Bioresource Technology 112 (2012) 1–9

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Review

Methanosarcina: The rediscovered methanogen for heavy duty biomethanation

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ARTICLE INFO

Article history:
Received 14 November 2011
Received in revised form 13 February 2012
Accepted 16 February 2012
Available online 25 February 2012

Keywords:
Anaerobic digestion
Methanosarcina
Methanosaeta
Syntrophic acetate oxidation
Biogas

ABSTRACT

Anaerobic digestion is an important technology in the framework of renewable energy production. The anaerobic digestion system is susceptible to perturbations due to the sensitivity of the methanogens towards environmental factors. Currently, technology is evolving from conventional waste treatment, i.e. the removal of pollutants, to very intensive biogas production from concentrated wastes, in the framework of bio-energy production. In the latter configuration *Methanosarcina* species appear to be of crucial importance. *Methanosarcina* sp. are, compared to other methanogens, quite robust towards different impairments. They are reported to be tolerant to total ammonium concentrations up to 7000 mg L^{-1}, salt concentrations up to 18,000 mg Na^{+} L^{-1}, a pH shock of 0.8–1.0 units and acetate concentrations up to 15,000 mg COD L^{-1}. The possibilities of *Methanosarcina* sp. as key organisms in specific types of anaerobic digestion systems are demonstrated in this review.

1. Introduction

The production of renewable energy from organic waste streams is one of the important aspects in the concept of sustainable development. It has been set forward by the European Union that by the year 2020 approximately 20% of the European energy demands should originate from renewable energy sources. The production of biogas from organic materials should contribute for about 25% towards the total budget of renewable energy sources (Holm-Nielsen et al., 2009). Anaerobic digestion can be considered as one of the most important techniques to convert organic waste streams into renewable energy in the form of methane (Holm-Nielsen et al., 2009). The reason that anaerobic digestion is a widely used technique can be contributed to the fact that, apart from the biogas production and organic waste stabilization, it has several other advantages, e.g. a low cell yield, a high organic loading rate, limited nutrient demands and low costs for operation and maintenance of the reactor system (Wijekoon et al., 2011).

The methanogenic archaea are responsible for the final and critical step of anaerobic digestion, as they produce valuable methane. One of the major drawbacks of anaerobic digestion is however the sensitivity of the methanogenic consortium to different environmental factors. An abrupt change in pH, an increase in salt or organic matter concentration, an alteration of the loading rate or the introduction of a toxic compound often causes system failure (Chen et al., 2008; Ma et al., 2009; Wijekoon et al., 2011). Overloading is a frequent problem in anaerobic digestion, since it leads to accumulation of fatty acids, as these are no longer efficiently removed by the methanogens. This is because of their low growth rates, compared to the acidogenic and acetogenic bacteria, which causes the uncoupling of the acetogenic bacteria and the methanogens (Gujer and Zehnder, 1983). Overloading thus causes both an accumulation of fatty acids to concentrations which may have a toxic effect on the methanogens (Ma et al., 2009). It also lowers the pH to suboptimal values, since the optimal pH range for methanogens lies between 6.8 and 7.5 (Appels et al., 2008; Gujer and Zehnder, 1983). Taking these aspects into account, anaerobic digestion in continuously stirred tank reactors (CSTR) commonly operates at organic loading rates (OLR) below their optimum capacity, to avoid overloading, and sludge retention times (SRT) in the order of 20 days or more, to avoid washout of the methanogens (Appels et al., 2008). It is however reported that *Methanosarcina* sp. have high growth rates (i.e. doubling times in the order of 1.0–1.2 days) and are tolerant to sudden changes in pH of around 0.8–1.0 units, caused by overloading, compared to the other methanogens, which have doubling times of minimum 4–6 days and tend to be affected by a pH shock of 0.5 units or even less (Conklin et al., 2006; Liu et al., 1985; Shin et al., 2011). *Methanosarcina* sp. are able to use both the acetoclastic

Abbreviations: CSTR, continuously stirred tank reactor; DIET, direct interspecies electron transfer; HM, hydrogenotrophic methanogenesis; OLR, organic loading rate; SAB, syntrophic acetogenic bacteria; SAO, syntrophic acetate oxidation; SRT, sludge retention time; TAN, total ammonia nitrogen; TIC, total inorganic carbon; TVA, total volatile acids; UASB, upflow anaerobic sludge blanket; VFA, volatile fatty acids.

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doi:10.1016/j.biortech.2012.02.079

and the hydrogenotrophic methanogenesis pathway, making them more tolerant to specific inhibitors of the acetoclastic pathway, such as fluoroacetate and methyl fluoride, compared to *Methanosaeta* sp. (Liu et al., 2011a; Thauer et al., 2008). They are tolerant to levels of ammonium up to 7000 mg TAN L^{-1} (total ammonia nitrogen) as well (Calli et al., 2005a; Schnurer and Nordberg, 2008). These *Methanosarcina* sp. are therefore able to achieve stable growth at low retention times (even as low as 4 days), high organic loading rates and high levels of e.g. ammonium (Conklin et al., 2006; Schnurer and Nordberg, 2008).

This review firstly examines the possibilities and opportunities of *Methanosarcina* sp. in anaerobic digestion by exploring its robustness against several forms of stress. Subsequently the potential of selective enrichment of *Methanosarcina* sp. on submerged cathodes is described. Finally it focuses on the characteristics of so-called heavy duty robust methanogenesis, i.e. a combination of syntrophic acetate oxidizing bacteria and *Methanosarcina sp*. These insights subsequently provide the information to aim at expanding the potentials of *Methanosarcina* in anaerobic digestion. These potentials are (1) genetically engineering of *Methanosarcina* sp. in order to even further enhance its tolerance to stress or to broaden its substrate pattern, (2) application of *Methanosarcina* sp. as inoculum in anaerobic digesters to strengthen the digester performance or to prevent upcoming failure and (3) development of an anaerobic digestion system which is particularly based on *Methanosarcina* sp. and which fits in the evolution of waste treatment systems to energy factories.

2. The possibilities of *Methanosarcina* sp. in anaerobic digestion

2.1. Methanosarcina: the robust methanogen

Anaerobic digestion is susceptible to different forms of disruption because of its delicate balance between the different microbial consortia in the anaerobic digestion process, from which the methanogens are most vulnerable (Chen et al., 2008; Ma et al., 2009; Wijekoon et al., 2011). *Methanosarcina* sp. differ however from other methanogens as they are often tolerant against different stressors (Calli et al., 2005a; Conklin et al., 2006; Shin et al., 2011; Thauer et al., 2008). The four most common forms of stress, i.e. ammonium toxicity, overcharging the loading rate and its related problems, high salt concentrations and temperature variation are discussed. The different characteristics of *Methanosaeta* and *Methanosarcina*, the two main acetoclastic methanogens, are summarized in Table 1.

2.1.1. Ammonium

The degradation of nitrogenous organic matter, mostly proteins, amino acids and urea during the anaerobic digestion process causes the release of ammonia into the aqueous solution (Chen et al., 2008). Total ammonia nitrogen (TAN) can be present in both the ammonium ion (NH_4^+) or the free ammonia (NH_3) form (Calli et al., 2005a; Chen et al., 2008; Schnurer and Nordberg, 2008). Their proportion depends mostly on the pH in the reactor, with the free ammonia form being the most inhibiting for the microbial community. The amount of free ammonia released increases with rising pH, for the same level of TAN (Chen et al., 2008; Schnurer and Nordberg, 2008).

The methanogens are in the majority of the anaerobic digesters the most susceptible with respect to high levels of TAN, i.e. exceeding 3000–4000 mg TAN L^{-1} (Chen et al., 2008; Schnurer and Nordberg, 2008). Yet in most cases *Methanosarcina* sp. seem to be more tolerant towards ammonium stress than other methanogens, particularly *Methanosaeta* sp., which are no longer detected at ammonium concentrations exceeding 3000 mg TAN L^{-1} (Schnurer and Nordberg, 2008). Two anaerobic digesters, one at 37 °C and the other at 55 °C, treating cattle excreta and olive mill wastes, were already dominated by *Methanosarcina* sp. at a concentration of 1300 mg TAN L^{-1} (Goberna et al., 2010). Systematically enhancing the ammonium concentration in 5 other anaerobic digesters with different seed sludge leads in all cases to a dominance of *Methanosarcina* sp. at a concentration of 2500 mg TAN L^{-1}. The removal efficiency remained above 90% in all five reactors at this level of TAN (Calli et al., 2005a). Two of these five reactors even managed to maintain a 93% COD removal efficiency at 6000 mg TAN L^{-1} (Calli et al., 2005b). The resistance of *Methanosarcina* sp. against high ammonium concentrations can be attributed to (1) their relative large cell size and spherical form and (2) their ability to grow in clusters, in contrast to other methanogens (Calli et al., 2005a,b; Goberna et al., 2010).

The large cell size and spherical form of *Methanosarcina* corresponds to a higher volume-to-surface ratio and this leads, together with the formation of clusters, to a much lower ammonia diffusion per unit of cell mass, compared to filamentous methanogens, thus inducing a higher tolerance to high concentrations of ammonia (Calli et al., 2005a,b; Goberna et al., 2010). This volume-to-surface ratio is for *Methanosarcina* sp. about 4–7 times higher than for *Methanosaeta* sp. (Calli et al., 2005a). As the ammonium concentration rises, single cells of *Methanosarcina* sp. can group together to form clusters thus lowering the ammonium toxicity. Reactor stability can therefore be correlated to the consistency of these clusters (Calli et al., 2005b). As long as the cluster formation is not disturbed by certain chemicals or high shear forces, the resistance to ammonium remains high.

2.1.2. Increase of the loading rate

Anaerobic digesters are vulnerable to overloading, which can disrupt their operational stability (Gujer and Zehnder, 1983). An increase of the loading rate leads to the accumulation of fatty acids eliciting toxic effects and causing the pH to drop to suboptimal conditions, which can cause a decrease in methanogenic activity (Appels et al., 2008; Chen et al., 2008; Gujer and Zehnder, 1983; Ma et al., 2009). Unlike most methanogens, *Methanosarcina* sp.

Table 1
Characteristics of *Methanosarcina* and *Methanosaeta*.

Parameter	*Methanosaeta*	*Methanosarcina*	Reference
μ_{max} (d^{-1})	0.20	0.60	Conklin et al. (2006), Gujer and Zehnder (1983), Qu et al. (2009), and Yu et al. (2006)
K_s (mg COD L^{-1})	10–50	200–280	Conklin et al. (2006), Gujer and Zehnder (1983), Qu et al. (2009), and Yu et al. (2006)
NH_4^+ (mg L^{-1})	<3000	<7000	Calli et al. (2005a), Nettmann et al. (2010), Schnurer and Nordberg (2008)
Na^+ (mg L^{-1})	<10,000	<18,000	Spanheimer and Muller (2008)
pH-range	6.5–8.5	5–8	Liu et al. (1985), Staley et al. (2011), Steinberg and Regan (2011), and van Leerdam et al. (2008).
pH-shock	<0.5	0.8–1	Liu et al. (1985)
Temperature range (°C)	7–65	1–70	Goberna et al. (2010), Simankova et al. (2001), Tang et al. (2008), and Xing et al. (2010)
Acetate concentration (mg L^{-1})	<3000	<15,000	Conklin et al. (2006), Hao et al. (2011), Liu et al. (1985), McMahon et al. (2004), Qu et al. (2009), Staley et al. (2011), Yu et al. (2006)