SAND2010-6442J
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Innovation article
Reviewers: Todd Lane
~ 1120 words
Photos: yes

Sandia using RapTOR technology for understanding algal pond collapse

Research will help commercial sector in making algae more viable, profitable as alternative fuel

By Mike Janes

As industry continues to find a profitable way to meet the nation's alternative energy objectives, more and more start-up companies, it seems, are growing production ponds of algae.

Algae are widely viewed as a potential source of renewable fuel, but the technology to mass-produce fuel-grade algae is still in the early stages. A major roadblock is the inability to produce large amounts of the greenish, chlorophyll-containing organisms.

Algae are commonly grown in raceway ponds, large, shallow, artificial ponds that serve as fields for algae crops. But when water is constantly re-used, mixed, or blended (a common practice at multi-acre algae production facilities), a problem emerges that companies aren't well-equipped to deal with: algal pond collapse.

It's really a farming or agricultural issue rather than a biotechnology issue, says Todd Lane, a molecular biologist and algae researcher at Sandia National Laboratories in California.

"Largely because of the way water is used at these facilities, there is a constant and very high risk that the ponds will collect a pathogen or predator in the water that will attack or feed on the algae," says Lane. Pond culture collapses, he says, are largely attributed to agents such as fungi, viruses, or predators like zooplankton.

“The organisms fall into these ponds and can crash a pond overnight,” he adds. “No one has identified many of the agents that are causing these pond crashes. You can’t develop countermeasures without understanding why something is happening. This is a complex problem with a lot of factors at play.” Pond crashes are often characterized by a sudden emergence of infection followed by rapid loss of algal biomass.

Most of today’s algae production companies, Lane says, simply don’t have the biotechnology know-how or the resources to identify pond crash agents and ensure their ponds are kept safe from attack. Consequently, pond crashes are now one of the primary factors that keeps those companies from successfully producing the amount of algae it will take for algal biofuels to be cost-effective.

Lane and his colleagues at Sandia, armed with a pathogen detection technology that they’ve honed through internal investments as well as a recent $800K grant secured through the Department of Energy’s Biomass Program, are now working to compare the environmental conditions and metagenomes of algal samples taken from normal ponds to those taken from ponds that have undergone collapse. “Metagenomes” are genetic material recovered directly from environmental samples.

In doing so, the goal is to develop a method for rapid characterization of the responsible predatory agents and either a device or a service for companies that will help make their algae ponds more productive.

Sandia’s winning DOE proposal, titled “Pond Crash Forensics,” will use pathogen detection and characterization technologies developed through the lab’s Rapid Threat Organism Recognition, or RapTOR, project. RapTOR was originally developed for homeland security purposes.

The bioterrorism threat space, explains Lane, is much bigger than most of us realize.

“When most people hear the word ‘bioterrorism,’ they think of agents like anthrax, smallpox, or ricin,” he says. “But those are the agents we know about. There is also a whole other realm of ‘unknown unknowns,’ lethal agents that could be weaponized from ordinary viruses or disguised to look harmless.”

Unlike known threats such as anthrax or smallpox, detection of an advanced agent would only occur when people begin showing symptoms. Every day that treatment is delayed, the lethality of the attack goes up exponentially. “If a novel attack occurs and our detection systems fail, we have limited time in which to identify and characterize the organism to be able to offer effective treatment,” says Lane.

History shows that identifying and characterizing a naturally occurring unknown organism is very difficult. The 1970s outbreak of Legionnaires’ disease took six months to characterize; nearly 30 years later, it still took weeks to characterize Severe Acute Respiratory Syndrome (SARS). Conventional DNA sequence-based detection systems failed to identify a recent outbreak of Ebola in Uganda because the virus had changed so much it was unrecognizable.

The RapTOR program seeks to solve the “unknown unknowns” problem by developing a tool to rapidly characterize a biological organism with no pre-existing knowledge. Sandia’s researchers, Lane says, are taking advantage of rapidly evolving molecular biology technology and the advent of ultra-high-throughput DNA sequencing in order to re-engineer time-intensive benchtop methods to be faster, easier, and automated.

That same tool can be used to quantify the “unknown unknowns’ at the root of pond crashes.

Sandia’s researchers will obtain samples of water from “freshly” crashed ponds and unaffected ponds grown in parallel at the same facility under similar conditions. They’ll focus on common production strains such as *Nannochloropsis* and *Neochloris* in primarily open pond architectures.

Sandia’s technical approach will combine modern methods of metagenomic analysis, advanced imaging, and field microbiology /

virology to identify, characterize and, when possible, isolate the agent causing a pond crash.

Metagenomic comparisions will be carried out by tailoring microfluidics-based technology to the problem of pond crashes. Sandia's RapTOR technology, says Lane, will supplant the cumbersome task of identifying and isolating the agent that has caused a crash by traditional microbiological methods. Instead, Lane and his colleagues will utilize ultra high throughput DNA sequencing to characterize and rapidly develop a genetic signature for the agent.

The technology will rapidly compare nucleic acids obtained from normal and diseased ponds, isolating those that derive from the pathogen or the algal response, and rapidly characterizing these nucleic acids by deep sequencing – *without requiring a prior knowledge of the agent*. Since the process is automated and high throughput, it allows the comparison of a large number of samples for statistical analysis and greater confidence in agent identification at minimal cost.

In addition to the standard chemical and physiological measurements, Sandia's research will also employ Sandia's unique capabilities in hyperspectral fluorescence imaging to characterize stress-induced fluctuations in algal photosynthetic pigments in both experimental and field systems. The project's first year will focus on the identification and isolation of those agents causing pond crashes, while during the second year of the project researchers will create pond crashes "on demand" for laboratory analysis and experimentation leading to countermeasure development.

Though specific collaborators have yet to be identified, Sandia's Lane says the project team will work with a number of academic and industrial partners to obtain samples from pond crashes.

"For the commercial sector involved in producing algae, this will save money since those companies will maintain better control over their ponds and experience less impact on their production schedules," says Lane. "We feel that we're in a good position to address this particular roadblock. It's a good niche for Sandia, and something that allows us to provide a service that will be of great benefit to the algal biofuel industry that will in turn greatly benefit the nation."

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

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