Report for 2004OH9B: Evaluating the Utility of Fluorescence In Situ Hybridizations as a Regular Process Monitoring Tool to Improve Reliable Wastewater Treatment

- Book Chapters:
    - Oerther, D.B., “Chapter 6: Biological Solutions,” in Environmental Solutions, Edited by N. Nemerow and F. Agardy, Reed Elsevier, (in press).
- Conference Proceedings:
    - Oerther, D.B., Jeyanayagam, S., de los Reyes, F., Noguera, D., Angenent, L., and Emrick, J., 2004, WEFTEC2004 Workshop #W117: FISHing in Activated Sludge Handbook, Water Environment Federation, 47 pages.
    - Smith, R.C., and Oerther, D.B., “Bacteria Breath: Using a Respirometer to Model Sewage Treatment,” Research/Scholarship Forum, University of Cincinnati, Mar. 4, 2005, p.31.
    - Zhang, K., and Oerther, D.B., “Identifying the Pioneer Bacterial Species Responsible for Irreversible Biofouling in Membrane Bioreactors,” Research/Scholarship Forum, University of Cincinnati, Mar. 4, 2005, p.33.
- Articles in Refereed Scientific Journals:
    - Smith, R., and D.B. Oerther, 2005, “Microbial community development in a laboratory-scale nitrifying activated sludge system with input from a side-stream bioreactor treating digester supernatant,” Water Science and Technology, International Water Association, (in press).
    - Oerther, D.B., (submitted June, 2005), “Integrating Molecular Biology Research, Teaching, and Professional Outreach in Environmental Engineering and Science,” Journal Environmental Engineering Science, Mary Ann Liebert, Inc., (in review).

Report Follows

Completion Report

Project Title.

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

Project Team.

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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, the **objective** of this project was 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.

The specific interdisciplinary tasks that were undertaken in this study included:

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

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

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

The research team that executed this project included: a tenure-track faculty member with experience in environmental biotechnology (Oerther); one international graduate student pursuing his doctoral degree (Zhang); and one domestic graduate student with more than seven years of prior employment as an environmental engineering consultant who returned to pursue his doctoral degree (Smith).

During one year of research, major project accomplishments included:

1. conducting a literature review to identify existing 16S rRNA-targeted oligonucleotide hybridization probes and experimental hybridization conditions to detect heterotrophic floc-forming bacteria, filamentous bacteria, and nitrifying bacteria in suspended growth activated sludge wastewater treatment systems operated to treat municipal sewage;
2. creating standard operating procedures for the use of 16S rRNA-targeted oligonucleotide hybridization probes for fluorescence in situ hybridization (FISH);
3. performing FISH with samples collected from representative sewage treatment systems;
4. disseminating the results of FISH as part of hands-on workshops for treatment plant personnel.

The most significant results of this study have been presented at international scientific gatherings and published in the archival peer-referred literature, including:

- Oerther, D.B., Jeyanayagam, S., de los Reyes, F., Noguera, D., Angenent, L., and Emrick, J., 2004, WEFTEC2004 Workshop #W117: FISHing in Activated Sludge Handbook, Water Environment Federation, 47 pages.
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In brief, we concluded from our literature review that existing oligonucleotide probes for heterotrophic floc-forming bacteria, filamentous bacteria, and nitrifying bacteria provide suitable coverage of the most abundant phylogenetically-defined bacterial populations anticipated to be found in municipal activated sludge sewage treatment. In particular, experimental results confirmed that a top-down phylogenetic approach targeting all bacteria, alpha subclass Proteobacteria, beta subclass Proteobacteria, gamma subclass Proteobacteria, actinobacteria, and cytophage-flavobacterium-bacteroides provided coverage of the major floc-forming heterotrophic bacteria, a range of genus and species-specific probes provided suitable coverage of filamentous bacteria, and suitable probes were available to identify ammonia oxidizing and nitrite oxidizing bacteria. Seasonal samples were collected each quarter for eleven municipal activated sludge sewage treatment plants located through metropolitan Cincinnati. FISH using existing 16S rRNA-targeted oligonucleotide hybridization probes was used to identify and

enumerate the predominant bacterial populations present in the samples. In general, the probe targeting beta subclass Proteobacteria successfully hybridized to nearly fifty percent of the total cells in all of the samples while more than half of the remaining cells hybridized with one of the remaining probes targeting floc-forming heterotrophic bacteria, filamentous bacteria, or nitrifying bacteria. These results lead us to conclude that existing 16S rRNA-targeted oligonucleotide probes recognized more than seventy-five percent of the total cells in municipal activated sludge systems. Using the probes targeting filamentous heterotrophic bacteria, the genus *Gordonia* was determined to be the most prevalent filamentous population in the eleven municipal activated sludge systems examined in this study. Furthermore, a transient seasonal increase in the relative abundance of *Gordonia* was detected during the warmest summer months. These results lead us to conclude that bacteria belonging to the *Gordonia* genus are the most significant filamentous bacteria populations inhabiting the municipal activated sludge systems in metro Cincinnati. Using the probes targeting nitrifying bacteria, the genus *Nitrosomonas* and the genus *Nitrospira* were identified as the most abundant ammonia oxidizing and nitrite oxidizing bacteria populations, respectively, in the eleven municipal activated sludge systems examined. Although nitrification is not required of most of the plants examined in this study, none the less populations of ammonia oxidizing and nitrite oxidizing bacteria were found to represent a total of nearly six percent of the total cell counts. To successfully transfer the technology of FISH from the research lab and into the day-to-day practice of environmental engineering, a number of hands-on workshops were convened at Cincinnati and throughout the state of Ohio. Each workshop included approximately ten participants who were plant operators, lab personnel, and consulting engineers working at public owned treatment works or local consulting firms. The day-long workshops included participants performing all of the experimental procedures for FISH using a set of phylogenetically-nested 16S rRNA-targeted oligonucleotide hybridization probes. Assessment of the effectiveness of the workshops to successfully transfer FISH from the research lab and into the field found that the hands-on laboratory exercises used in the workshop were highly desirable. Furthermore, participants strongly indicated a desire to receive additional background information or to participate in follow-up workshops indicating that the content and style of delivery for the workshops was appropriate and informative. The overall results of this study support our hypothesis that existing 16S rRNA-targeted oligonucleotide probes can be used successfully to track the most predominant floc-forming, filamentous, and nitrifying bacterial populations in municipal activated sludge wastewater treatment plants, and furthermore these molecular biology-based approaches can be successfully transferred to environmental practitioners through workshops including hands-on laboratory experiences.