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Dairy manure resource recovery utilizing two-stage anaerobic digestion – Implications of solids fractionation

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HIGHLIGHTS

- Dairy manure AD is economically uncompetitive.
- Research investigated AD of separate (fine/coarse) vs. combined solids.
- Combined solids AD generated enhanced VS destruction.
- Combined solids enriched for a more heterogeneous bacterial/archaeal consortium.
- Targeted AD of fat-rich solids could be an optimal approach for processing manure.

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ABSTRACT

Dairy manure management is increasingly becoming an environmental challenge. In this regard, manure anaerobic digestion (AD) can be applied to address environmental concerns; however, dairy manure AD remains economically uncompetitive. Ongoing research is focused on enhanced resource recovery from manure, including maximizing AD methane yield through a novel multi-stage AD configuration. Research presented herein centered on the hypothesis that separately digesting fine and coarse solids from fermented dairy manure would improve methane production; the hypothesis was disproven. While maximum methane concentration was realized on fine solids, combined solids AD yielded enhanced VS destruction. The diverse combined-solids substrate enriched for a more heterogeneous bacterial/archaeal consortium that balanced fermentation and methanogenesis to yield maximum product (methane). However, results suggest that targeted AD of the fat-rich fine solids could be a more optimal approach for processing manure; alternate (non-AD) methods could then be applied to extract value from the fibrous fraction.

1. Introduction

Based on the agricultural census, in 2007 there were 71,510 dairy operations in the United States housing 9.158 million cows and producing an estimated 500 billion pounds of wet manure yearly (Betts and Ling, 2009); additional cows have since been added to the working herds. Regarding byproducts management, as a legacy practice dairy manure is predominantly land applied to enhance forage crop production, with high levels of nitrogen, phosphorus, and other nutrients enhancing plant growth (USDA-NRCS, 2012). However, this approach is becoming increasingly problematic due in part to concerns regarding climate change caused by greenhouse gas (GHG) accumulation. Microbial metabolism of land applied manure releases significant amounts of methane and nitrous oxide to the atmosphere (EPA, 2014); both are potent GHGs (EPA, 2014). GHG emissions associated with dairy manure management account for 7.0% of agricultural sector emissions according to the most recent U.S. EPA estimates (EPA, 2014). Recognizing this concern, in January 2009 the Innovation Center (IC) for U.S. dairy announced a voluntary goal to reduce dairy GHG emissions 25% by 2020.

Beyond GHG emissions, challenges associated with conventional manure storage and land application practices include emission of unpleasant odors, potential nutrient migration to surface and ground water, and potential cross-contamination of crops with pathogenic organisms present in land-applied manure (Sahlstrom, 2003). These challenges have been exacerbated in recent years

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through consolidation of the U.S. dairy industry, which has increased manure densities (geographically). Public opposition to large dairy operations based on offensive odors, waste management, and environmental concerns has made it increasingly difficult for dairy operators to build new facilities or expand existing ones (Sanders et al., 2010).

Anaerobic digestion (AD) is an established waste treatment technology that can be applied to address many of the manure management environmental concerns. AD leverages an interdependent consortium of anaerobic microorganisms to break down complex organic wastes and produce a biogas consisting primarily of methane and carbon dioxide. The methane produced can be combusted to generate heat and electricity, which reduces GHG emissions through conversion of methane to carbon dioxide and by decreasing demand for fossil fuels. AD also provides pathogen reduction and can be used to produce EPA Class A or B biosolids (EPA, 2003). Finally, digestate is rich in ammonium, which is readily assimilated by plants when applied at agronomic rates, and it contains lower concentrations of volatile compounds responsible for objectionable odors than untreated manure (Betts and Ling, 2009; Weiland, 2010). The combination of pathogen and odor reduction, increased nutrient availability, and reduced GHG emissions make anaerobically digested manure a superior choice to untreated manure for land application.

In considering deployment of ADs at dairies, it is estimated that such installations are feasible at over 8000 U.S. dairy, swine, and poultry operations. However, only about 2% of the sites where AD is feasible actually have digesters installed (EPA, 2010). U.S. farmers generally avoid anaerobic digesters for a variety of reasons, with high initial cost and low or negative rate of return on investment being the most common (Faulhaber et al., 2012; Zaks et al., 2011). Indeed, studies have shown new AD projects will probably require some form of government market support in order to be profitable, such as carbon offset credits, low interest loans, or grants to compensate for low energy prices and the high initial cost of digester facilities (Faulhaber et al., 2012; Zaks et al., 2011).

If improvements could be made to the AD process, both in terms of improving digester methane yield and in developing complementary processes capable of producing additional revenue streams, its benefits could be more broadly realized. With this goal in mind, ongoing research has been exploring the potential for diversifying the commodity portfolio from manure waste streams (Coats et al., 2013). Central to the proposed integrated suite of processes is a novel two-stage anaerobic digestion process, which utilizes a fermenter and digester operated in series to separate manure hydrolysis and fermentation from methanogenesis in order to optimize the environment for the microbes responsible for each process (Coats et al., 2012). Within this integrated system, a portion of the volatile fatty acid (VFA)-containing liquid fraction generated in the fermenter is diverted from the digester and used as substrate in the production of polyhydroxyalkanoates (PHAs; a high-value bioplastic) by mixed microbial cultures (Coats et al., 2007; Wei et al., 2014), while the remaining liquid and residual solids are directed to an AD to produce methane (Coats et al., 2012).

With ongoing efforts to continuously enhance resource recovery from the proposed integrated suite of processes, investigations of this novel AD operation highlighted potentially useful process improvements associated with solids fractionation. Specifically, screening was applied to separate the carboxylate-rich liquid fraction from coarse, lignocellulosic residual solids (referred to hereafter as "coarse solids"). Applying this solids separation method, particles small enough to pass through the 1 mm mesh screen could be separately isolated by subsequent centrifugation; the residual solids material present in the screened effluent is hereinafter referred to as "fine solids." Preliminary investigations indicated the fine solids exhibited the potential to yield higher methane content biogas when digested separately from the coarse solids (~69% vs. ~55% (by volume)). Moreover, microbial population analyses demonstrated the ADs would select and enrich for fermenting bacterial consortia specialized in degrading the unique substrates (fine vs. coarse solids), thereby ensuring process stability (Briones et al., 2014). Building upon these investigations, the research presented and discussed herein focused on detailed interrogation of enhanced solids phase-separation AD. Research was driven by the hypothesis that separate AD of the two distinct solids streams (fine vs. coarse) would increase system-level methane production (both in concentration and yield) because the ADs would be microbially tailored to the respective substrates. Research objectives were to (i) comprehensively assess methane production potential of the separated fine and coarse residual solids fractions, (ii) assess overall performance of the contrasting respective AD configurations, (iii) evaluate the microbial populations in the AD systems, and (iv) make a final determination for combined vs. separate solids AD.

2. Methods

2.1. Experimental design

Two phase-separated AD systems, referred to as Systems 1 and 2, were operated to conduct the investigations (illustrated in Figs. 1a and 1b). Each "system" received the same volatile solids (VS) loading rate, with identical total operational volumes (20 L fermenter, 40 L digester, 60 L total). Reactor SRTs were also equivalent in each system (4 day fermenter, 20 day digester). Thus, the dominant factor which varied between the systems was the substrate. The substrate for System 2 was separated into two fractions, one containing primarily large, coarse solids, and the other small, fine solids, while the substrate to System 1 was not fractionated. System 1 (Fig. 1a) was operated as the experimental control and consisted of a fermenter (F6-c), and a digester (AD6-c) which received the centrifuge-separated (i.e., containing both coarse and fine particles) solids fraction of the fermenter effluent. System 2 (Fig. 1b) consisted of a fermenter (F3-c) and two independent digesters AD3-c and AD8, which were operated in parallel. AD3-c received the coarse solids fraction of the fermenter effluent, which was separated by screening. AD8 received the fine solids fraction, which was produced via centrifugation of the screened fermenter liquor. To ensure steady state conditions prevailed during operational performance assessment, both systems were allowed to equilibrate for 80 days (equivalent to 4 digester SRTs) prior to the start of data collection. Steady state conditions were defined based on observed stable AD biogas production and low effluent VFA concentrations, both of which were monitored regularly during the equilibration period. Once steady state conditions were achieved, data was collected during a subsequent 85 day operational period (equal to 4.25 digester SRTs). The length of this period was sufficient to identify statistically significant differences in performance between the two AD systems.

2.2. Source of manure

Raw dairy manure was collected from the floor of the University of Idaho dairy every 1–2 weeks, sampled for total and volatile solids content (TS/VS) at the time of collection, and stored at 4 °C until use. Collection of manure from areas of the dairy where it would likely be mixed with refractory lignocellulosic bedding material was avoided.

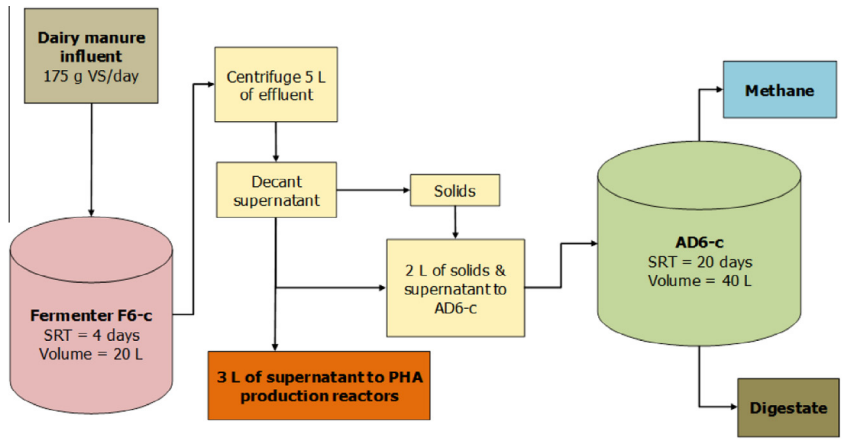

Fig. 1a. AD System 1 process flow chart (conventional two-phase AD).

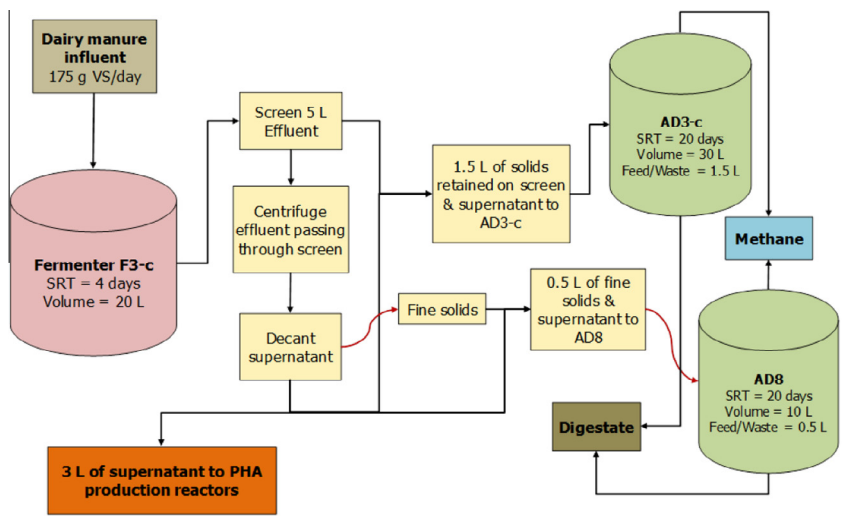

Fig. 1b. AD System 2 process flow chart (parallel two-phase AD).

2.3. Fermenter design and operation

Construction of both fermenters was identical. The reactor vessels were 22.7 L (6 gallon) HDPE buckets with valves installed in the base for wasting, and sealed with HDPE lids containing rubber gaskets. Both were operated at an active volume of 20 L, a 4-day SRT, and a target OLR of 8.75 g VS/L * d, with feeding/wasting conducted once per day. The target OLR was maintained based on VS samples collected from the raw manure, and the feed consisted of a mixture of raw dairy manure and sufficient tap water to bring the total feed volume to 5 L. Fermentation occurred at room temperature (22–25 °C). Mixing was accomplished using 9.53 cm (3.75 in.) diameter helical impellers driven by Oriental Motor (San Jose, CA, USA) USM315-401W 15-watt AC variable speed motors connected to 3GN35SA reduction gearboxes, and operated at a speed sufficient to provide uniform mixing. To minimize exposure of the fermenters to oxygen, gasketed bulkhead fittings were used to install draft tubes constructed of Schedule 40 1.9 cm (3/4 in.) PVC pipe through the lids and extending below the liquid surface. Each fermenter contained two tubes, one housing the impeller shaft and the other used for feeding. Biogas produced was vented through the lids via tubing connected to an airlock. The five liters of effluent produced daily from F6-c was centrifuged at 8000 rpm for five minutes at room temperature to separate liquid and solid fractions. Coarse solids were recovered from the F3-c effluent by screening through a strainer with a mesh size of approximately 1 mm (Norpro Inc., Everett, WA). The fermenter liquor passing through the strainer was centrifuged at 8000 rpm for 5 min at room temperature to recover the fine solids.

2.4. Anaerobic digester design and operation

Substrate for AD6-c was prepared using the separated solids from F6-c and sufficient centrifuged fermenter liquor to yield two liters. Substrate for digester AD3-c was batched using the solids retained on the strainer along with sufficient centrifuged fermenter liquor to yield 1.5 L of influent substrate, while that of AD8 consisted of the fine solids and sufficient centrifuged liquid to make 0.5 L. The remaining liquid effluent from both F3-c and F6-c was diverted to PHA production experiments (not part of this study).

Digesters AD3-c and AD6-c were operated at a 20-day SRT with active volumes of 30 L and 40 L, respectively. The reactors were constructed from Chem-Tainer 56.8 L (15 gallon) cone bottom HDPE tanks (West Babylon, NY, USA) fitted with a polypropylene lid and sealed using RTV silicone gasket sealant. Sections of 1.9 cm (3/4 in.) diameter PVC pipe were used as draft tubes to allow feeding and mixing while maintaining anaerobic conditions.

The digesters were mixed using 15.24 cm (6 in.) diameter two-blade impact-resistant plastic propellers driven by Oriental Motor (San Jose, CA, USA) BHF62AT-50 40-watt AC variable speed motors connected to 5GN3.6SA reduction gearboxes. The mixing intensity generated was sufficient for homogenization, although a floating scum layer approximately 5–7.5 cm (2–3 in.) thick developed on the surface of AD6-c. The digesters were heated by circulation of hot water through 15.24 m (50 foot) sections of 0.95 cm (3/8 in.) diameter copper tubing wrapped around the exterior of the tanks. The hot water cycling frequency was dictated by a programmable logic controller (PLC) connected to resistance temperature detector (RTD) probes installed in each AD, which maintained digester temperature at 35 °C.

Digester AD8 was constructed from a square 18.9 L (5 gallon) medium density polyethylene (MDPE) tank with a pyramidal base (Den Hartog Industries, Hospers, IA, USA). Feeding and mixing utilized 1.9 cm (3/4 in.) diameter PVC draft tubes to maintain anaerobic conditions. Mixing was provided by a 10.2 cm (4 in.) diameter three-blade stainless steel propeller driven by an Oriental Motor USM315-401 15-watt AC variable speed motor using a 36N5SA reduction gearbox (Oriental Motor, San Jose, CA, USA). The mixing speed was constant and of sufficient intensity to ensure homogenization. The digester was heated using two 12.7 cm × 25.4 cm (5 in. × 10 in.) silicone rubber heaters rated at 120 V/63 W (Watlow Electric Manufacturing Co., St. Louis, MO, USA). Heater cycling was controlled with a proportional/integral/derivative (PID) controller (Red Lion Controls, York, PA, USA) connected to a RTD temperature probe to maintain digester temperature at 35 °C.

Tedlar gas sampling bags (Smith Air Sample Supply, Mebane, NC, USA) were spliced into the gas exhaust lines of all digesters to allow the headspace volume to vary during feeding and wasting.

2.5. Analytical techniques

Digester gas production was quantified using wet tip gas meters (Wet Tip Gas Meter Co., Nashville, TN, USA). One meter was attached to each digester, although during the latter 48 days of the 85 day experiment the gas exhaust lines of AD3-c and AD8 were spliced together and the combined production was measured by a single meter.

Methane, carbon dioxide, and nitrogen content of the digester gas were quantified daily by gas chromatography using a Gow-Mac (Bethlehem, PA, USA) Series 550P Gas Chromatograph equipped with a thermal conductivity detector (TCD). The temperature of the column (Grace Davison Discovery Sciences, Deerfield, IL, USA, Alltech® Hayeseop® DB 100/120, 30 foot × 1/8 in. × 0.085 in., stainless steel) was held constant at 70 °C, and the injector temperature constant at 39 °C. The detector was operated at 160 °C with 180 mA current. Helium was used as the carrier gas at a flow rate of 22 mL/min, and 0.6 mL samples were injected using a gas-tight syringe (SGE Analytical Science, Austin, TX, USA).

Samples collected for quantification of VFAs were centrifuged at 15,000 rpm for 20 min and the supernatant was passed through a 0.22 μm PVDF syringe filter (Millipore Corp., Billerica, MA, USA). Filtered samples were acidified using 80–100 μL of 2 N HNO_3 depending on sample alkalinity in order to shift the pH to approximately 2 and VFA speciation to the volatile, protonated form. VFA concentrations were measured using a Hewlett–Packard (Palo Alto, CA, USA) 6890 series gas chromatograph and flame ionization detector. The column was a Grace (Grace Davison Discovery Sciences, Deerfield, IL, USA) AT™-AquaWax-DA (30 m × 0.25 mm) capillary column (PN 14537). Samples were injected using the Hewlett–Packard model 7679 auto-injector equipped with a 5 μL syringe using an injected volume of 0.5 μL in a 1:20 split ratio using helium as the carrier gas at a flow rate of 1.2 mL/min and nitrogen as the makeup gas. The temperature program used consisted of heating the oven to 50 °C for 2 min, ramp at 25 °C/min to 95 °C, then ramping at 10 °C/min to 150 °C, hold 3 min, ramping at 25 °C/min to 200 °C and hold for 12 min, for a total method length including cool-down of approximately 33 min.

Total and volatile solids were quantified in accordance with Standard Methods 2540G (APHA et al., 2012). A Thermo Fisher Scientific Accumet AP85 Waterproof pH/Conductivity Meter (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure pH, and was calibrated on a monthly basis.

Samples were collected on two occasions during the analysis period and shipped to the Dairy One Forage Laboratory (Ithaca, New York, USA) for quantification of acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), starch, crude fat, total phosphorus (TP), and crude protein. The methods and equipment used by the Dairy One laboratory are available at http://dairyone.com.

2.6. Comparative analysis of microbial populations

Microbial population analyses were conducted applying quantitative polymerase chain reaction (qPCR) based on 16S rDNA oligonucleotide primers following an approach similar to that described by Coats et al. (2012). Biomass samples were collected on four occasions from all digesters (operational days 14, 23, 29, and 38). Genomic DNA was extracted from each sample using the MO BIO PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA USA). 16S rDNA-based oligonucleotide primers were used to target the three principle orders of hydrogenotrophic methanogens (*Methanococcales* (*MCC*), *Methanobacteriales* (*MBT*), and *Methanomicrobiales* (*MMB*)), the two families of acetoclastic methanogens (*Methanosarcinaceae* (*MSC*) and *Methanosaetaceae* (*MST*)), all members of the domain Archaea and Bacteria, and prokaryotes (Table 1).

qPCR was performed on a StepOne Plus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using iTaq™ SYBR® Green Supermix with ROX (Bio-RAD Laboratories Inc., Hercules, CA, USA) with a total reaction volume of 25 μL. The qPCR process was performed under the following conditions: 3 min at 95 °C, 45 cycles of 30 s at 95 °C, 45 s annealing (annealing temperature varied from 55 to 60 °C depending on the primer set), and 30 s at 72 °C. Samples were prepared using 5 ng of total genomic DNA, quantified using a BioTek H1 hybrid multi-mode plate reader (BioTek, Winooski, VT, USA) and targeting a 500 nM final concentration of each primer per reaction. Annealing temperatures and primer concentrations were determined through an optimization process. All qPCR melting curves were evaluated to confirm a single melting peak, and agarose gel analysis confirmed one signal for each primer set. Amplification efficiencies were calculated for each primer set using baseline-corrected fluorescence data (StepOne software v2.0) and the LinRegPCR program (Ramakers et al., 2003). The cycle threshold was set at a constant value within the log-linear region across all samples for determination of quantification cycle (Cq) values, i.e., the cycle number at which the fluorescence value exceeded the threshold value. Multiple qPCR 96 well plates were processed and analyzed; average amplification efficiencies, calculated for each primer set and for each AD sample (Table 1), were consistent with that observed by others (Traversi et al., 2011; Yu et al., 2006). Comparisons of target gene quantities between samples were made by calculation of a relative expression ratio (RER) following an approach similar to that described by Čikoš and Koppel (2009) under the assumption that the 16S rDNA gene copy numbers for the groups being compared were identical. The RER for eubacteria was calculated relative to prokaryotes. Cq values for replicate samples on a given plate that differed by >±0.5 were discarded from the analyses.

Table 1
Primers and genetic sequences used in qPCR analysis.

Group	Primer	Sequence	Reference	Amplification Efficiency (%)
Methanococcales (*MCC*)	MCC 495F MCC 832R	TAA GGG CTG GGC AAG T CAC CTA GTY CGC ARA GTT TA	Lee et al. (2009), Yu et al. (2005)	AD3-c: 68.3–83.1 AD6: 71.2–83.1 AD8-c: 76.0–83.1
Methanobacteriales (*MBT*)	MBT 857F MBT 1196R	CGW AGG GAA GCT GTT AAG T TAC CGT CGT CCA CTC CTT		AD3-c: 80.1–88.4 AD6: 84.1–90.6 AD8-c: 82.2–92.4
Methanomicrobiales (*MMB*)	MMB 282F MMB 832R	ATC GRT ACG GGT TGT GGG CAC CTA ACG CRC ATH GTT TAC		AD3-c: 78.8–93.1 AD6: 92.7–87.9 AD8-c: 81.4–87.1
Methanosarcinaceae (*MSC*)	MSC 492F MSC 828R	GAA ACC GYG ATA AGG GGA TAG CGA RCA TCG TTT ACG		AD3-c: 81.3–92.3 AD6: 82.4–91.3 AD8-c: 79.3–85.1
Methanosaetaceae (*MST*)	MST 702F MST 862R	TAA TCC TYG ARG GAC CAC CA CCT ACG GCA CCR ACM AC		AD3-c: 57.7–77.4 AD6: 61.9–68.6 AD8-c: 60.6–69.7
All members of *Archaea*	ARC 349F ARC 806R	GYG CAS CAG KCG MGA AW GGA CTA CVS GGG TAT CTA AT	Takai and Horikoshi (2000)	AD3-c: 80.2–92.1 AD6: 84.5–87.5 AD8-c: 83.2–90.6
All members of *Bacteria*	BAC 338F BAC 515R	ACT CCT ACG GGA GGC AGC AG TTA CCG CGG CTG CTG GCA C	Huse et al. (2008)	AD3-c: 82.2–100.4 AD6: 86.8–97.7 AD8-c: 86.9–97.3
Prokaryotes	Prok 341F Prok 806R	CCT ACG GGR BGC ASC AG GGA CTA CYV GGG TAT CTA AT	Yu et al. (2005)	AD3-c: 69.8–81.5 AD6: 65.9–82.6 AD8-c: 69.7–76.5

2.7. Statistical methods

Single factor or two-factor ANOVA was used to establish differences in means using Microsoft Excel, with significance declared at $p < 0.05$.

3. Results

Two integrated two-stage AD systems were deployed for this study (Figs. 1a and 1b); as shown, the comparative systems ultimately included three separate ADs. The principle goal of this research was to develop an enhanced understanding of the potential and viability of separating fine and coarse residual solids from the fermenter for targeted, and enhanced overall, methanogenesis. Results presented and discussed herein represent data collected over an 85-day steady state operational period.

3.1. Comparative methane production

Typical methane content of AD-treated biomass ranges from 48% to 65% (Ward et al., 2008). Comparatively, previous investigations into this two-stage AD configuration revealed an average methane content of 54% (Coats et al., 2012). For these current investigations, methane content in the biogas over the analysis period was stable for all three digesters, both individually and for the combined system measurements (Table 2). The average methane content ranged from 57.1% (AD-3c; coarse solids only) to 64.4% (AD-8; fine solids) to 60.9% (AD-6c; combined solids); of note, AD of the fine solids generated methane content near the maximum expected for AD of agricultural wastes (Ward et al., 2008), while even the less digestible solids stream produced average concentrations. After an operational period of 38 days, the biogas exhaust lines of reactors AD3-c and AD8 (System 2) were spliced together, and biogas production from both digesters was measured using a single meter; this approach allowed measurement and characterization of the combined System 2 biogas, for enhanced direct comparison with System 1. During the operational period wherein biogas was combined from the System 2 ADs, the average methane content of AD6-c (System 1) was 61.3%, while that of System 2 was 59.9%; the difference was statistically significant ($p = 6.46 * 10^{-22}$).

Table 2
Comparative mean biogas production, composition and methane yield for the ADs. Confidence intervals represent one standard deviation. Values shown for AD3-c and AD8 are based on the first 37 days of data collection.

Reactor	Biogas (L/d@STP)	Methane content (%, v/v)	Methane (L/d@STP)
AD3-c	28.5 ± 1.9 (n = 36)	57.1% ± 0.7% (n = 30)	16.3 ± 1.0 (n = 36)
AD8	9.7 ± 1.2 (n = 35)	64.4% ± 1.0% (n = 30)	6.3 ± 0.8 (n = 35)
AD6-c (System 1)	37.2 ± 2.4 (n = 76)	60.9% ± 1.2% (n = 71)	22.7 ± 1.6 (n = 76)
AD3-c + AD8 (System 2)	35.0 ± 3.2 (n = 83)	59.9% ± 0.6% (n = 41)	20.8 ± 1.8 (n = 82)

In addition to methane content, the methane production from both systems was quite stable over the extended analysis period, an outcome that could be considered unexpected given that raw manure (an inherently heterogeneous substance) was used as the substrate. However, such stability reinforces the value of the two-stage AD configuration, as compared to the conventional single-state approach (i.e., raw manure), and reinforces original findings (Coats et al., 2012). Over the full analysis period, average methane production from System 1 (22.7 L/d) was statistically higher ($p = 6.82 * 10^{-6}$) than observed in System 2 at 20.8 L/d (Table 2 and Fig. 2).

Considering that methane production and yield values were actually highest for the combined solids System 1 – ultimately disproving the hypothesis driving this investigation – attention turned to further data interrogation for potential explanations as to why combined solids AD was more productive. The remainder of this paper focuses on discussion in this regard.

3.2. Volatile solids reduction and methane production

Care was taken to ensure the OLRs applied (on a VS basis) to each System were similar, so that differences in VS loading did

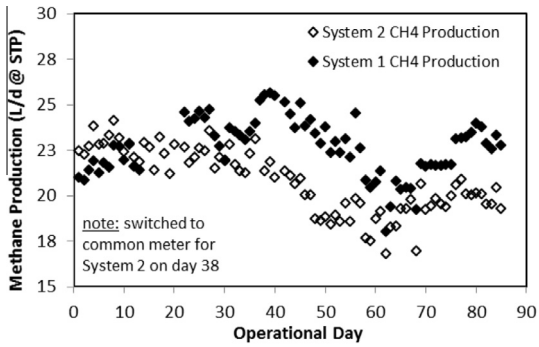

Fig. 2. Daily methane production over the analysis period for System 1 (AD6-c) and System 2 (AD3-c + AD8 combined).

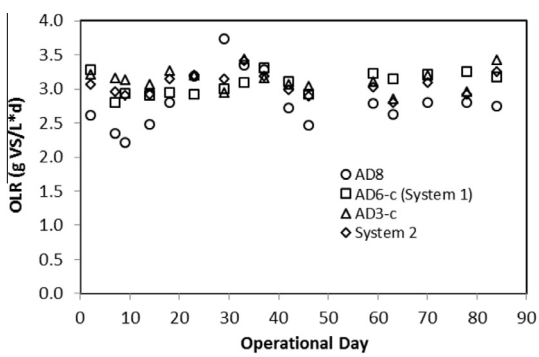

Fig. 3. Individual digester and System 2 (AD3-c + AD8) organic loading rates.

not influence the comparative operations and associated results. However, the digester OLRs were not regulated directly, and were instead controlled by the loading applied to, and the reactions occurring within, the upstream fermenters. Moreover, the production of fine solids by F3-c was variable, causing the loading rate of AD8 to fluctuate more than that of AD3-c and AD6-c (Fig. 3). Nevertheless, there was ultimately no statistical difference between the mass of VS applied to the two digestion systems or to their respective fermenters (Table 3).

While OLRs remained statistically the same, the observed VS destruction in System 1 was statistically higher than that of System 2 ($p = 0.02$; Table 4). Specifically examining the individual reactors, VS reduction averaged 14–17% in the fermenters (Table 4), and was subject to substantial uncertainty associated with variability in the composition of the raw manure. Note also that some of the fermenter VS reduction was due to biogas production; the methane content of biogas produced by F3-c averaged 45.8% ± 2.4% ($n = 10$), while that of F6-c averaged 42.9% ± 1.46% ($n = 5$), with the remainder consisting primarily of carbon dioxide. Observed AD VS reduction, at 30–35%, was slightly lower than reported values for raw dairy manure (El-Mashad et al., 2008; Kaparaju and Rintala, 2011) but comparable with previous research on this process configuration ((Coats et al., 2012); the OLR in this current study was also 24–31% lower than the previous study). However, total VS destruction across the fermenter and AD (exceeding 45%) was higher than typically observed for raw dairy manure, indicating the two-stage process achieves better overall capture of valuable carbon. Comparing VS destruction between digesters, AD8 (fine solids) and AD6-c (combined solids) exhibited similar reduction (approximately 34%; Table 5), while that of AD3-c (coarse solids) was only 30.4%. These comparative results indicate the readily biodegradable component of the coarse solids fraction was substantially smaller than that of the fine solids fraction, which could have adversely impacted methane production in the System 2 configuration. Moreover, since VS destruction in anaerobic digestion correlates directly with methane production, it would appear that separate digestion of the fine and coarse solids did not improve overall VS reduction, and instead the separation of solids streams appeared to adversely impact methanogenesis (these interpretations align with the methane results presented and discussed above).

Methane yield is a function of a range of factors, including the composition of the substrate (e.g., its biodegradability), and a range of values have been reported in the literature. Yields of 0.075–0.223 L CH_4/g VS applied were observed by Ogejo and Li (2010) digesting raw dairy manure, and Hawkes et al. (1984) reported yields of 0.166–0.204 L CH_4/g VS applied at SRTs from 5 to 15 days from screened dairy manure. The International Panel on Climate Change (IPCC) reports an optimal value for the ultimate methane production potential of raw dairy manure (i.e., optimal because that value would be generated by a biomethane potential (BMP) test) of 0.240 L CH_4/g VS applied (IPCC, 1997).

Table 3
Average solids characteristics and pH in feed and effluent of each reactor. Confidence intervals represent one standard deviation.

Reactor	TS (%)	VS (% of TS)	Total VS mass into or out of reactor per day (g)	pH
F3-c influent	15.7% ± 1.5% ($n = 16$)	83.0% ± 1.4% ($n = 16$)	179.3 ± 13.0 ($n = 16$)	7.4 ± 0.6 ($n = 16$)
F6-c influent	15.9% ± 1.6% ($n = 16$)	83.1% ± ± 1.4% ($n = 16$)	182.1 ± 13.1 ($n = 16$)	7.4 ± 0.4 ($n = 16$)
F3-c effluent	3.7% ± 0.3% ($n = 16$)	81.4% ± 1.3% ($n = 16$)	153.5 ± 8.8 ($n = 16$)	6.7 ± 0.1 ($n = 16$)
F6-c effluent	3.6% ± 0.2% ($n = 16$)	81.2% ± 1.1% ($n = 16$)	151.1 ± 6.8 ($n = 16$)	6.6 ± 0.1 ($n = 16$)
AD3-c influent	6.6% ± 0.3% ($n = 16$)	88.6% ± 1.3% ($n = 16$)	94.3 ± 4.8 ($n = 16$)	6.8 ± 0.2 ($n = 16$)
AD6-c influent	6.8 % ± 0.4% ($n = 16$)	85.7 ± 1.3% ($n = 16$)	123.2 ± 6.4 ($n = 16$)	6.8 ± 0.1 ($n = 16$)
AD8 influent	7.2% ± 1.1% ($n = 16$)	77.3% ± 1.6% ($n = 16$)	28.1 ± 4.0 ($n = 16$)	6.9 ± 0.1 ($n = 15$)
AD3-c effluent	5.0% ± 0.2% ($n = 16$)	85.5% ± 1.0% ($n = 16$)	65.5 ± 2.1 ($n = 15$)	7.3 ± 0.1 ($n = 16$)
AD6-c effluent	4.9% ± 0.2% ($n = 17$)	81.2% ± 1.6% ($n = 17$)	80.0 ± 2.3 ($n = 14$)	7.4 ± 0.1 ($n = 17$)
AD8 effluent	5.2% ± 0.4% ($n = 16$)	70.5% ± 1.4% ($n = 16$)	18.2 ± 1.1 ($n = 16$)	7.6 ± 0.1 ($n = 16$)

Table 4
Average organic loading rate and VS destruction in each reactor. Confidence intervals represent one standard deviation. Methane production normalized to grams VS applied, g VS destroyed, and liters of active digester volume. Values shown for AD3-c and AD8 are based on the first 37 days of data collection, while days 38–85 are reflected in the AD3-c + AD8 data. Confidence intervals represent one standard deviation.

Reactor	OLR (g VS/L * d)	VS Destruction (%)	L CH_4/g VS applied to digester	L CH_4/g VS applied to fermenter	L CH_4/g VS destroyed
F3-c	9.0 ± 0.7 (n = 16)	14.0% ± 7.1% (n = 16)	–	–	–
F6-c	9.1 ± 0.7 (n = 16)	16.8% ± 5.6% (n = 16)	–	–	–
AD3-c (coarse solids)	3.1 ± 0.2 (n = 16)	30.4% ± 4.2% (n = 15)	0.17 ± 0.01	N/A	0.55 ± 0.08
AD8 (fine solids)	2.8 ± 0.4 (n = 16)	34.2% ± 9.0% (n = 16)	0.22 ± 0.05	N/A	0.55 ± 0.24
AD6-c (System 1; combined solids)	3.1 ± 0.2 (n = 16)	34.9% ± 3.4% (n = 14)	0.18 ± 0.02	0.12 ± 0.01	0.52 ± 0.08
AD3-c + AD8 (System 2)	3.1 ± 0.2 (n = 16)	31.6% ± 4.2% (n = 15)	0.17 ± 0.02	0.12 ± 0.01	0.54 ± 0.11

Table 5
Average reactor influent/effluent composition on a percent of dry matter (percent) basis. Confidence intervals represent one standard deviation. (NDF = neutral detergent fiber; ADF = acid detergent fiber).

Sample Location (n = 2)	Crude protein	ADF	NDF	Crude Fat	Lignin	Starch
Fermenter influent	17.7% ± 1.5%	31.1% ± 4.3%	42.0% ± 0.4%	2.1% ± 0.1%	10.9% ± 2.7%	4.9% ± 2.1%
F3-C effluent	18.7% ± 3.8%	33.1% ± 1.9%	47.4% ± 3.5%	3.6% ± 1.0%	12.5% ± 2.4%	0.6% ± 0.4%
F6-c effluent	19.4% ± 2.3%	32.5% ± 1.0%	44.1% ± 2.0%	4.1% ± 0.2%	12.2% ± 0.4%	0.6 ± 0.4%
AD3-c influent	10.7% ± 0.4%	46.0% ± 0.5%	64.1% ± 4.2%	1.8% ± 0.1%	16.0% ± 0.9%	2.4% ± 0.1%
AD3-c effluent	12.5% ± 0.9%	54.1% ± 2.1%	64.1% ± 2.8%	1.2% ± 0.1%	23.2% ± 2.1%	0.5% ± 0.1%
AD8 influent	30.4% ± 0.1%	20.9% ± 0.1%	37.9% ± 1.2%	5.3% ± 1.1%	9.2% ± 0.4%	0.3% ± 0.3%
AD8 effluent	26.6% ± 1.3%	29.4% ± 2.0%	37.3% ± 1.4%	1.5% ± 1.6%	15.3% ± 1.5%	0.6% ± 0.1%
AD6-c influent	17.1% ± 1.8%	42.1% ± 4.1%	56.6% ± 4.8%	2.8% ± 0.8%	16.7% ± 3.2%	1.4% ± 1.1%
AD6-c effluent	15.8% ± 0.8%	46.1% ± 0.3%	57.9% ± 5.4%	1.3% ± 0.2%	21.7% ± 1.1%	0.4% ± 0.1%

Comparatively, the digestion systems examined in this research processed pre-fermented organic material remaining after diverting much of the soluble carbon (e.g., VFAs) to PHA production. Thus, as might be expected, the yields of System 1 and System 2 normalized to the VS mass applied to the fermenters (i.e., the mass of VS in the raw manure entering the system and available for combined PHA and CH_4 production) fall into the lower end of the range of yields per gram VS applied reported in the literature (Table 5); gross yields were approximately 50% of the IPCC optimal yield value. However, when methane production was normalized to the VS mass applied to each digester (Table 5), the values were comparatively in the higher end of those typically reported, indicating very effective digester performance. Moreover, methane yield on fine solids (AD8) was near the IPCC optimal, at 0.22 L CH_4/g VS applied. Conversely, methane yield on the coarse solids fraction (which, on a mass basis, was the dominant fraction), at 0.17 L CH_4/g VS applied, significantly reduced the overall average of the separate solids AD yield (compared with System 1). Combined with the VS destruction data, these results suggest that digesting coarse solids alone yields a less efficient biochemical system in terms of organics degradation, which contributed to disproving the research hypothesis. Note that, for this study, reported methane production volumes were adjusted to reflect the presence of biogas water vapor, and also are reported in units of standard temperature and pressure (STP). In contrast, most published biogas measurements in AD research are frequently not reported with such corrections; biogas data reported at ambient or elevated temperature/pressure would skew numbers higher, which lends additional credibility to the process and results detailed herein.

3.3. VFA analysis

Influent/effluent VFA concentrations are an effective and necessary process monitoring parameter associated with AD, and examination of such data can potentially explain differential process performance. A healthy AD should consume nearly all available and produced VFAs, with an effluent concentration less than 100–200 mg/L (Speece, 2008). Comparing Systems 1 and 2, average total effluent VFA concentrations were statistically similar between the two fermenters (p = 0.075). Each digester effectively removed nearly all VFAs present in the fermenter effluent (i.e., AD influent), along with any generated in the digester (Fig. 4); digester effluent VFA concentrations were well below the recommended 100–200 mg/L maximum (averages of 67 mg COD/L (AD3-c); 53 mg COD/L (AD6); 6.4 mg COD/L (AD8-c)). Of note, AD8 (fine solids) exhibited the best performance of the three digesters, with effluent VFA concentrations approaching non-detect. The average effluent VFA concentration of AD3-c (coarse solids) was somewhat higher than that of AD6-c (combined solids), although the difference was not statistically significant (p = 0.25). Combined, there was no statistical difference in the average mass of VFAs present in the effluent of the two AD systems (p = 0.86). Ultimately, the low effluent VFA concentrations suggest a healthy and syntrophic microbial culture in the two systems.

What is not reflected in the influent/effluent VFA data is the relative in-process potential to generate additional VFAs, specifically associated with the substrate; such potential could help explain the differential process performance (and associated hypothesis rejection). Indeed, characterization of the different solids fractions

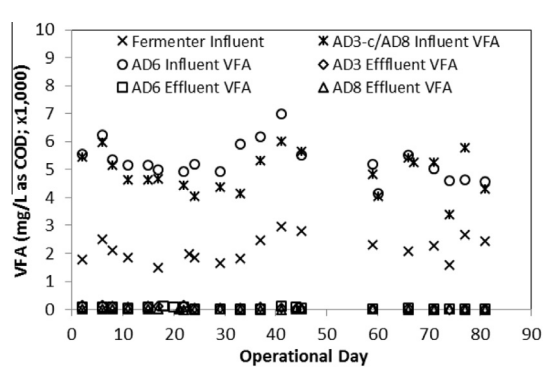

Fig. 4. Influent and effluent VFA concentrations of each reactor.

(Table 6) suggests that the difference in methane production between the three ADs was more likely due to the fermentative processes of hydrolysis and/or acidogenesis. Specifically, crude fat content was significantly higher in AD8 as compared with AD3-c, while influent solids to AD6-c were also relatively rich in crude fat. Fermentation of the crude fat fraction would be an efficient metabolism for generating methane precursors (acetate and hydrogen; both for acetoclastic and hydrogenotrophic archaea). Similarly, crude protein would generate quality methane precursors (principally acetate), and both AD8 and AD6-c exhibited protein degradation. Conversely, starch content (acetate production) would appear to have contributed more significantly to methane production in AD3-c. Thus, it would appear that the combined solids System 1 AD received a more heterogeneous mix of methane precursor substrate to support both acetoclastic and hydrogenotrophic archaea (note that additional microbial discussion follows in the next section), which ultimately led to improved methane production (as compared with AD3-c and AD8, which received a less heterogeneous solids substrate).

3.4. Microbial population comparisons

As a final interrogation of the respective ADs, and in further search of an explanation for differential process performance, the microbial populations were examined applying qPCR. Characterization of the microbial community within the respective systems can provide important insight into potential process functions, as demonstrated by previous studies into this two-stage AD configuration (Briones et al., 2014; Coats et al., 2012). For this investigation, samples for qPCR analysis of the microbial consortia were collected on days 14, 23, 29, and 38 from the three digesters and evaluated for archaeal species and bacteria. The relative expression ratio (RER) was calculated between each AD configuration for each targeted gene sequence in each reactor comparison combination; qPCR RER results are shown in Table 6. First considering hydrogenotrophic archaea, AD8 (fine solids) was very much more enriched in the hydrogen-utilizing *Methanomicrobiales*, and to a lesser degree *Methanococcales* (followed by AD6-c and, to a much lower degree, AD3-c). The higher relative concentrations of these hydrogenotrophs also correlated with elevated biogas methane content (Table 2); the methane content of AD8 was greater than that of AD6-c, which was greater than that of AD3. The larger hydrogenotrophic population would be expected to produce a biogas with a higher methane fraction, as these archaea utilize carbon dioxide during methane synthesis. Greater abundance of hydrogenotrophs thus appears to be correlated with the inclusion of the fine solids material in the digester feed (AD8; AD6-c) and the associated production of hydrogen through β-oxidation of fats, which, as noted, were present at elevated comparative levels in the fine solids (Table 6). The exception to this comparative archaeal observation was order *Methanobacteriales* (*MBT*), which appeared (across the four sampling periods) to generally exhibit similar concentrations in the three ADs, on average. Interestingly, recent research (Danielsson et al., 2012) found the rumen of dairy cows dominantly enriched with MBT; thus, it would be reasonable to expect the three ADs would generally exhibit similar MBT concentrations, given the source of substrate.

Regarding acetoclastic methanogens (which use acetate as the methane precursor), although as noted the overall VFA effluent concentrations were quite low for all ADs, effluent acetate concentrations in AD3-c were on average the highest, followed by AD6-c and AD8 (44, 31, and 5 mg/L, respectively). The maximum specific growth rate, μ_{max}, as well as the half-saturation coefficient, K_s, are higher in *Methanosarcina* than in *Methanosaeta* (Speece, 2008). Consequently, the presence of *Methanosarcina* tends to correlate with higher digester effluent acetate concentrations, while *Methanosaeta* is more likely to be found in digesters operated at lower acetate concentrations (De Vrieze et al., 2012). This relationship was well reflected by the qPCR data, with AD8 (lowest effluent HAc concentration) dominated by *Methanosaeta*, while AD3-c and AD6-c were dominated by *Methanosarcina*; from the RER perspective, AD8-c was minimally enriched for *Methanosarcina*. Finally, RER analysis of the archaeal population suggests that all three ADs, on average across the four sampling points, were comparably enriched with methanogens; these results align with the general consistency and productivity in methane from all three ADs.

In addition to methanogenic archaea, comparison of relative quantities of the domain Bacteria is of interest, as its members are responsible for the hydrolysis and subsequent fermentation of cellulosic material (Lynd et al., 2002; Thomas et al., 2011); a digester enriched with a larger bacterial population would be capable of an increased degree of cellulose degradation. In this regard, qPCR results suggest that bacteria were most prevalent in AD3-c (coarse solids), with AD8 and AD6-c generally exhibiting comparable concentrations. With the coarse solids being more difficult to degrade (being more enriched with ADF, NDF, and starch, all cellulose-based substrates; Table 6), these results suggest that the AD bacterial communities developed specific to substrate and overall process needs (i.e., less digestible substrate demands more bacteria); this interpretation also aligns with previous molecular interrogation of the respective AD configurations (Briones et al., 2014).

Table 6
Summary of RER comparisons between reactors. RER values for methanogens are relative to archaea; archaeal RER are relative to eubacteria; bacteria are relative to prokaryotes. Confidence intervals represent one standard deviation. Hydrogenotrophic methanogens: *Methanococcales* (*MCC*), *Methanobacteriales* (*MBT*), and *Methanomicrobiales* (*MMB*); the two families of acetoclastic methanogens (*Methanosarcinaceae* (*MSC*) and *Methanosaetaceae* (*MST*)). (N.D. = no data) (qPCR replicates: day 14, $n = 4$; day 23, $n = 7$; day 29, $n = 6$; day 38, $n = 4$).

Contrast	Operational day	MCC	MBT	MMB	MSC	MST	ARC	BAC
AD3-c vs. AD6-c	14	N.D.	0.67 (0.33)	0.26 (0.08)	0.65 (0.17)	0.63 (0.34)	1.66 (1.38)	5.42 (4.40)
	23	0.22 (0.06)	0.81 (0.24)	0.30 (0.18)	0.49 (0.15)	0.16 (0.16)	1.18 (0.29)	2.02 (0.28)
	29	N.D.	2.05 (0.19)	0.37 (0.05)	0.75 (0.06)	0.24 (0.03)	0.54 (0.07)	2.10 (0.56)
	38	0.24 (0.01)	0.91 (0.19)	0.92 (0.16)	5.97 (2.23)	1.72 (1.82)	0.55 (0.12)	3.08 (0.87)
AD8 vs. AD6-c	14	0.63 (0.01)	0.87 (0.22)	1.64 (0.27)	0.16 (0.14)	3.20 (1.67)	0.84 (0.31)	1.81 (0.20)
	23	1.43 (0.25)	2.14 (1.20)	8.40 (5.84)	0.48 (0.10)	N.D.	0.28 (0.24)	0.22 (0.05)
	29	N.D.	1.36 (0.19)	2.50 (0.10)	0.08 (0.02)	1.31 (0.78)	0.88 (0.10)	0.91 (0.09)
	38	0.39 (0.00)	0.73 (0.05)	2.12 (0.23)	0.08 (0.04)	2.95 (2.59)	1.91 (0.16)	2.34 (0.55)
AD8 vs. AD3-c	14	2.71 (1.19)	1.44 (0.40)	6.05 (0.22)	0.25 (0.05)	7.25 (5.78)	0.73 (0.43)	0.53 (0.36)
	23	6.75 (0.68)	3.41 (1.93)	41.2 (29.5)	1.33 (0.36)	N.D.	0.22 (0.19)	0.10 (0.02)
	29	13.38 (4.49)	0.67 (0.14)	6.99 (1.24)	0.13 (0.04)	6.47 (3.29)	1.65 (0.31)	0.45 (0.10)
	38	1.60 (0.05)	0.83 (0.14)	2.36 (0.38)	0.01 (0.00)	3.03 (1.75)	3.53 (0.65)	0.81 (0.30)
Summary (digesters)		8 > 6 > 3	6 ~ 8 ~ 3	8 > 6 > 3	6 > 3 > 8	8 > 6 > 3	6 ~ 8 ~ 6	3 > 8 ~ 6

4. Conclusions

Research presented herein centered on the hypothesis that separately digesting fine and coarse solids (vs. combined) from fermented dairy manure would improve methane production; the hypothesis was disproven. While maximum biogas methane concentration was realized on fine solids, combined solids AD realized enhanced VS destruction. The diverse combined-solids substrate enriched for a more heterogeneous bacterial/archaeal consortium that balanced fermentation and methanogenesis to yield maximum product (methane). However, results suggest that targeted AD of the fat-rich fine solids could be a more optimal approach for processing manure; alternate (non-AD) methods could then be applied to extract value from the fibrous fraction.

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