Water Research 123 (2017) 134–143

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Temperature regulates deterministic processes and the succession of microbial interactions in anaerobic digestion process

Qiang Lin [a, b], Jo De Vrieze [c], Chaonan Li [a], Jiaying Li [a], Jiabao Li [a], Minjie Yao [a], Petr Hedenec [b], Huan Li [a], Tongtong Li [a], Junpeng Rui [a], Jan Frouz [b, **], Xiangzhen Li [a, *]

[a] Key Laboratory of Environmental and Applied Microbiology, CAS, Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, 610041, China
[b] Institute of Soil Biology, Czech Academy of Sciences, Na Sádkách 7, CZ37005, České Budějovice, Czech Republic
[c] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, B-9000, Gent, Belgium

ARTICLE INFO

Article history:
Received 9 March 2017
Received in revised form
23 May 2017
Accepted 19 June 2017
Available online 20 June 2017

Keywords:
Microbial interactions
Modularity
Deterministic process
Anaerobic digestion
Temperature gradient

ABSTRACT

Temperature plays crucial roles in microbial interactions that affect the stability and performance of anaerobic digestion. In this study, the microbial interactions and their succession in the anaerobic digestion process were investigated at three levels, represented by (1) present and (2) active micro-organisms, and (3) gene expressions under a temperature gradient from 25 to 55 °C. Network topological features indicated a global variation in microbial interactions at different temperatures. The variations of microbial interactions in terms of network modularity and deterministic processes based on topological features, corresponded well with the variations of methane productions, but not with temperatures. A common successional pattern of microbial interactions was observed at different temperatures, which showed that both deterministic processes and network modularity increased over time during the digestion process. It was concluded that the increase in temperature-mediated network modularity and deterministic processes on shaping the microbial interactions improved the stability and efficiency of anaerobic digestion process.

1. Introduction

Anaerobic digestion (AD) can be considered as a comprehensively cooperative microbial process with various biochemical functions that engage in organic waste degradation and transformation for renewable energy production. Due to high diversity in substrate compositions, different functional micro-organisms are involved in the entire degradation process. Both the efficient conversion of complex substrates into methane through the AD food web (hydrolysis, acidogenesis, acetogenesis and methanogenesis), and the thermodynamic constraints require comprehensive interactions among micro-organisms. Especially, the methanogens traits that they are unable to utilize complicated substrates essentially drive the cooperation between methanogens and other decomposers in the AD process. Thus, the insights into the interactions among micro-organisms may provide essential information concerning the relationships between the microbial community and AD performances.

The co-occurrence network analysis is a robust way to deduce the potential interactions among micro-organisms (Faust and Raes, 2012). The topological features calculated based on a specific network have gradually became robust evaluations for the microbial interactions (Deng et al., 2012; Eldridge et al., 2015; Ma et al., 2016). The topological features not only provide descriptions of the network structure based on topology (Deng et al., 2012), but also represent some specific biological meanings (Eldridge et al., 2015; Ma et al., 2016). Based on these specific biological meanings, both the general (network-level) and the individual (node-level) topological features can to some extent indicate or explain some specific microbial processes. Especially, the microbial interactions analyses based on general topological features are capable to depict a global profile compared to a concrete

Abbreviations: AD, anaerobic digestion; OTU, operational taxonomic unit; SES, standardized effect size; PERMANOVA, permutational multivariate analysis of variance; PCoA, principal coordinates analysis; TS, total solid; HRT, hydraulic retention time; OLR, organic loading rate; VFAs, volatile fatty acids; VS, volatile solid; COD, chemical oxygen demand.

* Corresponding author.
** Corresponding author.
E-mail addresses: jan.frouz@czp.cuni.cz (J. Frouz), lixz@cib.ac.cn (X. Li).

http://dx.doi.org/10.1016/j.watres.2017.06.051
0043-1354/

microcosm exhibited by focusing on interactions between specific individuals (species or genes). In AD food web in which many functional micro-organisms are involved, the global profile contributes equally with the concrete microcosm to reveal microbial interactions.

The null model-based deterministic vs. stochastic processes have been comprehensively utilized in evaluating microbial assembly (Zhang et al., 2016; Zhou et al., 2014). Based on the niche theory, environmental pressure and species traits play crucial roles with respect to deterministic processes (Fargione et al., 2004). In contrast, based on the neutral theory, the random birth, death and dispersal, the low connectivity, and the priority effect caused by early-arriving species, would amplify the stochastic processes on microbial assembly (Zhang et al., 2016; Zhou et al., 2014). In AD systems, microbial interactions are also probably driven by an analogical niche theory that a necessary cooperation in the AD food web, methanogens traits and thermodynamic constraints strengthen the deterministic processes. In contrast, due to the important role of metabolite exchange in microbial interactions (Widder et al., 2016), an analogical neutral theory that a random and uneven dispersal of metabolites such as H_2, NH_4^+, fatty acids and other intermediates in the AD process probably strengthen the stochastic processes, also crucially influences the microbial interactions. However, little attention has been paid to deterministic vs. stochastic processes on microbial interactions, especially in the AD process.

Temperature greatly influences microbial community and the thermodynamic equilibrium of biochemical reactions in the AD process (Kim and Lee, 2016; Lin et al., 2016b). Hence, temperature probably plays crucial roles in microbial interactions. However, there are few studies referred to temperature effects on microbial interactions in the AD process. Our previous studies have revealed temperature effects on microbial compositions (Lin et al., 2016b) and microbial gene expressions (Lin et al., 2016a) in the AD process. However, these studies mainly focused on microbial diversity rather than microbial interactions to evaluate temperature effects. Thus, it is necessary for re-excavation of our previous data to further reveal temperature effects on microbial interactions in the AD process.

In this study, the combination with the analyses of co-occurrence network and deterministic vs. stochastic processes was used to investigate the temperature effects on microbial interactions in the AD process. Specifically, this study addressed three main points: (1) the variations of microbial interactions mainly assessed by network topological features at different temperatures; (2) the relationship between microbial interactions and methane production; and (3) the succession of microbial interactions along the AD process at specific temperatures.

2. Materials and methods

2.1. Anaerobic digestion experiment

The anaerobic digestion experiment was performed in a 2 L anaerobic flask containing 1.5 L of anaerobic sludge (total solid (TS) content of 8%), at 25, 35, 50 and 55 °C, respectively. At the start of digestion process, 450 mL of seed slurry (TS content of 8%) was inoculated. After daily CH_4 production reached its first peak in a digester, a semi-continuous mode was initiated that 150 mL of digestion slurry was replaced with same volume of fresh swine manure slurry (TS content of 8%) every three days (organic loading rate (OLR) of 2 g VS L^{-1} day^{-1}; hydraulic retention time (HRT) of 30 days). A more detailed description was given in the Supplementary information S1 (Lin et al., 2016b). Details about parameters at the start of fermentation were shown in Supplementary Table S1.

The collection of digestate samples was conducted in the initial period (24 h after inoculation), peak I (the time varied based on the temperature), and stable period (48 h after the second feeding) for the extraction of nucleic acid and the detection of AD dynamics (Lin et al., 2016b) (Supplementary information S2). The samples in all the three periods were used for 16S rRNA (gene) amplicon sequencing to measure active (present) microbial community, and the samples in the stable period were used for metatranscriptomic sequencing to quantify microbial gene expressions (Lin et al., 2016a, 2016b)(Supplementary information S3). The present microbial community represent all micro-organisms, including active, dormant and dead micro-organisms. The sequencing raw data were processed (Lin et al., 2016a, 2016b) (Supplementary information S4) and then the total 72 samples of microbial community detected based on 16S rRNA gene and 16S rRNA, respectively, and 12 samples of microbial gene expressions detected based on metatranscriptomic analysis were used for further analysis in this study. In this AD process, different periods represented distinguishing AD status, for example, the initial period indicated the initiation, peak I reflected the maximum potential and stable period represented the dynamic equilibrium. Besides the three periods, the other sampling points were used for the detection of AD dynamics. The AD performances had been described in detail in our previous study (Lin et al., 2016b), and were shown in Table 1 and Fig. S1.

2.2. Network construction and statistical analysis

The data of microbial community at operational taxonomic unit (OTU) level, and the metatranscriptomic data at gene expressions level were used for constructing co-occurrence networks, respectively. The co-occurrence networks were constructed based on the Spearman's correlation matrix, with *igraph* package (Csardi and Nepusz, 2006) in R (http://www.r-project.org/). All *p*-values for multiple testing were adjusted with the false discovery rate controlling procedure (Benjamini et al., 2006). The threshold of *p*-values was set at 0.001 for all networks constructions (Ma et al., 2016). The random matrix theory-based methods (Deng et al., 2012) were used to identify the thresholds of correlation coefficients (r) for different networks. The data from the stable period at different temperatures, based on the 16S rRNA gene ($r = 0.67$), 16S rRNA ($r = 0.64$) and metatranscriptome ($r = 0.81$) were used to construct three meta-networks, respectively, to dissect the effects of temperature on microbial interactions (including the interactions among micro-organisms and the gene co-expressions). The data from the initial period, peak I and the stable period, at each specific temperature, based on both 16S rRNA gene and 16S rRNA were used to construct eight meta-networks (Supplementary Table S2), respectively, to reveal the succession patterns of the interactions among micro-organisms over time during the AD process. The both network-level and node-level topological features (Supplementary Table S3) were calculated with *igraph* package in R (Csardi and Nepusz, 2006). The isolated nodes (degree = 0) were removed. The sub-networks at each temperature or each period were extracted from the meta-networks by preserving taxa present at each group, using *subgraph* function in the *igraph* package (Ma et al., 2016). Each sub-network with united constructive parameters in the meta-networks could be used to objectively identify and compare the effects of different temperatures and temporal dynamics on microbial interactions, without the bias caused by discrepant thresholds and other parameters for constructing multi-networks. It is considered that the taxa with high relative abundances probably play more crucial roles, and contribute more to systemic functions in AD systems than the rare taxa (Rui et al., 2015). To avoid a too high impact from rare taxa on each sub-network, the core taxa (taxa with least numbers to contribute to