

Understanding Patterns of Methane Production and Producers in Anaerobic Waste Water Treatment

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Current large-scale aerobic waste water treatment methods often produce large amounts of activated sludge waste and have high operational costs due to pumping and aeration. Anaerobic treatment, by contrast, produces less sludge waste, requires less energy, and has the potential for biogas recovery in the form of methane. Treatment of waste water with anaerobic baffled reactors (ABRs) has been studied and shows promise for full-scale, energy-neutral wastewater treatment. This study aims to assess methane production of microbial populations and quantify methanogenic DNA in ABRs in order to evaluate the future potential for self-powering anaerobic systems. The impacts of system disturbances, Chemical Oxygen Demand (COD), and temperature were also examined at different stages in the ABR treatment processes.

Figure 1: Overview of the Anaerobic Baffled Reactor Water Treatment Process:

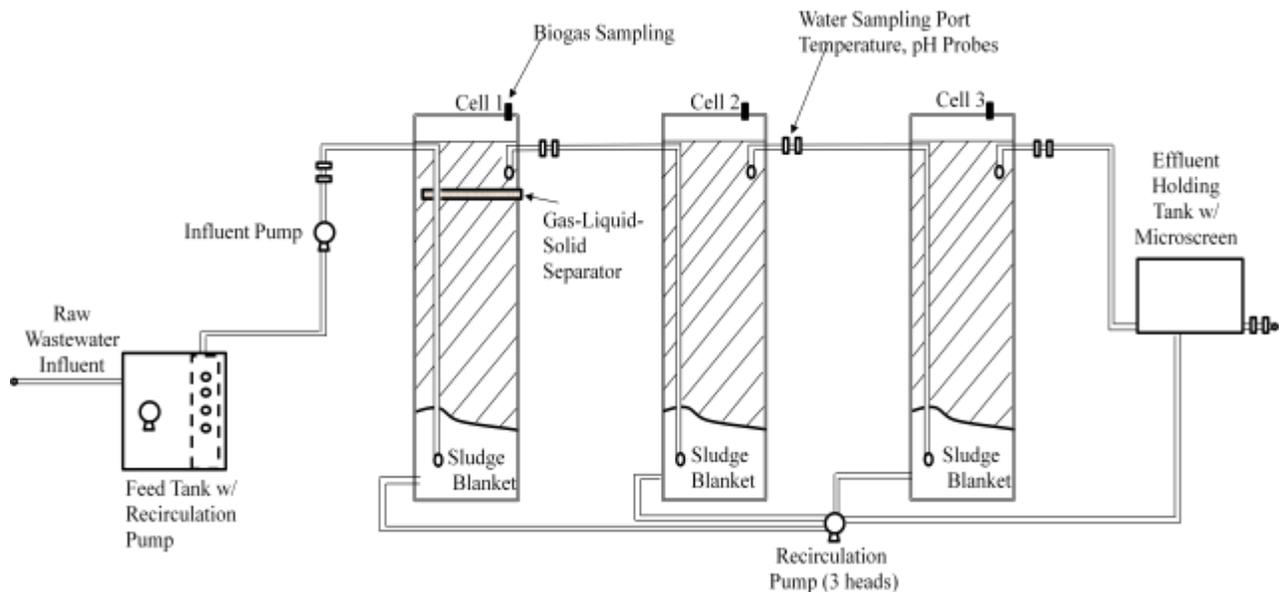
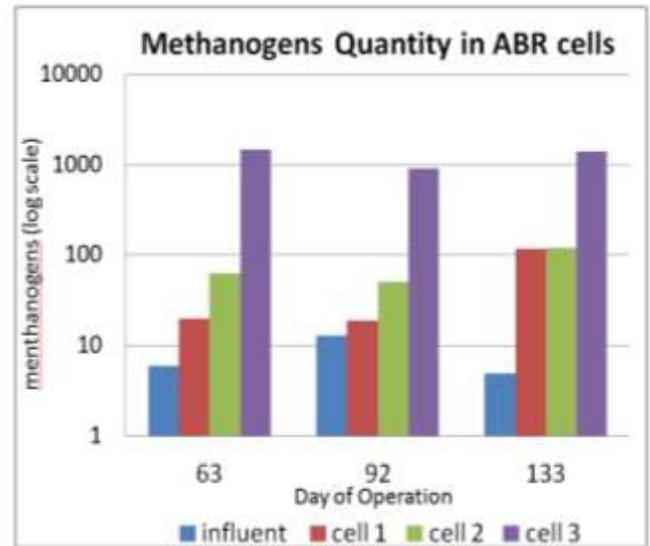
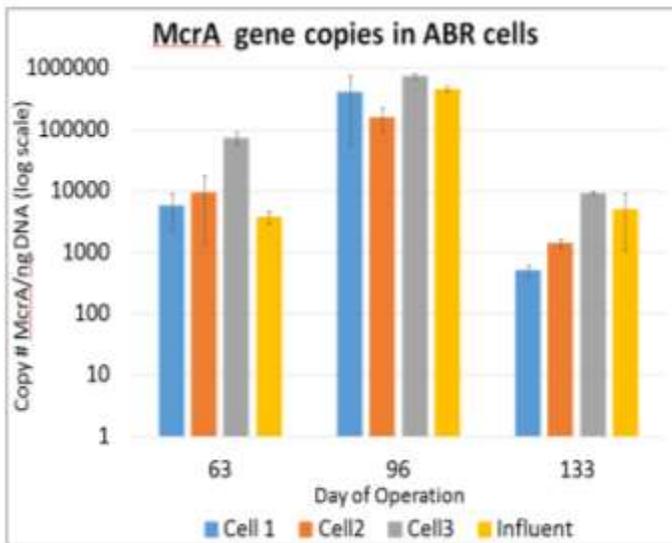


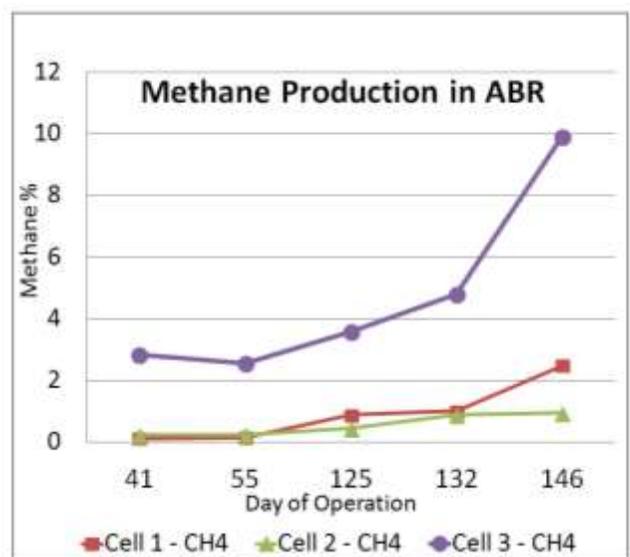
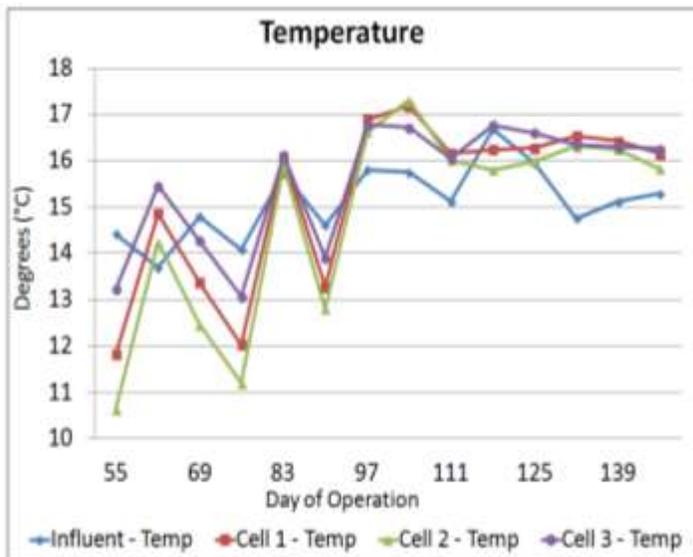
Figure one: No aeration or mechanical pumping is required in ABRs. They therefore involve little to no energy expenditure. Treatment in ABRs is achieved by anaerobic digestion by microbial communities (also known as sludge.) Settling of particulate matter and sludge occurs as a result of the force of gravity—this settled-out sludge results in the creation of a unique sludge blanket in the bottom of each cell—while treated water floats to the top and flows to the next cell for further treatment.

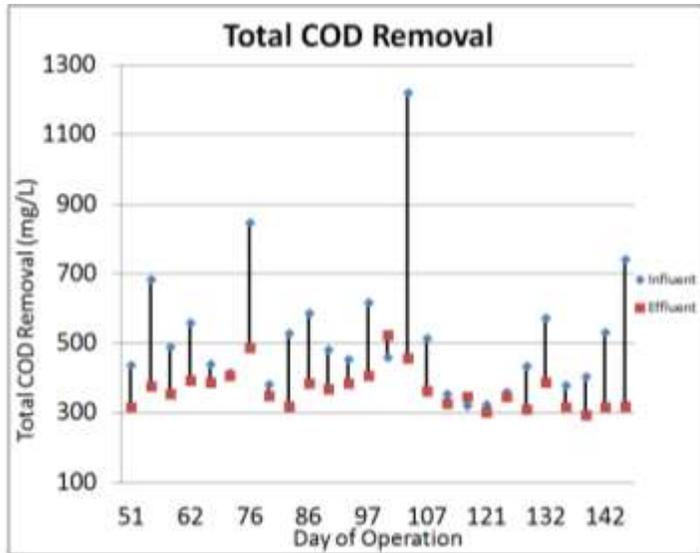
We hypothesized that Methanogenic populations and methane production will increase throughout the reactor (from influent to cell 1 to cell 2 to cell 3; rising temperatures will encourage methanogenic activity; disturbances in the ABR will impact the microbial and chemical functions of the system in the short-term, but will have a limited effect on long-term stability, and that, as time goes on, total COD removal will continue to approach EPA standards.

In order to test these hypotheses, we took samples from each cell and the influent (they had been originally taken in January, February and May and then preserved) and extracted the DNA. We then designed methanogenic DNA primers based on the sequences published by Pereyra et al. 2010 (*mcrA* optimized for qPCR) and Yu et al., 2005 (MSL812.) We then used these for Polymerase Chain Reactor (PCR) and quantitative (q) PCR. First, we tested the primers on non-historical samples to see if, indeed, we saw the presence of methanogenic DNA in our samples. Once this was confirmed in both our PCR results (by the presence of a band with the right florescence) and in our qPCR results, we then went on to run our primers on our extracted historical samples. After running qPCR, we then purified our samples and sent them out for sequencing at the University of Colorado - Boulder. In addition to this microbiological work, we also regularly assessed chemical metrics within the ABR such as Methane and Carbon-dioxide production overtime using gas-chromatography in mass-spectrometer, changes in temperature, and total biochemical oxygen demand.



The microbial analyses of the *mcrA* gene and the sequencing data show that the quantity of methanogens detected increase from the influent to cell 3. The sequencing taxonomic results showed that all archaeal species detected in sequencing were methanogens.





Overall microbial DNA, methane levels, and COD removal from influent cell 1 to cell 2 to cell 3 increased. COD removal consistently continued to approach EPA standards.

We concluded that the methanogenic populations in each of cells in the ABR differ. While the microbial communities seem to be impacted by disturbances, indicated by decreased COD removal and changes in methanogen DNA quantities, they seem to resume normal function shortly afterwards. Floating disturbances are largely thought to be due to biogas accumulation in reactors. The overall upward trend of both methanogen communities and methane production and temperature across the cells and over time indicate the potential for future biogas recovery and reuse applications, but further study is required to determine long term changes in these methanogens and their methane production levels in addition to assessing the system's ability to consistently meet EPA water treatment standards in regard to metrics other than COD, such as alkalinity, hydrogen sulfide removal, and others.