Researchers Strive to Mainstream Molecular Tools

Dr. Seuss probably wasn't thinking about genetic-based molecular tools when he wrote *Horton Hears a Who*, but the similarity between the story and the technology are striking. In the story, Horton hears a speck of dust talking. What he discovers is that an entire world lives in that speck. Only Horton — with his especially sensitive ears — could hear the Who's shouts.

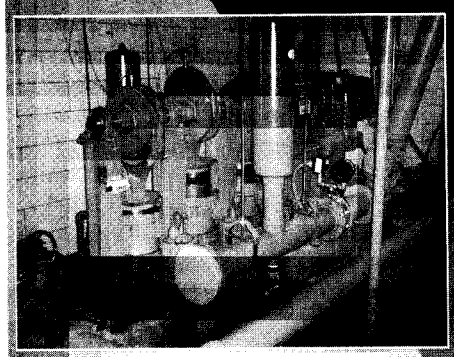
Everyone could see the speck and notice its movements, but without Horton's sensitive ears, they could not tell what was on it. Similarly, traditional wastewater measurements can assess the overall effect of organisms in a system, but finding a particular organism requires molecular tools that identify the organism by its genetic material.

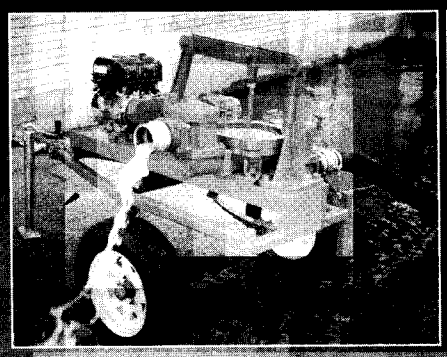
Molecular tools could produce quick and meaningful results, avoid indicator organism requirements, and determine whether pathogens are alive or dead, said Charles Bott, an environmental engineer at Parsons Corp. (Pasadena, Calif.). Despite such benefits, however, molecular tools still reside mostly in the research arena. Whether these methods ever will be viable for typical wastewater treatment has been a perennial discussion for researchers, noted Bruce Rittmann, an environmental engineering professor at Northwestern University (Evanston, Ill.).

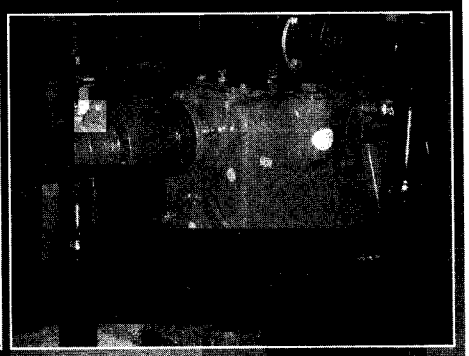
Quick Start

Molecular techniques were born in 1972 with the discovery of recombinant DNA and hybridization — the joining of two complementary strands of genetic material in which one strand is used as a probe to detect the other, said Daniel Oerther, assistant professor in the Department of Civil and Environmental Engineering at the University of Cincinnati. Then in 1983, Bott said, the polymerase chain reaction (PCR) — a technique to amplify a strand of DNA and detect a defined sequence of genes

NOTHING BEATS A WASTECORP. PUMP.







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— was developed, and a new method of protein analysis caused the molecular biology movement to take off.

The application of molecular tools to the wastewater treatment field has been evolving for the past 10 to 12 years, but in the last 2 to 5 years it has exploded, Oerther said. Currently, molecular techniques are used to identify which microorganisms are present, Rittmann said. The two classical methods are dot–blot hybridization and fluorescent *in situ* hybridization (FISH), he noted, while the two fingerprinting techniques are denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP).

In short, Rittman explained, the dot–blot method extracts a microorganism's RNA, attaches it to filter paper, and exposes it to genetic probes that stick to certain parts of the RNA. The method relies on a radioactive molecule attached to the probe to detect the microorganism.

The FISH method involves attaching a fluorescent molecule to the genetic probe and exposing the probe to microorganisms in the sample (*in situ*), he said. Researchers then use a fluorescent microscope to identify the microorganisms, Rittmann said. However, he noted, one must know which organisms are present in order to choose the proper genetic probes for either classical method.

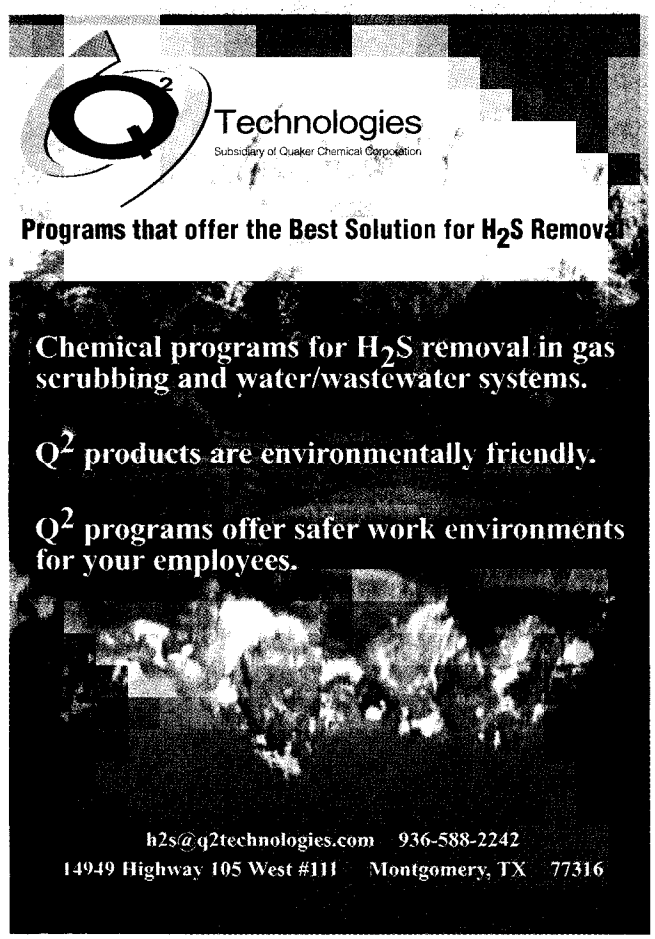
FISH also can help researchers learn about spatial relationships among different organisms, Rittman noted. FISH preserves the grouping patterns and aggregates of organisms in a treatment system, he said, and its results startled researchers a few years ago, when FISH revealed that nitrifying bacteria generally form extremely dense groupings inside larger aggregates of microorganisms.

The fingerprinting techniques, on the other hand, enable researchers to identify (or at least document) the DNA of any microorganism. "This deals with the reality that we've only isolated and sequenced a very tiny fraction of all the organisms that are interesting," Rittmann noted.

Early Leads

Some typical uses for molecular techniques in biological wastewater treatment applications include identifying filamentous bacteria as the cause of foaming and bulking, evaluating the effects of microbial stress responses, and understanding the microbiology of enhanced biological phosphorus removal, Bott said.

"One of the most exciting areas for molecular tools is the identification and evaluation of nitrifying bacteria," Bott said. "So, 'who's there, where are they,



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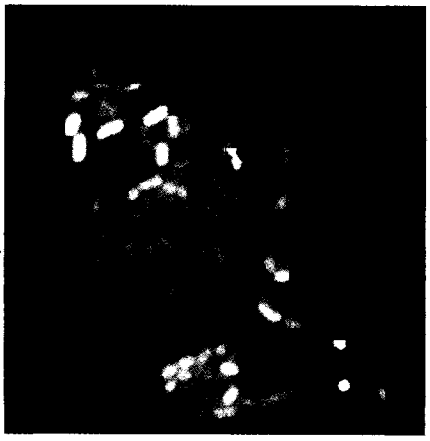
and what are they doing?' are the questions we're asking, and using these tools to answer."

Rittman agreed. In the past few years, he noted, researchers have been pushing beyond "who's there" type molecular tools to begin developing methods that determine what various bugs are doing.



COURTESY OF D.B. OERTHER, UNIVERSITY OF CINCINNATI.

This digital micrograph shows how fluorescence *in situ* hybridization can identify individual cells of *Acinetobacter calcoaceticus* (green) and their level of growth activity as measured by precursor 16S rRNA-targeted oligonucleotide probe hybridization (red).

"A microorganism can do many, many different things, but it doesn't do all of them at once," Rittman said. As a microorganism does a given task, it activates the needed DNA, and molecular tools can read the activated DNA strands, he noted.

Fast Pace

Molecular techniques are advancing very quickly. While current molecular tests readily identify microorganisms, they are not yet good at counting them, Rittmann said. However, in the last 2 or 3 years, researchers have begun to develop quantitative techniques. Also, kits are now available that make FISH-based testing much faster and easier, he said.

While new advancements are "filling in the holes," sometimes the information almost flows too quickly. "One of the great strengths and also one of the great challenges of the business is that it's changing so fast," Rittmann said. "It's great that we're getting these new techniques and more things to exploit, but on the other hand, it's hard to keep up."

The bigger problem, however, is making water quality professionals aware of the tools available. Understanding biology using molecular tools is fundamentally different than biology before molecular tools, Oerther said. While most scientific advancements evolve over generations of scientists, molecular biology has exploded onto the scene in only 25 years, he noted. The result of such quick development is that most people at the height of their careers today lack any formal training in this area.

Gaining Ground

To help move molecular tools out of the research lab and into practical use, researchers are taking molecular techniques to managers in the wastewater field.

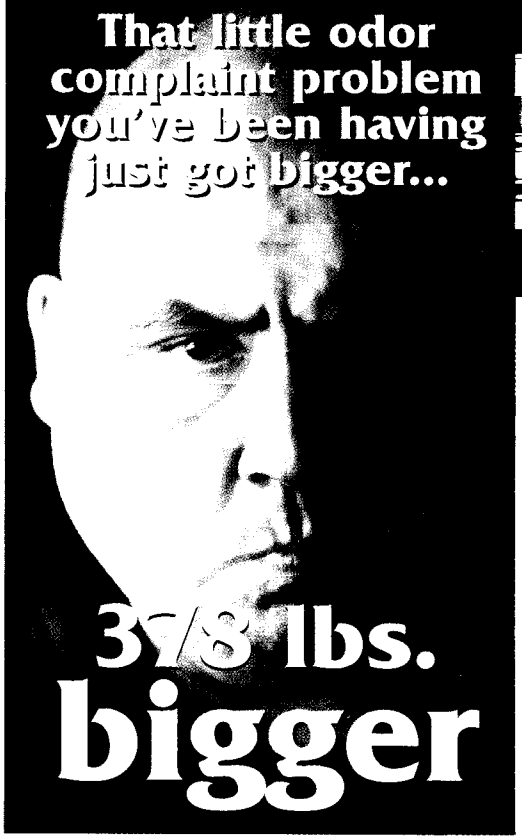
For example, Oerther and Nancy Love, an associate professor of civil and environmental engineering at Virginia Tech (Blacksburg), organized a workshop in 2002 so researchers and practitioners could discuss the merits of molecular tools. Funded by the National Science Foundation, the 2-day workshop had 15 participants who discussed four overarching questions:

- Can faculty establish careers working on molecular biology?
- How should molecular tools be standardized?
- How should molecular tools be taught?
- What are the practical applications of the molecular tools?

At press time, Oerther said the journal *Re/Views in Environmental Science and Bio/Technology* had accepted an article detailing the workshop results.

And last year, Rittman organized a special issue of the peer-reviewed journal *Water Environment Research* (September/October 2002), which included several papers on molecular techniques that had been presented at WEFTEC.01® in Atlanta.

WEFTEC.03® will feature several opportunities to learn more about the design and application of molecular tools in the wastewater treatment process. On



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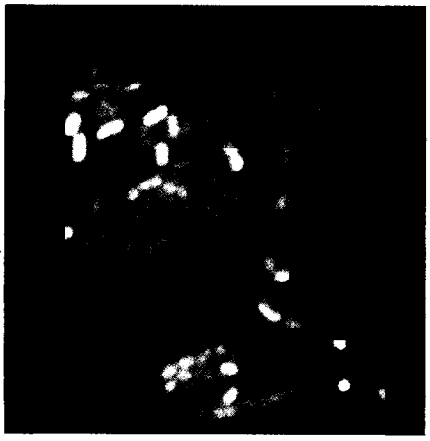

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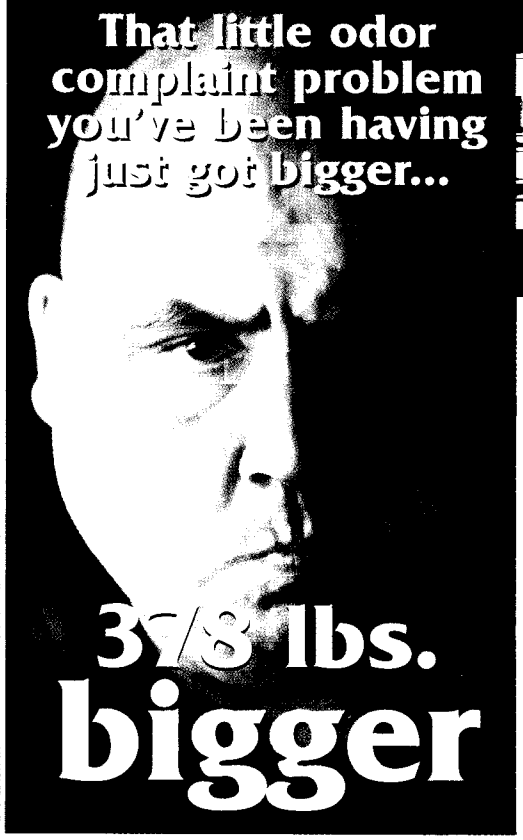

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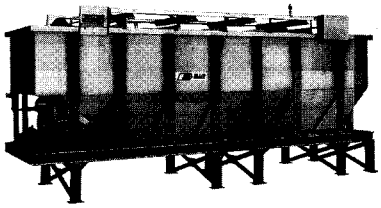

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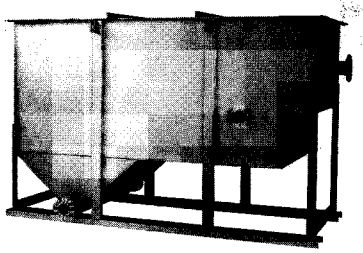

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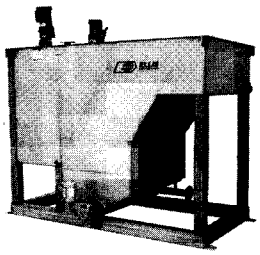

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Sunday, Oct. 12, Oerther will lead Workshop 115: FISHing in Activated Sludge, which will present basics, pros and cons, and potential uses of genetic techniques. The workshop is based on a 10-week molecular biology course geared toward postgraduate engineering students. In addition to interactive presentations, participants will be able to work with molecular tools to characterize wastewater samples from their own treatment plants, Oerther said.

WEFTEC.03 participants also can learn more about molecular tools by attending Session 20: Bulking and Floc Structure on Tuesday, Oct. 14, and Session 59: Application of Molecular Tools on Wednesday, Oct. 15. Bott, who will moderate both sessions, said that he is especially looking forward to the papers titled *Quantifying Species — Specific Filamentous Bulking Thresholds Using Molecular and Reactor Studies* and *A New Molecular Respirometry Method for Identifying Bacteria and Determining Their Growth State*.

— Steve Spicer, WE&T

Explorer Cousteau to Add an Ocean Perspective to WEFTEC.03

Jean-Michel Cousteau, president of the Ocean Futures Society (OFS; Santa Barbara, Calif.), a nonprofit marine conservation and education organization that he founded in 1999, will speak at the Opening General Session of WEFTEC.03®, the annual conference of the Water Environment Federation. The son of the late ocean explorer Jacques Cousteau, he has been exploring the ocean since he was 7.

Through OFS, Cousteau produces environment-oriented programs and television specials, public service announcements, multimedia programs for schools, Web-based marine content, books, and magazine and newspaper articles. He has produced more than 70 films. Cousteau has been awarded the Peabody Award, the Cable Ace Award, the Emmy, and the 7 d'Or, which is the French equivalent of the Emmy. He also travels worldwide to meet with leaders and policymakers and give public lectures.

In his work, Cousteau emphasizes the importance of the ocean to the survival of all life on Earth. Cousteau recently spoke out in support of *America's Living Oceans: Charting a Course for Sea Change*, a report of the Pew Oceans Commission (Arlington, Va.). The report, released in June, is a 3-year nationwide study of the oceans. Cousteau agreed with the report's assertion that some federal agencies that currently formulate policies and regulations affecting the ocean and watersheds should be consolidated into one independent federal agency to achieve "proper stewardship of resources."

"While the ocean is along our shores, we also need a new national commitment to improving the Mississippi River watershed system that has become a poisonous artery, creating a 'dead zone' in the Gulf of Mexico from the pollution the river carries to the sea," Cousteau said. "This shows the very real interconnectedness of people, our fresh water, and ocean environment."

OFS calls for the protection of coastal marine habitats, coral reefs, estuaries, and wetlands in order to ensure the ocean's quality of life — and thus peo-