Enhancing Greenhouse Gas Mitigation and Economic Viability of Anaerobic Digestion Systems: Optimizing Fermentation, PHA and AD reactors, and Enhancing Biomass Recovery for Commodity Production

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Project Overview

CH_4 and CO_2 emissions from dairy operations constitute ~2.5% of annual U.S. greenhouse gas (GHG) emissions, making dairies one of the largest industrial sources of GHGs in the U.S. Anaerobic digestion (AD) of dairy manure can reduce dairy CH_4 emissions while producing electricity. However, dairy ADs are severely constrained economically, principally due to low electricity rates. Our project is developing novel, integrated strategies to achieve a net reduction in dairy GHGs while producing value-added commodities. Commodities include bio-gas (CH_4), PHA-based bioplastics, carbohydrate production via algal polyculture for enhancing PHA production, and nutrient management also via optimizing PHA reactor conditions and algal polyculture. Additionally, we are developing an integrated system model that will provide a decision making tool for researchers, AD system developers/operators, and dairy owners. Lastly, we are expanding education in integrated agriculture systems for economically viable GHG management.

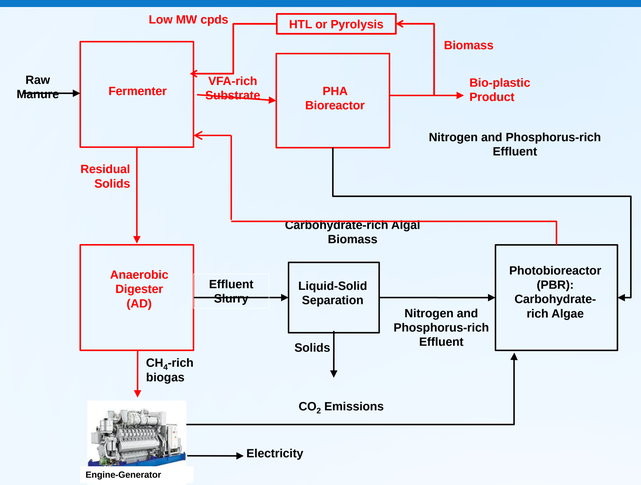


Figure 1-Integrated Dairy Manure Commodities Process: Focus on Fermentation, AD, PHA processes

Project Objectives: Focus on Fermentation, PHA and AD

- GHG MITIGATION and PRODUCTION OF VALUE ADDED COMMODITIES: To enhance dairy carbon (C) sequestration, this project is advancing a novel integrated manure-to-commodities system that (i) converts pre-fermented manure to bioenergy, (ii) sequesters carbon by converting volatile fatty acid (VFA)-rich fermenter supernatant to bioplastics and (iii) sequesters AD effluents (CO_2, nitrogen, phosphorus) by producing algae that can be harvested and internally recycled to enhance PHA production and enhance overall C-sequestration (iii is described in poster #2). This presentation describes our optimization of the AD and PHA processes as a function of modulating the 2 stage system, minimizing aeration costs in the PHA reactor, and exploring novel methodologies for sequestering additional carbon from the residual PHA-extracted biomass.
- SYSTEM INTEGRATION: Net effects of the Fermenter, AD, and PHA stages of our system are being integrated with the algae production stages via an integrated system model and web-accessible management decision tool (DAIRIEES). GHG reduction and C sequestration by the AD, PHA, Algae, and residual biomass processing stages (i.e. HTL and Pyrolysis) are being quantified and used to parameterize the DAIRIEES model. This model will allow us to integrate each component of the system and estimate utility of each stage for potential individual on-farm applications.

Research Progress

Anaerobic Digestion (AD): Maximizing CH_4 Production

Preliminary investigations on the fermenter effluent residual solids revealed (1) two distinct solids fractions (by size), one being fibrous and one being a fine fraction containing more lipids, (2) that AD of the finer fraction yielded a more CH_4-rich biogas, and (3) the methanogenic population was more enriched in hydrogenotrophic methanogens. Building upon the preliminary data, this research focused on the potential for enhancing the CH_4 yield of the two-phase, PHA production-coupled digestion system through separation of the two distinct fractions of fermented solids based on particle size, and digestion of each fraction in a separate reactor. Based on the preliminary data, it was hypothesized that the separate reactors would act as "selectors" for unique bacterial and methanogenic cultures optimized for the hydrolysis and methanation of each substrate, thereby increasing the methane yield in comparison to that of a single digester receiving the combined solids stream.

Table 1: Methane production normalized to grams VS applied, g VS destroyed, and liters of active digester volume.

Reactor	OLR (g VS/L*d)	VS Destruction (%)	L CH4/g VS applied
AD3-c (coarse solids)	3.1 +/- 0.2 (n = 16)	30.4% +/- 4.2% (n = 15)	0.17 +/- 0.01
AD6-c (System 1, combined solids)	3.1 +/- 0.2 (n = 16)	34.9% +/- 3.4% (n = 14)	0.18 +/- 0.02
AD8 (fine solids)	2.8 +/- 0.4 (n = 16)	34.2% +/- 9.0% (n = 16)	0.22 +/- 0.05
AD3-c + AD8 (System 2, separated solids)	3.1 +/- 0.2 (n = 16)	31.6% +/- 4.2% (n = 15)	0.17 +/- 0.02

Reactor	Biogas (L/d @ STP)	Methane Content (%)	Methane (L/d @ STP)
AD3-c (coarse solids)	28.5 +/- 1.9 (n = 36)	57.1% +/- 0.7% (n= 30)	16.3 +/- 1.0 (n = 36)
AD6-c (System 1, combined solids)	37.2 +/- 2.4 (n = 76)	60.9% +/- 1.2% (n = 71)	22.7 +/- 1.6 (n = 76)
AD8 (fine solids)	9.7 +/- 1.2 (n = 35)	64.4% +/- 1.0% (n = 30)	6.30 +/- 0.8 (n = 35)
AD3-c + AD8 (System 2, separated solids)	35.0 +/- 3.2 (n = 83)	59.9% +/- 0.6% (n= 41)	20.8 +/- 1.8 (n = 82)

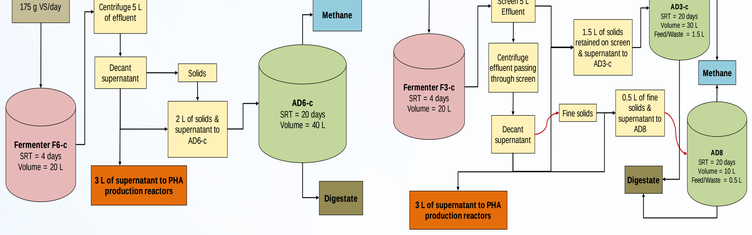


Figure 1. Configurations of two-stage integrated AD system 1 and AD system 2. AD system 1 received the combined solids stream post-supernatant removal to the PHA reactor. AD system 2 operated two Ads receiving separate solids fractions post-supernatant separation.

Table 2. Summary of Relative Expression Ratio comparisons between reactors. Confidence intervals represent one

	Relative Expression Ratio					
		Acetoclasts		Hydrogenotrophs		
Target Microbial Population	Bacteria	MSC	MST	MCC	MBT	MMB
AD3-c / AD6-c (n = 3)	0.7 +/- 0.5	2.7 +/- 4.1	0.2 +/- 0.1	0.4 +/- 0.2	0.5 +/- 0.2	0.5 +/- 0.3
AD8 / AD6-c (n = 3)	2.3 +/- 1.8	0.1 +/- 0.0	8.9 +/- 9.0	3.9 +/- 4.5	2.1 +/- 2.1	3.1 +/- 0.1
AD8 / AD3-c (n = 3)	5.8 +/- 4.9	0.2 +/- 0.1	53.0 +/- 54.4	9.1 +/- 7.1	4.3 +/- 3.7	14.8 +/- 17.1
Overall Population Sizes by Digester	8 > 6 > 3	3 > 6 > 8	8 > 6 > 3	8 > 6 > 3	8 > 6 > 3	8 > 6 > 3

Hydrogenotrophic methanogens: *Methanococcales (MCC), Methanobacteriales (MBT), Methanomicrobiales (MMB).* Acetoclastic methanogens: *Methanosarcinaceae (MSC), Methanosaetaceae (MST)*

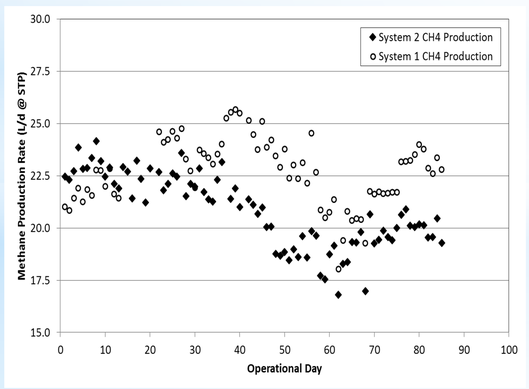


Figure 2. Daily methane production over the analysis period for System 1 (AD6-c) and System 2 (AD3-c + AD8 combined)

Results:

- In contrast to the hypothesized outcome, the average daily methane production from System 2 was statistically lower than that of System 1. In other words, the AD system receiving the combined fermenter effluent solids stream was more productive that the AD system processing the different solids fractions separately.
- ~80% of the methane produced occurred via fermentation of slowly biodegradable material.
- Both systems achieved essentially complete removal of all VFAs produced, indicating that methanogenesis was not rate-limiting in either case, and differences in performance were therefore most likely primarily due to dissimilarities in cellulose and hemicellulose hydrolysis rates, as these compounds account for the majority of the volatile solids in the digester feed.
- Although proteins and fats made up a much smaller fraction of the feed than fiber, differences in protein and LCFA hydrolysis rates could have contributed to the performance difference as well.
- Based on higher fat/protein content and qPCR results, the fine solids likely contained much of the fermentative biomass.
- Population analysis indicated bacterial population highest in AD8 and smallest in AD3-c, while AD6-c was more balanced.

Bioplastics Production and Reduced Carbon/Energy Footprint

PHA production on fermented dairy manure is an aerobic process. As such, the process can be expensive, associated with system aeration and the commensurate energy demand. For the commodity PHA to be price competitive, aeration costs must be reduced – but without compromising PHA production.

Investigations focused on understanding the relative effects of aeration on PHA production. Four bioreactors were operated and analyzed, with aeration control applied at oxygen mass transfer rates of 4, 8, 12, and 20 hr^{-1}.

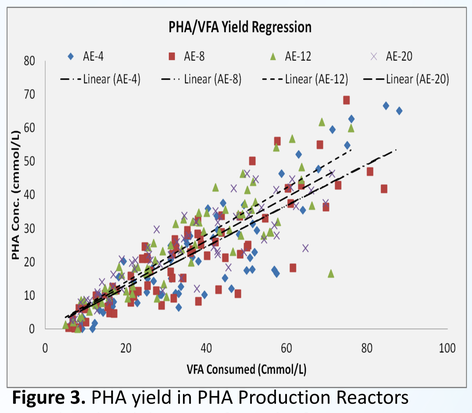


Figure 3. PHA yield in PHA Production Reactors inoculated with biomass from the four PHA Enrichment Reactors operated at different oxygen mass transfer rates (AE-4 = $4hr^{-1}$; etc.). There was no statistical difference in PHA yield between the different inocula, indicating that lower aeration rates can be maintained without compromising PHA production – thus reducing the overall process cost substantially.

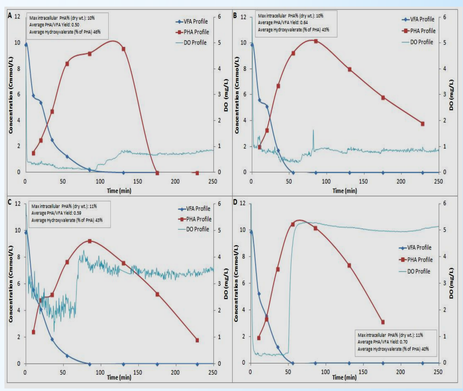
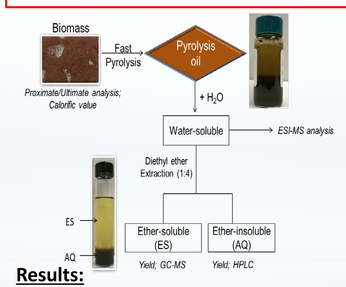
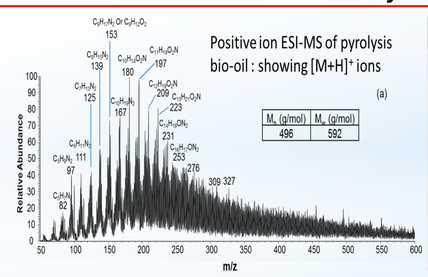


Figure 4. Relative effect of aeration control on PHA production. As shown, the PHA Enrichment Reactor can be aerated at an oxygen mass transfer rate as low as $4hr^{-1}$ without compromising PHA production.

Enhanced Biomass Recovery for Commodity Production





Residual biomass	
Pyrolysis bio-oil	26.0%
Bio-char	42.4%
Pyrolysis bio-oil pH	8.23
Bio-oil	100% water soluble
Ether-soluble (ES)	15%
Ether-insoluble (EI)	85%

Pyrolysis of Residual PHA Biomass

- Goal: Utilize residual biomass, after PHA extraction, to obtain fermentable compounds by fast pyrolysis. The pyrolysis products were fractionated and characterized.

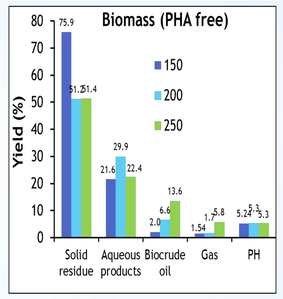
Results:

- The pyrolysis aqueous fraction has a substantial amount of water soluble low molar mass compounds that are suitable for fermentation into VFAs. The resultant VFAs can be converted to PHA.
- The pyrolysis biochar can be used as a sequestered carbon soil amendment or for converting into activated char

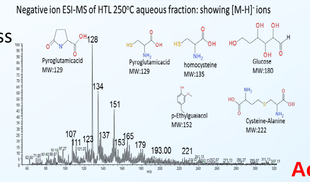
Hydrothermal Liquefaction of PHA extracted residual biomass

Goal:

- Utilize residual biomass, after PHA extraction, to obtain fermentable compounds after HTL treatment. Biomass was subjected to HTL at 150°C, 200°C and 250°C for 60 min.



Negative ion ESI-MS of HTL 250°C aqueous fraction: showing [M-H]- ions



Results:

- The HTL aqueous fraction has a substantial amount of low molar mass compounds that are suitable for fermentation into VFAs. VFAs can be converted to PHA

Conclusions:

- Methane production by system one (without size fractionating the residual fermenter solids outperformed the combined CH4 production from AD system 2.
- Operational power consumption for the PHA Enrichment Reactor can be minimized by operating at a minimum aeration rate while sustaining a mixed microbial consortium capable of hyper-PHA synthesis in a sidestream PHA Production Reactor.
- PHA yield of 60-70% can be sustained in the PHA Production Reactor (i.e. 60-70% of the organic carbon present in the VFAs is sequestered by a mixed microbial consortium as PHA.
- The PHA-extracted biomass represents a significant source of residual carbon in our system. Pyrolysis and HTL of this biomass produce 1) additional fermentable compounds to enhance PHA production and 2) biochar; two carbon pools with potential to enhance the GHG sequestration by our integrated system and increase system economic viability.
- These results are being compiled in the DAIRIEES model to quantify the net GHG sequestration and economic potential of our integrated system.

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