The Effects of Temperature on System Performance and Bacterial Community Structure in a Biological Phosphorus Removal System

Ufuk Goksin Erdal

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Civil and Environmental Engineering

Dr. Clifford W. Randall, Chair
Dr. James P. Wightman
Dr. Eugene M. Gregory
Dr. Gregory D. Boardman
Dr. Daniel Gallagher

February 1st, 2002
Blacksburg, Virginia

Keywords: activated sludge, biological phosphorus removal, temperature, cold stress, polyhydroxyalkanoates, glycogen, PAOs, GAOs, competition, energy metabolism, aerobic recycle, homeoviscous adaptation

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(ABSTRACT)

It is generally accepted that a decrease in temperature causes the rates of chemical and biochemical reactions to slow down, usually resulting in poorer performance of biological wastewater treatment systems. Despite this, early researchers repeatedly showed that excess biological phosphorus removal (EBPR) was more efficient at colder temperatures. Recent studies, however, have demonstrated that the reaction rates of EBPR processes decrease with temperature in accordance with Arrhenius' Law, resulting in an apparent contradiction in the literature. The objective of this study was to investigate the EBPR temperature controversy. The experimental systems used were two, lab-scale UCT configuration plants fed with acetate as the sole volatile fatty acid (VFA) source. The results showed that EBPR systems do perform more efficiently at colder temperatures, i.e., at 5^{o}C compared to 20^{o}C. The reason for better system performance was determined to be related to reduced competition for substrate in the non-oxic zones that results in an increased population of phosphate accumulating organisms (PAOs) relative to non-PAOs and, therefore, greater EBPR efficiency even though the reaction rates are slower. The proliferation of PAOs relative to non-PAOs at cold temperature indicates that some of the PAOs are psychrophilic, i.e., they have alternate biochemical pathways that give them a competitive advantage over bacteria dependent upon glycogen metabolism. The activated sludge acclimated to 20^{o}C had relatively high polyhydroxyvalerate (PHV) and glycogen contents relative to sludge acclimated to 5^{o}C. It was initially hypothized that there is a significant competition between PAO and glycogen accumulating organisms (GAOs) at 20^{o}C and cold temperature (5^{o}C) nearly eliminates this competition in favor of the PAOs. A series of batch test experiments revealed that despite similar acetate utilization by the sludges grown at the two temperatures nearly 30% less PHA was produced by the sludge taken from the 20^{o}C reactor, indicating that GAOs were a small fraction of the population at

20°C. Transmission electron microscopy pictures showed that the biomass acclimated to 20°C had a much more diverse bacterial population than the biomass acclimated to 5°C. However, no GAO population was detected in electron microscopy samples under any temperature conditions. The decreased P removal efficiency at 20°C was then attributed to the presence of fermentative or other non poly-P bacteria that are capable of utilizing substrate under anaerobic conditions.

PHA production greatly increased at 5°C, whereas glycogen metabolism substantially reduced. Even though glycogen is an essential requirement for EBPR mechanism, the EBPR microorganisms have the ability to adapt their metabolic pathways to environmental conditions and greatly reduce their need for glycogen. It is apperant that cold temperature inhibits some of the key enzymes in glycogen metabolism resulting in lower glycogen accumulation that in turn increases the EBPR performance. Therefore temperature not only exerts selective pressure on the dominant population but also alters the metabolic pathways of the EBPR process. Increased glycogen accumulation, as observed in this study at 20°C, may not be related to GAO proliferation as suggested by Filipe *et al.* (2001) instead it may be related to EBPR bacteria to efficiently use glycogen metabolism. Current models (Brdjanovic *et al.* 1997; Filipe *et al.* 2002) consider that GAO metabolism is an integral part of EBPR metabolism and the performance of EBPR processes depends on PAO/GAO fraction in the EBPR system. No GAO proliferation was observed even the A/O process was operated without P addition for more than 3 weeks at 10°C. Therefore such important concept should be further investigated before it is included in EBPR models.

EBPR stoichiometry was presumed to be insensitive to temperatures (Brdjanovic *et al*. 1997). However, observed stoichiometric values of PHA storage per unit glycogen utilization and PHA utilization per unit glycogen rephlenishment were quite different at different temperatures. Temperature, therefore, not only affects the kinetics of EBPR systems but also affects the EBPR stoichiometry.

Most prokaryotic cells have the ability to alter their cellular membrane fatty acid composition as temperature decreases to counteract the adverse effects of temperature on membrane fluidity (Becker *et al.*, 1996). This unique ability is known as "homeoviscous adaptation". In this study, homeoviscous adaptation by EBPR activated sludge was investigated for a series of temperatures ranging from 20^{o}C to 5^{o}C using one of the lab scale EBPR systems. The fatty acid analysis results showed that the unsaturated to saturated fatty acid ratio increased from 1.40 to 3.61 as temperature dropped from 20 to 5^{o}C. The increased cis-9-hexadecanoic acid (C16:1) at 5^{o}C strongly indicated the presence of homeoviscous adaptation in the EBPR bacterial community. Thus the cell membranes of the EBPR community were still in a fluid state, and solute transport and proton motive force mechanisms were operable even at 5^{o}C. It was concluded that loss of EBPR performance at low temperatures, as reported by McClintock *et al*. (1992) was not related to the physical state of the cellular membranes, but was probably caused by unsuitable operational conditions.

Even though the transport of volatile fatty acids (e.g. acetate) is an integral part of EBPR biochemistry and stoichiometry, this important concept has been ignored. Fleet (1997) concluded that acetate entry into bacterial cells in EBPR sludge was simple passive diffusion based upon the results of a single study (Baronofsky et al. 1984). However, this study showed that neither acetate nor propionate can cross the cell membrane via simple passive diffusion. The existence of apparent saturation curves when the substrate uptake rates (acetate and propionate) were plotted against the substrate concentrations suggested that transport of volatile fatty acids obey facilitated or active transport.

Following from the above results, an investigation of the impacts of operational conditions such as low solids retention time (SRT), presence of electron acceptors in the non-oxic zones, low anaerobic detention time, and lack of acclimation was performed. The results showed that the 'critical, i.e., wash-out' SRT increased as temperature decreased, but if the biomass was permitted to acclimate to the lower temperature, a major population shift would occur which would increase the capacity of the system for phosphorus (P) removal. When the 5 ^{o}C sludge was allowed to acclimate at a relatively

high SRT (18 d), the system's P-removal capacity greatly surpassed that of the 20 ^{o}C system. The decrease in EBPR performance because of the presence of nitrates in the non-oxic zones was determined to be greater than what would be predicted based on accepted stoichiometry.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my major advisor, Dr. Clifford W. Randall for his unending support and guidance. I thank him for being as understanding and humble as he has been all through these years, and for not just being an advisor but also providing me and my family with a warm home that was always open when needed. I am also thankful to my doctoral committee members, Dr. Jim Wightman, Dr. Eugene Gregory, Dr. Greg Boardman, and Dr. Dan Gallagher for their valuable advise and patience.

I would also like to thank Jody Smiley for her endless assistance with the instrumentation techniques and for her friendship that will always be remembered. I also thank Julie Petruska for her help with the laboratory procedures and safety issues. I would also like to thank Betty Wingate for always being there when help is needed with office related issues.

I would especially like to thank my family for their continuing support and encouragement for the completion of this study. I also thank my wife, Zeynep for her support, and my son, Ozan for his patience. Their positive attitude made it lot easier to work towards this degree.

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NOMENCULATURE

A/O: Anaerobic/oxic.

ATP: Adenosine three phosphate.

BOD: Biochemical oxygen demand.

BNR: Biological nutrient removal.

COD: Chemical oxygen demand.

DO: Dissolved oxygen

EBPR: Excess biological phosphorus removal

ED: Entner-Doudoroff.

EM: Electron microscopy.

EMP: Embden-Meyerhoff-Parnas.

FAME: Fatty acids metyl ester.

GAO: Glycogen accumulating organism.

MCRT: Mean cell residence time.

MLSS: Mixed liquor suspended solids.

MLVSS: Mixed liquor volatile suspended solids.

NAD: Nicotinamide adenine dinucleotide.

PAO: Poly phosphate accumulating organism.

PHA: Poly-hydroxy-alkanoates.

PHB: Poly-hydroxy-butyrate.

PHV: Poly-hydroxy-valerate.

SBR: Sequencing batch reactor.

SCVFA: Short chain volatile fatty acids.

TEM: Transmission electron microscope.

TCA: Tricarboxylic acid.

TP: Total phosphorus

UCT: University of Cape Town.

VFA: Volatile fatty acid.

VIP: Virginia Initiative Process.