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Comparison of anaerobic digestion strategies of nitrogen-rich substrates: Performance of anaerobic reactors and microbial community diversity

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ABSTRACT

In the present study, the application of different operating strategies on performance of three continuous stirred tank reactors digesting chicken manure at mesophilic temperature and constant organic loading rate (OLR) of $3.5\ g_{VS}\ L^{-1}\ d^{-1}$ was investigated. Control reactor (RC) and reactor (RH) with the decreasing hydraulic retention time (HRT) had the comparable specific biogas production (SBP) with maximum values of $334–351\ mL\ g_{VS}^{-1}\ _{(added)}$ during days 84–93, while the SBP from reactor with zeolites (RZ) was higher and achieved $426–432\ mL\ g_{VS}^{-1}\ _{(added)}$. Attachments of microorganisms to zeolite particles as the operational environment, exchanged cations released from zeolites as well as lower total ammonium nitrogen (TAN) levels observed in RZ ($6.2–6.3\ g\ L^{-1}$; days 71–93) compared to RC ($6.6–6.9\ g\ L^{-1}$; days 71–93) resulted in a more effective process in RZ. Moreover, microbial community structure and dynamics were comprehensively characterized using Illumina sequencing, pyrosequencing and T-RFLP analysis of 16S rRNA genes. Methanogenic archaeal activity was additionally assessed by the expressed *mcrA* genes encoding the alpha subunit of methyl-CoM reductase. Within the major class *Clostridia*, *Caldicoprobacter*, *Alkaliphilus*, *Gallicola*, *Sporanaerobacter* and *Tepidimicrobium* spp. were the notable bacteria developed during operation of all tested reactors. Archaeal communities were dominated by methanogens belonging to the genus *Methanosarcina* followed by the genus *Methanoculleus* during the experimental period. Results of this study indicate that attachment of microorganisms to the zeolite particles as the operational environment might have led to the higher microbial activity at high ammonia concentrations.

1. Introduction

A significant amount of animal manure is annually produced in Russia; however, in most cases, the uncontrolled storage of manures on agricultural lands results in the pollution of the atmosphere, soil and water resources. Such environmental pollution caused by various types of manures can be controlled by anaerobic digestion of agricultural organic wastes under both mesophilic and thermophilic conditions. Despite the fact that anaerobic conversion of residual biomass with the generation of biogas is not widely utilized in Russia, anaerobic digestion belongs to a more suitable method to treat various wastes resulting in minimization of their amount and obtaining energy in the form of energy-rich methane worldwide (Ye et al., 2013; Jia et al., 2015; Mazareli et al., 2016; Jensen et al., 2016; Li et al., 2016). Therefore, it is very important to develop and put in practice new effective biotechnologies to solve the problems associated with the growing levels of organic wastes.

Anaerobic conversion of chicken manure into methane-rich biogas has become increasingly attractive in the last decades as a good choice to minimize wastes accumulation and recover bioenergy (Nie et al., 2015; Niu et al., 2015; Wu et al., 2016; Sun et al., 2016). However, chicken manure contains high nitrogen concentrations due to the presence of uric acid and undigested proteins. Their microbial decomposition results in the formation and accumulation of toxic ammonia in anaerobic systems. The total ammonia nitrogen (TAN) levels include ammonium nitrogen (NH_4^+—N) and free ammonia nitrogen (FAN, NH_3—N) concentrations. Free ammonia is pH, temperature and TAN dependent and its excess concentration has been widely considered as the major cause of microbial consortia inhibition over the course of anaerobic digestion of nitrogen-rich substrates. Ammonia inhibition processes as well as processes to overcome ammonia toxicity on anaerobic

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microorganisms were investigated in several research works (e.g., Angelidaki and Ahring, 1994; Hansen et al., 1998); however, ammonia threshold varied in the described studies, indicating that various operating parameters as well as nature and composition of the used substrates can lead to different results. In addition, high sulfides concentrations are frequently detected in reactors digesting chicken manure (Belostotskiy et al., 2015; Ziganshina et al., 2015), which are toxic to various microorganisms and form unavailable complexes with metals necessary for microbial activity (Westerholm et al., 2015).

Food residues, bedding materials (e.g., lignocellulosic biomass) and feathers (Ziganshina et al., 2015) as well as antibiotic residues (Yang et al., 2014) are frequently detected in raw chicken manures; therefore, such variability in the composition of manures has significant impact on the decomposition and biogas generation characteristics of manure (Li et al., 2015). Besides composition of substrate, concentration of substrate added to anaerobic reactors is another significant parameter for the stability of whole anaerobic digestion process. Its increase results in accumulation of TAN, FAN and volatile organic acids in anaerobic digesters (Nie et al., 2015; Belostotskiy et al., 2015). There are several methods to handle the problem with ammonia inhibition process during anaerobic digestion of nitrogen-rich organic wastes, such as dilution of chicken manure with water (Bujoczek et al., 2000), co-digestion with some other livestock manures, straw and other substrates (Wang et al., 2012), reduction of ammonia levels (Abouelenien et al., 2010; Nie et al., 2015), acclimatization of microorganisms to high levels of ammonia (Fotidis et al., 2013), pH reduction (Ho and Ho, 2012) as well as application of natural and synthetic zeolites (Fotidis et al., 2014; Wang et al., 2015). For example, zeolites as the additives for anaerobic digestion can enhance the biogas production, are able to remove such undesired products as ammonia and can be immobilized by microorganisms preventing their wash out from the systems (Montalvo et al., 2012). Some described technologies have advantages over other for achieving higher rates of anaerobic digestion; however, additional research should be performed to overcome the ammonia inhibition and understand the microbial processes occurring during anaerobic digestion of nitrogen-rich substrates.

The understanding of microbial community structure and its functions during anaerobic treatment of chicken manure in biogas reactors is necessary for effective diversity management and digesters maintenance, leading to possible improvement in efficiency and stability of the complicated anaerobic process. The process stability and effectiveness in such systems with high biodiversity and complexity depend on multiple syntrophic interactions among bacteria and archaea. Their effective interactions are crucial to maintaining the anaerobic food chain and to prevent accumulation of high levels of molecular hydrogen and organic acids in reactors. Regarding ammonia level, which is the major toxicant, it was previously established that the aceticlastic methanogenic community is more sensitive to high TAN/FAN concentrations, and TAN values exceeding 2.8–3.0 NH_4—N g L^{-1} launch the bacterial syntrophic acetate oxidation (SAO) coupled to hydrogenotrophic methanogenesis as the dominant pathway for acetate catabolism (Schnürer and Nordberg, 2008; Fotidis et al., 2014). However, aceticlastic methanogenesis accomplished by acclimatized *Methanosarcinaceae* at high ammonium (5–7 NH_4—N g L^{-1}) concentrations was also demonstrated in batch tests (Fotidis et al., 2013), indicating the need for further investigation regarding the impact of various ammonia levels on the microbial community activity.

In order to maximize the anaerobic digestion processes, it is important to investigate the anaerobic microbiome and to clarify how these microbial communities respond to operational changes of biogas reactors. In the present study, we fed three continuous stirred tank laboratory reactors with chicken manure at constant OLR of 3.5 $g_{VS}\,L^{-1}\,d^{-1}$ and at 40 °C operating temperature. The influence of the substrate change, the HRT decrease as well as the addition of zeolites on the anaerobic process was investigated. Furthermore, microbial community structure and dynamics were comprehensively characterized using Illumina sequencing, 454 pyrosequencing and T-RFLP analysis of 16S rRNA genes. In addition, methanogenic archaeal activity was investigated based on the *mcrA* (methyl coenzyme M reductase) gene profiles. Correlations between the composition of the microbial communities and abiotic process parameters were also analyzed using multivariate data analysis.

2. Materials and methods

2.1. Feedstock

The chicken manures including a fraction of chicken feathers and wood shavings were obtained from a poultry farm located in the Zelenodolsky district, the Republic of Tatarstan (Russia) and stored in a refrigerator at +4 °C. The nitrogen-rich chicken manure from day 1 to day 49 had the total solids (TS) content of 74.5 ± 0.2% and volatile solids (VS) of 64.0 ± 0.5% and was collected from a place of uncontrolled storage of manures, whereas from day 50 to day 100 that chicken manure was replaced with fresh substrate collected directly from deposits under chicken cages of the poultry farm, which had the TS content of 45.5 ± 0.2% and VS of 39.4 ± 0.3%. Also, both chicken manures used in this study initially contained high TAN concentrations (up to 7.05 ± 0.3 g kg^{-1}), indicating that decomposition of uric acid occurred during storage of the substrates. After calculating the desired organic loading rate and hydraulic retention time values, the required amount of raw chicken wastes was diluted with tap water, thoroughly stirred and then added daily to anaerobic laboratory biogas reactors. Active inoculum adapted to chicken manure as monosubstrate was collected from biogas reactors investigated in our previous experiments (Ziganshina et al., 2015; Belostotskiy et al., 2015).

2.2. Experimental setup and procedures

Three continuous stirred tank laboratory reactors (CSTR) with an active volume of 10 L (total 12 L) were operated at 40 °C. The height of reactors was 33 cm and the diameter was 21 cm. All reactors were warmed by water circulation, agitated continuously at 60 rpm and fed semi-continuously (once a day) seven days per week. The effluent was removed prior feeding every day as well. The VS-OLR (volatile solids) was kept constant in all three reactors at 3.5 g L^{-1} d^{-1}. The first reactor served as a control reactor (RC) and operated at constant hydraulic retention time (HRT) of 35 days, whereas in the second reactor (RH) the HRT was decreased from 35 to 25 days during the entire experimental period (chicken manure was diluted with tap water). The third reactor (RZ) operated at constant HRT of 35 days was additionally supplied with 50 g of zeolite (molecular sieves "Merck 5704" 0.3 nm beads, ~2 mm) to remove NH_3 and NH_4^+ from the digestion mixture through adsorption and ion exchange, respectively, and to serve as supporting material for microorganisms. Zeolite with the following composition %: Si(SiO_2) – 17.8(38.0), A1($A1_2O_3$) – 17.1 (32.2), K(K_2O) – 24.7(28.9) was fixed in a porous nylon bag and added to reactor RZ through a special port after 49 days of the beginning of the experiments (on day 50). The configuration of this reactor presents such advantage as the applied zeolites can be replaced after maximum ammonia removal (Wang et al., 2011). Samples for microbial community analyses were taken at four distinct times: day 35, day 55, day 71 and day 93.

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