Evaluating the Utility of Fluorescence In Situ Hybridizations as a Regular Process Monitoring Tool to Improve Reliable Wastewater Treatment

Basic Information

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PI: Daniel B. Oerther, Ph.D., Tenure-Track Assistant Professor, Department of Civil and Environmental Engineering, University of Cincinnati

I. Executive Summary.

The microorganisms in the aeration basin are the most important yet least monitored part of a municipal activated sludge sewage treatment plant. Primarily, this is due to the inability of traditional monitoring technology to identify and quantify individual groups of microorganisms in a timely fashion. Therefore, we propose to develop "base-line" data demonstrating the value of regular process monitoring using a modern genetic tool - fluorescence *in situ* hybridization (a.k.a. **FISH**) - to track activated sludge microorganisms in municipal sewage treatment plants operated to remove solids, organics, and excess nitrogen. We expect that the information collected using FISH will empower plant operators to diagnose, alleviate, and most importantly avoid treatment process upsets. With the knowledge gained in this project, the water quality community will be able to move toward adopting state-of-the-art microbial treatment process monitoring technology with confidence. Ultimately this will lead to improved treatment process performance and enhanced protection of environmental water quality, which is a critical component for protecting raw water supplies identified as a research priority by the U.S. Geological Survey.

II. Introduction.

The stable performance of biological wastewater treatment systems hinges critically upon maintaining a diverse community of appropriate microorganisms including heterotrophic floc formers and filaments as well as autotrophic ammonia and nitrite oxidizing bacteria (AOB and NOB, respectively). Despite the reliance of the Nation's water quality professionals on microorganisms to treat more than 30 billion gallons of sewage each day, traditional methods for tracking "bugs", such as growth on a Petri dish or looking at them in the microscope, successfully identify less than 1% of microorganisms in the environment (Amann et al., 1995). In the past ten years, researchers in the emerging interdisciplinary field of environmental biotechnology have adapted and improved state-of-the-art molecular biology-based genetic methods for identifying and quantifying environmental microorganisms (Amann et al., 1998; Keller et al., 2002; Oerther and de los Reyes, 2001; 2002; Rittmann, 1998; 2002a; 2002b; Wilderer, 2002). The most universally accepted of these genetic methods targets 16S ribosomal ribonucleic acid (16S rRNA) using fluorescence *in situ* hybridization (FISH) to identify, enumerate, and localize evolutionarily-related groups of microorganisms directly in environmental samples (16S rRNA-targeted FISH as described in Oerther et al., 2002). Although academic and applied research using the 16S rRNA-targeted FISH approach has provided a significant amount of useful information regarding specific microbial groups including floc formers, filaments, AOB, and NOB (Grady and Filipe, 2000; Yuan and Blackall,

2002), as yet no one has demonstrated the value of FISH as a regular process monitoring tool for full-scale biological wastewater treatment systems (Oerther and Love, 2003). Therefore, **this important tool is currently not used on a regular basis to monitor treatment process performance.**

Historically, sewage treatment process performance has been monitored on a regular basis by measuring a limited number of priority pollutants including solids as well as organic pollution determined as five-day biochemical oxygen demand (BOD_5). Relying on the robust nature of microbial metabolism, traditional activated sludge treatment systems have provided adequate protection of environmental water quality for nearly a century (in particular since the passage of the Clean Water Act and the best management practice requiring a minimum of a secondary sewage treatment process). Moving into the 21st century, water quality professionals have been challenged with an expanding mission. Because of increasingly concentrated population centers and limited water supplies, treated sewage is used more and more as a raw water supply for the (in)direct production of potable drinking water. Thus, to track quality trends in raw water supply, it is of the highest priority that the water quality community find cost efficient ways to use the Nation's existing sewage treatment infrastructure to more consistently remove traditional pollutants to new lower levels and to remove an increasing number of pollutants (i.e., nutrients as well as personal care products and endocrine disrupting compounds). To effectively optimize existing activated sludge treatment process performance to meet increasingly stringent environmental regulations, water quality professionals need better information provided by improved process monitoring tools. Thus, the **significant question** posed in this project is to determine if the long-term value of 16S rRNA-targeted FISH is primarily as a research tool, or if FISH can be used as a regular process monitoring tool to track the diverse community of microorganisms in a municipal sewage treatment plant.

Since joining the Department of Civil and Environmental Engineering at the University of Cincinnati three years ago in the autumn of 2000, the Principal Investigator (PI), tenure-track Assistant Professor Daniel B. Oerther, Ph.D., has developed an active program of research and outreach focused upon the development, demonstration, and successful transfer to practice of effective microbial monitoring technology that is cost efficient, reliable, and easy to use by environmental professionals with minimal retraining. Although a new faculty member, Dr. Oerther's growing program at the University of Cincinnati has gained recognition as a leader in the State of Ohio and in the Nation in the emerging interdisciplinary field of environmental biotechnology (please see academic resume attached at the end of this application). Therefore, it is expected that the research plan outlined in this proposal will be successfully completed under Dr. Oerther's guidance. Furthermore, it is expected that the successful results of this research project will be broadly disseminated to the water quality protection community throughout the State of Ohio as well as the Nation. It is expected that the information collected using 16S rRNA-targeted FISH on a regular basis will empower plant operators to improve the reliable performance of biological wastewater treatment systems and to routinely meet increasingly stringent regulatory requirements. This project is expected to help 16S rRNA-targeted FISH "cross the threshold of acceptance," and for FISH to be

considered as a regular process monitoring tool in biological wastewater treatment facilities. The interdisciplinary team assembled for this project is well prepared to successfully complete the proposed research because we have a proven track record of working together to transfer to practice 16S rRNA-targeted FISH.

III. Project Objective and Tasks.

The overall **OBJECTIVE** of this project is determining if 16S rRNA-targeted FISH is a useful, regular process-monitoring tool in municipal sewage treatment plants operated to remove solids, organics, and excess nitrogen.

To accomplish this objective, the following **THREE TASKS** will be addressed over a one-year period:

> **Task One: Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to track major phylogenetic groupings of heterotrophic floc-forming microorganisms.**

Despite the acceptance of 16S rRNA-targeted FISH as a research tool, only a handful of systematic studies have been performed to demonstrate the value of regular process monitoring with FISH to track microorganisms in full-scale municipal sewage treatment plants (Biesterfeld and Figueroa, 2002; Oerther et al., 2001; de los Reyes and Raskin, 2002; Zilles et al., 2002a; 2002b). Documenting the success of a demonstration project is necessary to "cross the threshold of acceptance" and to show water quality professionals that 16S rRNA-targeted FISH is a useful regular process monitoring tool to track important groups of microorganisms. Because the phylogenetic diversity of heterotrophic floc-forming bacteria is enormous, it is experimentally infeasible to develop a suite of 16S rRNA-targeted oligonucleotide hybridization probes to just track "heterotrophic-floc forming microorganisms." Therefore, to address Task One, we propose to follow the "top-down" phylogenetic approach employed repeatedly by the research group of Prof. Dr. Rudolf Amann, Max Planck Institute for Marine Microbiology, Bremen, Germany (where the PI was a visiting scholar in 1998 and 1999, see Amann et al, 1995 as a representative publication). In the top-down phylogenetic approach, major groups of phylogenetically-related bacteria are targeted by individual oligonucleotide hybridization probes (i.e., probe S-D-Bact-0338-a-A-18 targets all Bacteria; probe S-P-Grps-1200-a-A-13 targets all Gram positive bacteria; probe L-Sc-bProt-1207-a-A-17 targets all bacteria in the beta subclass of the Proteobacteria, etc.). Changes in the relative abundance of these major groups indicate shifts in the structure of the microbial community. Ultimately, these changes in the overall structure of the microbial community can be correlated with the results of traditional process monitoring tools (e.g., solids and BOD_5) to develop relationships linking 16S rRNA-targeted FISH as a process monitoring tool with traditional process monitoring tools. *The expected outcome of Task One is "base-line" data establishing whether FISH should be used as a regular process monitoring tool for heterotrophic floc-forming bacteria.*

<u>Task Two:</u> Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to identify and enumerate major populations of filamentous microorganisms.

The simplest procedures for identifying filamentous microorganisms in samples of activated sludge use direct microscopic examination of morphology coupled with staining procedures (Eikelboom, 2000; Jenkins et al., 1993). Although these methods have been used to study filamentous microorganisms for more than a decade, reliable identification of filaments still requires a high level of experience on the part of the examiner. In contrast, filament identification using 16S rRNA-targeted FISH does not rely upon the experience of the examiner to recognize morphologic differences. Instead, molecular biology-based tools such as FISH are used to identify unique molecular signatures that are unambiguously linked to specific microbial populations (Zuckerhandl and Pauling, 1965). Previously, Wagner and co-workers developed a suite of 16S rRNA-targeted oligonucleotide hybridization probes to identify Gram negative filamentous microorganisms using FISH (Wagner et al., 1994). Following a similar approach, the PI collaborated with Dr. Francis de los Reyes, currently in the Department of Civil Engineering at North Carolina State University, to develop probes to identify important groups of Gram positive filamentous microorganisms including Mycobacteria (de los Reyes et al., 1997; de los Reyes et al., 1998; Oerther et al., 1999) as well as Microthrix (de los Reyes et al., 2002). These existing 16S rRNA-targeted oligonucleotide hybridization probes will be used in FISH assays to identify and enumerate major populations of Gram positive and Gram negative filaments. Samples with filaments that are not targeted by these existing probes will be preserved for potential follow-up analysis in future projects with the intention of developing additional probes targeting previously uncharacterized filamentous bacteria as documented in publications by the PI (Oerther et al., 2002; Iyer and Oerther, 2003). *The expected outcome of Task Two is linking filament identity and abundance with operating decisions and environmental conditions to begin to develop rationale "rules-of-thumb" relating filament occurrence to process performance.*

<u>Task Three:</u> Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to track bacteria responsible for nitrification.

The process of nitrification, the step-wise biochemical oxidation of ammonia-N to nitrite-N and ultimately nitrate-N, was first described more than one hundred years ago by Jean Jacques Theophile Schloesing (Schloesing and Muntz, 1877; Warrington, 1878; and Winogradsky, 1891). Today, an average of more than one hundred papers appear annually in the peer-reviewed scientific literature describing various aspects of "nitrification" AND "wastewater" (based upon a keyword search of the Institute for Scientific Information [ISI] Citation Database for 1997 – 2001). Although the nitrification process has been studied for more than 100 years, it still escapes our complete understanding. Perhaps even more astounding is that revolutionary discoveries involving nitrification still remain to be made. For example, during the past decade molecular biology techniques targeting 16S rRNA challenged the long-held "text book"

notion that *Nitrosomonas europaea* (Warrington, 1878) and *Nitrobacter winogradskyi* (Winogradsky, 1891) are the predominant AOB and NOB, respectively, in activated sludge systems (Grady et al., 1999; Madigan et al., 2002; Metcalf and Eddy, 2003; Rittmann and McCarty, 2001). Instead, 16S rRNA-targeted FISH and other molecular biology techniques showed that although *Nitrosomonas* spp. may be a predominant AOB, they are not the only ammonia oxidizing microorganism in activated sludge; furthermore, *Nitrobacter* spp. are rarely found in activated sludge systems and *Nitrospira* spp. are perhaps the more dominant NOB (Daims et al., 2001; Dionisi et al., 2002; Gieseke et al., 2001; Juretschko et al., 1988; Mobarry et al., 1996; Okabe et al., 1999; Purkhold et al., 2000; Sakano and Kerkhof, 1998; Sakano et al., 2002; Schramm et al., 1999; 1998; 1996; Wagner et al., 1995). In this project, 16S rRNA-targeted oligonucleotide hybridization probes will be used to document the identity and abundance of nitrifying bacteria over the year-long operation of a municipal sewage treatment plant operated to eliminate excess nitrogen to reduce nutrient loading on the environment. *The expected outcome of Task Three is documenting the stability of the nitrifying bacteria community in a full-scale municipal activated sludge sewage treatment plant during one-year of operation.*

IV. Benefits of the Project.

Environmental biotechnologies used to track environmental microorganisms are an area of evolving technology. The successful adoption of 16S rRNA-targeted FISH to track microorganisms in treatment processes should help to improve reliable process performance leading to improved trends in raw water quality. One of the primary obstacles to the successful use of FISH as a regular process monitoring tool is the lack of a successful demonstration project that can be cited as a definitive example that FISH provides much needed, highly useful information about the microorganisms in a wastewater treatment plant.

In addition, the successful results of the proposed research plan should provide a starting point for developing (semi-)automated techniques to make FISH into a routine process monitoring tool. This is important because the research in this project will lead to the use of FISH to monitor microorganisms in additional unit processes such as potable drinking water production as well as bacterial source tracking to identify pathogen pollution in the environment.

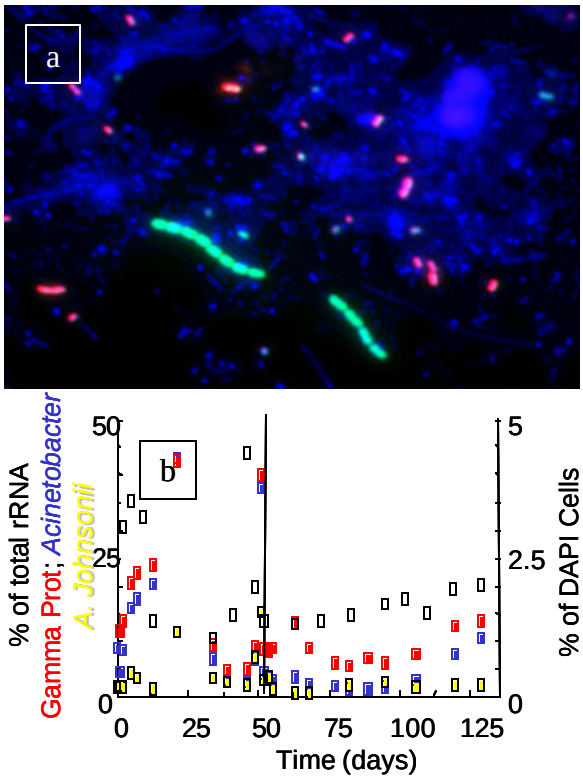
The proposed research plan will provide interdisciplinary training for one graduate student. Together with the PI and his existing research team of graduate and undergraduate students, the graduate student on this project will actively participate in regular monthly research group meetings as well as weekly one-on-one progress meetings with the PI. These meetings provide the student with an opportunity to present research results and defend the work in a public setting.

The results of the project will be broadly disseminated through active participation at local and international research conferences including the Water Environment Federation Technical Exposition and Conference and the general meetings of the American Chemical Society and the International Water Association. These meetings will provide

an excellent learning experience for the student researcher, and they will afford the PI with an opportunity to discuss the results of the project with other faculty and practitioners in the water quality community. The PI anticipates submitting the successful results of the research project to peer reviewed journals such as Applied and Environmental Microbiology, Biotechnology and Bioengineering, and Water Research.

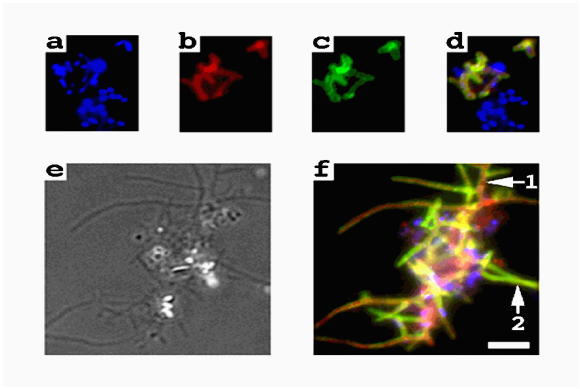
V. Preliminary Results.

Preliminary results relevant to each of the Three Tasks described above in Section III Project Objective and Tasks are presented below.



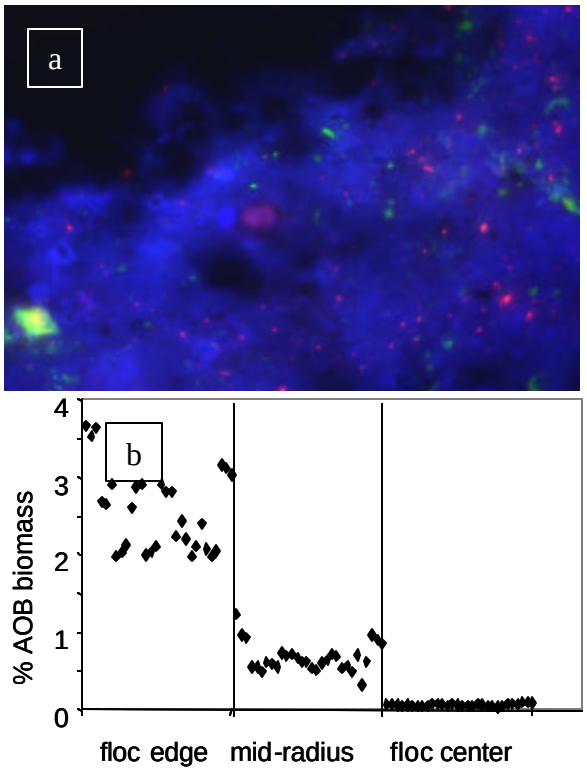
Task One: To track floc-forming heterotrophic bacteria in a 5-liter lab-scale activated sludge sewage treatment system during one hundred and thirty days of operation, the PI used 16S rRNA-targeted oligonucleotide probe hybridizations with the FISH assay as well as with quantitative hybridizations of membrane immobilized total RNA extracts. The hybridization results followed a "top-down" phylogenetic approach with all microorganisms detected using a universal probe, S-*-Univ-1390-a-A-18, while phylogenetically-nested groups of bacteria were detected using probe L-Sc-gProt-1207-a-A-17 (targeting the gamma-subclass of the Proteobacteria), probe S-G-Acin-0659-a-A-24 (targeting the genus *Acinetobacter*), and probe S-S-A.john-0451-a-A-22 (targeting *Acinetobacter johnsonii* type strain). Representative results of FISH are provided in Figure 1 panel A; while summary results collected during the one hundred and thirty day operation of the lab-scale reactor are provided in Figure 1 panel B. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 1a, blue cells). Hybridization results with a general probe targeting all of the genus *Acinetobacter* (Fig. 1a, green and red cells) versus hybridization results with a specific probe targeting just *A. johnsonii* type strain (Fig.1a, red cells) showed that *Acinetobacter* represented less than 5% of DAPI stained cell counts (Fig. 1b, open square symbols reported on right y-axis), and *A. johnsonii* was not the only *Acinetobacter* present in this sample. The summary results of quantitative membrane hybridizations showed a similar trend with the abundance of *A. johnsonii* (Fig.1b, filled yellow square symbols reported on left y-axis) less than or equal to the abundance of the genus *Acinetobacter* (Fig. 1b, filled blue square symbols reported on left y-axis) as well as the abundance of the gamma subclass of the Proteobacteria (Fig. 1b, filled red square symbols reported on left y-axis). Of note, the contribution of *Acinetobacter*-related

microorganisms to the total community 16S rRNA pool (Fig. 1b, values on left y-axis) was an order of magnitude higher as compared to the contribution of *Acinetobacter*-related biomass (Fig. 1b, values on right y-axis). These results strongly suggest that heterotrophic floc-forming bacteria such as *Acinetobacter*-related microorganisms can make a disproportionately large contribution to the overall microbial metabolic activity as compared to biomass levels suggesting that the net yield value of *Acinetobacter*-related microorganisms is lower as compared to the net yield value of other microorganisms in this particular lab-scale activated sludge system. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate representative heterotrophic floc forming microorganisms using a top-down phylogenetic approach. These results strongly support the capabilities of the PI to complete the research proposed in Task One.*



Task Two: To track filamentous bacteria in a full-scale municipal sewage treatment plant during one year of monitoring, the PI used 16S rRNA-targeted oligonucleotide probe hybridizations with the FISH assay as well as with quantitative hybridizations of membrane immobilized total RNA extracts. In addition, to track individual cells immunostaining with antibodies was performed. The complementary molecular biology methods used in this year-long study (16S rRNA-targeted FISH; membrane hybridizations; and immunostaining) were selected to specifically target a phylogenetically-coherent population of filamentous Mycobacteria, namely the *Gordonia amarae*-like organisms (formerly known as the *Nocardia amarae*-like organisms or Nocardiaforms). Representative results of whole cell FISH combined with antibody immunostaining are provided in Figure 2 panels A-F; while summary results collected during the year-long sampling campaign for the full-scale system are provided in Figure 2 panel G. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 2a, 2d, and 2f blue cells). Hybridization results with a general probe targeting all of the genus *Gordonia* (Fig. 2b, 2f red cells) versus antibody staining (Fig. 2c, 2f green cells) were combined to provide two independent methods confirming the identity of *Gordonia* cells in mixtures of pure cultures (Fig. 2d, yellow cells) as well as in samples of activated sludge (Fig. 2f, yellow cells). A comparison of the year-long summary results with the membrane hybridizations (Fig. 2g, filled blue circle symbols reported on the left y-axis) with the results of FISH (Fig. 2g, filled red triangle symbols reported on the right y-axis) and whole cell antibody immunostaining (Fig. 2g, open red

triangle symbols reported on the right y-axis) show that the abundance and activity of the genus *Gordonia* increased during the warmer summer-time months (July through October). In contrast to the results discussed above for *Acinetobacter*-related microorganisms, the abundance of *Gordonia* (Fig. 2g, filled and open red triangle symbols reported on the right y-axis) was at least one order of magnitude greater than the relative activity of the *Gordonia* biomass (Fig. 2, filled blue circle symbols reported on the left y-axis). These results strongly suggest that some filamentous bacteria may have high levels of total biomass although they only contribute a minimal amount to the overall metabolic activity of the activated sludge microbial community. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate heterotrophic filamentous microorganisms using a phylogenetically-targeted approach. These results strongly support the capabilities of the PI to complete the research proposed in Task Two.*



Task Three: To track autotrophic nitrifying bacteria inside individual activated sludge flocs removed from a full-scale municipal sewage treatment plant, the PI used 16S rRNA-targeted FISH and compared the results with measurements of chemical microenvironments determined with microelectrodes. The microelectrode measurements (not reported here) showed that dissolved oxygen levels decreased moving toward the center of individual flocs while ammonia levels dropped, nitrite levels rose, and nitrate levels rose, respectively. As expected, FISH results targeting ammonia and nitrite oxidizing bacteria showed a greater abundance of AOB and NOB at the edge of the flocs as compared to the interior. Representative results of FISH are provided in Figure 3 panel A; while summary results of FISH for ten individual flocs are provided in Figure 3 panel B. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 3a, blue cells). Hybridization results with probe S-*-Nso-1225-a-A-18 targeting ammonia oxidizing members of the beta subclass of the Proteobacteria (primary the genus *Nitrosomonas* and relatives) (Fig. 3a, red cells) corrected for measurements of autofluorescence (Fig. 3a, green speckles) showed that *Nitrosomonas*-related AOB represented less than 5% of DAPI stained cell counts. Summary results collected for hybridizations of thin cryo-sections of individual flocs showed a strong correlation between location in the floc and the abundance of AOB (Fig. 3b) suggesting that the abundance of AOB decreased as the levels of dissolved oxygen decreased (moving from the floc edge to the floc center). These results strongly suggest a spatial organization of individual microorganisms inside individual activated

sludge flocs according to microenvironments measured using microelectrodes. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate autotrophic nitrifying microorganisms using 16S rRNA-targeted FISH. These results strongly support the capabilities of the PI to complete the research proposed in Task Three.*

Collectively, the results reported in this section, V. Preliminary Results, effectively demonstrate that the PI has the experience, expertise, and facilities necessary to undertake the proposed research project including sampling for floc forming and filamentous heterotrophic microorganisms as well as autotrophic nitrifying bacteria. Furthermore, the PI has demonstrated that he can successfully monitor both lab-scale and full-scale activated sludge sewage treatment systems during prolonged operation. Thus, it is expected that the PI will successfully complete the proposed research tasks in the proposed timeframe of one-year.

VI. Methods, Procedures, and Facilities.

The procedures for 16S rRNA-targeted fluorescence in situ hybridizations (16S rRNA-targeted FISH) are employed daily in the regular operation of the PI's laboratory at the University of Cincinnati. A brief description of the procedure (adapted from Oerther et al., 2002) follows:

> For most applications of FISH with metabolically active samples (i.e., grab samples of activated sludge mixed liquor collected fresh and processed immediately), often one milliliter of a cell suspension provides adequate biomass for all subsequent analyses. Samples are typically fixed using 4% (wt/vol) paraformaldehyde prepared in a solution of 1x phosphate buffered saline for either a short duration (one minute, typically for Gram positive microorganisms) or long duration (12 hours, typically for Gram negative microorganisms) or fixation with paraformaldehye is eliminated from the procedure (typically for Gram positive microorganisms). After fixation, sample preservation is carried out by storing the samples in an equal volume of ethanol and 1xPBS with storage at -20^{o}C for up to one year before analysis. Fixed, stored samples are typically immobilized on a Heavy Teflon Coated microscope slide (Catalog Number 10-619, Cel-Line/Erie Scientific Co. [800]258-0834) for processing. Alternatively, samples could be filtered using 0.2 micron nominal pore size Sudan Black prestained nylon filters and each filter can be subsequently hybridized, washed, and analyzed. Immobilized cells are air dried, and dehydrated by passing through a 50%, 80%, and 100% ethanol bath for 1 min each. Unlike traditional membrane hybridizations where the hybridization step is performed at low stringency and the subsequent wash step is performed at high stringency; for FISH both the hybridization and wash steps must be performed at high stringency to reduce non-specific background fluorescent signal. Traditionally, the level of formamide in the hybridization buffer is used to control stringency for the hybridization step while the level of sodium chloride in the wash buffer is used to control stringency for the wash step. An appropriate hybridization solution (containing between 0% of 70% formamide) is placed on top of each sample of fixed bacteria. At least 50 ng / 10 microliters of 16S rRNA-targeted fluorescently-

labeled oligonucleotide hybridization probe is added to the hybridization cocktail. The microscope slide is placed in a hybridization chamber and incubated for 2 hrs at 46°C. The washing step is carried out using high stringency with the substitution of sodium chloride for formamide in the wash buffer. Washing is performed at 48°C for at least 30 min. After washing, each slide is rinsed with distilled water and air dried in the dark to avoid unnecessary fluorescence quenching due to exposure to bright light.

Hybridized slides (or filters) are subsequently stored at –20°C until they are analyzed using epifluorescence or confocal laser scanning microscopy. The PI's laboratory is well equipped to visualize samples. We have exclusive use of three epifluorescence microscopes including an upright Nikon model E-600, an upright Nikon model E-400 and an inverted Nikon model TE-2000. A slow scan cooled charged coupled device (CCD) camera is available to capture digital images and Metamorph software is routinely used to perform semi-automated digital image analysis. In addition to epifluorescence capability, the PI's laboratory has recently been equipped with a state-of-the-art Nikon model C-1 Confocal Laser Scanning Microscope equipped with lasers for green, red, and infrared excitation. This microscope was provided through a grant from the Ohio Board of Regents to the PI, and is available for the exclusive use of the PI's research team. The typical cost for hourly-use of the shared CLSM available in the College of Medicine at the University of Cincinnati is $150/hr. We expect to use the CLSM for approximately 5 hrs/wk during this project. Therefore, because we have exclusive use of our own CLSM system, we are saving $39,000 of user-fees that would have otherwise been associated with this project.

Samples of mixed liquor will be collected weekly throughout one year of operation from a municipal sewage treatment plant in Greater Cincinnati. The PI has been working with the Greater Cincinnati Metropolitan Sewer District for the past three years. In particular, the PI has used 16S rRNA-targeted FISH to examine heterotrophic floc forming and filamentous microorganisms as well as autotrophic nitrifying bacteria present in samples collected from the Mill Creek and the Muddy Creek Wastewater Treatment Plants. The Mill Creek plant operates two parallel conventional activated sludge treatment basins to process a daily average flow of 120 million gallons of sewage and a peak capacity of approximately 400 million gallons per day during wet weather events. The influent characteristics include 150 mg-chemical oxygen demand per liter of flow and 25 mg-N per liter of flow. The Muddy Creek plant operates a single pass activated sludge system with a suspended growth nitrification system to process a daily average flow of 14 million gallons of sewage and a peak capacity of approximately 60 million gallons per day during wet weather events. The influent characteristics include 60 mg-chemical oxygen demand per liter of flow and 10 mg-N per liter of flow. These two plants are easily accessible by public transportation from the campus of the University of Cincinnati. The Greater Cincinnati MSD shares all process performance data as well as log books of operator observations with the PI as part of ongoing research. Therefore, the PI has adequate access to the necessary full-scale municipal sewage treatment plants as demonstration sites for determining the value of regular process monitoring using 16S rRNA-targeted fluorescence in situ hybridization.

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Zuckerkandl, E., and Pauling, L., 1965, Molecules as Documents of Evolutionary History, J. Theor. Biol. 8:357.

CURRICULUM VITA DANIEL B. OERTHER

EDUCATION

1995	B.A.	Biological Sciences	Northwestern University, Evanston, IL
1995	B.S.	Environmental Engineering	Northwestern University, Evanston, IL
1998	M.S.	Environmental Engineering	University of Illinois, Urbana-Champaign
2001	Ph.D.	Environmental Engineering	University of Illinois, Urbana-Champaign

PROFESSIONAL EXPERIENCE

1995 – present	Professional Engineer Intern, State of Illinois (061-026304)
1995 – 2000	Graduate Assistant, Department of Civil and Environmental Engineering, University of Illinois, Champaign, IL
1998 – 1999	Visiting Scholar, Max Planck Institute for Marine Microbiology, Bremen, Germany
2003	Visiting Scholar, Marine Biological Laboratory, Woods Hole, MA
2000 – present	Assistant Professor, Department of Civil and Environmental Engineering, University of Cincinnati, OH
2003 – present	Adjunct Assistant Professor, Department of Biological Sciences, University of Cincinnati, OH

HONORS AND SCHOLARLY AWARDS

- Richard S. and Mary E. Engelbrecht Fellowship, 1997, University of Illinois
- First Place, James M. Montgomery Consulting Engineers Master's Thesis Award, 1999, Association of Environmental Engineering and Science Professors (AEESP)
- Neil Wandmacher Teaching Award for Young Faculty, 2002, University of Cincinnati
- Elected membership in Sigma Xi, The Scientific Research Society, 2002
- CAREER Award, FY2003-2008, National Science Foundation
- Ohio Young Engineer of the Year, 2003, Ohio Society of Professional Engineers
- Selected one of the most influential people in Cincinnati under 35 years old, *The Creative Class*, Cincinnati Magazine, May 2003, p. 74-75

REPRESENTATIVE SCHOLARLY WORKS (from a list of 118 works)

- **Oerther, D.B.**, Pernthaler, J., Schramm, A., Amann, R., and Raskin, L., 2000, "Monitoring Precursor 16S rRNA of *Acinetobacter* spp. In Activated Sludge Wastewater Treatment Systems," *Appl. Environ. Microbiol.*, 66:2154-2165.
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- **Oerther, D.B.**, 2002, "Introducing Molecular Biology to Environmental Engineers Through Development of a New Course," *CEE: Chemical Engineering Education*, 36(4):258-263.
- **Oerther, D.B.**, and de los Reyes III, F., 2002, "Molecular Methods in Biological Systems," *Water Environ. Res.*, 74(4):CD-ROM, 23 pages.
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- Saikaly, P., Stroot, P.G., Butler, R., and **Oerther, D.B.**, "Dynamic Model of Activated Sludge: Linking Process Performance with Identity and Abundance of Individual Microbial Populations," Ohio Water Environment Association, Annual Meeting, Columbus, OH, June 25-28, 2002, p. 9.
- Stroot, P.G., and **Oerther, D.B.**, "A New Molecular Respirometry Method for Identifying Bacteria and Determining Their Growth States," Water Environment Federation, Proceedings of Research Symposium, WEFTEC, Los Angeles, CA, October 11 – October 15, 2003, CD-ROM, 6 pages.
- **Oerther, D.B.**, and Love, N.L., 2003, "The Value of Applying Molecular Biology Tools in Environmental Engineering: Academic and Industry Perspective in the U.S.A.," *Re/Views in Environmental Science and Bio/Technology*, (in press).

REPRESENTATIVE COMPLETED RESEARCH PROJECTS (and significant findings)

- National Science Foundation, "SGER: Rapid Identification, Enumeration, and Characterization of Mycobacteria and Nocardioforms in Environmental Samples," $99,294, September, 2001 – November, 2002. The objective of this project was to develop a microfluidic device to isolate colonies of slow growing environmental Mycobacteria in water samples. The successful prototype device detected environmental Mycobacteria in five minutes as compared to conventional techniques that require up to ten days to complete. Our device represents a significant advancement in cost effective monitoring for environmental Mycobacteria. Six conference proceedings and two refereed papers were produced from this project.
- US Environmental Protection Agency, "Testing the Resource Ratio Theory as a Framework Supporting Bioremediation Strategy for Clean-up of Crude Oil-Contaminated Environments," $256,109, April, 2001 – April, 2003. The objective of this project was to use a well-described theory from ecology to predict the optimum dose of fertilizer to enhance the bioremediation of crude-oil in soils and sediments. Experimental results were highly correlated with the literature and provided a much needed conceptual framework for understanding the dose-response relationship among the application of fertilizer and the successful remediation of oil. Three conference proceedings and one refereed paper were produced from this project.
- CH2M Hill, "Evaluating Wastewater Treatment Using Molecular Tools," $15,300, June, 2001 – August, 2001. The practical objective of this project was to identify the cause of poor sludge separation in an industrial wastewater treatment plant. Molecular biology analyses predicted that Paenibacillus-like microorganisms were responsible. Lab-scale bioreactor operation confirmed the molecular biology results. Three conference proceedings and two refereed papers were produced from this project.