Bioresource Technology 137 (2013) 41–50

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Methanosarcina domination in anaerobic sequencing batch reactor at short hydraulic retention time

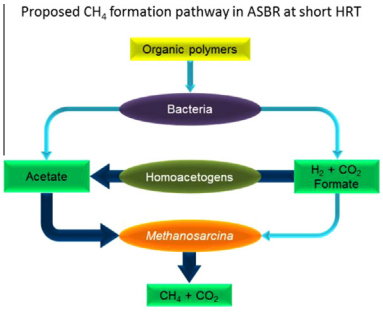
Jingwei Ma [a], Baisuo Zhao [a], Craig Frear [a], Quanbao Zhao [a], Liang Yu [a], Xiujin Li [b], Shulin Chen [a,*]

[a] Department of Biological Systems Engineering, Washington State University, Pullman, WA 99164, USA
[b] Center for Resources and Environmental Research, Beijing University of Chemical Technology, Beijing 100029, China

HIGHLIGHTS

- ASBR has potential to establish a *Methanosarcina* dominated methanogenic community.
- Microbial community showed higher diversity when reactor performance was superior.
- Homoacetogenesis plus aceticlastic methanogenesis was speculated as main pathway.
- 16S rRNA or *mcr*A clone library alone cannot provide complete community structure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 February 2013
Received in revised form 14 March 2013
Accepted 17 March 2013
Available online 22 March 2013

Keywords:
Microbial community structure
ASBR
Clone library
T-RFLP
Methanogenic pathway

ABSTRACT

The *Archaea* population of anaerobic sequential batch reactor (ASBR) featuring cycle operations under varying hydraulic retention time (HRT) was evaluated for treating a dilute waste stream. Terminal-Restriction Length Polymorphism and clone libraries for both 16S rRNA gene and *mcr*A gene were employed to characterize the methanogenic community structure. Results revealed that a *Methanosarcina* dominated methanogenic community was successfully established when using an ASBR digester at short HRT. It was revealed that both 16S rRNA and *mcr*A clone library could not provide complete community structure, while combination of two different clone libraries could capture more *archaea* diversity. Thermodynamic calculations confirmed a preference for the observed population structure. The results both experimentally and theoretically confirmed that *Methanosarcina* dominance emphasizing ASBR's important role in treating low strength wastewater as *Methanosarcina* will be more adept at overcoming temperature and shock loadings experienced with treating this type of wastewater.

1. Introduction

High activity of methanogens is important for maintaining efficient anaerobic digestion and avoiding accumulation of volatile fatty acids (acetate) and hydrogen, two of the most important methanation substrates. *Methanoarchaea*, or methanogens involved in anaerobic digestion, cover four orders (Liu and Whitman, 2008). All species within three of these orders, *Methanobacteriales*, *Methanococcales*, and *Methanomicrobiales*, are hydrogenotrophic methanogens reducing CO_2 to CH_4 using H_2 or formate. The other order, *Methanosarcinales*, comprising two families, *Methanosaetaceae* and *Methanosarcinaceae*, is unique. The former family contains a single genus, *Methanosaeta*, an exclusively acetotrophic species which utilizes acetate as its sole energy source, while the latter one, including *Methanosarcina* and several other genuses, can metabolize both hydrogen and acetate as energy source (Boone et al., 1993).

Methanogens, along with other hydrogenotrophs and acetotrophs compete for acetate and hydrogen, forming complex *Archaea* community structures, which vary under different anaerobic environments (Table 1). Affinity to substrate (K_s), growth rate (μ_{max}),

* Corresponding author. Tel.: +1 509 335 3743; fax: +1 509 335 2722.
E-mail address: chens@wsu.edu (S. Chen).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.03.101

Table 1
Typical competitive microbes in anaerobic digester.[a]

Competitive substrate	Acetate			H_2		
Microbes	Methanosarcina	Methanosaeta	Syntrophic acetate-oxidizing bacteria	Homoacetogens	Hydrogentrophic methanogens	Sulfate reducing bacteria
K_s[b]	3.0–4.5	0.49–0.86	–	6 μM or 800 Pa	4–8 μM or 550–1100 Pa	2 μM or 250 Pa
Threshold (mM)	0.62–1.2	0.005–0.069	>0.2	320–710 nM or 43–95 Pa	23–75 nM or 3–10 Pa	6.8 nM or 0.9 Pa
Y[c]	1.1–3.1	1.1–1.4	–	–	0.6–6.4	5.8
k[d]	11.4	9.4	–	–	–	–
μ_{max}[e]	0.55–2.04	0.08–0.69	–	–	0.70–3.46	1.44
Products	CH_4, CO_2	CH_4, CO_2	H_2, CO_2	Acetate	CH_4	H_2S
$\Delta G^{0'}$[f]	−31.0	−31.0	+104.6	−104.6	−135.6	−152.0
Favored conditions	High acetate concentration	Low acetate concentration		High hydrogen concentration, low temperature	Low hydrogen concentration, high temperature	
Note	*Methanosarcina* will outcompete *Methanosaeta* with average acetate concentrations higher than 1.9 mM			Homoacetogens are better adapted to grow at lower temperatures. The critical temperature is 20–25 °C Methanogens will be inhibited by sulfate reducing bacteria when H_2 concentration is below 5 μM		

[a] Data from Robinson and Tiedje (1984), Daniels et al. (1984), Cord-Ruwisch et al. (1988), Zinder (1993), Conklin et al. (2006), Demirel and Scherer (2008).
[b] Half-saturation constant (mM).
[c] Cell yield (g cell/*rnx*).
[d] Maximum specific substrate utilization rate (g COD/g cell·d).
[e] Maximum specific microbial growth rate (d^{-1}).
[f] Gibbs free energy under standard conditions (kJ/*rnx*).

and substrate utilization rate are the key factors governing the dominance of species within a microbial community. As noted, *Methanosarcina* are the most versatile methanogens (Zinder, 1993), offering methanation processes, which when compared to hydrogenotrophic as well as aceticlastic methanogens (*Methanosaeta*), are higher in substrate utilization rate, growth rate and cell yield while exposed to an environment with relatively high acetate and hydrogen concentration (Daniels et al., 1984). Thus *Methanosarcina* is favored under conditions in which a high input of organic matter leads to rapid accumulation of acetate and hydrogen (Zinder, 1993). Consequently, digesters dominated by *Methanosarcina* are more capable of handling increased loads and therefore would be less prone to upset by feeding increases; promoting more stable digestion (Conklin et al., 2006). Previous research has repeatedly shown that *Methanosaeta* dominance was found in most steady state anaerobic digesters, such as CSTR (continuous stirred tank reactor) and UASB (upflow anaerobic sludge blanket) (McHugh et al., 2003; Raskin et al., 1995; Schmidt and Ahring, 1999; Sekiguchi et al., 1998). Raskin et al. (1995) investigated 21 conventional sewage anaerobic digesters with a wide variation in digester design and operating conditions by means of molecular probes, and found that *Methanosaeta* sp. dominated in all digesters. Their dominance was consistent with the low acetate concentrations present in all of the digesters conditions, which provided competitive advantage for *Methanosaeta* sp. due to their low K_s and threshold compared to *Methanosarcina* sp. (Table 1). McHugh et al. (2003) examined six granular digesters of varying scale, design, feedstock, and temperature and the dominance of *Methanosaeta* sp. was discovered across all digesters, indicating these filamentous, acetate-utilizing methanogens had a crucial role in the formation and maintenance of stable anaerobic granules. Correspondingly, it was generally assumed that *Methanosaeta* sp. improved granulation and resulted in more stable and higher rate reactor performance (Schmidt and Ahring, 1999).

Methanosarcina outcompeting *Methanosaeta* though has been reported under certain operating conditions (short hydraulic retention time (HRT) or high acetate concentration) (Leclerc et al., 2004; Mladenovska et al., 2003). Leclerc et al. (2004) reported the dominance of *Methanosarcina* sp. in fluidized-bed, fixed-film, ASBR and CSTR, although detailed digester information was not provided. Prevalence of *Methanosarcina* sp. in biofilm reactors has also been reported by Schmidt and Ahring (1999). They concluded that *Methanosarcina* sp. formed biomass clumps consisting of large numbers of individual cells surrounded by a thick polymeric wall, as opposed to the filamentous type biofilm consisting of long multicellular rod-shaped *Methanosaeta* sp.; noting that the immobilization process of *Methanosarcina* sp. was even faster than that of *Methanosaeta* sp. The stratification of methanogens along the height of an UASB reactor was noticed with *Methanosaeta* sp. predominating in the top of the granular layer while *Methanosarcina* sp. primarily presented in the bottom of the reactor, concepts which are consistent with acetate concentration distribution in an UASB (Schmidt and Ahring, 1999). The presence of *Methanosaeta* ensured a better performance due to their low acetate threshold, however, in granules where *Methanosarcina* sp. were the only acetate-utilizing methanogen present, a syntrophic acetate-oxidizing system was found (Schmidt and Ahring, 1993).

Operation of an ASBR, with its infrequent feeding and intermittent mixing protocol, creates a high acetate concentration and dynamic condition within each cycle (Ma et al., 2013; Wang et al., 2011). Additionally by being able to uncouple HRT and SRT (sludge retention time), ASBR often employ uniquely short HRT, adapted to treat low strength waste streams while still treating high flow rates. Although above advantages for *Methanosarcina* dominance have been studied in UASB and CSTR, limited data exists showing methanogenic community dynamics in ASBR digester. The goal of this research was to investigate that if the unique hydraulic regime of ASBR may select *Methanosarcina* as the dominant species, which can lead to a more efficient and more stable anaerobic digestion process. In this study, the diversities of methanogens in ASBR digesters operated at different HRT were compared with T-RFLP (Terminal-Restriction Length Polymorphism). The composition of the methanogenic community of the digester with the best methane production performance was then revealed by sequence analysis of the partial 16S rRNA gene (~800 base pairs (bp)) and functional gene marker (*mcrA* gene, about 400 bp) generated from two constructed clone libraries. The