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CHARACTERIZATION OF METHANOSAETA IN SLUDGE DIGESTERS

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Characterization of Methanosaeta in Sludge Digesters

In the laboratory of the Department of Civil and Environmental Engineering, at University of Washington, in Seattle, where I spent the time for doing my thesis, anaerobic treatment processes, notably anaerobic sludges digesters, were studied over many years. In the recent years, the key role of acetoclastic methanogens has been explored. This trophic group is less diverse than others in the digestion processes, yet are responsible for about 70% of the energy flow from complex substrate to methane and seem to be intimately involved in process upsets and failure. Digester failure can be very serious, the sludge is only partially treated, it results odorous and putrescent, methanogens activity reduces causing reduction in methane production and accumulation of acetate. So better understanding of the causes of digester instability can lead to better process monitoring and new approaches for maintaining stability and avoiding process upsets.

The present thesis represents a part of this work, in fact one of the major findings of this experiment is developing Polymerase Chain Reaction (PCR) to identify and characterize the two genera of acetoclacstics methanogens, *Methanosaeta sp.* and *Methanosarcin sp.*

Several researchers have attempted to quantify the methanogens in wastewater treatment processes using molecular methods, but few have attempted PCR method, that was employed in this study for targeting *Archaeal 16S rRNA* genes.

This study used acetoclastic enrichments that were maintained in two CSTR reactors. The reactors were originally seeded with sludge from the West Point municipal treatment anaerobic digesters in Seattle, Washington, and then fed an anaerobic mineral media containing acetate as carbon source to enrich for the acetoclastic methanogens. One reactor was fed once hourly and the other reactor was fed acetate once daily. These reactors were routinely monitored for pH and acetate concentration and were continuously mixed with magnetic stirrers, maintained at 35°C.

From the reactors, two enrichments samples were collected from which PCR-ready genomic DNA was isolated and its features were analyzed through a spectrophotometer. It was used to determinate the concentration and the purities of the DNA, contamination by protein and by phenol was determinate.

Once genomic DNA has been extracted from the two samples, the particular gene was targeted and amplified by PCR to confirm the presence of the organisms, depending on primer specificity. PCR is a whole genome amplification technique, in which the DNA sample is sheared chemically or physically, universal priming regions are ligated to the ends of the sheared DNA fragments, and the resulting sequences amplified by limited cycles of isothermal amplification. After 30 or often more cycles, samples that contained gene sequences, complementary to the oligonucleotide primer sequences, were visualized on an agarose gel stained with ethidium bromide. Subsequently the TOPO TA cloning kit from Invitrogen for TOP10 chemically competent *Escherichia coli* cells was used to developed clones from cleaned PCR products, according to the instructions provided with the kit spreading 50 µl and 100 µl of the clone mixture to the LB-plates containing kanamycin. Plates were grown over night at 37°C, and clones were picked to individual wells in a 96-well plate containing 75 µl of LB media with kanamycin. 96-well plates were incubated around 24 hr at 37°C. Once growth was observed, 75 µl of 15% glycerol was added to the wells and the solutions were mixed by pipeting up and down and the trays were stored at -80°C.

Sequences were screened using ABI 3730 XL high-throughout capillary DNA Analyzer and the phylogeny was inferred by comparing sequences to information in Gene Bank using a Basic Local Alignment Search Tool (BLAST) of the databases at National Center for Biotechnology Information (NCBI). The BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Obtained sequences were introduced into the BLAST search that demonstrated the presence of only *Methanosaeta* genus within the two different enrichment reactors, from which the sample were extracted.

The detected microorganisms belonged primarily to *Methanosaeta concilii* and *uncultered Methanosaeta* spp. and this confirms the theory, developed by precedent research, about *Methanosaeta concilii* is the only mesophilic species within the *Methanosaeta* genus, given that the reactors were maintained at 35°C.

Thanks to information gathered from this study, it has been possible to construct a phylogenetic tree of *Methanosaeta*, in which eight samples of mine, four came from daily-fed reactor and four came from hourly-fed reactor, were placed and confronted with the whole *Methanosaeta* genus.

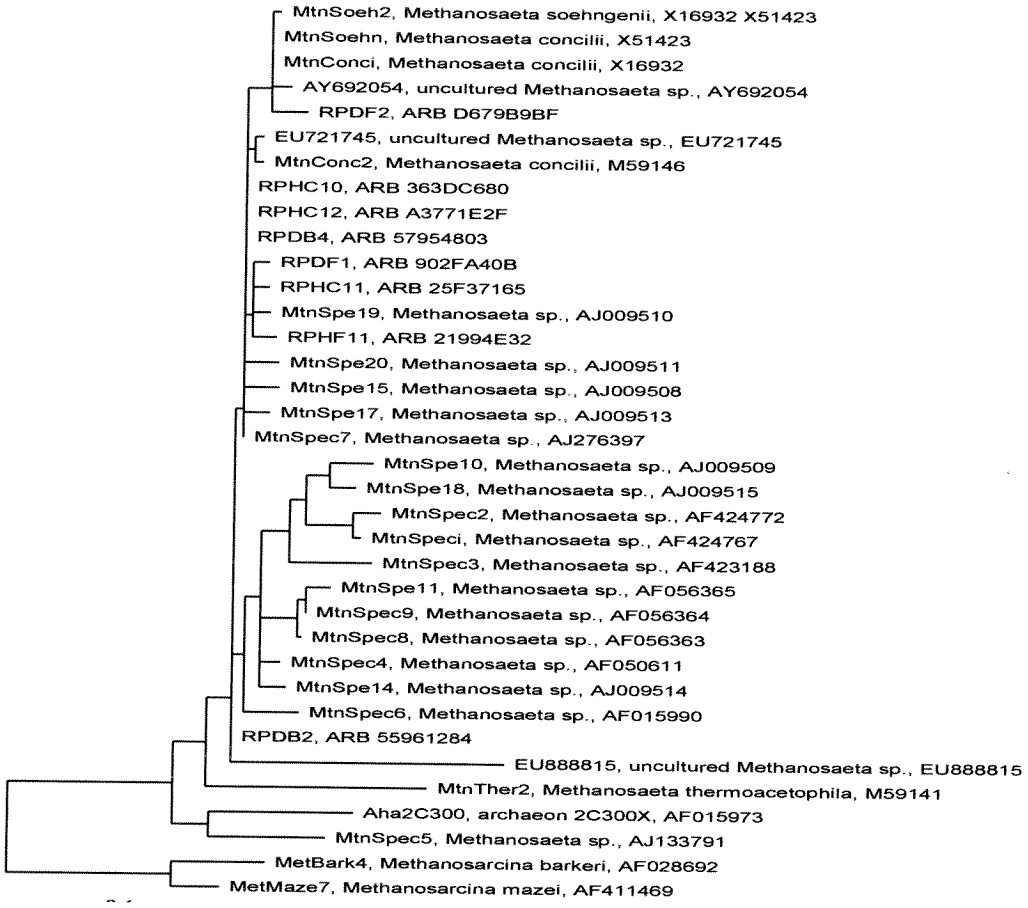

Figure 1. Methanosaeta tree

The presence of acetoclastic methanogens inside the reactors can assure methane production from the reactors.

In the future it will be possible to select *Methanosaeta* or *Methanosarcina* as dominant genera in reactors varying acetate concentration. In fact at acetate concentrations below about 240 mg/L, *Methanosaeta* grows faster than *Methanosarcina* and should predominate in a well mixed anaerobic digester. Typical mesophilic anaerobic digesters operate at concentrations well below 240 mg/L acetate and are dominated by *Methanosaeta.*

To validate the experimental data described, in the second part of this elaborate, a mathematical model was proposed to simulate the biochemical processes prevailing in a anaerobic digestion reactor fed with sewage sludge and the organic fraction of municipal solid waste.

This model is based on the Anaerobic Digestion Model n°1 of the International Water Association, using a surface-based kinetics to model the organic waste disintegration and conversion to carbohydrates, proteins and lipids.

The proposed mathematical model is based on differential mass balance equations for substrates, products and bacterial groups involved in the anaerobic process and includes the biochemical reactions of the substrate conversion and the kinetics of microbial growth and decay.

The model was developed, taking into account isotope ^{13}C value, to describe the dynamics of methanogenic population during mesophilic anaerobic digestion of putrescible solid waste and municipal solid waste. Three groups of methanogens were considered in the model including unified hydrogenotrophic methanogens and two acetoclastic methanogens *Methanosaeta sp.* and *Methanosarcina sp.* Hydrolysis/Acidogenesis and Acetigenesis/Methanogenesis were included in the simplified model as the two possible rate-limiting steps of the overall anaerobic digestion process.

The hydrolysis/acidogenesis of different waste fractions, including putrescible waste, was described as simple first-order reactions; the Monod functions with single or two

substrates were used to describe substrate utilization, product formation and biomass growth.

Several set of simulations were carried out, varying, from the initial and parameter values, acetate concentration, hydraulic load to validate the experimental data shown in this study. Moreover a sensitivity analysis of model was implemented to understand the input variables from which the mathematical model depends.

The model dynamics describe the experimental data reasonably well and show how *Methanosarcina sp.* concentration increase and dominate over *Methanosaeta sp.* when the acetate concentration is high and when the reactor is fed with a high hydraulic load, during instability condition.

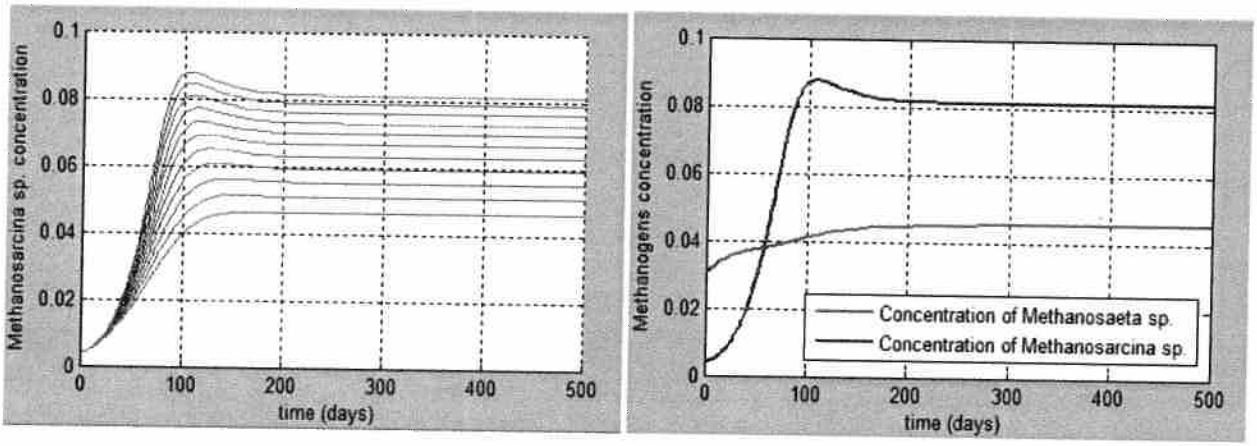

Figure 2. Time profile of acetoclastic methanogens under hydraulic load increase

The mathematical model proposed can be implemented to assess the degradation of sewage sludge and putrescible solid waste in anaerobic digestion and makes possible to predict the amount of sludge which can be treated and the methane can be generated. Besides the model can be used to study and control anaerobic digester stability, predicting the efficiency of the one already in service and avoiding digester failure and upset.