated and is still unknown. Despite this uncertainty, the transport of acetate is generally assumed to occur by either carrier mediated or active transport (Becker *et al.*, 1996). This assumption is probably made because volatile fatty acids are considered to be charged molecules in the neutral pH range and cell membranes are impermeable to charged species. However, Baronofsky *et al*. (1984) showed that the transport of acetic acid into *Clostridium thermoaceticum* was by passive diffusion over a concentration range of 0 to 150 mM and over a pH range of 5 to7. Hume *et al*. (1993) found that uptake of acetate in the caecum and colon of prairie voles was primarily passive over the acetate range of 10-50 mmol/L However acetate uptake was found to be carrier-mediated in both regions when the acetate concentration was higher, i.e., 100 mmol/L.

Comeau and Wentzel (1986), Mino (1987), and Smolders (1994), have proposed an active transport mechanism for acetate in their current EBPR models. It was also proposed that the energy requirement for active transport was obtained through the hydrolysis of ATP. Therefore, a certain amount of P-release would be required for active transport of acetate in each model. However, if passive diffusion occurs, no P release would be associated with the acetate transport process and the model predictions would be in error. As has been presented, acetate transport is an integral part of the anaerobic stoichiometry of the EBPR process. In addition, the study of the transport mechanism may provide essential information for temperature research, because, if the transport of acetate is passive, the rate of acetate diffusion will be heavily influenced by solution temperature. Reduced acetate uptake in EBPR studies may simply be related to the limitation of acetate transport at cold temperatures. Further investigation of the SCVFA transport mechanism(s) is needed to further EBPR model development and temperature research.

j. What are the effects of pH on EBPR performance?

It has been reported that solution pH has two major impacts on EBPR processes. In the anaerobic stage, it has been proposed that high pH causes more P release because acidification of cells occurs due to high proton entry. The immediate H+ expulsion is carried out by the symport movement of $H_2PO_4^-$ species (Smolders *et al*., 1994). The pH value of 5 was found to completely deteriorate EBPR performance, and it was proposed that this occurred because the pH gradient was dissipated (Fleit, 1995; Smolders *et al*., 1994). Similarly, Liu *et al.* (1996) proposed that the optimum pH range for EBPR is between 6.5-8.0, which is very typical for full scale and bench scale operation. On the other hand, high pH may increase the precipitation of phosphate in EBPR systems and reduce the PAO population (Beatens *et al*., 1999). It was proposed by Filipe *et al.*, (2001) that pH effects can cause a population shift in EBPR systems. Contradictory to the studies performed by Smolders *et al*. (1994) and Liu *et al*. (1996), this study suggests that a pH value of 7.25 or less can cause proliferation of GAOs, thereby suggesting an even more narrow pH range for PAO dominance. However, no evidence was given to support the postulated GAO dominance in their study.

The potential effects of pH on EBPR would appear to be significant. However, considering that the pH of wastewaters is typically adjusted to near the neutral range to enhance the growth of more sensitive organisms such as nitrifiers, it is unlikely from practical stand point that pH will significantly affect EBPR performance in most applications.

k. How important is the metabolism of glycogen to EBPR?

The involvement of glycogen in EBPR biochemistry was first proposed by Mino *et al.* (1987), and they proposed a biochemical model that incorporated this feature and separated it from the pre-existing Comeau-Wentzel Model (1986). Since then, other models developed by Smolders *et al.* (1996) and Pereira *et al.* (1998) also have included

glycogen metabolism as an EBPR mechanism. Both the Mino and the Smolders models suggest that the reducing power for PHA formation is solely generated by the breakdown of glycogen through glycoloysis. Pereira *et al.* (1996) performed an investigation of EBPR pathways using tagged acetate and, based on the results, proposed a model that incorporates utilization of both the TCA cycle and glycolysis for the generation of reducing power in the EBPR anaerobic stage. A careful examination of past studies revealed that glycogen was never measured during any of the reported temperature studies. The absence of these measurements makes it hard to develop a complete picture of EBPR biochemical pathways and mechanisms. Helmer and Kunst (1997) proposed that PAOs are pyschrophilic organisms, but concluded, based on their results, that, relatively little storage of glycogen could be expected at cold temperatures. Liu *et al.* (1997) concluded that PAOs can store energy in the form of Poly-P and that glycogen storage is of minor importance for EBPR. However, the issue is not convincingly resolved by the literature, and the effects of temperature upon EPBR glycogen metabolism are somewhat unknown at present. Additional research is needed to determine the fate of glycogen during EBPR under different temperatures.

CONCLUSIONS

Although there have been several attempts to develop biochemical models of EBPR processes, there is no consensus model as yet, and there is much disparity regarding the reactions proposed for the anaerobic stage of EBPR systems. The reactions are complex, and studies of the effects of temperature upon this set of complex reactions have resulted in inconsistent and disparate results. A considerable fraction of the inconsistent results can be explained by the use of different substrates, different operating conditions, and the application of different analytical techniques as mentioned previously by Brdjanovic and coworkers (Brdjanovic *et al.,* 1997). Even when this explanation accepted, however, it does not explain why different studies that used the same substrate and operating conditions obtained opposing results, e.g., Brdjanovic *et al.* (1997) vs. Brdjanovic *et al.* (1998) and Panswad *et al.* (2000). It appears that the application of improper SRT values at 5^{o}C (Brdjanovic *et al.,* 1997 and Beatons *et al.,* 1999) caused the EBPR performance to be partially or completely lost. It is necessary to fully understand the experimental conditions of each study, and compare only those that used similar procedures. In addition to SRT, other experimental factors that need to be considered and compared are: temperature acclimation vs. nonacclimation, utilization of different substrates, improper system operation such as excessive recycle of nitrates to the anaerobic stage, failure to monitor all of the important parameters, etc. With respect to the latter, changes in the fluidity of the bacterial cell wall membranes with temperature have never been investigated. Also, the measurement of vital parameters such as cellular glycogen, PHA and Poly-P were ignored in most of the temperature studies. A complete picture of EBPR mechanisms cannot be obtained without the measurement of internal storage products.

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