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Bioaugmentation of overloaded anaerobic digesters restores function and archaeal community

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ABSTRACT

Adding beneficial microorganisms to anaerobic digesters for improved performance (i.e. bioaugmentation) has been shown to decrease recovery time after organic overload or toxicity upset. Compared to strictly anaerobic cultures, adding aerotolerant methanogenic cultures may be more practical since they exhibit higher methanogenic activity and can be easily dried and stored in ambient air for future shipping and use. In this study, anaerobic digesters were bioaugmented with both anaerobic and aerated, methanogenic propionate enrichment cultures after a transient organic overload. Digesters bioaugmented with anaerobic and moderately aerated cultures recovered 25 and 100 days before non-bioaugmented digesters, respectively. Increased methane production due to bioaugmentation continued a long time, with 50–120% increases 6 to 12 SRTs (60–120 days) after overload. In contrast to the anaerobic enrichment, the aerated enrichments were more effective as bioaugmentation cultures, resulting in faster recovery of upset digester methane and COD removal rates. Sixty days after overload, the bioaugmented digester archaeal community was not shifted, but was restored to one similar to the pre-overload community. In contrast, non-bioaugmented digester archaeal communities before and after overload were significantly different. Organisms most similar to *Methanospirillum hungatei* had higher relative abundance in well-operating, undisturbed and bioaugmented digesters, whereas organisms similar to *Methanolinea tarda* were more abundant in upset, non-bioaugmented digesters. Bioaugmentation is a beneficial approach to increase digester recovery rate after transient organic overload events. Moderately aerated, methanogenic propionate enrichment cultures were more beneficial augments than a strictly anaerobic enrichment.

1. Introduction

Bioaugmentation is the practice of adding specialized microorganisms to biological systems for improved performance (Nyer and Bourgeois, 1980; Rittmann and Whiteman, 1994; Hairston et al., 1997; Maier et al., 2000; Deflaun and Steffan, 2002; Mulligan, 2002; Evans and Furlong, 2003). The approach has been used for hazardous waste remediation as well as

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aerobic waste treatment, but not in full scale for anaerobic, methanogenic systems. In aerobic wastewater treatment, bioaugmentation has resulted in more reliable nitrification, improved sludge settling, enhanced grease degradation and accelerated transformation of xenobiotic organic contaminants (Rittmann and Whiteman, 1994).

Although bioaugmentation of full-scale anaerobic digesters has not been reported, it has been studied at laboratory scale to increase methane production from animal manure (Angelidaki and Ahring, 2000), distillery wastewater (Savant and Ranade, 2004), lipid-rich wastes (Cirne et al., 2006), sewage sludge mixed with pig manure (Bagi et al., 2007) and cellulose (Nielsen et al., 2007; Weiss et al., 2010). Furthermore, bioaugmentation has decreased the recovery time of anaerobic digesters stressed by hydrogen sulfide (O'Flaherty et al., 1999; O'Flaherty and Colleran, 1999) and oxygen (Schauer-Gimenez et al., 2010). Production of individual bioaugmentation cultures, each enriched to degrade a specific substrate, would be time consuming. It may be more practical to target key, ubiquitous intermediates to improve anaerobic digestion. Acetate and propionate are reasonable targets since chronically elevated concentrations are often observed in anaerobic digesters during periods of low COD removal and low biogas production (Smith and McCarty, 1990). Adding propionate-utilizing enrichment cultures that can convert acetate and propionate to methane may lead to improved digestion.

Previous methanogenic bioaugmentation research involved adding strictly anaerobic cultures. Digesters bioaugmented with anaerobic propionate enrichment cultures after organic overload recovered approximately 25 days before non-bioaugmented digesters (Tale et al., 2011). In addition, benefits of bioaugmentation continued for more than 12 solids retention times (SRTs) after the transient overload. However, adding facultative or aerotolerant methanogenic cultures (i.e., cultures exposed to oxygen that produce measurable amounts of methane) to anaerobic digesters may be more practical and effective. Aerotolerant cultures consistently exhibited higher methanogenic activity before as well as after heat and freeze drying in air and after being held under conditions simulating 20 years of storage subsequent to drying (Bhattad et al., 2010; Zitomer, 2013). This is beneficial when considering the production of commercial, dried bioaugmentation products; aerotolerant biomass can be easily processed in ambient air and still retain methanogenic activity.

The comprehensive review by Botheju and Bakke (2011) describe beneficial effects of adding limited oxygen during growth of methanogenic biomass. Methanogenic mixed cultures exposed to limited aeration still produce significant amounts of methane (Zitomer, 1995), and some low-aeration biomass exhibited 20% higher specific methanogenic activity (SMA) values than control cultures maintained anaerobically (Zitomer and Shrout, 1998). The higher SMA of aerotolerant cultures may result in a superior outcome when used for bioaugmentation to increase methane production rate.

In this study, bioaugmentation of anaerobic digesters with methanogenic, aerotolerant cultures enriched for propionate degradation was investigated as a method to reduce recovery time following a transient organic overload. Results of bioaugmentation with aerotolerant and anaerobic cultures were compared. Differences in microbial community structure due to bioaugmentation were also determined.

2. Materials and methods

2.1. Enrichment cultures

Biomass samples from 14, full-scale anaerobic digesters were assayed for SMA against propionate (Tale et al., 2011). The highest SMA value was observed for biomass from an upflow anaerobic sludge blanket (UASB) digester treating brewery wastewater (City Brewery, LaCrosse, Wisconsin). Because of its high activity, this biomass was subsequently enriched by feeding propionate under four conditions that differed based on air addition rate, with individual oxygen loading rates of 0, 25, 125 and 225 mg O_2 per L of reactor per day (mg O_2/L-day). These oxygen loadings were equivalent to 0, 10, 50 and 90% of the COD organic loading rate (OLR), and cultures were designated as Enrichment 0, Enrichment 10, Enrichment 50 and Enrichment 90 (E0, E10, E50 and E90), respectively. Each Culture was maintained in triplicate.

Enrichments were maintained in 750 mL serum bottles containing 150 mL of culture. Every day, 10 mL was removed via plastic syringe and replaced with an equal volume of medium to maintain an SRT and hydraulic residence time (HRT) of 15 days. All enrichments received 0.17 g propionate/L-day (0.25 g COD/L-day) in basal nutrient medium. Before feeding, the volume of biogas in each enrichment was measured at 35 °C and atmospheric pressure by inserting a needle and glass syringe with wetted glass barrel through the serum bottle septa. The excess gas was then released, wasting and feeding were completed, an appropriate volume of air was added using a syringe, and the bottles were placed on a shaker (150 rpm) in a temperature-controlled room at 35 ± 3 °C. The culture volumetric air doses were 0, 14, 68 and 124 mL air/L-day (35 °C, 1 atm), respectively. These enrichment cultures were subsequently used to bioaugment organically overloaded digesters.

2.2. Anaerobic digesters

Anaerobic digesters were 160 mL serum bottles containing 50 mL of active volume incubated on a shaker table in a temperature-controlled room at 35 ± 2 °C. Seed biomass was taken from a laboratory-scale anaerobic, methanogenic system fed non-fat dry milk for over three years and originally seeded with biomass from a municipal anaerobic digester stabilizing primary sludge (South Shore Water Reclamation Facility, Oak Creek, Wisconsin, USA). Digesters were operated at a 10 day SRT and HRT by removing 5 mL of digester contents every day and adding an equal volume of feed. Volatile suspended solids concentration of 4.5 ± 0.1 g/L was maintained in the digesters. Wasting and feeding were performed by inserting a needle with a plastic syringe through serum bottle septa. Biogas production was measured daily by inserting a needle with a glass syringe and wetted glass barrel through serum bottle septa, and excess biogas was wasted to the atmosphere or used for biogas methane content analysis.