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The effect of anaerobic biomass drying and exposure to air on their recovery and evolution

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form
1 May 2014
Accepted 12 May 2014
Available online 22 May 2014

Keywords:
Methanogenic activity
Anaerobic digestion
Drying methods
Biodegradation rate
mcrA
Wastewater treatment

ABSTRACT

The main goal of this study was to test the effect of various drying methods of granular anaerobic biomass on biomass survival, potential and rate of methane re-production, and structure. This may facilitate the development of drying methods to preserve excess anaerobic biomass in dry form for re-inoculation of existing digesters after process failure or wash out or for the start-up of new digesters. To that end, anaerobic granular biomass was collected from an up-flow anaerobic sludge blanket (UASB) reactor. The biomass was dried using two alternative methods: oven with air circulation at 50 °C for 24 h (DAO), and vacuum rotary evaporator at anaerobic conditions (DAN). For comparison, the control was a biomass with no drying (WET). Biomass samples were tested for specific methanogenic activity using synthetic wastewater. The microbial communities were also tested for viability using the LIVE/DEAD kit, and total biomass was initially quantified by qPCR targeting 16S rRNA genes. In addition, the *mcrA* functional gene was used s a target for the detection of the most abundant methanogens. Basic bacterial morphology classification was done by VIT® gene probe technology using a fluorescence microscope. Dried DAN and DAO biomasses required approximately four operational runs to recover their initial methanogenic activity compared to WET biomass. LIVE/DEAD results showed clear increases in the proportions of the viable biomass of the total bacterial communities over time, especially for the DAN and DAO samples. A comparison of the qPCR results of both DAN and DAO to the WET biomass showed that the methanogenic *mcrA* gene fraction of the total biomass population of 16S rRNA gene concentrations decreased moderately by about 17.2% in the samples of DAO and by approximately 6.7% in the samples of DAN over all runs after Run1.

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http://dx.doi.org/10.1016/j.watres.2014.05.010
0043-1354/

1. Introduction

The anaerobic digestion of organic wastes and wastewater is considered to be a sustainable technology due to its low energy consumption and it's potential to produce methane as a bio-energy source (Fiss and Smith, 2007). Many agricultural and industrial wastes are ideal candidates for anaerobic digestion because they contain high levels of easily biodegradable materials. But problems such as low methane yield and process instability are often encountered in anaerobic digestion, preventing this technique from being widely applied (Chen et al., 2008). The limited knowledge of the microbial ecosystem involved has meant that many field-scale anaerobic digesters have been empirically set up and operated with over-dimensioned volumes or excessively low organic loading rates to avoid system failure (Connaughton et al., 2006; Fernandez et al., 2000).

The running and stabilizing of an anaerobic digester are directly related to the microbial community and types of substrate used for anaerobic digestion. Several models have been developed for the kinetics analyses of the biogas production processes for particular types of biomass and substrate. The Gompertz model and the Chen and Hashimoto model are well known for modeling the kinetic behavior of anaerobic digestion process (Chen and Hashimoto, 1980; Jagadish et al., 2012). The Gompertz equation can easily estimate the kinetic parameters, biogas yield potential, lag phase duration, and the maximum biogas production rate for wheat straw pretreated substrates co-digested with cattle manure in batch assays. This model fits experimental measurements well (Krishania et al., 2013).

Methanogenic communities play a key role in the maintenance of stable and efficient digestion processes. An increased understanding of anaerobic microbiology (i.e., community composition) and how that affects digester functionality may promote the development and efficiency of customized methanogenic cultures for enhanced anaerobic bioprocesses. The makeup of the inoculation biomass and the composition of the wastewater being treated significantly influence both the microbial community in, and the performance of, anaerobic digestion processes. With the development of culture independent molecular techniques, researchers have been able to follow changes in the methanogenic community of anaerobic digesters exposed to organic shock loads (Steinberg and Regan, 2011). Lee et al. (2010) used molecular and statistical tools to demonstrate that during the start-up of a batch anaerobic digestion of whey permeate, methanogenic communities changed dynamically, showing both qualitative and quantitative structural shifts in their communities. Methanogens can be surveyed and monitored using genes and transcripts of *mcrA*, which encodes the α subunit of methyl coenzyme M reductase, the enzyme that catalyzes the final step in methanogenesis (Morris et al., 2014). McMahon et al. (2004) revealed that digesters whose communities were not able to consistently attain stability of function in the past were more tolerant and resilient to an overload or shock than communities that had always functioned well.

Our central hypothesis posits that tolerant methanogenic cultures can survive even after drying and handling and exposure to oxygen. Moreover, the surviving methanogenic cultures will exhibit higher methanogenic activity than cultures developed under strict anaerobic conditions, irrespective of which drying method is used. Viable methanogens have been detected in dry, aerobic environments, such as dry reservoir sediment, dry rice paddies and aerobic desert soils (Mayer and Conrad, 1990; Mitchell and Baldwil, 1999; Peters and Conrad, 1995), findings suggesting that methanogens possess mechanisms that ensure their long-term survival in a desiccated state. Anderson et al. (2012) observed that dried *Methanosarcina barkeri* exposed to air at room temperature did not lose significant viability after 28 days. In fact, the exposure of *M. barkeri* to air after desiccation even appeared to improve the recovery of viable cells compared with that of desiccated cells that were never exposed to air. Desiccated *M. barkeri* was also found to be more resistant to higher temperatures, and although their resistance to oxidative conditions such as ozone and ionizing radiation was not as robust as in other desiccation-resistant microorganisms, the protection mechanisms are likely adequate to maintain cell viability during periodic exposure events. The main goal of this study was to test the effect of various drying methods of granular anaerobic methanogens on their survival, potential and rate of methane re-production, and diversity. This may enable the development of drying methods to preserve excess anaerobic biomass in dry form for the re-inoculation of existing digesters after process failure or for the start-up process of new digesters.

2. Materials and methods

2.1. Drying of anaerobic biomass

Anaerobic granular biomass was collected from a well operated, up-flow anaerobic sludge blanket (UASB) bio-reactor used to treat the wastewater of a citrus-based soft drink factory (PRIGAT) at kibbutz Givat Haim, Israel. The biomass, which was kept at room temperature with UASB effluents, was analyzed for its water content (13.1%) and volatile solid (VS) (79.7%). Three types of pretreatment were conducted prior to immobilization of the anaerobic biomass as can be seen in Table 1.

2.2. Methanogenic biomass re-cultivation – batch experiments

Three identical lab-scale reactors with volumes of 2850 mL each (active volume of 2800 mL and headspace of 50 mL) were operated in parallel in a batch mode to monitor the dynamic changes in the composition of the biomass communities and in methanogen viability. This experiment was conducted in a large volume reactors in order to minimize the effect of sampling on the mass balances. The first reactor was inoculated with 76.1 g of WET biomass, and the second and the third reactors were inoculated with 10 g of DAO and DAN biomass, respectively. The 76.1 g of wet biomass corresponds to about 10 g of dry biomass, which means that the three systems were started at the same amount of biomass (on the basis of dry matter). The water contents of these two dried biomasses were 7.74% and 9.81% for DAO and DAN, respectively.