New bacteria from anammox wastewater treatment

March 31 2016

Removal of nitrogen from wastewater is crucial to maintain water quality. A novel wastewater treatment technology based on "anammox" bacteria, capable of removal of nitrogen compounds anaerobically, has greatly improved this process. After a decade of operation of a full scale anammox reactor, researchers of the Radboud University and BE-Basic have studied the microorganisms in that a reactor and found unexpected diversity in this man-made system, including some "microbial dark matter". The work, published on March 31 by the journal *Nature Communications*, can help improving the performance of anammox reactors.

Dutch wastewater treatment company "Waterstromen" installed one of the world's first anammox reactors in 2006 in Olburgen, The Netherlands, to clean nitrogen from wastewater of the nearby Aviko french fries factory. In theory, anammox reactors can remove nitrogen from wastewater cheaper and more energy efficient than traditional methods. In practice, the reactor even exceeds expectations, and also removes most of the remaining carbon from the wastewater. As the microbes in the reactor kept performing well over time, this bioreactor has been used to seed other wastewater treatment plants, from England to China, where the biomass was used to start the largest anammox reactor in the world. This required several tonnes of biomass to be flown all the way to China, as the culture died during transport per ship; an investment illustrating the value of this treatment technology. Currently, anammox technology has been adopted in over 100 wastewater treatement plants worldwide, and this number is quickly increasing.

Municipal wastewater treatment

After a decade of stable operation, the time was ripe to check how the Olburgen biomass is really doing. "The perforance of the microbes in this reactor is stellar, so more insight in the composition of this culture will help us further improve anammox technology and also apply it to municipal wastewater treatment" Mike Jetten, professor of microbial ecology at Radboud University explains.

New technique

Until now, studies of anammox reactors focused on the known microbes system. These are aerobic, ammonium munching, nitrite producing organisms that depend on oxygen, and anaerobic ammonium munching (anammox) organisms that instead produce nitrogen gas, but for whom oxygen is toxic. These two types of organisms can coexist in tight clumps of biomass, where the aerobes sit on the on the outside and use all the oxygen, leaving the inside devoid of oxygen, and suitable for anammox. Using the new technique metagenomics, microbiologist Daan Speth, lead author of the study, investigated which other organisms could thrive in the anammox reactor, and what their role could be. Speth recently obtained his PhD from the Radboud University, in part on this work.

23 organisms

"There is such a tremendous diversity of microorganisms, and we really know only a tiny fraction of what is out there. Under the microscope these organisms look a lot alike, so indentification by eye is tricky. On top of this, many microbes aren't even named, so we use DNA sequencing to classify them. For this study we have taken all DNA in the reactor, and sorted it per species based on specific properties of the sequences. Using this technique, we could classify the 23 most abundant organisms in the reactor and gain insight in their function in the system."

Metagenomics

To do this, Speth and his colleagues did separate analyses on both the aerobic part of the biomass floating in the liquid, and the anaerobic biomass in the core of the clumps. This helped them identifying and classifying the DNA. "This way of using metagenomics is still in its infancy, and this is one of the first studies to apply it to a complete ecosystem".

The researchers were surprised to find that most species they dentified were very dissimilar to known species. "Most microbes we know a lot about are still those that grow well on agar plates, but this work again illustrates that there is a whole world out there that doesn't like agar all that much".

Microbial dark matter

Five of the species detected in the anammox reactor belong to the "microbial dark matter", the largely uncharted areas of the tree of life. "We can't say much about these organisms, even though we know their DNA sequence." Speth explains. "All we can confidently say is their genomes are tiny, and seem to miss all sorts of 'essential' genes. My interpretation is that these are microbial parasites, feeding off others in the reactor. Time and more studies will tell if that's true, and whether it's a good or a bad thing."

Metagenomics in ecology

Where classical genetics and genomics involves the DNA of a single organisms, metagenomics looks at a mixture of the DNA of an entire microbial ecosystem. This way, the genes present in an ecosystem can be studied, which gives an indication of the processes that could occur there. "Until a few years ago, this was the limit of metagenomic analysis, but now we can start assigning these processes to the microbes catalyzing them. This is a big step towards understanding the way a system functions, and manipulating it. These advance are possible because of revolutionary improvement of DNA sequencing technology and strategies for analysis" Speth says. "On top of better understanding of the system, this provides a window into the capabilities of the 'microbial dark matter' without growing them in the lab. We will need to grow them eventually, to test the theories we get from our metagenomics work."

More information: Daan R. Speth et al. Genome-based microbial ecology of anammox granules in a full-scale wastewater treatment system, *Nature Communications* (2016). DOI: 10.1038/ncomms11172

Provided by Radboud University