Modeling and Analysis

Biofpr Biofuels Bioproducts & Biorefining

An integrated two-stage anaerobic digestion and biofuel production process to reduce life cycle GHG emissions from US dairies

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Abstract: Over 9 million dairy cows generate an estimated 226 billion kg of wet manure annually in the USA. To help mitigate dairy greenhouse gas (GHG) emissions associated with the degradation of this organic-rich waste, manure can be processed via anaerobic digestion (AD) to methane and ultimately electricity. This potential value of AD has generated high-level dairy-industry support for broad-scale technology deployment; however, on-the-ground AD realization has been impeded by process stability/reliability concerns and poor economics. Considering these challenges but recognizing that AD represents a fundamentally sound manure-management approach, an interdisciplinary research team has completed proof-of-concept investigations on an integrated process that will concurrently improve manure management economics and reduce dairy GHG emissions. The integrated processes center on a two-stage fermentation/AD system that can generate methane quantity/quality comparable to conventional single-stage AD. Molecular level investigations confirm that the AD is highly enriched with a unique and synergistic microbial population which yielded a more resilient and stable process. Beyond AD, algae grown on nitrogen/phosphorus-rich AD supernatant in a photobioreactor yielded biomass concentrations approaching 1.0 g L^{-1}; despite an apparent growth lag/inhibition associated with excess organic acids and ammonia, algae growth was significant. Environmental life cycle assessment (LCA) demonstrated that the two-stage AD configuration coupled with algae production can reduce GHG emissions by approximately 60% as compared with a traditional anaerobic lagoon. The end result is a manure-management platform that can increase US dairy viability and sustainability. Ongoing investigations are aimed at process refinement with an ultimate commercialization goal.

Keywords: anaerobic digestion; algae; methane production; life cycle assessment

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Introduction

Dairies and environmental emissions

Over 9 million dairy cows generate an estimated 226 billion kg of wet manure and approximately 5.8 billion kg of carbon dioxide (CO_2) equivalents annually in the USA.[1] These emissions constitute an estimated 7% of the 2005 greenhouse gasses GHGs; see Table 1 in the USA[2] and make dairies one of the largest single industry sources.[3] Recognizing this concern, in January 2009 the Innovation Center (IC) for US Dairy announced a voluntary goal to reduce dairy GHG emissions 25% by 2020.[4] Beyond dairy GHG emissions, manure nutrient management is also a challenge, as each tonne of manure contains approximately 6.6 kg of N and 1.1 kg of P.[5] Manure land application – a common practice – can yield excess P in the soil,[6] which can contribute to advanced surface water eutrophication associated with water run-off. Ground water nitrate concentrations have also been found to be elevated associated with manure land application.[7] Recognizing these potential water quality risks associated with land application of manure, the US EPA has strengthened rules associated with feedlot operations.[8] As US dairies look to the future, it will be critical to remedy these environmental emissions in order to remain economically competitive; US consumers certainly desire a positive outcome in this regard, not only for environmental protection but also to ensure that our dairy product supplies remain local.

Toward environmentally effective dairy manure management

Historically lagoons and/or pits have been the most common form of manure management, storage, and/or treatment at dairies[9] principally due to ease of construction and operation. However, these technologies do little to mitigate GHG emissions or to sequester phosphorus and nitrogen. Nutrient management practices have conventionally centered on agronomic land application, although this practice is becoming increasingly difficult for dairy operations associated principally with water quality concerns. More recently, recognizing the intrinsic value of this organic-rich waste and the need to reduce GHG emissions, the trend has been to use dairy manure for the production of electricity through anaerobic digestion (AD).[10] In the process of AD, micro-organisms convert manure to a CH_4-rich biogas which can be burned in a generator to produce electricity. Not only can AD generate energy, the process

Table 1. Summary and explanation of nomenclature and abbreviations.

Abbreviations and Nomenclature	Explanation
Anaerobic Digestion (AD)	Engineered biological process wherein organic-rich substrate is mixed with a consortium of fermenting bacteria and methane forming archaea, with the ultimate bio-production of a biogas rich in CH_4 and CO_2.
ADE	Anaerobic digester effluent
Biogenic	Carbon emissions associated with the CO_2 cycle for plant growth and subsequent utilization and food
DOC	Dissolved organic carbon
Eutrophication	Excess input of nitrogen and phosphorus to a natural water body (surface or groundwater), leading to excess algal growth and eventual reduction in dissolved oxygen.
GHGs	Greenhouse Gases, including CH_4, CO_2, and N_2O.
GWP	Global warming potential; metric to assess the potential for GHGs to induce a change in earth's climate
HRT	Hydraulic retention time; the average time a liquid slurry resides inside a reactor
IC	Innovation Center for U.S. Dairy
LCA	Environmental life cycle analysis
P	Phosphorus
PBR	Algae photobioreactor
PCR	Polymerase chain reaction; a molecular method employed to generate large quantities of copied DNA
N	Nitrogen
RT	Retention time
SRT	Solids retention time; the average time microorganisms reside inside a biological reactor
VFAs	Volatile fatty acids (e.g., acetate, propionate, etc.)

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can help reduce dairy GHG emissions via the conversion of CH_4 to CO_2. The US Environmental Protection Agency (EPA) has identified approximately 6800 candidate dairies for AD and estimates that AD biogas could be used to generate >6,800 GWh yr^{-1} in power, roughly equivalent to the average annual electricity usage of 500 000 to 600 000 homes.[10] In other words, manure AD-to-electricity could displace up to 2.4% of US electricity demand while simultaneously reducing the dairy industry's GHG footprint.[2] Recognizing this real potential for ADs to mitigate dairy GHG emissions while producing a valuable commodity, the IC has proposed construction of 1300 new dairy ADs as a centerpiece in meeting its targeted GHG reduction goal.

Despite IC support behind broad dairy AD deployment, unfortunately manure AD technology is not yet sufficiently economical, reliable, or stable to realize widespread use at dairies. Based principally on low electricity prices but also considering the one-dimensional aspect of AD, it has been suggested that AD alone as a commodity production strategy is not anticipated to develop significant economic traction for over 10 years.[11] As evidence, nationally there are only approximately 140 ADs in use (as of 2012); only 2% of candidate facilities employ this technology while only approximately 5% of the energy potential is realized.[10] Ultimately, the economic constraints of a dairy manure-to-power-only implementation plan, based on current technologies, intrinsically curb the developer investment that is necessary to achieve the IC's goals. Novel strategies for improving AD technology, enhancing economics, and further reducing the environmental footprint are necessary to enhance the likelihood of achieving the ICs AD target.

Limits to conventional AD practices

Conventionally AD is used to process raw organic matter in a single- or two-stage configuration.[12] In a single-stage system, all three synergistic AD microbial metabolisms – hydrolysis, fermentation, and methanogenesis – occur concurrently within a single tank. However, CH_4 production inefficiencies arise associated with maintaining environmental and growth conditions that intrinsically compromise individual metabolism efficiencies to ensure overall process stability.[13] Fermenting bacteria prefer a pH of 5.2–6.5 and exhibit a doubling time of 2–4 d, whereas methanogens prefer a pH of 6.6–7.6 and realize a doubling time of 3.5–4 d.[12, 13] Thus, single-stage ADs typically operate at a compromising neutral pH. Beyond pH, and perhaps more importantly with regard to maximizing value recovery from manure, single-stage AD is limited by hydrolysis rates[14] and thus not all the high-value organic matter can be recovered.

Two-stage AD was developed to remedy some single-stage inefficiencies.[15] In this process configuration hydrolysis and fermentation occur in one tank, and VFA-rich liquor is transferred to a second-stage reactor for methanogenesis.[16] The two-stage configuration allows for semi-optimization of the hydrolysis/fermentation and methanogenesis metabolisms, thereby potentially enhancing methane production. However, while two-stage AD can enhance process stability, ultimately this configuration also leaves significant amounts of high value organic matter undigested associated with necessarily shorter retention times (RTs) in the hydrolysis/fermentation tanks.

A dairy manure solution: Integrated bio-energy production

Despite the challenges to AD, the technology remains a logical centerpiece for manure management because it can generate renewable, base-load energy while reducing GHG emissions from dairies. The fundamental challenge is to advance a broader, integrated process scheme that diversifies the commodity portfolio while concurrently mitigating environmental emissions and enhancing overall process stability. In response to this challenge, a multidisciplinary research team has advanced the concept presented and discussed herein focused on a suite of technologies that collectively maximize resource recovery for bio-energy production from manure (Fig. 1).

Novel two-stage AD operations

The proposed integrated resource recovery process centers on a novel two-stage AD process configuration.[17] As illustrated (Fig. 1), manure is first fermented to produce an effluent rich in VFAs; fermenter liquor characterization is provided elsewhere.[17, 18] In contrast to all conventional AD operating schemes, the downstream AD does not receive the VFA-rich supernatant. Rather, in our configuration, the VFA-rich supernatant fraction generated in the hydrolysis/fermentation stage would be recovered to produce other commodities (e.g. bioplastics[19], biofuel[20]), leaving the partially biodegraded, thickened manure to be digested for the synthesis of CH_4-rich biogas and electricity production. To the best of our knowledge this concept of anaerobically digesting thickened pre-fermented organic matter (that is largely depleted of readily hydrolysable carbohydrates and is also without most of the VFA-rich supernatant) has only once been investigated

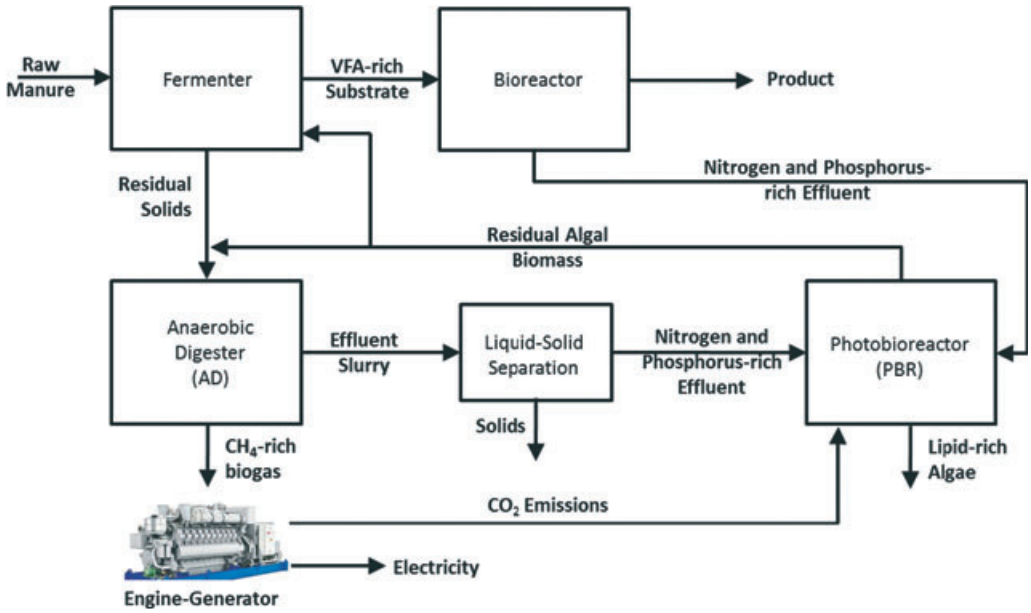

Figure 1. Proposed dairy manure to biofuel/biopower/bioproducts process. The two-stage anaerobic digestion-centered process integrates processes to maximize recovery and conversion of valuable manure constituents to multiple high value commodities.

and documented;[17] preliminary process investigations, however, were highly encouraging.

Enhanced AD stability through balanced microbiology

A central operational challenge with conventional AD is the estimated order-of-magnitude differential between fermentation and methanogenesis kinetics.[21] Readily fermentable manure, once hydrolyzed, is rapidly converted to VFAs, which can result in an excess of methanogenic substrate that can induce process upset/failure. Both conventional single- and two-stage AD operations are susceptible to process upset associated with imbalanced process kinetics. As a contrast, our configuration can improve process stability by achieving better balance in microbial ecology and process stoichiometry between the fermenting microbes and methanogenic archaea. Specifically, by partially oxidizing manure through pre-fermentation, the rate of VFA production in the AD will be reduced. The goal is a synergistically balanced microbial consortium that generates and consumes substrate at rates that maintain relative equilibrium, resulting in a more stable, robust, and reliable AD process. Moreover, if we can concurrently enrich the consortium with the methanogen *Methanosarcina,* the AD process should become more substrate-flexible in that CH_4 could be produced from diverse substrate such as acetate and/or H_2/CO_2 without the need to select for functionally distinct populations.[22]

Upcycling AD effluent for algae production

Incorporating an algal CO_2 capture stage within the integrated system can further reduce the dairy GHG footprint and generate a high value commodity. AD effluent rich in nitrogen and phosphorus can be used to support algal growth; to enhance algal growth rates CO_2-rich combustion gas can be diverted to the algal photobioreactor (PBR). This approach can mitigate a problematic component of AD systems, provide additional GHG mitigation through carbon assimilation, and improve AD system economics by generating an additional commodity (i.e. algal lipids for biofuel production, or slow-release fertilizer[23]).

The use of algal production systems for nutrient recovery from animal waste management systems is not novel,[23–27] and the concept of producing lipid-rich algae as a means of producing biodiesel or jet fuel has been extensively investigated.[28–30] What sets our strategy apart is that we physically connect algal production to dairy wastewater treatment for biofuel production and carbon sequestration. Photosynthetic rates from a variety of algal species (e.g. *Chlorella vulgaris* strain UTEX 2714, *Chlorella* sp. UTEX 2168, *C. zofingiensis* UTEX-B32, *Botryococcus braunii*) suggest that algal C-fixation and subsequent biomass conversion to biofuels could significantly impact the GHG footprint of dairies and other animal waste management systems while treating a problematic nutrient rich

wastestream. Compared to conventional algal biofuel systems this approach can minimize costly nutrient import, reduce water use, and improve AD system economics.

Although AD effluents present a viable opportunity as a waste-based feedstock for algal production, the physiochemical characteristics of the effluent poses unique challenges for algal cultivation. N, P, pH, bacterial loads, VFAs, and dissolved organic carbon content (DOC) (both quality and quantity) can vary in the AD effluent based on the composition of the input waste and the AD design and operational conditions. Each of these factors can affect algal production rates, community structure, and corresponding lipid production. For example, high VFA concentrations and elevated NH_4 can inhibit algal growth,[24] while chromophoric DOC compounds can inhibit photosynthesis by reducing light penetration.[31] A key factor in achieving the deployment of waste nutrient-dependent algal cultivation systems is to understand the influence of the quality of the AD effluent as an algal cultivation medium and nutrient source.

Life cycle analysis

A primary desired outcome of the proposed integrated manure management configuration is to reduce dairy GHG emissions (toward achieving the IC goals). In this regard, life cycle analysis (LCA) can be applied to assess the GHG implications. LCA as an analytical tool is applied to develop a metric with which to compare, contrast, and evaluate processes and products in regards to their potential environmental impacts from cradle to grave.[32] At its core, an LCA is a graded model, with inputs of energy and raw materials and outputs of waste or emissions; potential environmental impacts are assessed for each emission associated with the inputs and outputs. With appropriate weighting scales, processes can then be quantitatively compared as a whole from an environmental perspective. When applied comparatively, LCA can be used to analyze the differences in environmental impact between multiple processes that accomplish similar tasks or functions. The value of LCA lies in the ability to make more educated and informed decisions in regard to broad environmental impact when considering alternative processes.

Results and discussion

The purpose of this paper is to present a systems-level assessment to mitigate GHG emissions from dairy operations based on a systematic, integrated process for utilizing dairy manure to generate bio-based fuels and chemicals in a sustainable manner. What follows are preliminary results collected by the research cohort in support of the described integrated manure upcycling process. The objectives of these investigations were to (i) establish viability of the proposed integrated AD and algal processes, (ii) develop preliminary results pertaining to the underlying process fundamentals that are critical to ultimate full-scale process implementation, and (iii) characterize the integrated process on its potential to reduce GHG emissions relative to the IC's goals.

Two-stage AD biogas and methane production

Initial research into this novel two-stage AD process focused on validating the potential to actually achieve CH_4 production on manure that had been fermented (i.e. much of the readily degradable carbohydrates hydrolyzed and fermented to VFAs) and that was without most of the VFA-rich liquid fraction (diverted to another biological process).[17] Two pilot-scale experimental AD systems were designed and operated (fed raw manure from the University of Idaho dairy); E1 was a conventional single-stage system (i.e. the control AD) while E2 represented our two-stage system. Both ADs were operated in a completely mixed, batch-fed (24-h cycle) mode under mesophilic conditions (35–39°C) and at a solids retention time/hydraulic retention time (SRT/HRT) of 20 days. Over an 85-day operational period, the average biogas production for E1 and E2 was statistically identical ($p = 0.06$; student *t*-test), averaging 54.5 ± 9.1 and 51.8 ± 7.9 L/d, respectively, while the biogas CH_4 content in E2 (54%) was statistically higher than in E1 (51%);[17] overall, CH_4 content was typical of manure ADs.[33] Note that E2 was organically loaded at a slightly higher rate than E1 (Table 2) due to negligible VS destruction in the upstream fermenter (which was expected, given the 4-day SRT/HRT and the associated anaerobic thermodynamics[34]). While volatile solids destruction in E1 (43.7%) was slightly higher than E2 (40.6%), VS destruction for the fermenter-AD system in E2 averaged 51.6% (i.e. increased particulate carbon conversion via the two-stage configuration). Collectively these results suggest that the microbial consortium in E2 was more metabolically robust in order to produce the comparable biogas and CH_4 quantities from lower quality and less organic matter.

Having demonstrated the feasibility of our two-stage AD configuration, two new ADs were evaluated to further characterize process potential. Digesters E3 and E4 were operated at 20 and 30 d SRTs/HRTs, respectively (both ADs coupled to a fermenter, again operated at a 4-day

Table 2. Summary performance statistics for the laboratory-scale experimental pilot-scale anaerobic digesters E1–E4. For E1 and E2, OLRs (g VS/L-d) were calculated as the average of 25 discrete measurements collected over an 85 day operational assessment period,[17] while the OLRs for E3 and E4 were calculated as the average of 279 and 258 samples collected over a 365-day operational assessment period. The number of samples collected for the respective biogas/CH_4 measurements were as follows: E1 (n = 76), E2 (n = 76), E3 (n = 132), E4 (n = 145). Typical values per Speece[12] and Khanal.[13]

	"Typical"	E1	E2	E3	E4
AD OLRs (g VS/L-d)	1.6–4.8	3.7	4.2	3.4	3.1
AD OLRs (VS + VFAs; gC/L-d)	–	1.9	2.3	1.8	1.7
L biogas/g VS destroyed	0.75–1.12	0.84	0.76	0.87	0.85
L CH_4/g VS destroyed	0.4–0.78	0.43	0.41	0.49	0.46
L biogas/g VS applied	–	0.37	0.31	0.30	0.34
L CH_4/g VS applied	–	0.19	0.17	0.17	0.19
L biogas/L-d	–	1.36	1.3	1.02	1.07
L CH_4/L-d	–	0.70	0.71	0.57	0.58

SRT/HRT and additive to the AD SRTs). Performance details are summarized in Table 2. Of note, while the organic loading rate (OLR) realized in E3 and E4 was lower as compared with E2, biogas production and CH_4 yield normalized to gVS destroyed was actually higher. Although strict control was exerted in maintaining OLRs for all pilot-scale ADs (i.e. regular VS analysis of the manure), ultimately the realities of feeding real manure, which would exhibit variable organic content (and thus would reflect full-scale AD operations), resulted in slightly different OLRs between systems. On a gross basis, over a 365-day operational period, the average biogas production for E3 and E4 averaged 43.6 (53.4% CH_4) and 42.9 L/d (54.1% CH_4), respectively. Similar to that observed in E1 and E2,[17] biogas production over an operational cycle was relatively stable and constant (Fig. 2). Beyond the demonstrated ability to produce a CH_4-rich biogas from partially oxidized organic matter, in considering all three pilot-scale ADs (E2-E4), the two-stage ADs exhibited excellent process stability. In contrast, the single-stage AD (E1) regularly experienced upsets, typically resulting in tank overflows. The enhanced process stability was likely a consequence of both receiving a more consistent organic substrate and a more balanced microbial ecology between fermenting bacteria and methanogenic archaea.

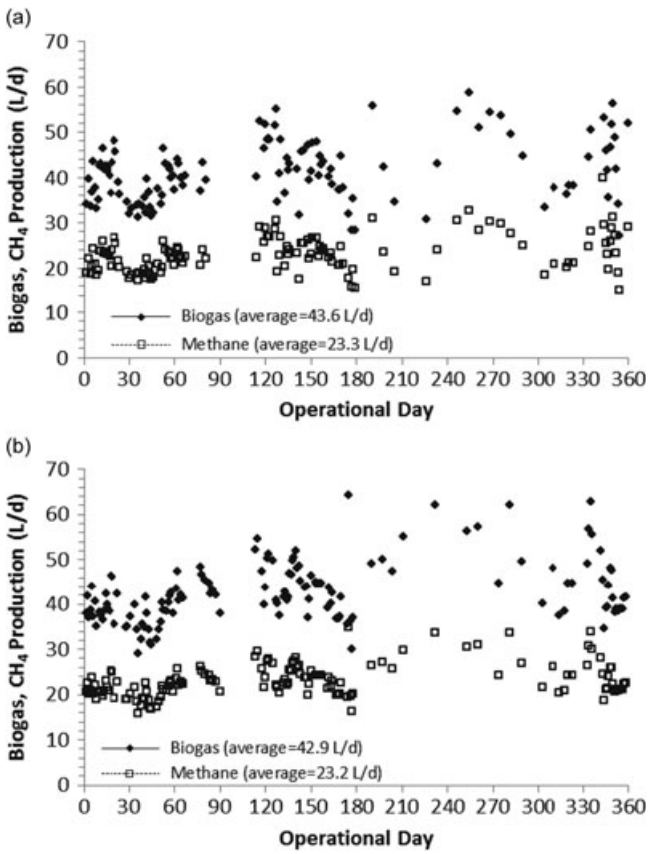

Figure 2. Daily biogas and methane production for pilot-scale ADs E3 (a) and E4 (b) for continuous operations over a year-long operational period.

AD microbial ecology

Methane is synthesized in ADs by hydrogenotrophic and acetoclastic methanogens, of which there are four distinct orders. Methanogens of the hydrogenotrophic orders (i.e. *Methanococcales* (MCC), *Methanobacteriales* (MBT), *Methanomicrobiales* (MMB)), which use H_2 and CO_2 for CH_4 synthesis, are broadly recognized as being the most diverse in regards to species, principally due to favorable bioenergetics.[12] Conversely, acetoclastic

Table 3. Relative fractions of methanogens in the pilot-scale ADs E1–E4 as measured using quantitative PCR on DNA extracted from AD biomass.

Target Group	16S rDNA Copy Number	Relative Quantity (%) in Pilot-Scale ADs			
		E1	E2	E3	E4
Methanococcales (MCC)	2.86	n.d.	n.d.	n.d.	n.d.
Methanobacteriales (MBT)	2.5	0.7	0.6	2.7	1.4
Methanomicrobiales (MMB)	2.25	6.7	3.2	8.5	3.3
Methanosarcinales (MSL)	2.8	63.2	65.7	69.3	69.2
Methanosarcinaceae (Msc)	3	26.9	82.1	28.7	53.7
Methanosaetaceae (Mst)	2	0.2	0.1	0.7	0.2
Archaea	2.65	–	–	–	–

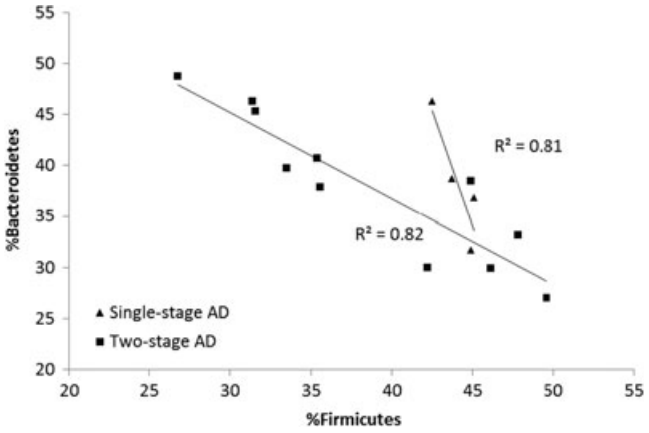

Figure 3. Scatter plot of percentages of the phyla *Firmicutes* and *Bacteroidetes* based on 40,000 high-quality sequence reads per sample of 16S rRNA gene from biomass samples collected from pilot-scale ADs E1–E4 at 2–3 different time points.

methanogens use acetate to produce methane and are combined into a single order (*Methanosarcinales* (MSL)) that has been further subdivided into two principle families (*Methanosarcinaceae* (Msc), and *Methanosaetaceae* (Mst)).[13] Quantitative polymerase chain reaction (qPCR) analysis of archaea in the lab-scale ADs (E1-E4) revealed that the systems were highly enriched with the methanogen family Msc (Table 3), which includes versatile methanogens capable of utilizing various electron donor substrates (the fraction of all methanogens is in relation to the total archaeal population). More importantly, digesters E2–E4 (our two-stage configuration) exhibited a much larger fraction of Msc as contrasted with the 'control' AD (E1), validating that our AD process configuration enriched for a much more robust population of methanogens.

As discussed, bacteria also play a critical role in successful AD operation, providing the necessary substrate for CH_4 synthesis. To better understand the potentially stabilized link between bacteria and methanogens in our AD systems, we recovered DNA from 2–3 time points during the operations of bioreactors E1-E4 and analyzed the biomass using next-generation sequencing technology (Ion Torrent; Life Technologies Corp.). Bacterial identities within each community were determined by classification of 40 000 high-quality DNA sequence reads per sample using the 'Classifier' tool of the Ribosomal Database Project (Release 10; http://rdp.cme.msu.edu/). All ADs were dominated by two major phyla, *Firmicutes* and *Bacteroidetes* (Fig. 3); the sum of both phyla comprised 72–89% of all bacteria. Conventional AD E1 had a narrow percentage range of *Firmicutes* (42–45% of bacteria) while *Bacteroidetes* in E1 ranged from 32–46% of bacteria. A distinguishing feature of the *Firmicutes* in E1 was the abundance of class *Erysipelotrichia*, which was a dominant member of the *Firmicutes* only in the single-stage reactor (5–40% of *Firmicutes* in single-stage *vs.* 1–15% of *Firmicutes* in two-stage AD). In mice and humans, this class of bacteria is correlated with a high-fat, high-carbohydrate diet, while diets high in fiber and low in fat are characterized by higher *Bacteroidetes* dominated by *Prevotella*.[35, 36] In our two-stage bioreactors, both *Prevotellaceae* and *Flavobacteria* were dominant when *Firmicutes* decreased ($R^2 = 0.54$). Thus, the two-stage system was flexible in terms of bacterial dynamics, which probably reflects the selection of bacteria better suited to process recalcitrant, fibrous substrate. Such flexibility in the bacterial community reinforces the archaeal population observations, which tended to be dominated by *Methanosarcinaceae*, the only methanogenic family that includes members capable of both acetoclastic and hydrogenotrophic methanogenesis. Results in our study bear a striking similarity to findings in the human gut microbiome, which raises the future research endeavor of relating bioreactor performance to microbial ecology, just as the human microbiome project seeks to link microbial ecology to human health using next-generation sequencing technology.

An enhanced understanding of the AD microbial population can be complemented with functional-level molecular analyses to further understand how the microbial ecology can be manipulated to enhance CH_4 production and improve AD stability. In this regard, we conducted preliminary proteomic investigations using AD biomass from the pilot-scale ADs E3 and E4. Using electrophoretic

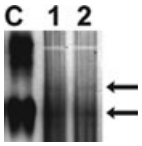

Figure 4. Gel electrophoresis and activity staining for peroxidase (ligninase). Arrows indicate bands with peroxidase activity. Lane C-peroxidase control; Lanes 1 and 2–E4 protein samples at 50 and 100mg loading, respectively.

separation coupled with xylanase activity staining we observed a family 43 xylanase band. Additional staining for lignin peroxidase revealed protein bands with peroxidase activity (Fig. 4). Although their role in lignin degradation in the ADs is unknown, the presence of these activities implies an active pathway for lignocellulose breakdown – which would partially explain the process success given the lignocellulose-rich pre-fermented substrate fed to the AD. To further understand functional aspects of the respective consortia, we applied functional gene primers and polymerase chain reaction (PCR) and detected the presence of dsrA (sulfate reduction to H_2S), soxB (sulfur oxidation of H_2S to sulfate), amoA (ammonia oxidation), and narG (nitrate reduction to reduced nitrogen oxide GHG) genes. One recurring issue with AD is the cycling and inter-conversion of N and S; it is important to understand how these processes contribute not only to nutrient levels and odor, but also to generation and consumption of GHGs. For example, if nitrate reduction is a prevalent process, it would be expected that reduced gaseous forms of N, namely NO and N_2O, would be produced as intermediates. But, if ammonia and NO_X oxidation pathways are encouraged in the reactor, GHG intermediates could be reduced. Likewise, the cycling pathway of S can be governed to control H_2S production as well as SO. The presence of these genes infers a capacity for sulfur and nitrogen conversion in the reactor, which can be governed to reduce GHG emissions.

Engineered systems relying on microbial processes have commonly been designed by viewing the biological compartment as a 'black box'; as long as the desired outcome was achieved, there was little concern for the specific microbial structure. In regard to microbial structure and the black box, our process configuration (specifically the second-stage AD) is similar to single-stage AD in that it demands both fermentation and methanogenesis. However, unlike the conventional single-stage AD, the reduced quantity of readily biodegradable carbohydrates fed from the fermenter to the AD will certainly affect both the structure and function of these respective microbial populations. As we advance and further interrogate our two-stage AD system we will leverage these integrated molecular methods to establish metrics that help us look beyond the black box to more fully understand how operating criteria affect the AD structure-function and thus enhance operational stability and reliability.

Algae production

Preliminary investigations were conducted to validate the potential to use AD effluent (ADE) for algal production. Each PBR was amended with 5% supplemental CO_2 to mimic the use of AD combustion gases as a supplemental CO_2 source. Liquid ADE was collected after a brief settling period to remove residual solids, diluted with tap water, and then used as a cultivation media for a suite of algal species in bench-scale PBRs. A key factor in achieving the deployment of waste nutrient-dependent algal cultivation systems is understanding the influence of the quality of the ADE as a cultivation medium and nutrient source. In particular is the apparent need for dilution of ADE to make it a suitable nutrient and water source for algal cultivation. It has been shown that an increase in ADE dilution rate (10x, 15x, 20x, 25x) relates to an increase in algal growth rate (0.282, 0.350, 0.407, 0.409 day^{-1}).[37] Fortunately, ADE dilution would not increase the overall volume of wastewater produced because algal system effluent would be the diluent for incoming ADE. However, a recent report from the US Department of Energy (DOE) specifically identifies the need for additional research and development of economically viable algal harvesting, dewatering, and water and nutrient reuse technologies.[38] With advancements in these areas the production of additional wastewater from an AD system could be minimized and the economic potential of algal cultivation realized.[38]

In addition to ADE quality and dilution, using wastewater as a nutrient source for algal production presents another challenge: the presence of potential competing organisms. ADE contains a multitude of microorganisms and therefore we can assume competition for nutrients exists. However competition may not necessarily limit the potential for autotrophic production. *Chlorella sp.* can be involved in mutualistic and/or commensalistic relationships with heterotrophs when cultivated with bacteria and/or fungus; relationships that can result in higher biomass concentrations (OD_{680} and chlorophyll) than when cultivated alone.[39]

Results from our experiments indicate that effluent from our two-stage AD system can support significant quantities of algal production by both known species and undefined wastewater treatment facility (WWTF)-derived algal consortia (Fig. 5). Optical density and chlorophyll

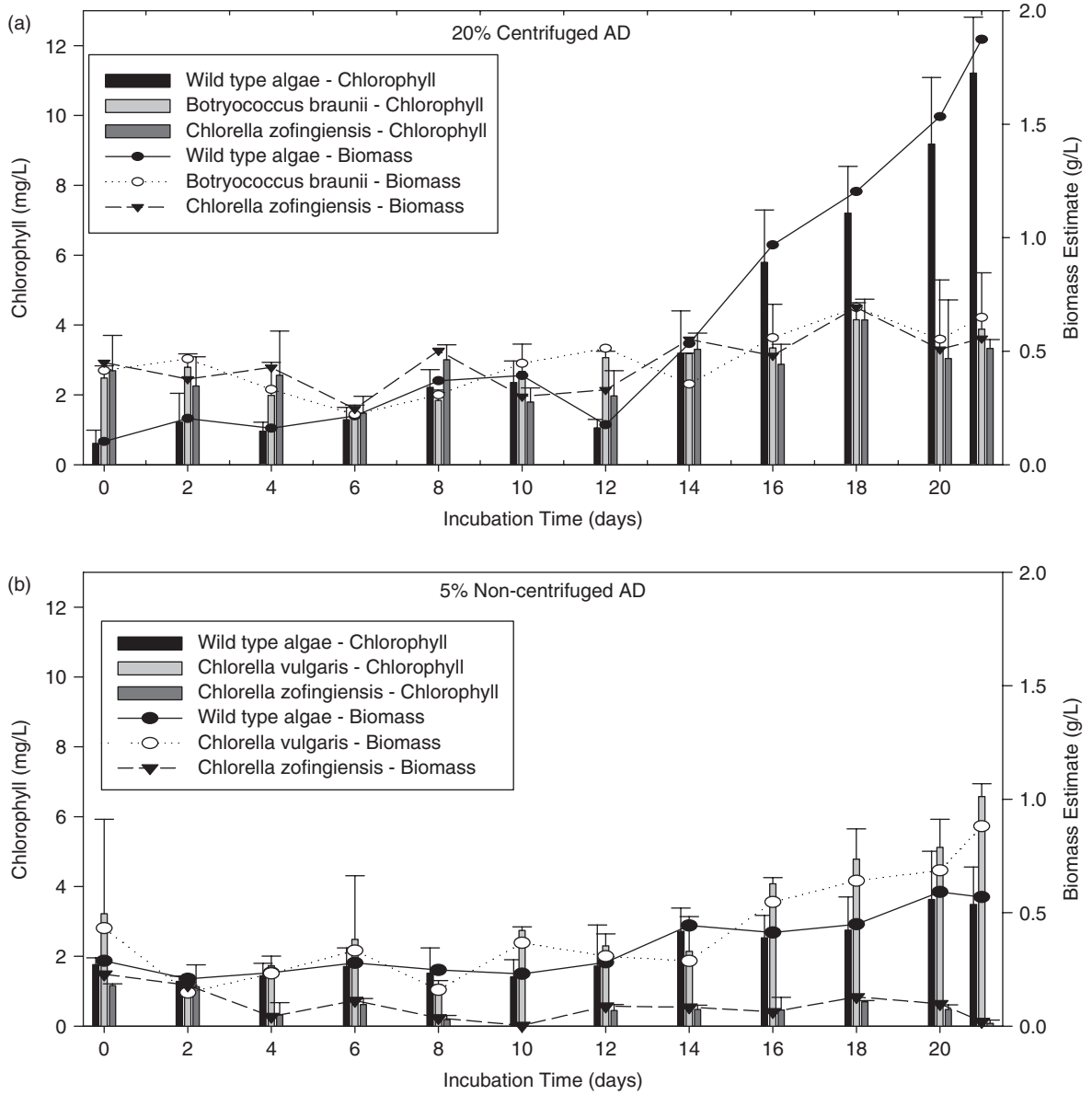

Figure 5. (a) Growth of known and wild type algae on 20% centrifuged AD effluent between 0 and 21 days of incubation. (b) Growth of known and wild type algae on 5% AD effluent between 0 and 21 days of incubation. There is a prolonged lag phase between 0–14 days that correlates with initially high VFA levels (data not shown), followed by a rapid increase in algal production. There is also a clear difference in the level of algal growth among the two known pure algal cultures (*Chlorella zofingiensis* and *Botryococcus braunii*) and the wild algae isolated from wastewater treatment facilities (wild type). Bars = total chlorophyll (mg/L), lines = biomass (g/L).

were measured as indicators of algae growth during 21-day incubations when grown on 40%, 20%, and 5% AD effluent dilutions. The high concentration ADE systems of 40% and 20% produced minimal and sporadic algal growth likely due to high turbidity as well as high concentrations of inhibitory compounds. When a centrifuge pre-processing step was implemented for the 20% ADE, thereby reducing turbidity, significant algal growth was observed (Fig. 5). All species displayed a 12–14-day lag phase, with the algae isolated from a local WWTF resulting in the highest final biomass with a range of 1.82 to 1.98 g L^{-1}. Similarly, when grown on non-centrifuged 5% ADE, chlorophyll and biomass increased significantly after a 10–12-day lag period resulting in a maximum biomass concentration for the *Chlorella vulgaris* species, with a biomass range of 0.79 to 0.96 g L^{-1} on day 21. These

results correlate well with literature sources that report a 21-day biomass range of 1.47 to 1.71 g L^{-1} for Chlorella grown in various AD dilutions (Fig. 5).[24] The initial lag period in algal growth correlated with elevated levels of VFAs and NH_4 in the original effluent (e.g. 314–559 mg L^{-1} acetate and 761 mg L^{-1} NH_4). After VFA depletion and NH_4 oxidation, algal growth rates reflected those reported in the literature. ADE also contains particulate and chormophoric compounds that absorb and scatter light in the same wavelengths as photosynthetic pigments inside the algal cell, thereby inhibiting growth. The absorbance and scattering of the ADE was decreased by various means, primarily centrifuging and/or diluting with water. The dilution method also simultaneously reduced the NH_4^+ and VFA concentrations below inhibitory levels. To reduce algal growth lag times, site specific optimization based on the quality of the ADE (e.g. VFA, NH_4^+ content; chromophoric character) may be required.

The amount of lipid production by algal cultures was dependent on the strain selected and the cultivation conditions. When grown on defined media (i.e. Chu 13) the strains employed generated lipids between 35 and 56 mg g^{-1} of dry mass; similar ranges of lipid production are commonly observed for *Chlorella* strains under standard cultivation conditions.[40] Based on these observations, an appropriately scaled PBR supporting an algal community growing at the rates we have observed (approximate generation time of 0.36 day^{-1}) would be CO_2 limited at a flux rate of 0.57 L of CO_2 per L of culture volume per day during the log phase of growth. Based on the observed growth rates, lipid production potential, and typical triglyceride make up of the lipids of the algal species we employed,[24] we estimate a range of lipid yield between 42.7 and 104.7 mg L^{-1}. Efficient C-sequestration and lipid production will likely be dependent on separating log and stationary phase growth. However, appropriate scaling of a primary and secondary PBR to match log and stationary phase growth rates, respectively, to the level of CO_2 produced by the AD and electrical generation (i.e. CH_4 combustion) will be necessary. Scaling and PBR design characteristics will also need to account for the AD effluent characteristics to account for potential inhibitory compounds and/or biotic competitors present in the effluent.

Two-stage AD GHG reduction potential

Potential GHG reduction associated with our two-stage process can be assessed in part by examining a carbon mass balance for the ADs (Table 4). With the biogas burned to produce electricity, all CH_4 would be converted to CO_2; as contrasted with conventional lagoon storage of manure wherein the resulting CH_4 would be emitted unaltered into the atmosphere, the GHG footprint associated with avoided CH_4 emissions is reduced by over 90% (assuming a CH_4:CO_2 equivalence of 25:1[41]). While the fermentation component of the two-stage AD process generates no carbon credits due to the microbial thermodynamics that yield reduced end products (i.e. VFAs;[34] a carbon mass balance on the E3 and E4 fermenters confirmed this theoretical premise (data not shown)), downstream utilization of the VFA-rich effluent stream generated in the fermenter (e.g. for bioplastics[19] or biofuel[20]) would further sequester carbon and reduce the GHG footprint. CO_2 from engine-generator emissions could be used to stimulate algal production and further reduce the system GHG emissions, the magnitude of which is noted in the LCA analysis detailed below.

Beyond a process carbon balance, the potential of our two-stage AD configuration to reduce GHG emissions

Table 4. Summary of pilot-scale AD carbon balance analysis. Influent solids and volatile fatty acids (VFAs), effluent solids and VFAs, and biogas (CH_4 and CO_2) were included in the analysis.

	E1	E2	E3	E4
	Average, gC (number of samples)			
Influent Solids	73.2 (n = 25)	87.5 (n = 25)	70.1 (n = 279)	64.6 (n = 258)
Influent VFAs	1.9 (n = 25)	4.8 (n = 25)	3.5 (n = 96)	2.2 (n = 93)
Effluent Solids	42.4 (n = 25)	52.5 (n = 25)	46.7 (n = 295)	37.7 (n = 279)
Effluent VFAs	0.6 (n = 25)	0.9 (n = 25)	0.8 (n = 92)	0.4 (n = 83)
Biogas (CO_2)	15.5 (n = 21)	13.3 (n = 21)	9.9 (n = 132)	10.6 (n = 145)
Biogas (CH_4)	16.3 (n = 21)	15.7 (n = 21)	12.5 (n = 132)	12.5 (n = 145
Balance	0.3	9.9	3.7	5.6
	0.7%	10.7%	5.0%	8.4%

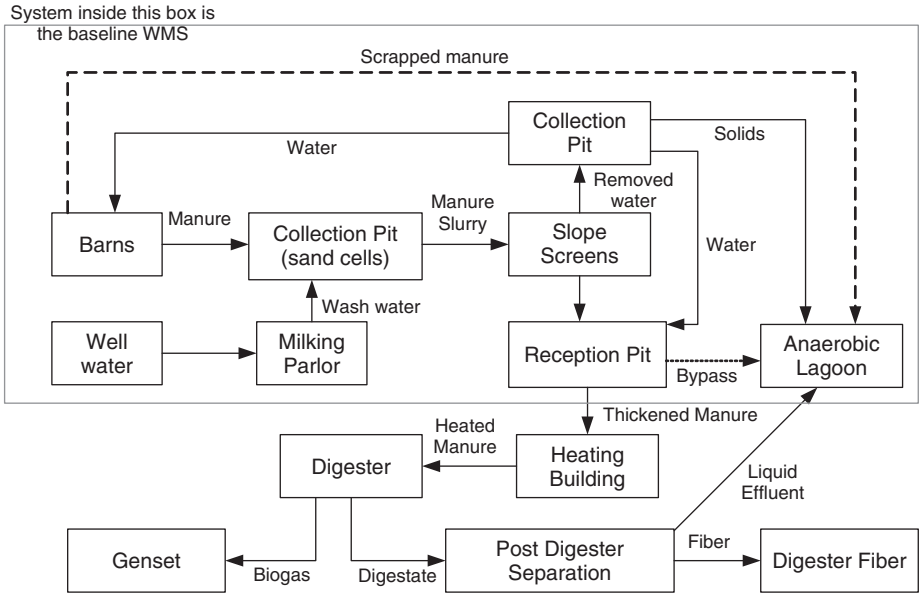

Figure 6. Schematic process diagram illustrating key elements considered in developing the LCA. The solid line boundary is the system prior to retrofitting with AD.

relative to both conventional lagoon and single-stage AD designs was also assessed using LCA. Figure 6 illustrates the modeled process. Specific to our proposed process (Fig. 1), the LCA compared GHG emissions (CH_4, N_2O, and CO_2) from a baseline manure management model (anaerobic lagoon (AL)) versus three existing full-scale single-stage AD systems, the pilot scale single-stage AD (E1), and the pilot scale two-stage AD (E2). The full-scale ADs included in this analysis (referenced as AD-1, AD-2, and AD-3) are located in the Pacific NW region of the USA (specific locations not disclosed due to confidentiality agreements) and consist of free stall dairies with Holstein milk cows. The AD systems are hybrid flow mesophilic plug-flow digesters. Following an average residence time of approximately 38 days, effluent is pumped through a fiber separation system, with the fiber used as cattle bedding. Liquid effluent is stored in an AL for ultimate land application. AD biogas is processed through a regenerative bio-scrubber and an air-to-gas heat exchanger to remove moisture, and then burned in engine generators to produce electricity. Each AD system is also equipped with a flare system that combusts the biogas if the engine generators are not in operation. AD operating parameters are summarized in Table 5. To estimate parameters for the LCA not otherwise collected by the operations staff, the full-scale ADs in this study were modeled using SuperPro Designer® modeling software. GHG emission calculations were conducted using the Intergovernmental Panel for Climate Change (IPCC) tier 2 method.[42] Manure characteristics and manure management system characteristics used in this study are reported in the 2011 *US Greenhouse Gas Inventory Report*.[43]

The intrinsic challenge in comparing data from full-scale operations with laboratory systems is factoring in the 'up time' of the ADs (i.e. operational time producing CH_4-rich biogas). While our lab-scale ADs experienced nearly 100% up time, the full-scale systems experienced operational challenges that ultimately reduced their up time. AD1, AD2, and AD3 experienced up times of 80%, 85%, and 86%, respectively. Moreover, the three full-scale ADs did not always use all produced CH_4 for electricity production (Table 4). The amount of CH_4 used depended on the capacity to use biogas during the peak methane production and also power purchase agreements.

The LCA focused on the global warming potential (GWP) reduction metric to assess the potential to reduce GHG emissions with our two-stage AD configuration. CH_4, CO_2, and N_2O emissions for the AL system were estimated using tier 2 Intergovernmental Panel on Climat Change (IPCC) guideline for GHG emissions calculations and were consistent with the methodology used by the US EPA.[44] Fuel used in vehicles for necessary AD operations were also included in total GHG emissions, as was a displacement of this fuel via production of algal lipid-based biodiesel. The VS produced by the herd was estimated using animal waste management field handbook.[45] For the baseline model, 100% of the manure was handled by AL WMS. For the AD models, the average manure collection values reported by operations

Table 5. Pertinent data and operational parameters for the anaerobic digesters evaluated in the LCA. All numbers after ± indicates 95% confidence interval of measured statistic.

	AD1	AD2	AD3	E1[a]	E2[a]
# of Cows	6,200	6,500	12,000	NA	NA
AD Volume (m^3)	20,400	20,400	30,500	0.04	0.04
Fermenter volume	NA	NA	NA	NA	0.02
VS produced by herd (MT/yr)	15,000	15,700	29,100	NA	NA
AD Influent Quantity (MT/yr)	169,500	168,700	355,600	0.73	0.91
Organic Loading Rate (g VS $L^{-1}day^{-1}$)	1.0	1.4	1.4	3.7[b]	3.5[b]
AD influent volatile solids (%)	4.4 ± 0.3[c]	6.1 ± 0.4	4.4 ± 0.3	7.4±0.8	6.7±0.7
Manure fed into AD in annual basis (%)	49.1 ± 3.1	64.8 ± 3.7	54.0 ± 1.8	NA	NA
AD hydraulic residence time (days)	39	45	19	20	20[c]
AD effluent VS (%)	2.3 ± 0.3	3.6 ± 0.4	3.3 ± 0.1	4.2±0.5	4.0 ± 0.4
Fermenter VS destruction (%)	NA	NA	NA	NA	18.5 ± 5.3
AD VS destruction (%)	47.7 ± 8.7	40.5 ± 7.3	25.9 ± 5.1	43.7 ± 8.1	40.6 ± 6.7
Biogas produced (m^3 /kg VS destroyed)	0.74	0.81	1.77	0.84	0.76
CH_4 produced (m^3/kg of VS applied)	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03
Methane in biogas (%)	57 ± 1.0	59 ± 1.2	50 ± 0.5	51 ± 1.1	54 ± 1.2
Biogas utilization by genset (%)	61 ± 1.0	54 ± 3.1	87 ± 1.1	NA	NA
Biogas used to produce electricity (%)	61	54	87	NA	NA
Genset efficiency (%)	31	35	39	NA	NA
Average electricity produced (kWh/day)	8,700	9,300	26,700	NA	NA
Electricity/m^3 of biogas (kWh/m^3)	1.8	1.9	1.8	NA	NA
AD down time (%)	20	15	14	NA	NA
Gensets down time (%)	12	20	20	NA	NA

[a] Pilot-scale data obtained from Coats *et al.*[17]
[b] The design organic loading rate for both digesters was 3.6 g VS $(L\text{-}d)^{-1}$. The deviation is due to the real variability that is experienced in using animal manure, which is inherently a heterogeneous substrate.
[c] HRT of 4 days in fermenter and HRT of 16 days in digester.

staff were used. CH_4 and N_2O emissions were included in the GHG calculation for each WSM as prescribed by the EPA.[43] Biogenic CO_2 emissions were excluded from the models per recommendations specified by IPCC.[42] CH_4 was either used to produce electricity or flared.

To assess reduction in GWP, each full-scale AD configuration was compared against the baseline AL. The pilot-scale ADs were then paired with each full-scale AD and compared against the baseline AL. GHG emissions for the pilot scale units represent anticipated potential benefits when up-scaled. Results are tabulated in Table 6. Note that the higher GWP reduction for AD2 is primarily because of the higher percentage of manure being fed to the AD; for every percentage increase in manure discharged to the AD, the GWP reduced by an additional 0.6%. As would be expected, simply processing manure through AD significantly reduced the GWP (by 36–46%) as indicated by the AD1-AD3 results. Similarly, the performance of pilot unit E1 was effectively the same as the full-scale operational digesters (all were single-stage ADs). However, analyses suggest that when the pilot-scale two-stage AD (E2) is up-scaled, the GHG emission reduction can be even more pronounced; LCA estimates indicate that the additional reduction in GWP for our two-stage process relative to AD1–AD3 would be 6.7–10.2% (maximum reduction of 56.2%), which is a significant incremental improvement. The enhanced GHG reduction was attributed to the higher combined fermenter and digester VS destruction associated with the two-stage configuration. Any VS remaining post-digestion would produce CH_4 that would not be recovered for electricity and would be emitted as a GHG.

Algal production results showed that lipid production varied from 0.55–1.08 g $(L\text{-}d)^{-1}$ of E2 digester effluent when the

Table 6. Comparison in potential GHG reduction, as measured by GWP, under actual (AD1, AD2, AD3) and scale up (E1–AD1, E1–AD2, E1–AD3, E2–AD1, E2–AD2, E2–AD3) conditions (kg/year/animal) associated with implementation of AD at dairies.

	CH_4	N_2O	CO_2e	GWP (% reduction)
Baseline	41.6	0.4	1,000	0
AD1	25.6	0.3	640	36.5
AD2	21.4	0.3	540	46.0
AD3	24.2	0.3	600	40.1
E1-AD1	25.7	0.3	640	36.6
E1-AD2	21.0	0.3	540	46.7
E1-AD3	23.1	0.3	580	42.3
E2-AD1	24.0	0.2	570	43.2
E2-AD2	18.8	0.2	440	56.2
E2-AD3	21.3	0.2	510	49.4

algae was grown on ADE diluted to 5%. Thus, the LCA was expanded to include the use of ADE from AD E2; data for algae production energy use was obtained from the EPA,[46] and all required nutrients were assumed to be provided by the ADE. Data for biodiesel transesterification and transportation were assumed to be same as soybean biodiesel.[47] Extending the laboratory lipid results to full-scale production, it was estimated that the E2–AD1 scenario could produce biodiesel at 146.8–288.4 L per day; at an average rate of 217.6 L day^{-1}, the equivalent GHG emission reduction would be 25.6 kg CO_2e per year-animal from replaced petroleum diesel fuel. Similar analysis for E2–AD2 and E2–AD3 resulted in 298.5 and 459.3 L of biodiesel per day, respectively, which reduced dairy GHG emissions by an additional 2.5-5.4%. After inclusion of algal system, the total reduction of GHG for E2–AD2 remained highest at 59.7%.

Conclusions

The purpose of this manuscript was to present a systems-level assessment for an industrial platform producing multiple commodities from manure while concurrently reducing dairy nutrient and GHG emissions. The integrated processes center on a two-stage fermentation/AD system to produce a CH_4-rich biogas that can be burned to produce electricity. Nitrogen and phosphorus-rich supernatant from the AD is used to grow algae biomass in a photobioreactor. Based on collaborative, interdisciplinary investigations of this integrated manure upcycling technology, key conclusions are as follows.

- Our two-stage AD configuration receiving pre-fermented manure is more resilient and stable than conventional single-stage AD receiving raw manure.
- Pre-fermented manure can be anaerobically digested to produce quantities and yields of CH_4-rich biogas comparable to that generated in single-stage AD receiving raw manure.
- The two-stage AD is more highly enriched with *Methanosarcinaceae*, a group of methanogens that can utilize both acetate and H_2 to produce CH_4. Moreover, the AD microbial population is more highly enriched with bacteria that can ferment the partially digested substrate.
- Algal production for the purpose of biofuel generation is feasible using two-stage AD effluent as a nutrient/CO_2 source. Production of biodiesel from AD effluents could generate an additional commodity that may positively influence overall AD system economics.
- Environmental life cycle assessment demonstrated that the integrated configuration can reduce GHG emissions by approximately 60% as compared with a traditional anaerobic lagoon.

Having established overall process potential and the ability to positively affect dairy GHG emissions, ongoing investigations are aimed at process refinement with an ultimate commercialization goal. Included in these on-going investigations will be appropriate techno-economic analyses to establish commercial viability of the integrated suite of technologies.

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References

1. Liebrand CB and Ling KC, *Cooperative approaches for implementation of dairy manure digesters*. USDA, Washington DC, pp. 28 (2009).
2. Cuellar AD and Webber ME, Cow power: the energy and emissions benefits of converting manure to biogas. *Environ Res Lett* **3**:1–8 (2008).
3. USEPA, *Inventory of US Greenhouse Gas Emissions and Sinks: 1990–2009*. #430-R-11-005 (2011).
4. USDA and The Innovation Center for U.S. Dairy, MOU for Reducing Dairy GHG Emissions. USDA, Washington DC (2009).
5. USDA-NRCS, *Agricultural Waste Management Field Handbook.* USDA, Washington DC (2012).
6. Hristov AN, Hazen W and Ellsworth JW, Efficiency of use of imported nitrogen, phosphorus, and potassium and potential for reducing phosphorus imports on Idaho dairy farms. *J Dairy Sci* **89**:3702–3712 (2006).

7. Wang SJ, Fox DG, Cherney DJR, Klausner SD and Bouldin DR, Impact of dairy farming on well water nitrate level and soil content of phosphorus and potassium. *J Dairy Sci* **82**:2164–2169 (1999).
8. EPA, *Revised National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitations Guidelines for Concentrated Animal Feeding Operations in Response to the Waterkeeper Decision; Final Rule*, 40 CFR Parts 9, 122, and 412. EPA, U.S. Government Printing Office, Washington, DC, USA (2008).
9. Key N and Sneeringer S, *Climate Change Policy and the Adoption of Methane Digesters on Livestock Operations*. USDA, pp. 40 (2011).
10. US EPA, *Market opportunities for biogas recovery systems - improved performance at competitive costs*, AgSTAR program U.S. EPA, Washington, DC, USA (2010).
11. Zaks DPM, Winchester N, Kucharik CJ, Barford CC, Paltsev S and Reilly JM, Contribution of anaerobic digesters to emissions mitigation and electricity generation under US climate policy. *Environ Sci Technol* **45**:6735–6742 (2011).
12. Speece RE, *Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries*. Nashville, Tennessee, USA (2008).
13. Khanal SK, *Anaerobic Biotechnology for Bioenergy Production*. Ames, Iowa, US (2008).
14. Veeken A, Kalyuzhnyi S, Scharff H and Hamelers B, Effect of pH and VFA on hydrolysis organic solid waste. *ASCE J Environ Eng* **126**:1076–1081 (2000).
15. Pohland FG and Ghosh S, Developments in anaerobic stabilization of organic wastes - the two-phase concept. *Environ Lett* **1**:255–266 (1971).
16. Massey ML and Pohland FG, Phase separation of anaerobic stabilization by kinetic controls. *J Water Pollut Control Fed* **50**:2204–2222 (1978).
17. Coats ER, Ibrahim I, Briones A and Brinkman CK, Methane production on thickened, pre-fermented manure. *Bioresource Technol* **107**:205–212 (2012).
18. Coats ER, Gregg M and Crawford RL, Effect of organic loading and retention time on dairy manure fermentation. *Bioresource Technol* **102**:2572–2577 (2011).
19. Coats ER, Loge FJ, Wolcott MP, Englund K and McDonald AG, Synthesis of polyhydroxyalkanoates in municipal wastewater treatment. *Water Environ Res* **79**:2396–2403 (2007).
20. Huber GW, Iborra S and Corma A, Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem Rev* **106**:4044–4098 (2006).
21. Tomei MC, Braguglia CM, Cento G and Mininni G, Modeling of anaerobic digestion of sludge. *Crit Rev Environ Sci Technol* **39**:1003–1051 (2009).
22. De Vrieze J, Hennebel T, Boon N and Verstraete W, Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresource Technol* **112**:1–9 (2012).
23. Mulbry W, Westhead E, Pizarro C and Sikora L, Recycling of manure nutrients: Use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresource Technol* **96**:451–458 (2005).
24. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J *et al.,* Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technol* **101**:2623–2628 (2010).
25. Mulbry W, Kondrad S, Pizarro C and Kebede-Westhead E, Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresource Technol* **99**:8137–8142 (2008).
26. Kebede-Westhead E, Pizarro C and Mulbry W, Treatment of dairy manure effluent using freshwater algae: Elemental composition of algal biomass at different manure loading rates. *J Agric Food Chem* **52**:7293–7296 (2004).
27. Wilkie A and Mulbry W, Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresource Technol* **84**:81–91. (2002).
28. Tang H, Chen M, Garcia M, Abunasser N, Ng K and Salley S, Culture of microalgae Chlorella minutissima for biodiesel feedstock production. *Biotechnol Bioeng* **14**:23160 (2011).
29. Lam M and Lee K, Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol Adv* **Dec 6**, 2012 May-Jun;30(3):673-90. doi: 10.1016/j.biotechadv.2011.11.008 Epub ahead of print (2011).
30. Heredia-Arroyo T, Wei W and Hu B, Oil accumulation via heterotrophic/mixotrophic Chlorella protothecoides. *Appl Biochem Biotechnol* **162**:1978–1995 (2010).
31. Green SA and Blough NV, Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnol Oceanogr* **39**:1903–1916 (1994).
32. Hauschild MZ, Assessing environmental impacts in a life-cycle perspective. *Environ Sci Technol* **39**:81A–88A (2005).
33. Ward AJ, Hobbs PJ, Holliman PJ and Jones DL, Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technol* **99**:7928–7940 (2008).
34. Grady Jr CPL, Daigger GT, Love NG and Filipe CDM, *Biological Wastewater Treatment*. pp. 991 (2011).
35. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA *et al.,* Linking long-term dietary patterns with gut microbial enterotypes. *Science* **333**:105–108 (2011).
36. Zhang CH, Zhang MH, Wang SY, Han RJ, Cao YF, Hua WY *et al.,* Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME Journal* **4**:232–241 (2010).
37. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J *et al.,* Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technol* **101**:2623–2628 (2010).
38. ANL; NREL; PNNL. Renewable Diesel from Algal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model. ANL/ESD/12-4; NREL/TP-5100-55431; PNNL-21437. Argonne, IL: Argonne National Laboratory; Golden, CO: National Renewable Energy Laboratory; Richland, WA: Pacific Northwest National Laboratory. U.S. DOE, Oak Ridge, TN, USA (June 2012).
39. Watanabe K, Takihana N, Aoyagi H, Hanada S, Watanabe Y, Ohmura N *et al.,* Symbiotic association in Chlorella culture. *FEMS Microbiol Ecol* **51**:187–196 (2006).
40. Demirbas A, Production of biodiesel from algae oils. *Energ Source* **31**:163–168 (2008).
41. Bare JC, Norris GA, Pennington DW and McKone T, TRACI: The tool for the reduction and assessment of chemical and other environmental impacts. *J Ind Ecol* **6**:49–78 (2002).
42. IPCC, *Emissions From Livestock and Manure Management*. Kanagawa, Japan, pp. 673 (2006).
43. EPA, *U.S. Inventory of Greenhouse Gas Emissions and Sinks: 1990–2009*. EPA, Washington, DC, pp. 199–225 (2011).
44. EPA, *ANNEX 3: Methodological Descriptions for Additional Source or Sink Categories*. EPA, Washington, DC, pp. 199–225 (2011).
45. NRCS, *Agricultural waste characteristics*. NRCS, Washington, DC, pp. 8–10 (2008).

46. EPA, *Algae biodiesel lifecycle GHG results and impact calculations (Base Case)*, EPA-HQ-OAR-2005-0161-3174 (2010).
47. Pradhan A, Shrestha D, McAloon A, Yee W, Haas M and Duffield J, Energy life-cycle assessment of soybean biodiesel revisited. *Trans. ASABE* **54**:1031–1039 (2011).

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