Deciphering the microbial ecology in bio-gas reactors for optimizing the anaerobic digestion process - DTU Orbit (26/10/2019)

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Anaerobic digestion (AD) is a microbial mediated process where organic compounds are degraded to biogas (CH4 and CO2). AD occurs in many natural anoxic environments and is an essential step for global carbon circle. Engineered AD systems, i.e. biogas reactors, enhance methanogenic activity by applying empirical operational conditions, in order to accelerate the methane production for energetic purposes. In Denmark, biogas produced from AD has a considerable share in renewable energy with the expectation to expand. Thus, the more effective operation of biogas plants will significantly benefit Denmark’s sustainable development. As AD relies on complex microbial activity, a more comprehensive understanding of the AD microbial consortia and their activity provides the fundamental knowledge for process control and optimization.

In AD, the microbial metabolisms are mostly thermodynamically constrained and the obligatory syntrophy is an essential intermediary step. Thus, the majority of AD microbiota remains uncharacterized since in the past it was mainly investigated using cultivated-based methods. The advent of more powerful sequencing technology (i.e. next generation sequencing, NGS) and newly developed bioinformatic methods enable researchers to perform in-situ analyses on uncharacterized microbial communities. The applications of NGS technology were proved to be effective tools to reveal AD microbial ecology. However, the detailed mechanisms of microbial activity are still far from fully elucidated due to the intricacy of AD process.

This Ph.D. project relied on comprehensive investigations of microbial communities in order to optimize the AD process and elucidate the fundamental metabolisms. Specifically, in the case of process optimization, 16S rRNA amplicon sequencing was used to identify, analyse and solve the operational challenges during the start-up of thermophilic up-flow anaerobic sludge blanket (UASB) reactors. To elucidate the microbial metabolisms, genome-centric metagenomics was applied to characterize methanogenic communities degrading a set of defined substrates. In addition, the Ph.D. study also expands the understanding of AD microbial ecology by proposing and characterizing a novel Candidatus species ubiquitously present in AD systems.

The start-up of thermophilic UASB reactors was investigated in lab-scale reactors inoculated with mesophilic granules. After increasing the operational temperature from mesophilic to thermophilic, volatile fatty acids (VFAs) and alcohols were found as the main digestion products. Methane production, on the other hand, only initiated after bicarbonate addition as external pH control. The dynamicity of microbial community composition in the granules during the temperature shift suggested that the majority of the mesophilic microbes could not tolerate the thermophilic conditions. Moreover, it was demonstrated that the fermentative thermophiles first evolved in the liquid phase of UASB reactor and then were encapsulated in the granular structure of the sludge. The growth of these bacteria rapidly restored the hydrolysis, acidogenesis and acetogenesis in the reactor. On the contrary, the thermophilic methanogens grew much slower than fermentative bacteria leading to severe process imbalance (i.e. accumulation of VFAs and alcohols). Thus, the evolution of thermophilic methanogens was recognized as the biological ‘bottleneck’ during the temperature transition. To overcome the identified obstacle, bioaugmentation, i.e. provision of exogenous microbes, was proposed to accelerate the microbial community adaptation. The best strategy found to perform bioaugmentation was the injection of axenic methanogenic cultures. This practice significantly increased the thermophilic methane production rate by 40% compared with the control reactor (i.e. without bioaugmentation). The enhancement of methane production was attributed to the evolution of exogenous Methanothermobacter thermotrophicus and the concomitant growth of its syntrophic partners in the granular structure. The positive effects brought by bioaugmentation were persistent in UASB reactor due to the retention of the microbes in the granular sludge.

For the investigation of the basic microbial metabolism and ecology, methanogenic microbial communities were enriched in a lab-scale continuous stirred-tank reactor (CSTR) fed with synthetic feedstocks. In the experiment, the substrates used were stepwise simplified (i.e. polysaccharide, monosaccharide, short chain fatty acids, acetate) to mimic the four steps of AD process. During the continuous operation, the microbial community was substantially simplified, because the microbes that could not metabolize the specific compounds were washed out. The overall microbial community consisted of only 35 metagenome assembled genomes (MAGs) (31 bacterial and 4 archaeal). The abundance of these MAGs dramatically varied in the communities adapted to different substrates. The shifts in microbial community composition indicate that MAGs have specific functional roles in AD food chain and their roles cannot always be physiologically defined in accordance with 4 AD steps. Moreover, the explicit degradation pathways were reconstructed from the functional annotation of MAGs. It is notable that, a novel glucose degradation model was proposed with the syntrophic activity of Clostridiaceae sp. and Methanoculleus thermostophilus. In this model, acetate is not produced as intermediate compound. The genome-centric metagenomics reveals a considerable number of MAGs that could not be taxonomically assigned to characterized species. A MAG extracted from co-assembly of 8 AD metagenomes was especially emphasized due to its ubiquity in AD system and its high abundance under specific conditions. From the functional annotation and gene expression profile, it is confirmed that this MAG performs hydrogenotrophic methanogenesis in AD system and is found dominant from the reactors where H2 was added. This genome is present in 40 different samples from both full-scale and lab-scale AD reactors. The MAG was found in higher abundance during thermophilic reactor operations with relatively short hydraulic retention times. The phylogenetic assignment was based on 400 conserved genes and on 16S rRNA genes. The two methods concordantly showed that this MAG is closely related to Methanoculleus bourgensis MS2T.

However, the average nucleotide identity between M. bourgensis MS2T and the selected MAG was only 89%, which is too low similarity to assign the MAG at the species level. Thus, we propose a novel Candidatus species. According to the metabolic traits, it is named as Candidatus Methanoculleus thermohydrogenotrophicum. sp. nov.

Overall, the results from this Ph.D. study bring new knowledge on the AD process based on NGS technology. Practically,
the gained information regarding microbial community composition and dynamicity was directly used to solve technical challenges in AD operations. Fundamentally, deeper insights into the microbial metabolisms and ecology substantially expanded the current understanding of AD. The revealed knowledge provides pivotal prerequisites for future AD process control and optimization.

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