Bioresource Technology 172 (2014) 83–90

Contents lists available at ScienceDirect

Bioresource Technology

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

Comparison of metabolomic profiles of microbial communities between stable and deteriorated methanogenic processes

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HIGHLIGHTS

- Stable and deteriorated methanogenic processes were compared.
- Intracellular metabolisms within complex microbial communities were shown.
- Clear differences in the metabolites were observed in the processes.
- Carbon flux in glycolysis and glutamate production were increased in stable process.
- Pyruvate, acetyl-CoA, and succinate were accumulated in deteriorated process.

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 9 August 2014
Accepted 11 August 2014
Available online 19 August 2014

Keywords:
Metabolomic analysis
Intracellular metabolites
Central metabolism
Microbial community
Methanogenic process

ABSTRACT

Central metabolite profiles from glucose in microbial communities during methanogenic process were compared between a stable methanogenic reactor (MR) and a deteriorated reactor (DR). The concentrations of intracellular metabolites related to the Embden–Meyerhof and pentose phosphate pathways, with the exception of pyruvate, remained high in the MR, showing increased carbon flux in the glycolysis pathway during stable methanogenesis. Extracellular acetate temporarily accumulated in the MR, consistent with higher ATP level in the MR. Intracellular concentrations of the intermediates in the reductive branch of tricarboxylic acid cycle, malate, fumarate, and succinate were higher in the DR. Low NADH/NAD^+ ratio both in the MR and DR would suggest NADH consumption during acetate and lactate/succinate production in the MR and DR, respectively. Intracellular glutamate levels were higher in the MR, correlating with lower NADPH/$NADP^+$ ratio concentrations in the MR. These findings contribute to a better understanding of the metabolic state during stable methanogenesis.

1. Introduction

Anaerobic digestion, such as methane fermentation, produces renewable biogas from wastes (Risberg et al., 2013; Weiland, 2010). Methane fermentation involves a complex series of metabolic reactions that can be divided into at least four phases, hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanogenesis by anaerobic microorganisms (Angelidaki et al., 2011; Weiland, 2010). During hydrolysis and acidogenesis, organic compounds are broken down into CO_2 and organic acids, which are further degraded by acetogenic bacteria into acetate, CO_2, and hydrogen. These substrates are then preferentially utilized by methanogens to produce methane (Mountfort and Asher, 1978; Stams and Plugge, 2009).

Under the deteriorated methanogenic process, intermediates, organic acids such as acetate, butyrate, and propionate accumulate (Angelidaki et al., 2003). Small subunit ribosomal DNA sequence-based methods have been used to examine changes in microbial community structure during deterioration of the methanogenic process (Hori et al., 2006; Sasaki et al., 2010). These analyses have revealed such deterioration is associated with a decrease in the proportion of methanogenic archaea among the total microbial population. This imbalance between different microbial groups results in the accumulation of organic acids, because their degradation is coupled to hydrogen consumption by hydrogenotrophic methanogens and acetate consumption by aceticlastic methanogens

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http://dx.doi.org/10.1016/j.biortech.2014.08.054
0960-8524/

(Hassan and Nelson, 2012; Sasaki et al., 2011). The reduced activity of these methanogens results in decreased methane gas production, leading to further deterioration of methanogenic activity. However, changes in the intracellular metabolites produced by microorganisms during this deterioration have not yet been clarified.

Metabolomics studies are useful for elucidating the intracellular pathways that produce certain metabolites within a single microbial species and have the potential to bridge the gap between phenotype and genotype. The fermentation of monosaccharides by microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* (Zhang et al., 2010) has been used to produce biofuels and chemicals (Toya and Shimizu, 2013). Although microbial communities found in methanogenic reactor are composed of diverse assemblages of microorganisms, most species, particularly bacteria that contribute to hydrolysis, acidogenesis, and acetogenesis/dehydrogenation degrade monosaccharides through the Embden–Meyerhof (EM) and/or pentose phosphate (PP) pathways. In anaerobic conditions, monosaccharides frequently enter an incomplete tricarboxylic acid (TCA) cycle after glycolysis (Feng et al., 2009; Trotter et al., 2011). Metabolomics can be applied to the analysis of microbial communities to clarify the central metabolic processes that are involved in the production of organic acids from monosaccharides during methane fermentation under stable and deteriorated conditions. Although nuclear magnetic resonance and gas chromatography-mass spectrometry (GC–MS)-based metabolomics approaches have been used to examine the extracellular metabolic dynamics during the anaerobic fermentation of glucose and cellulose-based substrates (Date et al., 2012; Yang et al., 2014), these approaches cannot sensitively identify intracellular metabolites.

In the present study, GC–quadrupole-MS (GC-Q-MS) and liquid chromatography triple-stage quadrupole MS (LC-QqQ-MS) were employed to examine the methane fermentation process by focusing on a wide range of metabolites involved in the EM and PP pathways, and TCA cycle. Methane fermentation was conducted at a pH of approximately 7.5 using glucose as the major carbon source until stable conditions were achieved, and deterioration was then induced by decreasing the pH to approximately 5.0. The reactor performance and intracellular metabolite profiles of microorganisms were compared under the stable and deteriorated methanogenic conditions.

2. Methods

2.1. Seed sludge and feed material

The supernatant from a 20% aqueous solution (wt/vol) of manure collected from a Japanese black cattle farm in Iwate Prefecture, Japan, was used as seed sludge. Synthetic medium containing (per 1 L) 10 g glucose (1%, wt/vol), 1.0 g yeast extract, 2.0 g $NaHCO_3$, 1.0 g NH_4Cl, 0.1 g KH_2PO_4, 0.2 g K_2HPO_4, 0.1 g $MgCl_2{\cdot}6H_2O$, 0.1 g $CaCl_2{\cdot}6H_2O$, 0.6 g NaCl, 10 mL Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) medium 141 trace element solution, and 10 mL DSMZ medium 141 vitamin solution, was used as feed material. The total organic carbon (TOC) concentration of the synthetic medium was 4399 mg-C L^{-1}.

2.2. Operation of anaerobic reactors

For pre-cultivation, two types of reactors, a stable methanogenic reactor (MR) and deteriorated reactor (DR), containing 250 mL (working volume) seed sludge, were initially operated at 55 °C and pH 7.5 with stirring at 200 rpm in duplicate. Pre-cultivation using a synthetic medium was conducted at a hydraulic retention time (HRT) of 20 and 15 days for 35 and 30 days, respectively. The reactor operation was conducted in semi-continuous mode by

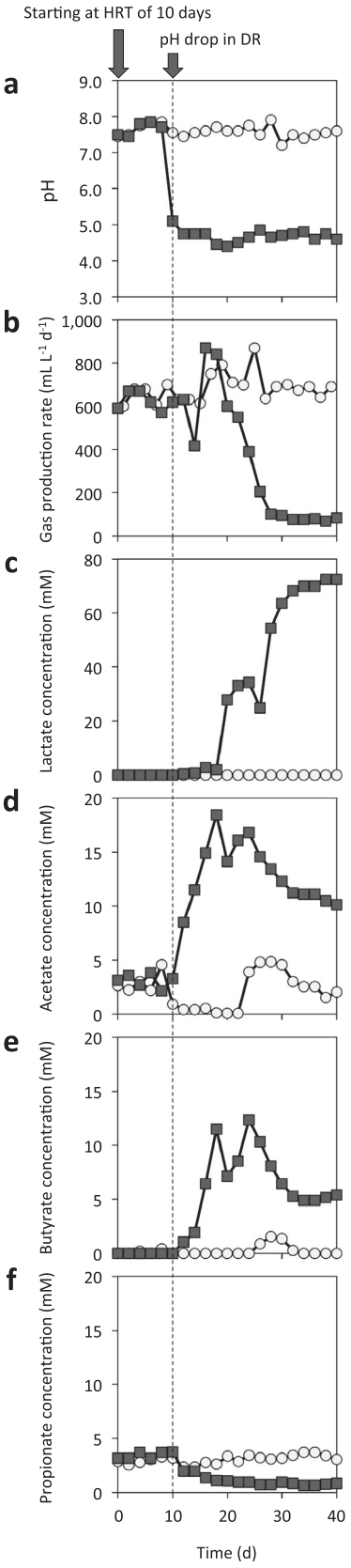

Fig. 1. Average values of pH, gas production, and extracellular organic acid accumulation in the stable methanogenic reactor (MR; ○) and deteriorated reactor (DR; ■) operated in duplicate. (a) pH, (b) gas production rate (mL L^{-1} day^{-1}), (c) lactate concentration (mM), (d) acetate concentration (mM), (e) butyrate concentration (mM), and (f) propionate concentration (mM) were analyzed throughout the operation period at an HRT of 10 days.