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Shift of pathways during initiation of thermophilic methanogenesis at different initial pH

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ABSTRACT

To investigate the metabolic pathways during the initiation of methanogenesis from acid crisis, the influence of initial pH (5.0–6.5) on thermophilic methanogenic conversion of 100 mmol/L acetate was monitored based on the isotopic signature and selective-inhibition method combined with analysis of the microbial structure. The results showed, lower pH extended the lag phase for methanogenesis which was inhibited at pH 5.0 throughout the incubation. At initial pH 6.0–6.5, methanogenesis was primarily initiated via acetoclastic methanogenesis (AM), with the fraction of the hydrogenotrophic pathway (f_{mc}) accounting for 21–22% of total methane formation. Conversely, at initial pH 5.5, the dominant pathway shifted to syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (SAO-HM), with f_{mc} rising to 51% and the abundance of syntrophic acetate-oxidizing bacteria increasing remarkably. Methanogenesis could initiate independently via SAO-HM pathway when AM pathway was inhibited. Acetate-oxidizing syntrophs could function as the initiation center of methanogenesis from low-pH crisis.

1. Introduction

Cost-effective and robust anaerobic digestion technology is now being widely applied to recover bioenergy from biowaste; however, there are still various technical barriers to overcome. The accumulation and inhibition of volatile fatty acids (VFAs) is one of the most concerned problems inducing process deterioration. Especially for the easily biodegradable materials, rapid fermentation and slow methanogenesis can quickly lead to high VFA concentrations (up to tens in g/L) and low pH (to a minimum ranging between 4.0 and 5.5). It takes a long time for the recovery of stable methanogenesis, since methanogens are highly sensitive to environmental factors and grow slowly.

The inhibition of methanogenesis by high concentrations of VFAs might be due to the undissociated form of acids, which are speculated to act as uncouplers of the membrane proton gradient or protonophores at low external pH (Fukuzaki et al., 1990). pH is thus suggested to be an important factor determining the toxicity. In addition, methanogens and bacteria also differ in their optimal pH on growth (Kotsyurbenko et al., 2004). VFA concentration coupled with pH has been recognized as a major selecting pressure strongly influencing methanogenic community structures (Vavilin et al., 2008).

To a large extent, the initiation of methanogenesis from acid crisis depends on the rapid degradation of acetate, which is the product of acidogenesis and acetogenesis, and the immediate substrate as a methanogenic precursor. The transformation of accumulated acetate to methane lies in strengthening the acetoclastic methanogenesis (AM) operated by the acetotrophic methanogens, such as *Methanosaetaceae* and *Methanosarcinaceae*, or the tandem reactions of syntrophic acetate oxidation (SAO) from acetate to H_2/CO_2 and the subsequent hydrogenotrophic methanogenesis (HM) conducted by the syntrophs of acetate-oxidizing bacteria and hydrogenotrophic methanogens (Karakashev et al., 2006). The latter syntrophic pathway, which is also known as SAO-HM, has been observed in various anoxic or anaerobic environments (Hattori, 2008; Conrad and Klose, 2011; Hori et al., 2011). Both acetate-utilizing groups (responsible for AM and SAO-HM) could break through the inhibition from the anaerobic acid phase and function as the methanogenic initiation center. Their contributions might be highly related to the acidic status of their habitats, especially the pH level. *Methanosarcina barkeri*, a mixotrophic species, was found to be critical to overcome the low pH and high VFA conditions in landfills (Staley et al., in press; Vavilin et al., 2008); nevertheless, the minimal pH in their research was around 6 or 7. Lü et al. (2010) reported the initiation of methanogenesis via the SAO-HM pathway from extremely acidic status with pH around 5, indicating that acetate-oxidizing syntrophs may play a significant role under these conditions. At even lower pH (3.8), Kotsyurbenko et al. (2007) found a shift in the dominant methanogenic pathway from AM (at pH 6.0) to HM for acetate turnover, as well as the

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