NRCS Conservation Innovation Grant

Final Report September 30, 2013

Prepared by

J. M. Hackney

&

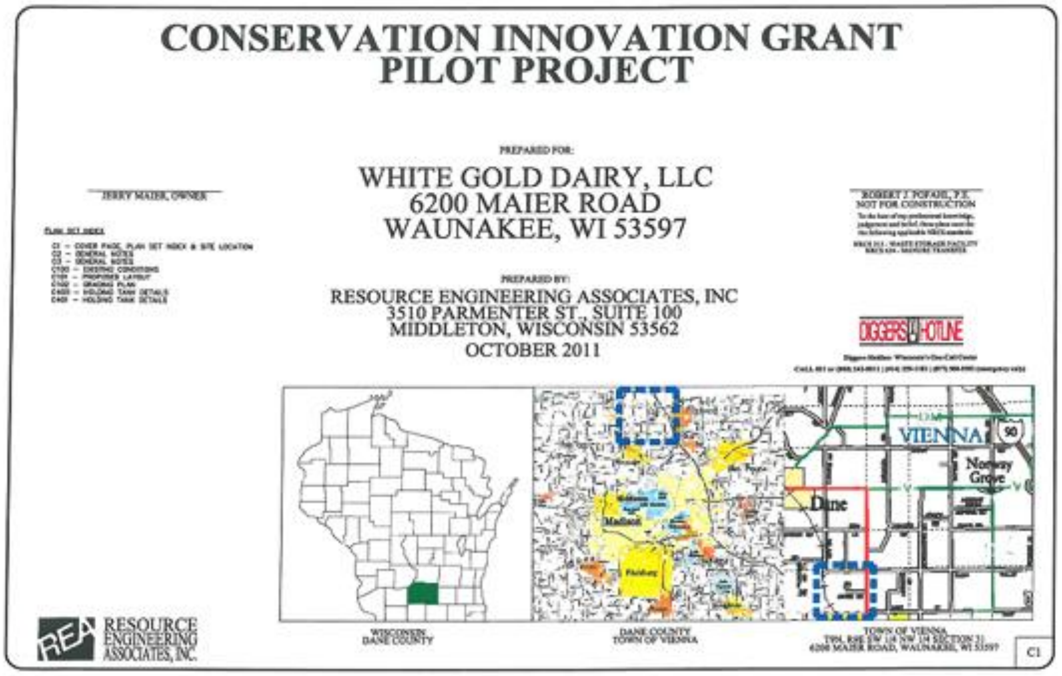
H. Tony Hartmann

Great Lakes Ag Energy, LLC

www.greatlakesagenergy.com

Grantee Name: Great Lakes Ag Energy, LLC	
Project: Use of a Constructed Wetland and a Mass Culture of Attached, Filamentous Algae to Complement Methane-digester Technology in Managing Dairy-farm Phosphorus Runoff	
Agreement Number: 69-3A75-10-161	
Project Director: John M. Hackney (PI) & H. Tony Hartmann (Project Mgr.)	
Contact Information: H. Tony Hartmann	**Phone Number:** 608-215-4446 **E-Mail:** thartmann@greatlakesagenergy.com
Period Covered by Report: 10/1/10-9/30/13	

Table of Contents



CONSERVATION INNOVATION GRANT
PILOT PROJECT

PREPARED FOR:

WHITE GOLD DAIRY, LLC
6200 MAIER ROAD
WAUNAKEE, WI 53597

PREPARED BY:

RESOURCE ENGINEERING ASSOCIATES, INC
3510 PARMENTER ST., SUITE 100
MIDDLETON, WISCONSIN 53562
OCTOBER 2011

JERRY MAIER, OWNER

PLAN SET INDEX
C1 – COVER PAGE, PLAN SET INDEX & SITE LOCATION
C2 – GENERAL NOTES
C3 – GENERAL NOTES
C100 – EXISTING CONDITIONS
C101 – PROPOSED LAYOUT
C102 – GRADING PLAN
C400 – HOLDING TANK DETAILS
C401 – HOLDING TANK DETAILS

ROBERT J. POFAHL, P.E.
NOT FOR CONSTRUCTION

DIGGERS HOTLINE

RESOURCE ENGINEERING ASSOCIATES, INC.

WISCONSIN
DANE COUNTY

DANE COUNTY
TOWN OF VIENNA

TOWN OF VIENNA
T9N, R9E SW 1/4 NW 1/4 SECTION 31
6200 MAIER ROAD, WAUNAKEE, WI 53597

C1

Project location; near the beginning of the Lower Rock River Watershed, on the Maier ‘White Gold’ Dairy – Approximately 200 miles from the confluence of the Rock and Mississippi Rivers

Southerly view over algae trough toward dairy (duckweed pond visible to right)

1. Executive Summary

NRCS-CIG project #69-3A75-10-161 demonstrated the use of aqua-cultural cropping to manage digested manure derivatives and organic substrate wastewater (‘centrate’) liquid on a Wisconsin dairy farm, after processing in a complete-mix, ‘community’ anaerobic digester (AD). This pilot-scale study also demonstrated nutrient sequestration by a sediment basin and constructed wetland’s emergent vegetation (built to NRCS & Wisconsin-DNR codes), as a complement to the ‘upstream’ photosynthetic production by an assemblage of attached filamentous algae. The constructed wetland system additionally received storm surface runoff from the dairy’s farmstead, and prevented release into the local watershed. (Total phosphorus levels were measured from 3 mg/L to below 1 mg/L throughout the project’s Lower Components.)

Indoor and outdoor algal-culture systems were to use nutrient media comprised of diluted digestate fractions from the Dane Community anaerobic digester, then flowing through the balance of the system to the constructed wetland. Wastewater flow through the cascading system was re-engineered to detour mid-stream, after a meeting with the W-DNR resolved that ‘manure derivatives’ treated in the project’s Upper Component features would be considered point-source discharge. Liquids treated in the Upper Components were ultimately irrigated onto an adjacent field of alfalfa, before field runoff was treated in the project’s Lower Components – a sediment basin and wetland.

The original intent was to judge the effectiveness of the project by focusing upon the extent of phosphorus clearance from water samples and the degree to which phosphorus is sequestered into vegetative biomass and wetland soils. The project was based on two previously demonstrated aquaculture technologies, though success in applying these technologies to treated waste from a Wisconsin dairy operation was confounded by the variety of high-potency FOG and carbohydrate wastes that the digester AD operators brought in to produce a greater volume of biogas, and subsequent renewable electricity.

A series of chemical and physical analysis on process materials from the AD provided insight as to potential change in NPK&S, and on transfer of these macronutrients to the system outputs. There was no clear indication of N, P, or K loss, but S was lower in concentration as output, due to volatilization of H_2S gas. For all NPKS there was a clear division in concentrations going into the centrate and solid fractions that result from centrifugal separation of the digestate slurry exiting the digestion tanks. Macronutrients went into fiber at a higher rate. These were the primary manure derivatives used in nutrient media for growing algae and duckweed.

Investigating the use of manure derivatives to grow attached filamentous algae and floating duckweed, we encountered 2 major hurdles; centrate (liquid fraction) was too loaded w/ revived organisms and organics to serve as a viable medium, even at high dilution rates. Light & heat intensity exasperated problems w/ revived organisms. Compost tea water was more successful. As a result we identified optimal periods when growth was maximized; primarily the spring and fall months. Indoor growth was supplemented w/ LED grow lights. Using appropriate dilutions of compost tea water we were able to provide a NH4-N loading rate of 0.5 g/NH_4-N/m^2/day. This led to an average algal production rate ranging from 4.5 to 5.0 g (dry wt.)/m^2/day, with a high of 5.8/m^2/day. Upon analysis we found a nutrient uptake rate of 0.0025 g NH4-N/m^2/day.

The economics of using aquatic plants in this pilot (specifically *attached filamentous algae*) to sequester macronutrients year around, were found to be $17,000-$18,000 per pound of P sequestered (only slightly lower outdoors) under optimal growing conditions. Assumptions included a 10-year amortization of capital costs, and one full time equivalent (FTE) farm hand to operate year ‘round indoors, with an additional FTE during the 4 mo. outdoor season. If plant material was dried to optimal moisture level (not friable), and shredded, it would in theory be 100% available for recycling as organic, dry fertilizer. Duckweed, at up to 25% starch may serve as a fermentable sugar.

2. Project Background (Description)

Agriculture is identified as the leading source of pollution for more than 40% of impaired U.S. water bodies, and farm runoffs of nitrogen (N) and phosphorus (P) are linked specifically to the damage in half of these instances. Although supplements of N and P are required to improve crop yields, livestock-manure or commercial-fertilizer applications that exceed agronomic rates create major sources of ground- and surface-water contaminations. The likelihood of nutrient runoff following application of manure (or effluent from anaerobic manure-digester systems) is determined by factors such as soil type, manure-loading rates, characteristics of solids, degree of plant incorporation, and the timing of applications relative to storm runoff events. P runoff is particularly damaging to freshwater habitats, and is widely recognized as the limiting nutrient for undesirable plant growth (chief driver of aquatic eutrophication).

For producers, P-based standards can prove problematic, as there is an incongruence between the N:P ratio of manure (*ca*. 3:1) and uptake by crops (*ca*. 8:1). Farming areas having high animal densities and high levels of excess nutrients increasingly are those regions with shortages of land available for manure spreading at agronomic rates. Producers in such areas frequently are required to transport manure long distances off-farm, at considerable expense. The work undertaken in this project directly addressed such problems by applying new aqua-cultural technologies for sequestering P into plant biomass; demonstrating P reductions using both emergent wetland plants and cultures of attached filamentous algae. The emergent wetland plants were cultivated in a 22,000 s.f. ‘constructed wetland’, designed to process farmstead and field runoff that originally entered the local watershed. The attached filamentous algae was grown year-around in a greenhouse facility clearing manure nutrients from water prior to its drainage to the harvestable bog, where it would then support growth of other species of fast-growing, aquatic plants.

The harvestable bog in our project was built to hold its water at a consistent depth, supporting growth by emergents across the entire surface. In theory, the basin could be drained (sump pump) at will, providing access to machinery used to cut the vegetation at the dried sediment surface. This design would promote collection and removal of vegetative matter at periodic intervals, and providing a tool for harvesting P that has been sequestered into angiosperm tissues. Emergent species are recognized as vessels for the transient storage of nutrients in wetland environments, absorbing nutrients during the growing season and then releasing large amounts at senescence. Ultimately duckweed was the species of choice, for it’s rapid uptake of nutrients, and potential value as a starch crop.

Attached filamentous algae (often termed "algal turfs") consist of heterogeneous, multi-species assemblages of algae with relatively low statures, high biomass-specific growth rates, and rapid vegetative regeneration from intact basal cells, provided they are not damaged by grazing or mechanical harvest. Cultures of attached filamentous algae most often are grown on porous substrata, under intense illumination in shallow-water troughs that provide high-velocity currents. Such currents disrupt nutrient diffusion barriers that otherwise would establish around individual filaments, thus promoting high rates of nutrient uptake by cells. These uptake rates are often coupled to high levels of biomass accumulation, and cultures require frequent harvests to avoid self-shading or blocked circulations of media. (Basal cells typically are protected in the pores of the substratum when the surface is mechanically scraped for harvest.) Numerous studies have documented high productivities by commercial cultures of attached filamentous algae growing on wastewater from fish-farming operations [8], agricultural runoffs, domestic sewage, and industrially contaminated groundwater. When grown on preparations of livestock-manure specifically, cultures of attached filamentous algae have demonstrated production rates from 5.5 g dry wt./m^2/day, 25 g dry wt./m^2/day, depending upon the nutrient loading rates utilized. Cultures grown on manure preparations have also shown P-clearance rates ranging as high as 77% [13]. Dried algal biomasses harvested from cultures grown on livestock manure hold potential for use as a slow-release fertilizer or as a source of fatty acids for biofuel production. It is noted that most published studies involving field culture of attached filamentous algae have been conducted in subtropical climates or, in temperate climes, have been limited to the warmest months of the calendar year. Thus a major objective was to adapt attached filamentous algal culturing to the climate of the Upper Midwest; utilizing excess heat and energy produced by a methane-digester facility to raise temperatures and artificially illuminate the cultures during the coldest periods of the year.

Our pilot-scale project was established on a Concentrated Animal Feeding Operation (CAFO), a dairy farm that has been placed under a P-based, comprehensive nutrient management plan (CNMP). A goal then, was to demonstrate that significant P sequestration may be achieved on-farm through both of these aquaculture technologies, and to identify the eventual scale-up that would be required for processing that portion of the CAFO's P load that currently is taken off-farm for land spreading.

During the course of this study, the Wisconsin State Legislature made revisions to statutes NR 102 and 217, which address quality standards for surface waters and discharged effluents. The revisions essentially cut allowable P levels by a factor of 10, though promulgation of these new standards has been delayed. Advisors within local agricultural and environmental communities consider that an eventual promulgation of these standards is likely, and that such legislation will eventually establish a P-trading system involving both producers and municipal water utilities throughout the state. This project then, could also facilitate new aqua-cultural systems monitoring clearance of P from waters and sequestration of P into sediments and plant tissues. It could also help establish basis for payments in a future Wisconsin P-trading scheme.

The project commenced in the autumn of 2010, with visits to algae growing sites in and around the Chesapeake Bay area. Cultivation systems known globally by different names, High-Rate Algal Ponds (HRAPs), Algal-Turf Scrubbing (ATS), and Algae Wheel technology were evaluated for best-practices, and adaptation to an Upper-Mississippi River Basin dairy operation. Ultimately, our NRCS-CIG backed project spawned 'Phyto-Aquatic Nutrient Recycling' (PANR).

Original project action plan and timeline

Task 1	Engineering/ Design Turf-Algae Culture Facility & Harvestable Bog; Q1 through end of January in Q2
Task 2	Engineering/ Design Storm Water Sediment Basin, Constructed Wetland & Pond; Q2
Task 3	Construction of Turf-Algae Culture Facility & Harvestable Bog; Q2 through end of April Q3

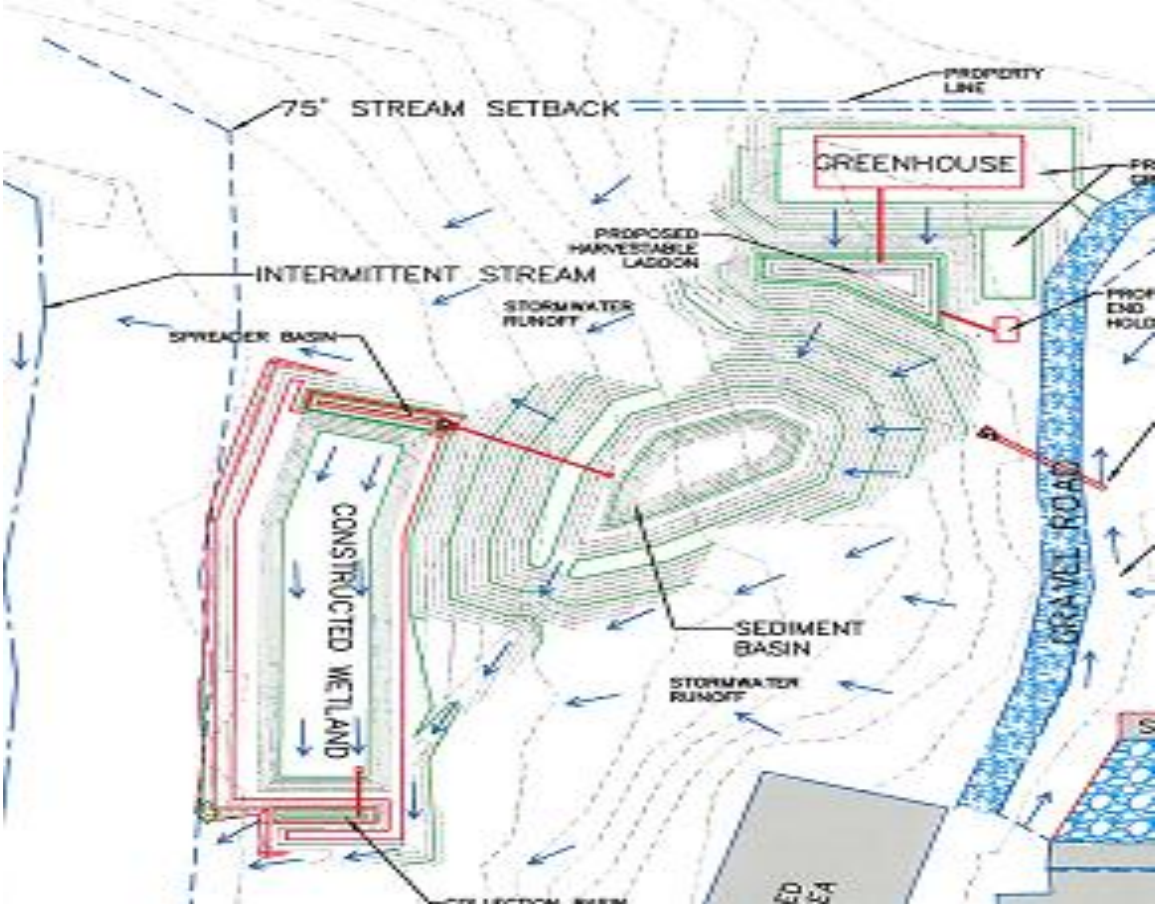
Task 4 Construction of Storm Water Sediment Basin, Constructed Wetland & Pond; March of Q2 through end of Q3
Task 5 Operational Turf-Algae Culture Facility & Harvestable Bog; Q3 through Q8
Task 6 Operational Storm Water Sediment Basin & Pond; June of Q3 through Q8
Task 7 Design/Develop Sampling & Testing Plan; Q2
Task 8 Water Sampling & Data Collection; May of Q3 through Q8
Task 9 Operational Constructed Wetland; March of Q6 through Q8
Task 10 Data Analysis & Conclusions; Q5 through Q8
Task 11 Formulate Educational/Training Program; May of Q7 through Q8
Task 12 Prepare and Present Final Report; Q8

3. Engineering & Construction

The project was originally planned for a large dairy (Crave Brothers), approx. 30 miles east of the eventual location, but also in the Rock River Watershed. After securing permission from the NRCS, the project site was changed to the White Gold Dairy, immediately adjacent to the new Dane Community Digester. This change afforded greater access with improved proximity, and preserved Clear Horizons, LLC as a project partner since they are the digester owner/operator in both cases. All civil engineering work, and most site drawings were executed by primary project partner, Resource Engineering Associates (REA). [All civil engineering reports can be found in the appendices attached hereto.]

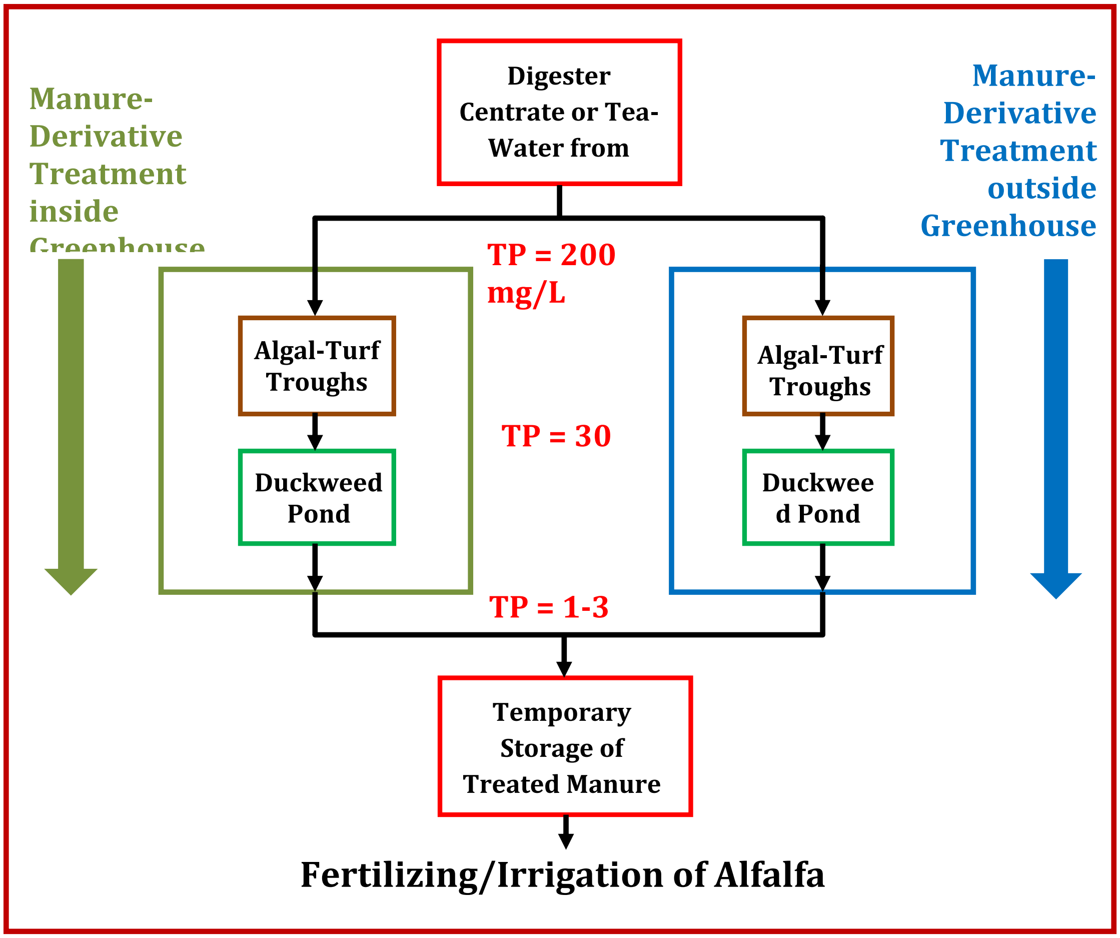
Site Plan

Drainage/flow follows the natural topography from upper right (northeast) to lower left (southwest). Major site features; Farmstead lower right; Digester upper right; Irrigation field (alfalfa) right (east); Final drainage to watershed lower left].



With the change of venue came the opportunity to utilize a much more favorable elevation/grade change throughout the project site. Furthermore, it provided the opportunity to demonstrate nutrient runoff and drainage water best practices on a piece of ground that what largely unused and marginal for crop production due the steepness of the hillside and highly variable soils.

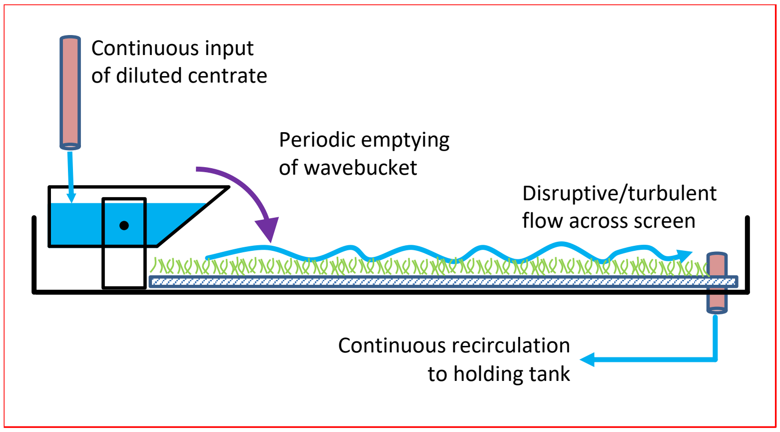
All of the components originally planned were built on the new site, which can be broken down into two primary components; The Upper Section directly handled 'manure derivatives' with its corresponding stricter set of rules, while the Lower Section, was composed of the physically larger elements, handling 'field runoff' and built to existing NRCS specifications. The flow diagram below illustrates the planned movement of liquids through the Upper Section of the project, with estimates of phosphorus removal (in red);

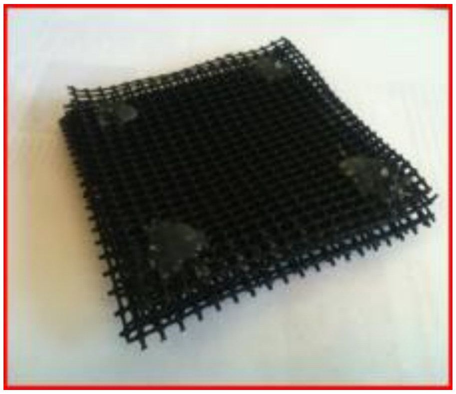


For reasons explained in the coming sections, the movement of liquid through the upper components of the system did not take the continuous, cascading route originally anticipated. Beginning-to-end processing had been planned to take advantage of both gravity and the natural properties of flowing liquid; in the end however, the challenges of dealing with *point source* vs. *non-point source* discharge, and creating an economic, year 'round, conditioned space added to more manual labor and altered engineering. Changes in biological activity from recirculating liquid growth medium was further exacerbated by the hottest summer on record for Dane County, with several weeks of 100+ degree temperatures.

Indoor Trough Designs

Project PI, John Hackney, designed the first, smallest, and most versatile trough used in the project. All major components were made of plastic and plexiglass; a substrate (algae growing) deck that fit within a standard feed trough created a platform for removable plastic mesh for easier algal harvesting. Conceptual drawing, and photos below (with more in Appendix #5);





This trough proved versatile, and easy to use, though it's volume was small, and it was more susceptible to rapid temperature change on the hottest summer days. Made from a simple plastic watering trough, it could be very economic if mass-produced, while lending itself to greater portability/versatility.

MATC student (large) trough design

With the project program connection to Madison (Area Technical) College, Dr. Ken Walz proposed that his engineering students tackle a new generation, larger, more sophisticated trough as part of their spring (2012) semester course work. They started by drafting the following description of the challenge;

Problem Statement

Great Lakes Ag Energy LLC, in conjunction with Clear Horizons LLC at the Dane County Manure Digester site, will be growing algae in a greenhouse using phosphorus-rich liquid centrate removed from methane-producing anaerobic digesters. This algal turf will reduce the detrimental levels of phosphorus in the centrate, allowing it to then be irrigated as grey-water, a nutrient and pathogen-reduced, non-point source discharge, as per applicable law, thereby reducing water pollution. Madison College has been recruited to assist in developing this project. Our objective is to design and implement a trough growing system that maximizes algal growth and thus phosphorus sequestration, while minimizing electrical consumption and startup costs. The system will utilize a centrate/*H_2O *mixture holding tank, turbulent flow of recirculating centrate mixture in growing troughs, and upon phosphorus depletion, discharge into storage cistern or duckweed propagation pond for further nutrient* *filtration. The system will also incorporate supplemental lighting, potential CO_2 aeration of the centrate mixture, and easy removal/cleaning of the algae growing screens. This construct must last for at least two years, and be readily adjustable for experimentation of production variables.*

With that, they set to work and created a wooden-framed, CO_2 enriched, partially LED-lit trough, with more than twice the surface area of the project's smaller, workhorse, indoor trough.

When fully operable it was as productive as the smaller trough, though less 'watertight'. (White-colored, vinyl panels were originally glued into the wooden structure, with a shingling overlap.) After retrofitting with HDPE (rubber pond-liner) we had a more usable product. It was however, still a bit leaky and had a tendency to overheat more quickly with the 'aftermarket', black rubber liner. A larger, 5 gallon wave bucket was the functioning front-end, creating the desired wave surge by filling approx. every 30 seconds to the tipping point (utilizing a 3 phase, 1/2 hp, 'lawn sprinkler' pump). The advancement with this trough was a CO_2 gas-sparging column designed to charge the liquid medium, and accelerate plant growth.

Outdoor Trough & Harvestable Bog Design

Moving outdoors in the warmer months (April-September) the team first installed a 30,000 gallon ‘harvestable bog’. The resulting duckweed pond/hydroponic feature, with approx. the same surface area as the footprint of the greenhouse, was lined with a WDNR-approved HDPE (rubber), pond-liner product. The fast-growing, native duckweed was found to be easy to propagate and ‘transplant’ from the greenhouse *refugium*. The only challenge came on windy days was the plants were pushed readily across the surface, opening up significant opportunity for other species to compete more favorably for sunlight.

The 2 outdoor troughs each had a 4’x30’ screen-covered deck (utilizing the same plastic screen product as the indoor troughs), set into the hillside with a 2% grade/downhill slope from top to bottom. They were also lined with the HDPE pond-liner.


Photos:

1) 3’ of P200 rock/sand ($P_{200} > 20\%$) brought in and laid on 5 sides to create protective barrier in case of spills/seepage of ‘manure derivative’ (top left)
2) Leveling/grading (top right)
3) Rack (top of platform) for 10 gal. stainless steel wave buckets and terminal end of PVC (divider) recirculation pipe (lower left)
4) Sump-pump in pit provided continuous (re-) circulation of centrate or tea-water for PANR processing (lower right)
5) 60 mil HDPE - liner used in all wet areas, per WI-DNR code

Ultimately, each component of the Upper section was built as planned, though added expense was incurred to comply with all Wisconsin DNR (WDNR) regulations for handling manure, or manure derivate. Additional precautions included; HDPE lining of all outdoor features, 3’ thick, 200-grit fill material underneath and on all sides of the outdoor trough, as well as a 1,600 gallon, concrete storage cistern to aggregate all liquids before irrigation on the adjacent alfalfa field.

Lower Component Features

For the most part, components of the Lower section were built as planned, and to NRCS standards. (Fully outlined in civil engineering documentation for the project, which can be found in the Appendices of this report.) Non-point source nutrient management for ‘field runoff’ best practices were demonstrated throughout.

Sediment Basin (WDNR Code 1064)

The largest surprise/challenge in this part of the construction was the discovery of a dry spring on the steepest grade, and subsequent sandy soils surrounding the sediment basin. 40 bags of bentonite spread on the bottom of this feature did improve runoff retention, but ultimately, the drought of 2012 meant there was very little liquid in the site’s largest feature for much of the research period.

Constructed Wetland (NRCS Code 656)

The constructed wetland was built by Snyder Excavating & Blacktop, LLC. It was excavated, graded with earthen ramparts, and prepared for planting by project volunteers. In 2012, we irrigated plantings with well water on several occasions during the hottest part of summer. Susan Priebe of Aquaterra (Wetland plant contractor) visited the site in September and offered a positive assessment, although some species fared much better in the prolonged heat/drought, i.e. – sedges, rushes, and wool grass.

4. Analyses of Digester Process Materials & Manure Derivatives

Establishment, growth, and biology of filamentous-algae systems

Background

A major project goal was to investigate potential uses of manure-derivatives as nutrient media for growing aquatic vegetation. At the Dane Community Digester, three forms of manure-derivatives are generated by anaerobic digestion of wet dairy-manure *via* complete-mix operations: 1) **digestate**, collected as slurry by pumping from tanks following *ca*. 30-days of digestion and methane production, 2) **centrate**, produced as the liquid fraction of digestate's centrifugal separation, which occurs immediately after slurry collection, and 3) **fiber-solids**, the solids fraction of this same centrifugal separation. These derivatives are formed by the anaerobic digestion of two types of feedstock: **dairy-cattle manure** (*ca*. 90% of total feedstock mass) and **substrate** (food-processing and other industrial wastes, forming ≤ 10% of total feedstock mass). The centrate and the fiber-solids fractions are the final two process materials exported from the digester system, and thus are considered to have potential for nutrient-media preparation. Centrate was speculated to show promise at appropriate dilutions, and fiber-solids had been identified from previous practices as providing enriched "compost-tea" solutions after soakings of the dry mass in fresh water (1).

These feedstocks and manure-derivatives constitute a set of **anaerobic-digester process materials**. Digester operators sample these process materials periodically and subject them to physical and chemical analyses so as to develop and maintain effective management strategies. Results of process-material analyses conducted during our study period are examined thoroughly in this section of the report. Examination provides opportunity not only to observe whether the Dane Community Digester functions as expected, based on previous studies of complete-mix digester systems(2), but also to trace movement of plant nutrients and organic compounds through stages of the anaerobic-digestion process. Such monitoring improves prediction of the utility that centrate dilutions and compost-tea preparations could hold for future aquaculture projects.

Findings

General procedures and measures of physical characteristics. Values for analyses of materials associated with varied stages of anaerobic digestion at the Dane Community Digester are listed in **Table 1** and illustrated in Figures 1-9. All values were measured by state-licensed (Wisconsin) analytical laboratories under contract to Clear Horizons, LLC, for routine reports on the system's performance over a period that encompassed the current study. Table 1 lists averages, minima, and maxima for physical and chemical values collected during routine examinations on 35 separate samples each of **raw-manure** (compiled from the three participating dairies), **substrate** (compiled from multiple deliveries prior to its mixing with manure), **digestate** (immediately upon pumping from digester tanks but prior to solids/liquid separation ("SLS")), and **centrate** (immediately following SLS). Dried fibrous materials are not subjected to physical/chemical analyses on such a routine basis at the Dane Community Digester, but Table 1 also includes data from this period on four separate samples of **fiber-solids** that were collected after SLS and additional waste-heat drying.

Physical and chemical measurements were conducted following standardized methods of manure and/or soil analysis (3,4). Total-N content was determined following the macro-Kjeldahl method (5), whereas NH_4-N and NO_3-N were measured by ion-selective electrodes (6). Determinations of P as P_2O_5, K as K_2O, and Total-S concentrations required breakdown of organic matter for solubilization of the elements. This digestion was performed using nitric acid with peroxide on a heated block-digester, which was followed by reflux with nitric and hydrochloric acids, filtering with deionized water, and finally measurement using inductively coupled plasma optical emission spectrometry (7,8). Determinations of Total-P were conducted on separate samples, utilizing the vanado-molybdophosphoric acid method of digestion and spectrophotometric readings at 470 nm (3). Volatile-Acid determinations were coupled with titrations for alkalinity measures. Samples were titrated with standard sulfuric acid through two stages (initially to pH 5.1, then from pH 5.1 to 3.5), and the bicarbonate and total volatile fatty-acid concentrations were calculated directly from appropriate titration volumes (9). Total Solids were calculated as the portion of the total mass after thorough drying at 103 °C, and Volatile Total Solids were calculated as the portion of Total Solids that were determined to be volatile, based upon combustion at 550 °C.

Although tests on these material samples provided reasonable averages and in most cases relatively narrow ranges between maxima and minima, these data were not subjected to further statistical analyses. This was due in part to our lack of direct participation in the laboratory analyses, in part to the variation in number of replicate samplings provided for different process materials. We instead interpret these data as describing general shifts in physical and chemical parameters through process stage and as indicating general macronutrient concentrations that would become available to plants through media prepared from centrate and fiber-solids. All values in Table 1 are presented as concentrations measured on the five process materials. No firm conclusions may be reached about mass balances beyond the stage of digestate collection, because the system becomes open to the environment at that point, and total weights and volumes of neither fiber-solids nor centrate were available for relevant calculations.

Table 1. Average, minima, and maxima concentration values from physical and chemical analyses of biomasses under various stage of processing at the Dane Community Digester system. Biomasses are listed as materials from different stages in the anaerobic-digestion process. **Raw-manure** and **substrate** refer to samples of dairy-cattle manure and to samples of food-processing and other industrial wastes (90% and 10% of the system's total feedstock inputs, respectively). **Digestate** refers to slurry pumped from digester tanks as system output, following an average 30-day dwell time under anaerobic digestion. Recovered digestate is subjected to centrifugal solids/liquid separation to produce liquid **centrate** (which exits the system through return to participating dairies) and partially dried **fiber-solids** (which are further dried with excess gen-set heat and exported from the watershed as animal bedding or soil -amendment materials). Values for raw-manure, substrate, digestate, and centrate are calculated from 35 separate analyses of material samples. Values for fiber-solids are collected from four separate analyses of material samples.

Process stage	**Dry Matter** %	**Moisture** %	**pH**	**Alkalinity** (lbs/1000 gallons)	**P as P_2O_5** (lbs/1000 gallons)	**Total-P** (lbs/1000 gallons)	**Total-N** (lbs/1000 gallons)	**NH_4-N** (lbs/1000 gallons)	**NO_3-N** (lbs/1000 gallons)	**K as K_2O** (lbs/1000 gallons)	**Total-S** (lbs/1000 gallons)	**Total Solids** (lbs/1000 gallons) (mg/L)	**Volatile Total Solids** (lbs/1000 gallons) (mg/L)	**Chemical Oxygen Demand** (lbs/1000 gallons) (mg/L)	**Volatile Acids** (lbs/1000 gallons) (mg/L)
Raw-manure	7.03 Min = 5.25 Max = 11.01	92.97 Min = 88.99 Max = 94.75	7.01 Min = 6.8 Max = 7.5	66.63 Min = 49.87 Max = 133.69	8.42 Min = 3.83 Max = 13.21	4.77 Min = 3.26 Max = 7.82	20.96 Min = 1.92 Max = 29.05	9.77 Min = 0.835 Max = 24.22	0.1420 Min = 0.0835 Max = 0.3758	23.19 Min = 9.41 Max = 30.78	2.62 Min = 1.15 Max = 3.89	543.6 Min = 359.05 Max = 835.00 65,100 Min = 43,000 Max = 100,000	26.97 Min = 17.37 Max = 44.00 3,230 Min = 2,080 Max = 5,270	717.4 Min = 268.4 Max = 2,260 85,921 Min = 32,140 Max = 270,700	41.14 Min = 14.06 Max = 56.96 4,927 Min = 1,684 Max = 6,821
Substrate	45.97 Min = 30.44 Max = 75.66	54.03 Min = 24.34 Max = 69.56	4.9 Min = 4.5 Max = 5.1	< 0.1420	2.94 Min = 0.16 Max = 11.63	1.37 Min = 0.12 Max = 3.46	4.11 Min = 0.4 Max = 16.6	1.25 Min = 0.5845 Max = 3.34	0.1420 Min = 0.0835 Max = 0.4175	22.50 Min = 0.80 Max = 71.09	1.03 Min = 0.20 Max = 3.95	1,204 Min = 192.1 Max = 3,173 144,200 Min = 23,000 Max = 380,000	154.5 Min = 19.21 Max = 433.4 18,500 Min = 2,300 Max = 51,900	6,331 Min = 43.57 Max = 15,846 758,200 Min = 5,218 Max = 1,897,800	93.52 Min = 59.93 Max = 122.9 11,200 Min = 7,177 Max = 14,714
Digestate	5.43 Min = 4.60 Max = 5.98	94.57 Min = 94.02 Max = 95.40	7.7 Min = 7.5 Max = 8.0	94.20 Min = 76.54 Max = 170.61	8.45 Min = 6.91 Max = 9.57	4.23 Min = 3.27 Max = 6.00	23.19 Min = 20.75 Max = 28.22	10.77 Min = 5.01 Max = 23.38	0.1253 Min = 0.0835 Max = 0.8267	27.43 Min = 23.15 Max = 30.49	2.40 Min = 1.80 Max = 2.88	447.6 Min = 400.80 Max = 634.60 53,600 Min = 48,000 Max = 76,000	17.54 Min= 15.03 Max = 26.72 2,100 Min = 1,800 Max = 3,200	767.2 Min = 302.8 Max = 8,831 91,877 Min = 36,260 Max = 1,057,600	12.95 Min = 6.16 Max = 20.67 1,551 Min = 738.3 Max = 2,476
Centrate	3.61 Min = 2.92 Max = 7.35	96.39 Min = 92.65 Max = 97.08	7.9 Min = 7.4 Max = 8.2	90.52 Min = 71.86 Max = 161.54	5.24 Min = 1.11 Max = 13.13	2.81 Min = 2.07 Max = 4.40	20.53 Min = 5.8 Max = 33.2	10.94 Min = 5.01 Max = 22.55	0.1336 Min = 0.0835 Max = 0.334	26.49 Min = 5.80 Max = 35.14	2.22 Min = 0.41 Max = 11.10	288.9 Min = 258.85 Max = 484.30 34,600 Min = 31,000 Max = 58,000	6.68 Min= 5.85 Max = 11.69 800.0 Min = 700.0 Max = 1,400	371.5 Min = 232.1 Max = 467.9 44,495 Min = 27,790 Max = 56,040	12.37 Min = 7.30 Max = 17.08 1,481 Min = 874.2 Max = 2,045
Fiber-solids	28.45 Min = 25.90 Max =	71.55 Min = 69.80 Max =	7.8 Min = 7.8 Max = 7.8	145.08 Min = 141.87 Max =	80.12 Min = 77.49 Max =	N.A.	67.97 Min = 65.97 Max =	25.89 Min = 23.38 Max =	0.1253 Min = 0.0835 Max =	34.19 Min = 33.44 Max =	9.50 Min = 8.85 Max =	2,255 Min = 2,188 Max = 2,321 270,000 Min=262,000	461.8 Min = 446.7 Max = 496.0 55,300 Min=53,500	580.2 Min = 568.8 Max = 600.8 69,480 Min=68,125	N.A.

Table-1 values indicate variations in dry-matter/moisture percentages between the five materials. Raw-manure varies from 5-11% dry matter, which closely matches literature values for dairy-cattle waste$^{(10)}$. Substrate dry-matter concentrations are much higher (average 46%), but the low proportion of substrate within the total feedstock mix (≤ 10%) contributes to even lower average levels of dry-matter (*ca*. 5%) in output digestate. This drop likely reflects general solubilization of feedstocks and an overall loss of biomass to the gas phase during the digestion process. After the digestate slurry is pumped from the digester tanks, SLS decreases dry-matter in liquid centrate (to *ca*. 3%) and generates a fiber-solids fraction with *ca*. 30% dry-matter. The Dane Community Digester utilizes gen-set waste heat for drying fiber-solids more thoroughly following SLS, to *ca*. 50% dry-matter.

Variations in average pH and alkalinity concentrations through materials at selected process stages are plotted in **Figure 1**. Average pH values increase notably from raw-manure (representing system input) to digestate (representing system output), and remain relatively constant into the centrate and fiber-solids fractions produced by centrifugal SLS. Average alkalinity values follow a similar pattern, though a definite division in concentrations is noted between centrate and fiber-solids, likely attributable to the tendency of alkaline salts to precipitate onto fibrous materials during SLS. The general increase in both pH and alkalinity during the 30-day dwell time may be linked in part to CO_2 production by microbe metabolism. CO_2 in solution promotes acidification within the liquid phase, but passage of CO_2 into the gas space at the top of each tank effectively removes this acidity, thereby increasing pH. Plots in Fig. 1 indicate generally increasing values of pH (about one pH unit) and alkalinity concentrations (*ca*. 50 lbs./1000 gallons) between input of system feedstocks, collection of digestate output, and SLS of the digestate into centrate and fiber-solids.

Measures of macronutrient concentrations. Monitoring macronutrient concentrations at these same stages of the digestion process provides opportunity to identify movement by these elements between process materials or loss of the elements' mass during the 30-day period of digestion. Plots of average Total-N concentrations (**Figure 2**) fail to indicate a clear change in averages between system input (raw-manure) and output (digestate), suggesting no loss of nitrogen within the closed digester tanks. However, much higher relative concentrations of nitrogen are apparent in the fiber-solids fraction of separated digestate than in the liquid centrate, which suggests a greater tendency by nitrogen compounds to adhere to fibrous materials during centrifugation than to remain soluble. **Figure 3** provides a potentially more detailed view of nitrogen movement by examining the compounds ammonia (NH_4-N) and nitrate (NO_3-N). The anaerobic conditions required for successful functioning of digester systems would predict reduction of nitrate to ammonia. Substantial decrease in nitrate concentration appears to occur between system input and output, but such a drop is not matched by ammonia increase. Ammonia is highly volatile and might exhibit movement into the gas space at the top of each digester tank, but unfortunately the compositions of these gases were not analyzed here. From the plots in Figs. 2 & 3, we presume no net loss of nitrogen during the digestion process, but perhaps observe reduction of NO_3-N to NH_4-N. The somewhat more pronounced differences in average concentrations between the liquid and solid phases that follow digestate-separation suggest a tendency by ammonia to adhere to fibrous material, whereas nitrate remains soluble in the liquid centrate.

Figure 1. Variations in average pH and alkalinity values through selected stages of anaerobic digestion at the Dane Community Digester. The category of "raw-manure" represents the majority of the system's feedstock inputs and "digestate" refers to the system's output after completion of digestion. Following removal from digester tanks, digestate slurry is subjected to centrifugal solids/liquid separation to produce the category of "centrate" (liquid fraction) and "fiber-solids" (solid fraction). Plots of both measures represent concentration averages and display general increases from the system's inputs to the output and separated fractions.

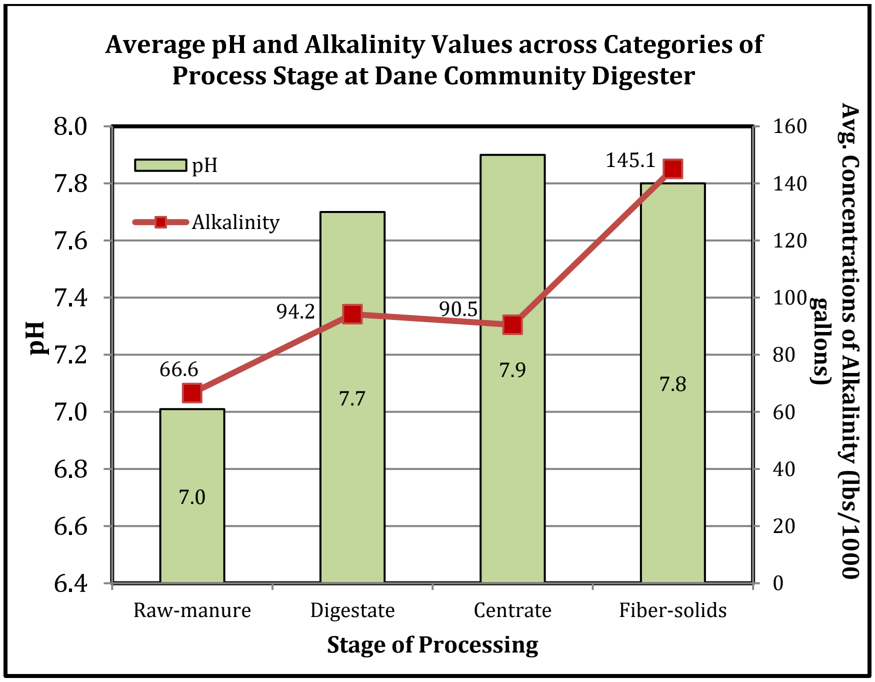


Figure 2. Variations in average concentration of Total-N through selected stages of anaerobic digestion at the Dane Community Digester. Plots show little if any change in concentration between system input (raw-manure) and output (digestate), suggesting no loss or gain of nitrogen within the closed system of a digester tank. Following centrifugal SLS of digestate, however, relatively lower concentrations of nitrogen are associated with liquid centrate than with the fiber-solids fractions.

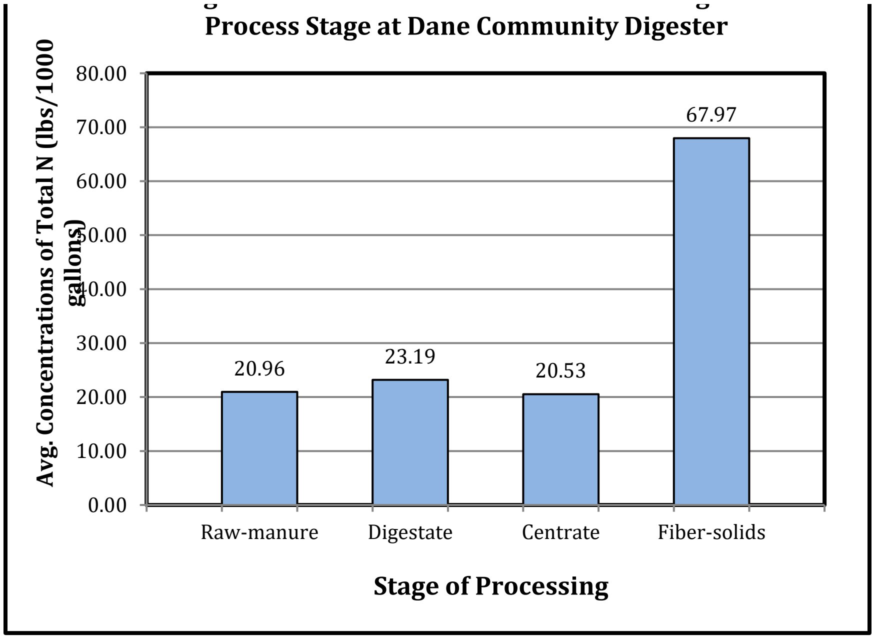
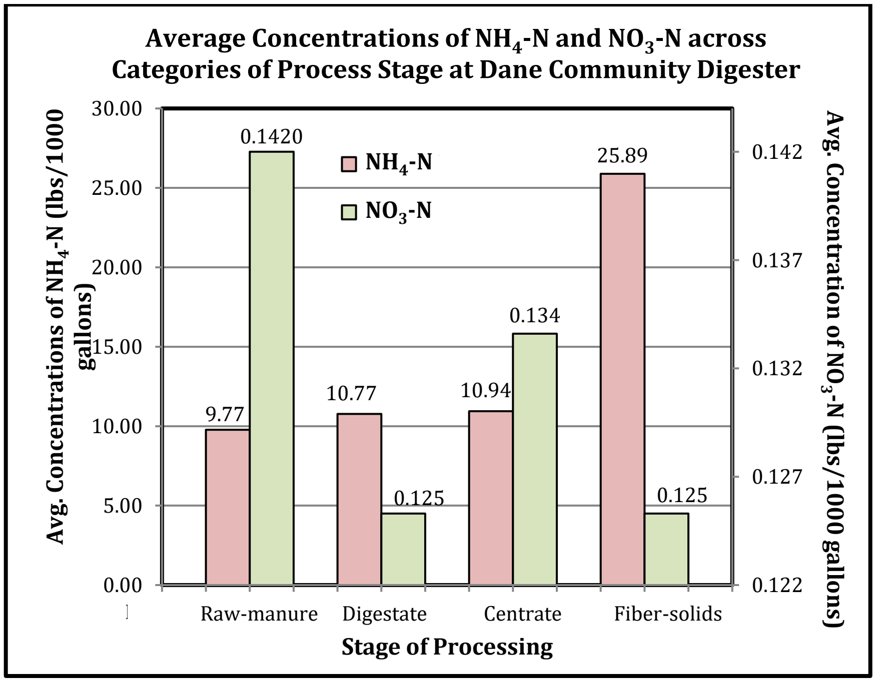
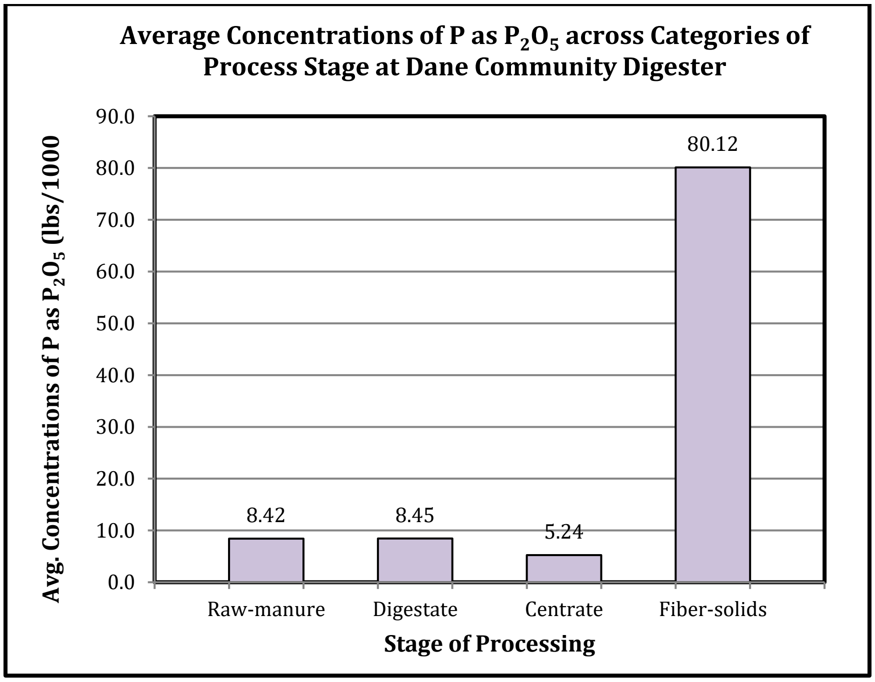


Figure 3. Variations in average concentration of NH_4-N and NO_3-N through selected stages of anaerobic digestion at the Dane Community Digester. Plots show little if any change in NH_4-N concentrations between system input (raw-manure) and output (digestate), but a substantial drop in NO_3-N concentrations in this same comparison. Following centrifugal SLS of digestate, a higher concentration of NH_4-N is noted for fiber-solids than for centrate, but concentrations of NO_3-N drop between centrate and fiber-solids.



Average concentrations for the macronutrient phosphorus in the form of P_2O_5 (*i.e.*, orthophosphate (PO_4^{3-})) are plotted for the same process stages in **Figure 4**. These averages also show no clear change in the element between the two forms of system inputs and the output. Again, we recognize no loss of this macronutrient during the anaerobic-digestion process. A marked division in concentration between liquid centrate and fiber-solids is readily apparent, however, and this is likely attributable to the strong tendency of the phosphate ion to leave the soluble state and bind tenaciously to particulate matter (10). Similar plotting for the macronutrient K as K_2O (**Figure 5**) suggests only a small, probably insignificant, increase in concentration averages between inputs and outputs. When compared to plots for the three other macronutrients analyzed here, a much less pronounced difference in potassium concentrations is apparent between averages for centrate and those for fiber-solids. This may derive from a higher solubility of potassium ions, which would serve to retain more of this element in the liquid state (centrate). Finally, average concentrations for Total-S (**Figure 6**) again show no marked difference in averages between raw-manure input and digestate output, but a clear division between the low levels in centrate and the elevated levels in fiber-solids.

Figure 4. Variations in average concentration of P as P_2O_5 through selected stages of anaerobic digestion at the Dane Community Digester. Plots show little if any change in P concentrations between system input (raw-manure) and output (digestate), but a substantial difference between concentrations found in the liquid fraction of centrifugally separated digestate (centrate; 5.24 lbs/1000 gallons) and the solids fraction of the separation (fiber-solids; 80.12 lbs/1000 gallons).



Average Concentrations of P as P_2O_5 across Categories of Process Stage at Dane Community Digester

Figure 5. Variations in average concentration of K as K_2O through selected stages of anaerobic digestion at the Dane Community Digester. Plots show little if any change in K concentrations between system input (raw-manure) and output (digestate), and only a small difference between concentrations found in the liquid fraction of centrifugally separated digestate (centrate; 26.49 lbs/1000 gallons) and the solids fraction of the separation (fiber-solids; 34.19 lbs/1000 gallons).

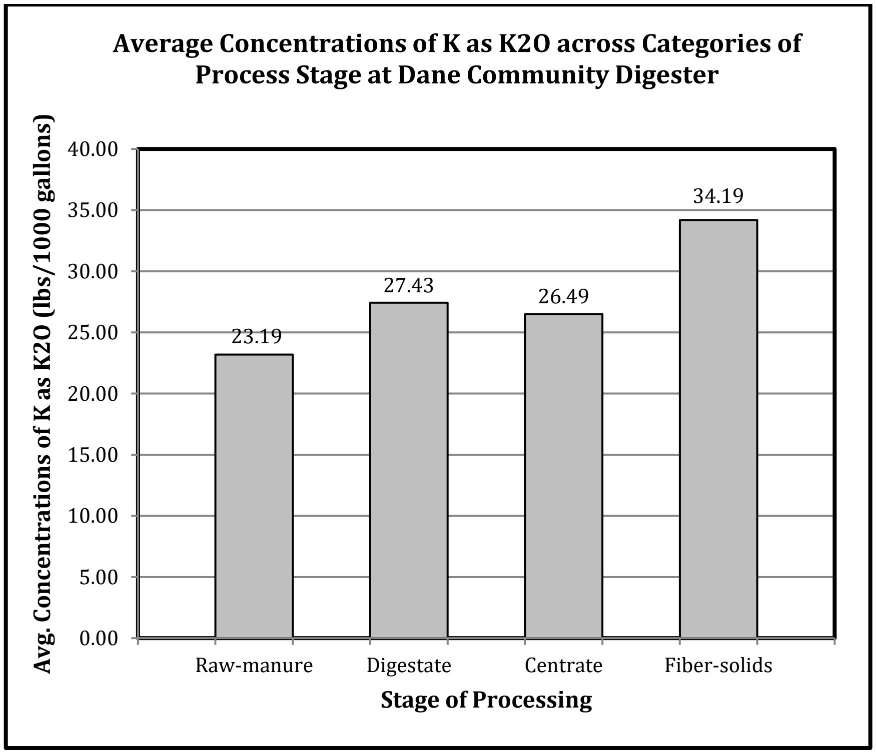
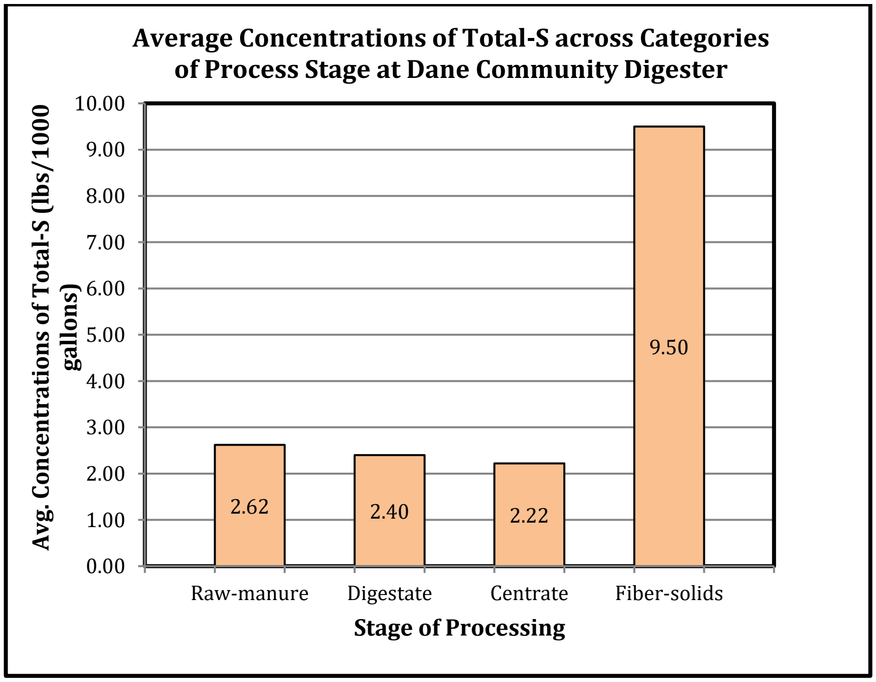


Figure 6. Variations in average concentration of Total-S through selected stages of anaerobic digestion at the Dane Community Digester. Plots suggest little change in S concentrations between system input (raw-manure) and output (digestate). However, a clear division appears between concentrations found in the liquid fraction of centrifugally separated digestate (centrate; 2.22 lbs/1000 gallons) and the solids fraction of the separation (fiber-solids; 9.50 lbs/1000 gallons).



The above analyses of average macronutrient concentrations at four selected process stages appeared to show clear differences in element distributions between the liquid and solid phases of centrifugally separated digestate. This identifies the relative levels of N, P, K, and S that are returned to farms as centrate destined for field applications or are bound to fiber-solids for ultimate export from the watershed. However, these particular plots failed to demonstrate clear changes in concentration occurring between system inputs and output inside the closed digester-tanks proper. As explained, the Dane Community Digester routinely processes substrates (food-processing and other industrial wastes, as *ca*. 10% of feedstock inputs) in addition to manure, but the substrates' impact on digestate macronutrient concentrations was not illustrated by comparing these four process stages in Figs. 2-6.

Public discussions in Wisconsin and elsewhere have highlighted at least occasional misunderstandings concerning the fate of macronutrients that enter anaerobic digesters as dairy-cattle manure. Frequently, it is assumed (incorrectly) that anaerobic digestion actually *consumes* phosphorus in some way, thereby decreasing the element's ultimate environmental impact. Data collected in the current study provide opportunity to examine more closely whether detectable changes in phosphorus or any of the other macronutrients occur within closed digester tanks. Table 1 presents averages for both raw-manure and substrate inputs, and for digestate output. By adjusting concentration averages for both raw-manure and substrates to account for dilutions effects, a more accurate picture of macronutrient movements during digestion may be plotted.

Figure 7 presents dilution-adjusted Total-N averages for raw-manure and substrate and the original average for digestate. The plot indicates that *ca*. 20% increase in Total-N concentrations occurs between the combined feedstocks and the single output (digestate) measured at the end of the 30-day period of anaerobic digestion. A similar increase is shown by adjusted averages for NH_4-N in these materials (**Figure 8**), but a slight decrease is observed among adjusted averages for NO_3-N (**Figure 9**). Although unanalyzed for statistical significance, these

Figure 7. Variation in average concentrations of Total-N between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentration for mixed inputs (19.27 lbs/1000 gallons) appears to increase by *ca*. 20% in the digestate pumped from closed digester tanks after 30-day period of anaerobic digestion.

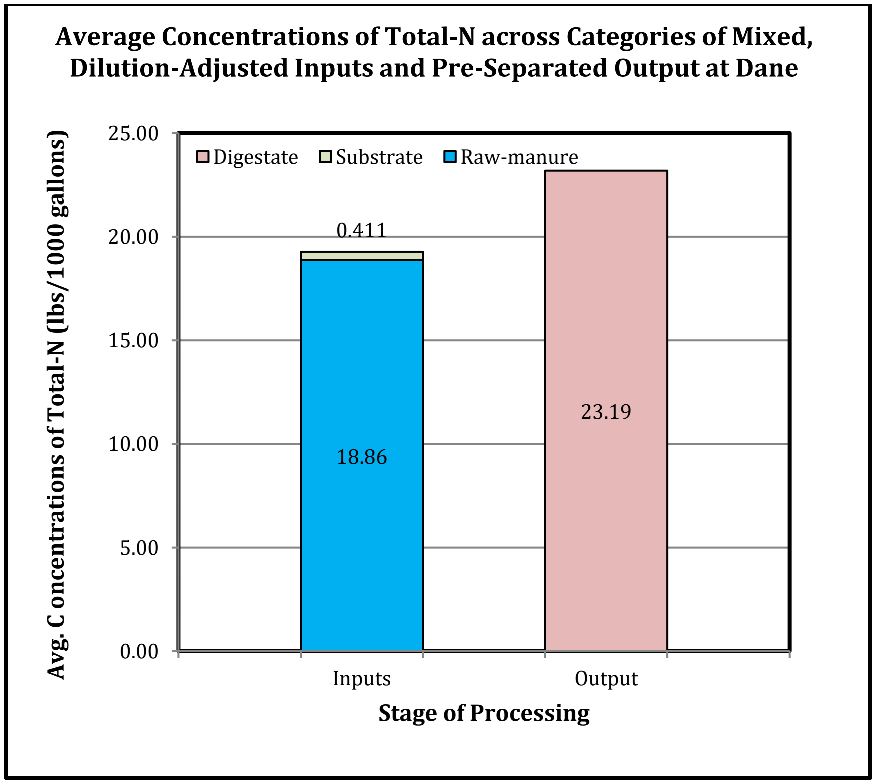


Figure 8. Variation in average concentrations of NH_4-N between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of ammonia for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentration for mixed inputs (8.92 lbs/1000 gallons) appears to increase by *ca*. 20% in the digestate pumped from closed digester tanks after 30-day period of anaerobic digestion.

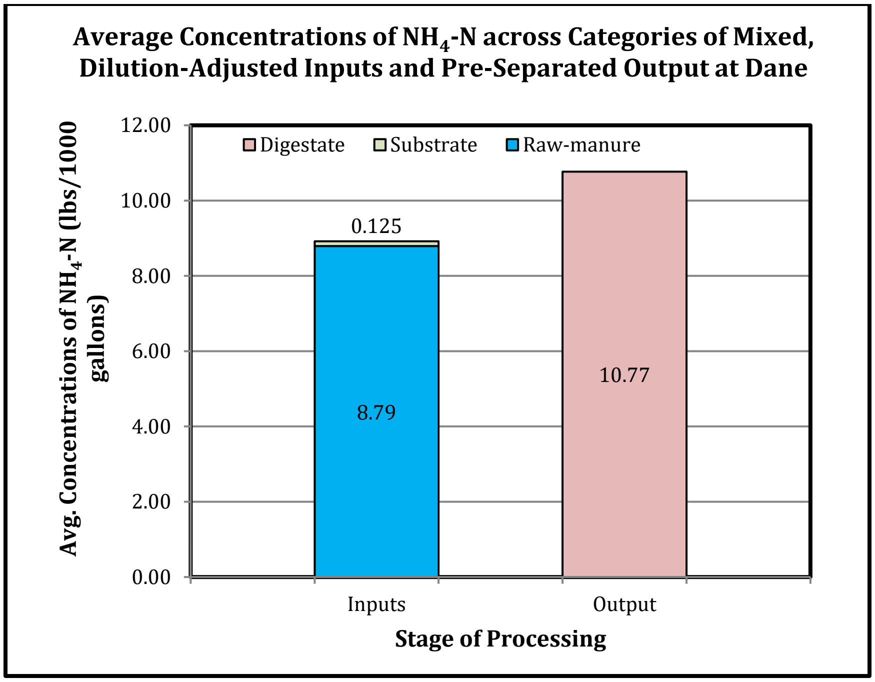
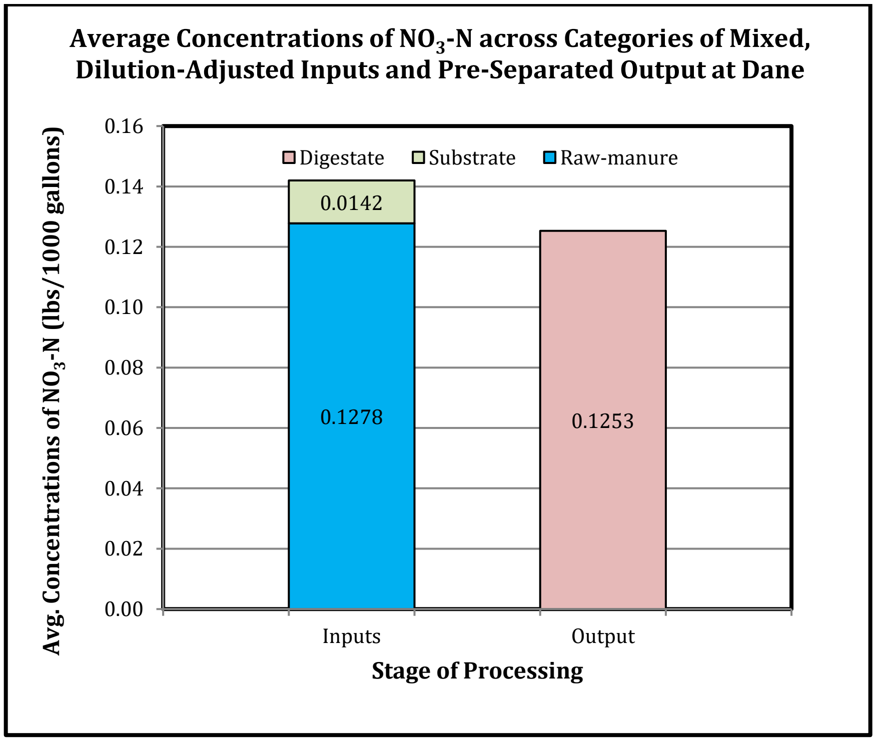


Figure 9. Variation in average concentrations of $N0_3$-N between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of nitrate for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentration for mixed inputs (0.142 lbs/1000 gallons) appears to decrease by *ca*. 4.4% in the digestate pumped from closed digester tanks after 30-day period of anaerobic digestion.



calculations suggest reliability based upon the relatively large number of replicates (35) gathered on the two system inputs and the single output. The apparent changes in nitrogen concentrations within the closed system of digester tanks might be adequately explained by a documented 9.5% loss of input biomass (volatile loss of carbon to CO_2 and CH_4) during methanogenesis at the Dane Community Digester facility(11), which would affect concurrent decrease in total volume of output digestate. Furthermore, as discussed above, the chemical/physical environment maintained within digester tanks may promote reduction of nitrate, increasing even further the concentrations of ammonia seen in the output digestate in Fig. 8. Such conversion in nitrogen form would explain declining nitrate concentrations for output in Fig. 9, though this decrease would have needed to counter the reduction of digestate volume stemming from biomass loss.

Similar dilution-adjusted averages for concentrations of P as P_2O_5 are presented in **Figure 10** and show an increase (*ca*. 7.4%) for concentrations in output digestate. This increase is less pronounced than those for nitrogen, but may also be linked to the phenomena of biomass loss and decreased digestate volume. The plot of dilution-adjusted averages for Total-P concentrations (**Figure 11**) shows a slight decrease in output digestate. This decrease is likely insignificant in the context of these data collections, and probably derives from inaccuracies in analytical methods. Detection of a valid decrease would contradict Fig. 10, because values in this plot show that Total-P concentrations surpass those of P as P_2O_5. Any increase displayed in Fig. 10 that derives from volatile biomass loss should be matched by an increase in Fig. 11. We suggest that these data provide no firm evidence of changing P concentrations during the 30-day period of digestion within tanks.

Phosphorus and the other macronutrients analyzed in this study are continuously mineralized by metabolic breakdown of digester feedstocks and then sequestered back into new organic compounds during microbe growth. The mineralized form of P is orthophosphate (PO_4^{3-}), the form most readily available for uptake by photosynthesizers. Fertilizer-industry conventions present phosphate in units relating to “P as P_2O_5”, based upon elemental P contributing 43.64% of the compound’s total weight. For biomasses, weights of Total-P account not only for P in the form of P_2O_5, but also for P in biological compounds such as the energy-carrying compounds ATP and ADP, nucleic acids, coenzymes, and phospholipids. Consequently, weights of Total-P should surpass those for P as P_2O_5. Comparing averages for the two forms of P in Table 1 demonstrates that Total-P weights, measured after complete chemical digestion of biomass samples, are *ca*. 10-30% greater than weights of elemental P in the mineralized form. **Table 2** compiles those values used to calculate these comparisons. Because Total-P wasn’t measured on fiber-solids, only averages for P as P_2O_5 are presented for these dried materials.

Figure 10. Variation in average concentrations of P as P_2O_5 between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of P as P_2O_5 for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (7.87 lbs/1000 gallons) appears to increase by *ca*. 7.4% in the digestate pumped from closed digester tanks after 30-day period of anaerobic digestion.

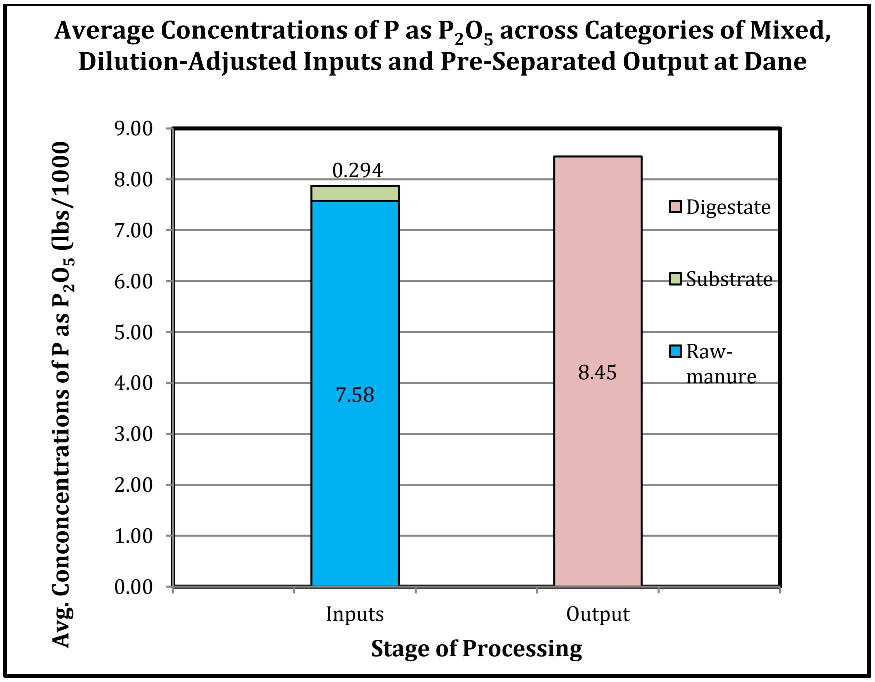


Figure 11. Variation in average concentrations of Total-P between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Total-P for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (4.43 lbs/1000 gallons) appears to decrease by *ca*. 4.5% in the digestate pumped from closed digester tanks after 30-day period of anaerobic digestion.

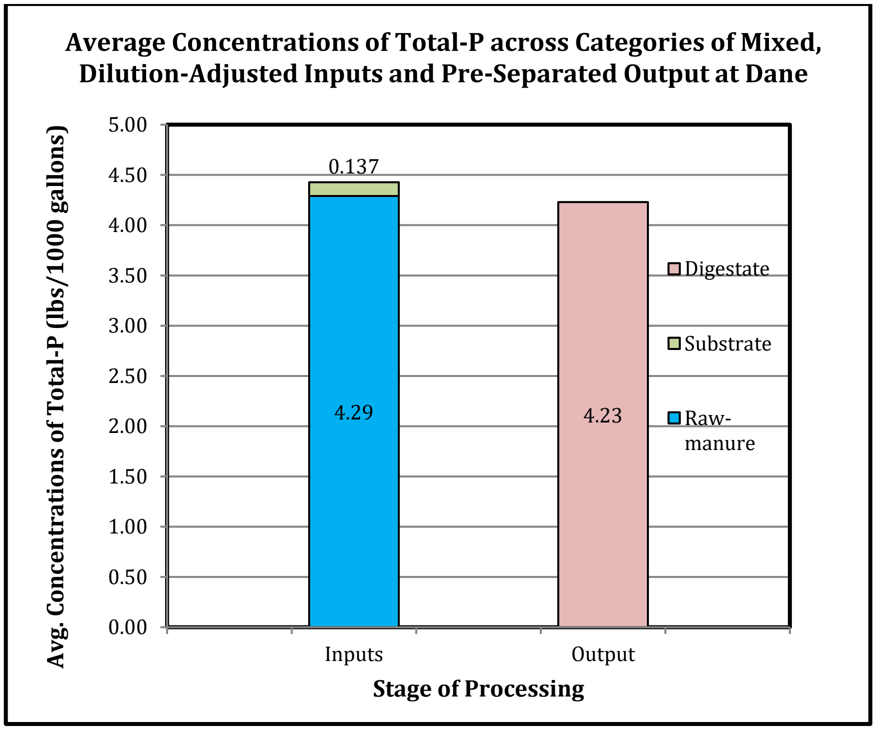


Table 2. Ratios of Total-P mass to P-as-P_2O_5 mass for materials collected at different stages of anaerobic digestion at Dane Community Digester. Values for mass of inorganic P_2O_5, mass of elemental P within P_2O_5, and mass of Total-P (including both organic and inorganic forms) are listed in calculation of ratios. Mass values for fiber-solids are limited to P_2O_5 measures, which precludes ratio calculation. Weights of Total-P are shown to exceed those for P as P_2O_5 by *ca*. 10-30%.

Stage of Processing	Mass of Inorganic P_2O_5 (lbs./1000 gallons)	Mass of Elemental P within P_2O_5 (lbs./1000 gallons)	Mass of Total-P (Elemental; Organic + Inorganic Forms) (lbs/1000 gallons)	(Mass of Total-P) / (Mass of P within P_2O_5) %
Input feedstock (raw-manure)	8.42	3.67	4.77	128.1
Input feedstock (industrial substrate)	2.94	1.28	1.37	107.0
System output (digestate)	8.45	3.69	4.23	114.6
Solids/liquid separation (liquid centrate)	5.24	2.29	2.81	122.7
Solids/liquid separation (fiber-solids)	80.12	34.96	N.A.	N.A.

Dilution-adjusted values of K as K_2O are plotted in **Figure 12**, and show an increased concentration of about 19% at stage of digestate recovery. The magnitude of this increase is similar to those for Total-N and NH_4-N, and could be linked in part to decrease in digestate volume stemming from carbon loss. **Figure 13** plots dilution-adjusted averages for concentrations of Total-S. A decrease in concentration of more than 29% is noted between input feedstocks and digestate output. This pronounced drop in Total-S concentration is probably linked to production of hydrogen-sulfide (H_2S) gas during methanogenesis. H_2S is formed by the chemical reduction of sulfur in organic compounds and passes from the liquid slurry to the gas phase at the top of each digester tank. Biogas is typically composed of 50-65% CH_4, 40-50% CO_2, and < 1% H_2S(12). Because H_2S combines with water to form sulfuric acid, however, measures must be taken to remove even this small amount of the gas to avoid damage to pumps and other equipment at digester-facilities.

The plots of macronutrient concentrations in Figs. 2-13 were generated to examine 1) potential changes in N, P, K, and S concentrations during anaerobic digestion in the closed systems of complete-mix digester tanks, and 2) the manner by which these macronutrient concentrations might divide between liquid-centrate and fiber-solids fractions following SLS of the output digestate slurry. Based on these graphics, we find no evidence that masses of N, P, or K are lost during the 30-day digestion period, though concentrations of these elements in output digestate may increase slightly due to ongoing volatile carbon loss to the tank gas spaces. Concentrations of S in output digestate show a clear decline, which is likely connected to generation of H_2S gas that subsequently moves from liquid to gas phases inside each tank. Although nitrogen shows no evidence of loss inside the closed systems, it likely undergoes change in chemical form as a result of NO_3-N reduction to NH_4-N. Examination of macronutrient movements following centrifugal separation of digestate into liquid centrate and fiber-solids demonstrates generally higher concentrations dividing into the solid phase. The one exception to this trend might be potassium, which is known to produce salts of high solubilities.

Table 3 summarizes concentrations of the macronutrients analyzed in this study. Following conventions established for commercially prepared fertilizers, nitrogen is presented as concentration of Total-N, phosphorus as concentration of P_2O_5 (in addition to Total-P concentration), and potassium as concentration of K_2O. Each nutrient shares the same unit of concentration (lbs/1000 gallons), which allows direct computation of N:P:K ratios. Examination of ratios demonstrates that values for raw-manure alone or raw-manure/substrate mixtures (2.5:1:3) are well within the range of values published for dairy-cattle (14). The ratio for centrate reflects a marked increase in N and K concentrations relative to the ratios for feedstocks. The ratio for fiber-solids, in contrast, shows increase in P and decrease in K relative to the feedstock ratios. These shifts in concentration are consistent with the relative solubilities for K (high solubility and tendency to migrate towards liquid fractions) and P (low solubility, tendency for strong particulate binding and migration towards solid fractions).

Figure 12. Variation in average concentrations of K as K_2O between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of K as K_2O for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (23.12 lbs/1000 gallons) appears to increase by *ca*. 19% in the digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.

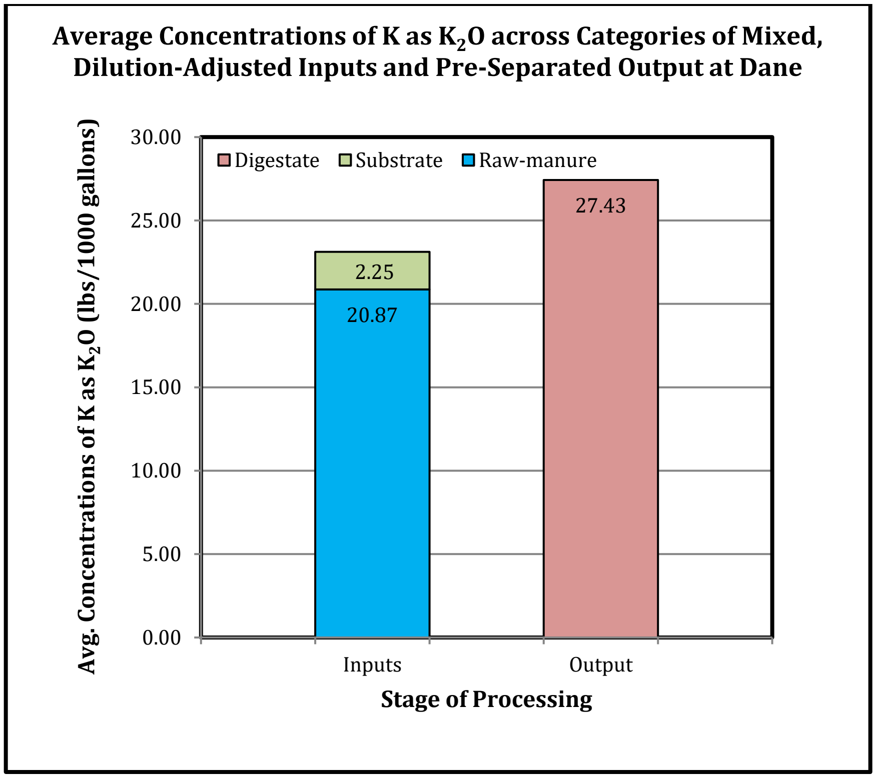


Figure 13. Variation in average concentrations of Total-S between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Total-S for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (3.39 lbs/1000 gallons) appears to decrease by *ca*. 29% in the digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.

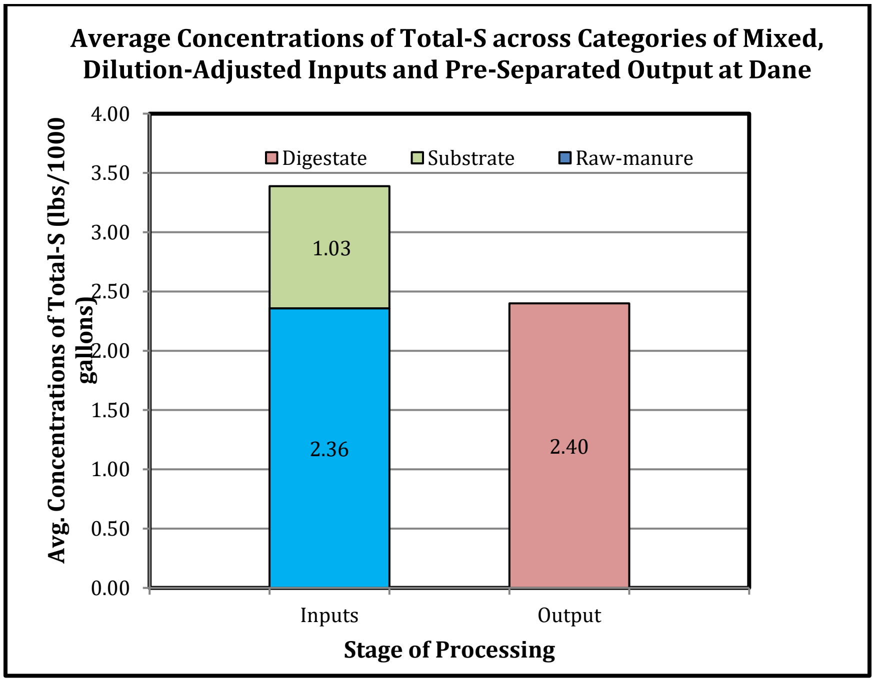


Table 3. Average concentrations of the macronutrients N, P, K, and S in system feedstocks and in separated liquid- and solids-fractions at the Dane Community Digester. Values of average concentrations are utilized to calculate N:P:K ratios, which may be employed to judge potential concentration of nutrients into separated fractions during the course of digestion.

Concentrations					
Process material	**Total-N** (lbs/1,000 gallons)	**P as P_2O_5** (lbs/1,000 gallons)	**K as K_2O** (lbs/1,000 gallons)	**Total-S** (lbs/1,000 gallons)	**Total-P** (lbs/1,000 gallons)
Raw-manure **(liquid)**	20.96	8.42	23.19	2.62	4.77
Combined feedstocks **(liquid; raw-manure + substrates; dilution-adjusted)**	19.27	7.87	23.12	2.46	4.43
Centrate **(liquid)**	20.53	5.24	26.49	2.22	2.81
Fiber-solids **(solid)**	73.14	80.12	31.10	9.50	N.A.
N:P:K ratios					
	Total-N	**P as P_2O_5**	**K as K_2O**		
Raw-manure **(liquid)**	2.5	1	3		
Combined feedstocks **(liquid; raw-manure + substrates; dilution-adjusted)**	2.5	1	3		
Centrate **(liquid)**	4	1	5		
Fiber-solids **(solid)**	2.5	2.5	1		

Measures of micronutrient concentrations An opportunity arose in our study to measure concentrations in centrate of at least seven different elements classified as plant micronutrients (generally accepted as encompassing those nutrients present in plant tissues in quantities ranging from 5-200 parts *per* million, or less than 0.02% tissue dry weight). As part of a study assessing dried algal filaments as a potential form of renewable fertilizer, the digester process material centrate (generated as the liquid fraction of digestate SLS) was subjected to Total X-Ray Fluorescence Spectroscopy ("TXRF"). TXRF enables detection of extremely low levels of elements from Sodium to Uranium in the periodic table (excluding Niobium to Technetium). (Inclusion of the macronutrients P, S, and K in this range of elements allows a direct comparison to concentrations compiled in Table 1.) TXRF operates on a principle of detecting all fluorescence emitted as a result of an X-ray beam reflecting at a very slight angle to the sample preparation. TXRF is based on internal standardization, and thus is calibrated through addition of an element not present in the sample material. In these studies, gallium was found not present in any samples, and therefore was chosen as the internal standard. Analyses of centrate were performed using a Bruker S2 PICOFOX spectrometer.

Table 4. Compilation of values from TXRF spectroscopic analysis of centrate samples. Data were collected on concentrations of seven elements classified as plant micronutrients (Cl, Cu, Fe, Na, Ni, Mn, and Zn) and of three elements classified as macronutrients (P, K, and S).

Element	Concentration in liquid centrate, (mg/kg)	Element	Concentration in liquid centrate (mg/kg)
P	81.6	Ni	0.098
S	182.219	Cu	10.274
Na	n.d.	Zn	17.621
Mg	n.d.	Ga	10.0
Si	n.d.	As	n.d.
K	2,388.697	Se	0.082
Cl	598.403	Br	0.874
Ca	391.11	Rb	0.851
Ti	1.462	Sr	2.007
Cr	0.075	Ba	0.822
Mn	6.989	Pb	n.d.
Fe	43.345		

The values presented in **Table 4** show that the elements Cl, Cu, Fe, Na, Ni, Mn, and Zn were all examined for concentrations in centrate. Of these recognized micronutrients, all but Na (not detected) and Ni (0.098 mg/kg) were passed along to the centrate from the anaerobic-digestion process in substantially high concentrations (from 7 to 600 mg/kg). These concentrations suggest that centrate could be developed into a compound fertilizer that supplies these elements to depleted soils. Concentrations of the macronutrients K and S, when converted to appropriate units, compare favorable to values listed in Table 1. When measured by TXRF, phosphorus concentrations (0.68 lbs/1000 gallons) are *ca*. 25% of those listed in Table 1.

Measures of organic-compound concentrations. The last columns of Table 1 list averages, minima, and maxima for the mass of organic compounds found in materials recovered from varied stages of anaerobic digestion. This mass of compounds was measured using several different analyses: 1) Concentrations of organic compounds were described indirectly by analyzing **Chemical Oxygen Demand** ("COD"), a measure of the weight of oxygen consumed by organic material when the sample is fully oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions. 2) **Total Solids** were measured by weighing the mass that remains after drying at 103 °C for 24 hours. 3) In turn, the mass of organic material within the resulting sample of dried Total Solids was measured in terms of carbon content, as **Volatile Total Solids**, by calculating the amount of material lost after heating at 550 °C for 1 hour. 4) **Volatile Acids** content was measured on liquid materials in conjunction with alkalinity titrations, to describe content of low-molecular-weight organic compounds.

It is understood that **COD** provides an indirect measure of total organic-compound mass. Mass of **Total Solids** provides a measure of both total suspended solids ("TSS", the solids that remain intact when added to water) and total dissolved solids ("TDS", the fraction that appears as solid material only after the sample is completely dried). Both TSS and TDS can include organic matter. Measures of **Volatile Total Solids** cut across the TSS and TDS categories by directly measuring the amount of organic-matter mass lost during combustion. Finally, the mass of **Volatile Acids** provides measure of a certain category of organic matter. Inside digester tanks, saprophytic bacteria continuously hydrolyze complex organic compounds to simpler forms through fermentation. These simpler molecules include short-chain volatile fatty acids such as acetate, propionate, butyrate, and other products of fermentation such as CO_2 and H_2. Archaean methanogens can directly process acetate, CO_2, and H_2 in their production of methane, and saprophytic bacteria continually supply these specific materials by breaking down longer-chain fatty acids alongside other, more-complex compounds. However, a too-rapid production of volatile acids will overwhelm Archaean ability to consume acetate, CO_2, and H_2. When this occurs, the alkaline buffer capacity is exhausted, and the pH drops to such extent that neither the methanogens nor the saprophytes can process materials inside the digester system. Concentration of volatile acids and pH levels are both closely monitored by digester operators to assure that alkalinity is not exhausted and the system is not in danger of "going sour" and shutting down(12). Concentrations of volatile acids also provide a direct measure of organic compounds that may be most easily consumed as "food" by the Archaeans, for metabolic conversion to methane.

Examination of values for organic-compounds in Table 1 show perhaps a higher-than-expected ratio of Total Solids to Volatile Total Solids, for both the raw-manure and substrate input feedstocks. In many instances of anaerobic-digester management, values for this ratio comparison show that 75% of the Total-Solids mass is volatile organics, with the remaining 25% of mass composed of ash (13). Instead, the ratios of organic material to Total Solids in the present study show 5% for raw-manure and 13% for the substrates. The explanation for these ratio values is uncertain, but probably is partly related to high levels of inert materials coming in with the industrial substrates, ongoing changes to cattle-feed compositions, and to the possible inclusion of suspended, particles of sand derived from the sand-separation operation conducted on bedded manure at one of the contributing dairies.

Values for organic-compound analyses do show a consistent concentrational decline between feedstock inputs and digestate output, as would be expected. **Figure 14** plots averages for dilution-adjusted values of COD, demonstrating a decreased concentration of *ca*. 40% in the amount of O_2 consumed by organic compounds at stage of digestate recovery, which reflects indirectly upon the anaerobic processing of organics in the digester tanks. **Figure 15** illustrates a similar drop in values for a direct measure of mass for suspended plus dissolved solids, with averages for dilution-adjusted Total-Solids values declining *ca*. 27% in samples of system output. The averages for dilution-adjusted concentrations of Volatile Total Solids (**Figure 16**) drop by *ca*. 56% between system input feedstocks and the single digestate output, which gives our first direct measure of the extent to which organic compounds are consumed during methane production. Volatile Acids, constituting a specific form of organic compound that is most readily processed during methanogenesis, are plotted as dilution-adjusted concentrations in **Figure 17**, and illustrate *ca*. 72% decrease in concentration by the time of digestate collection. The diminished concentrations shown by these graphs affirm an ongoing solubilization of materials within the system, and show that this particular fraction of feedstocks is the one most-directly metabolized throughout the 30-day period of digestion. Also demonstrated by Table 1 data and Figs. 14-17 is a residual portion of organic compounds contained within the volume of collected digestate. This residue of organic materials was not entirely expected during the early days of project planning. The organics clearly migrate to the liquid centrate and fiber-solids fractions generated during SLS of digestate. Table 1 values show about 1.5-times more organics as measured by COD go into fiber-solids than into centrate. This magnitude of division increases markedly for organics when measured as mass in solids form, as might be expected. About 8-times more Total Solids migrate into the fiber-solids fraction than go into centrate. About 69-times more Volatile Total Solids go into the fiber-solids fraction than go into centrate. Similar calculations are not available for Volatile Acids, as concentrations of these organics could not be measured on samples of fiber-solids. We believe that the demonstrated residue of organic compounds in the digester system's output could impact the utility of both centrate and fiber-solids as growth media for aquatic plants.

Figure 14. Variation in average concentrations of Chemical Oxygen Demand between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Total-S for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (153,149 mg O_2/L) appears to decrease by *ca*. 40% in digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.

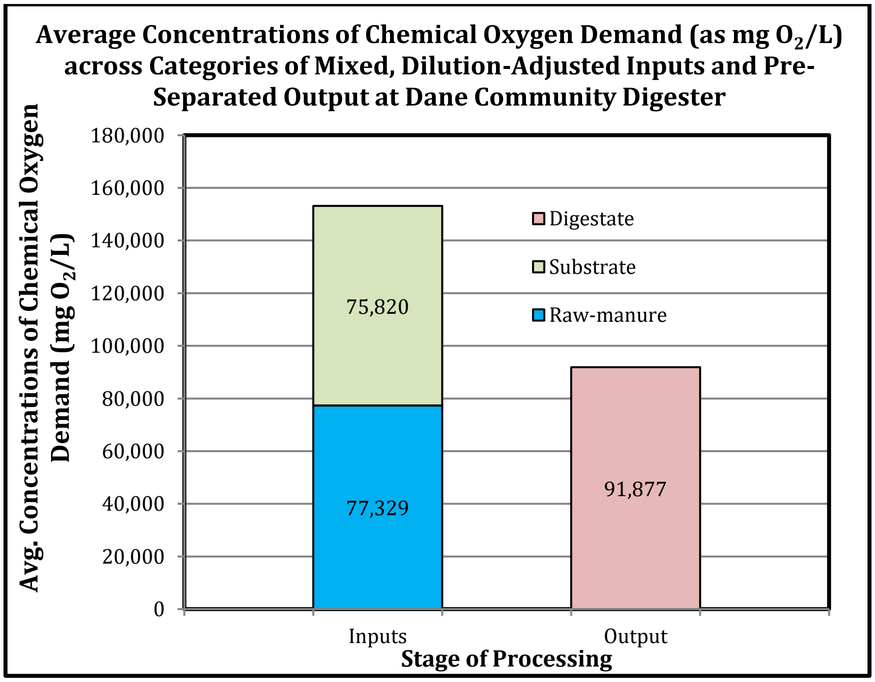


Figure 15. Variation in average concentrations of Total Solids between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Total Solids for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (73,010 mg /L) appears to decrease by *ca*. 27% in digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.

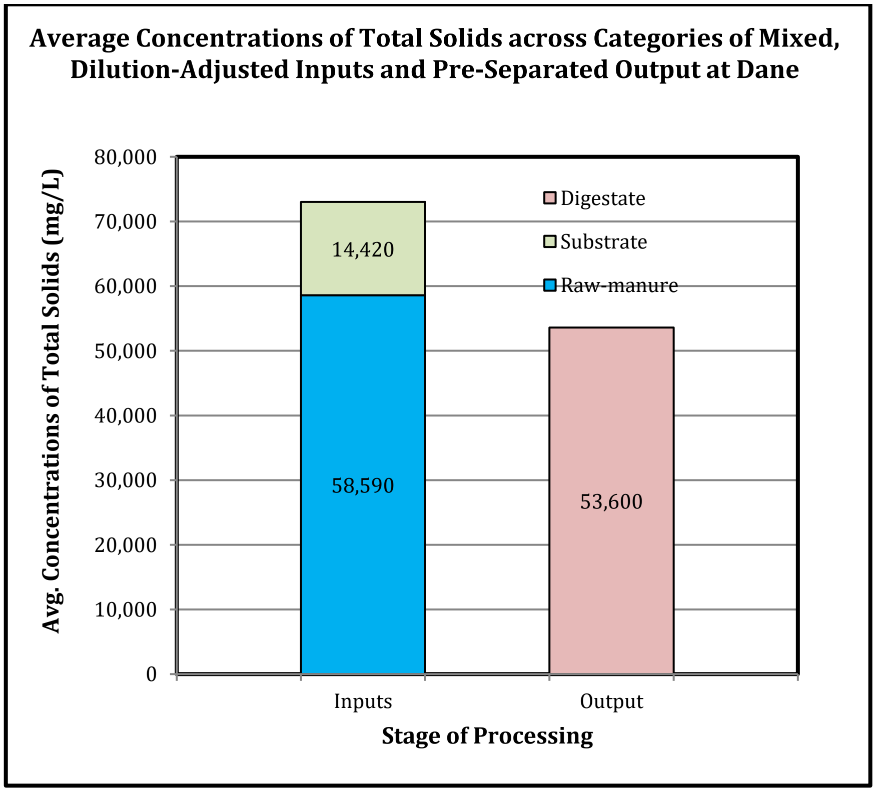


Figure 16. Variation in average concentrations of Volatile Total Solids between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Total Solids for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (4,757 mg /L) appears to decrease by *ca*. 27% in digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.

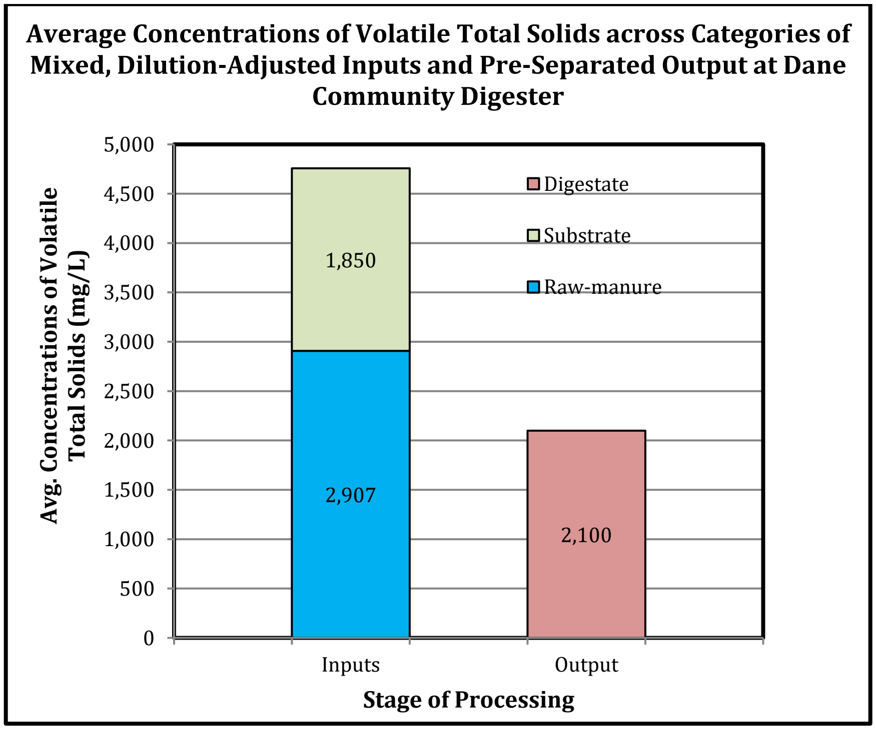
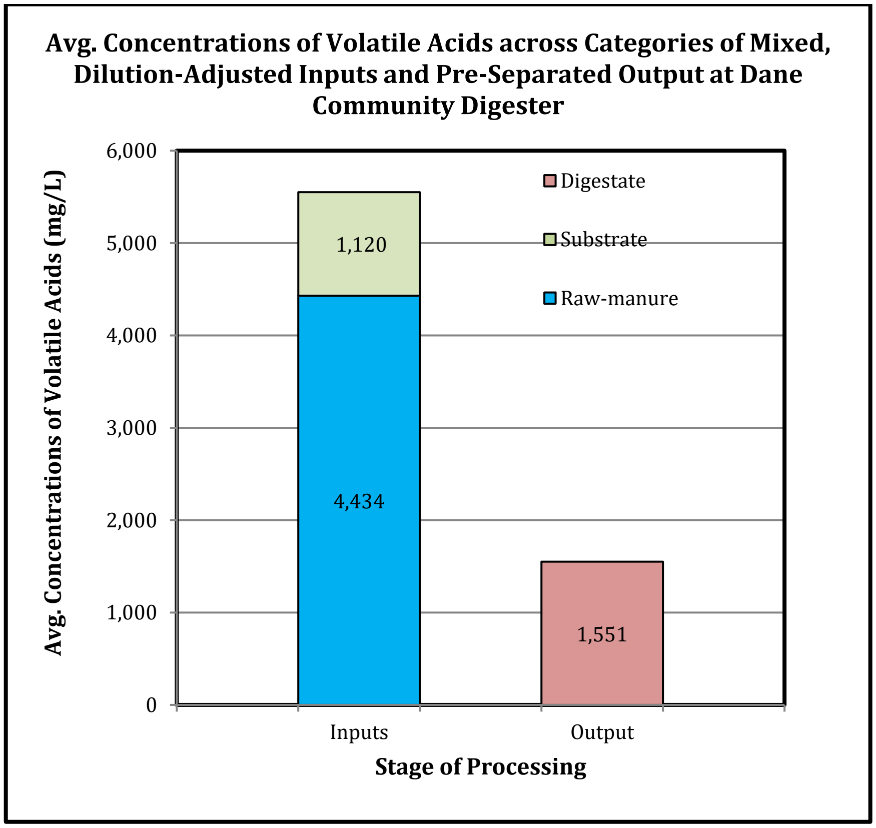


Figure 17. Variation in average concentrations of Volatile Acids between dilution-adjusted feedstock inputs and system output at the Dane Community Digester. Averages show changes between combined concentrations of Volatile Acids for mixed inputs (90% raw-manure plus 10% substrate) and samples of the single output (digestate). Total average concentrations for mixed inputs (5,554 mg /L) appears to decrease by *ca*. 72% in digestate pumped from closed digester tanks after the 30-day period of anaerobic digestion.



Analysis of centrate's biochemical methane potential. Demonstration that significant concentrations of organic compounds were migrating from output digestate to the two SLS fractions prompted questions regarding the impact of an extend period of digestion. Consequently, a study of centrate's Biochemical Methane Potential (14) ("BMP") was undertaken both to confirm elevated levels of organics transfer and to measure the additional volumes of methane potentially generated from replicate samples of centrate. Three centrate samples were collected bi-weekly from the Dane Community Digester during January and February, 2013. 300-mL volumes of samples were incubated for 25 days on a model "AMPTS II" benchtop digester system. Centrate samples were recognized to contain concentrations of organic compounds both for direct processing by methanogenic Archaeans to CH_4 and CO_2 and for further breakdown by saprophytic bacteria to food molecules used in Archaean methanogenesis. Centrate also was recognized as containing syntrophic cultures of these Archaeans and saprophytic bacteria. Such characteristics also applied to a control solution of non-separated digestate slurry taken from a plug-flow digester on a dairy farm in southwestern Wisconsin. Measures of Total Solids and Volatile Total Solids were taken on all samples following standardized methods of manure and/or soil analysis (3,4), and COD analyses were performed using the Hach DRB 200 heating block with Hach COD reagent vials.

Results of BMP analysis on the control and the three centrate samples are summarized in **Table 5**. The three centrate samples were demonstrated to produce varying final volumes of CH_4, which varied from 22-59% of the volume produced by the digestate control. The volumes of CO_2 accompanying methane production ranged from 7-25% of the volume produced by the digestate control. The percent contributions by CH_4 to the total volume of gases produced ranged from 73-96%, surpassing the 75.4% contribution measured on the control.

Table 5. Measures of CH_4 and CO_2 production collected during BMP analysis on centrate samples taken from the Dane Community Digester and on an unseparated digestate control taken from a plug-flow digester in southwest Wisconsin. Volumes of gas were produced during 25-day incubations on a benchtop-scale digester unit.

Sample	Final volume CH_4 produced (mL)	Final volume CO_2 produced (mL)	Final volume of total gas production (mL)	% CH_4	% CO_2
Control digestate (slurry from WI plug-flow digester)	637	208	845	75.4	24.6
Centrate 1/15/2013	138	52	189	72.8	27.2
Centrate 1/31/2013	376	43	419	89.8	10.2
Centrate 2/15/2013	312	14	326	95.8	4.2

The values for CH_4 production measured on the three centrate replicates and control are plotted over the course of the 25-day incubation period in **Figure 18**. As shown here, the three centrate samples produced CH_4 at their maximum rates well before the control digestate, which did not begin leveling-off its production until (presumably) the final day of incubation.

Figure 18. Plots of CH_4 production by samples of centrate and control digestate during 25-day BMP incubations. Plots show that maximum rates of CH4 production were achieved by the three test samples before the rate demonstrated by the control. Final volumes of gas produced by the test samples were 22-59% of that associated with the control.

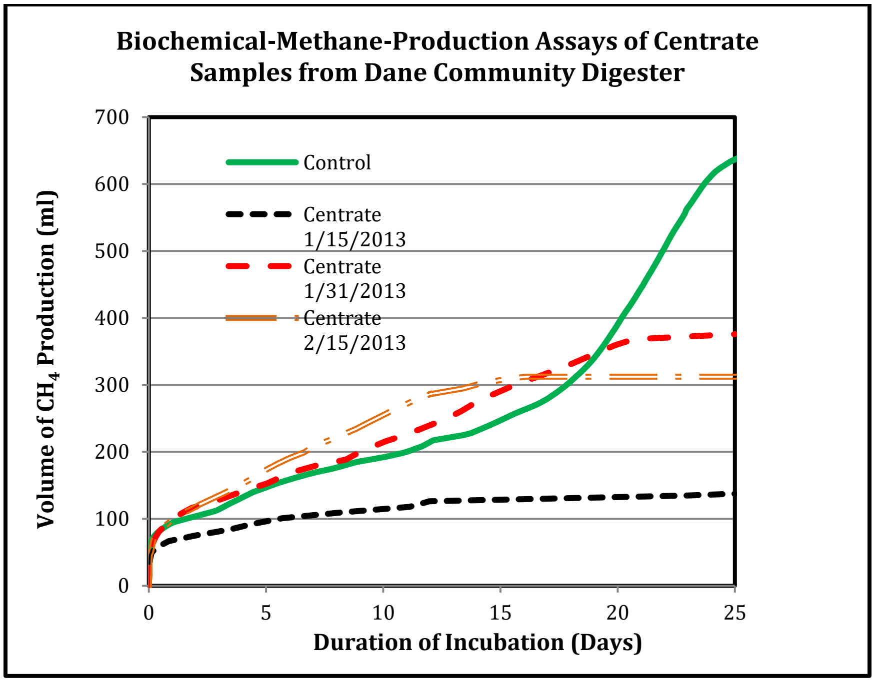


Table 6. Averages of organic-content measurements taken on samples of centrate from the Dane Community Digester and on an unseparated control sample of digestate from a plug-flow digester system. Analyses of COD were conducted before materials were subjected to BMP incubations and analyses of Total Solids and Volatile Total Solids were made both before and after BMP incubations.

Sample	Pre-BMP analyses			Post-BMP analyses	
	Chemical oxygen demand (mg/L)	Total Solids (mg/L)	Volatile Total Solids (mg/L)	Total Solids (mg/L)	Volatile Total Solids (mg/L)
Control digestate (slurry from WI plug-flow digester)	66,933	43,000	32,852	37,700	27,766
Centrate (SLS liquid fraction from Dane Community Digester)	44,267	28,000	18,284	25,700	16,052

Several analyses of organic-compound concentrations in the centrate samples and control are compiled in **Table 6**, which lists averages for analysis of COD before the BMP incubations and averages for Total-Solids and Volatile-Total-Solids measured both before and after the BMP incubations. The posted averages show COD levels to be similar to the averages listed in Table 1 for this material. The average concentration of COD for the digestate control, however, is lower than the posted average digestate value (91,877 mg/L) in Table 1, though it falls well within the posted range (36,260 to 1,057,600 mg/L). The Table 6 Total-Solids averages for both the control and the replicate test samples are lower than the corresponding digestate and centrate averages in Table 1, and this trend is exhibited for both before and after the BMP incubations. However, Table 6 averages for Volatile Total Solids are markedly larger than the corresponding digestate and centrate averages in Table 1, again for measurements both before and after the BMP incubations. These large differences in Volatile Total Solids averages raises questions about the accuracy of analytical procedures followed either in this BMP study or in our own larger study, though Table-1 values were provided by certified analytical labs. It perhaps can be summarized that Table 6 presents measures of COD and Total Solids concentrations that could be reasonably expected for digestate and centrate collected from complete-mix digester systems, given variations in feedstock compositions. The averages for Volatile Total Solids, however, are probably higher than what should be expected, though these values decreased when measured on post-BMP materials.

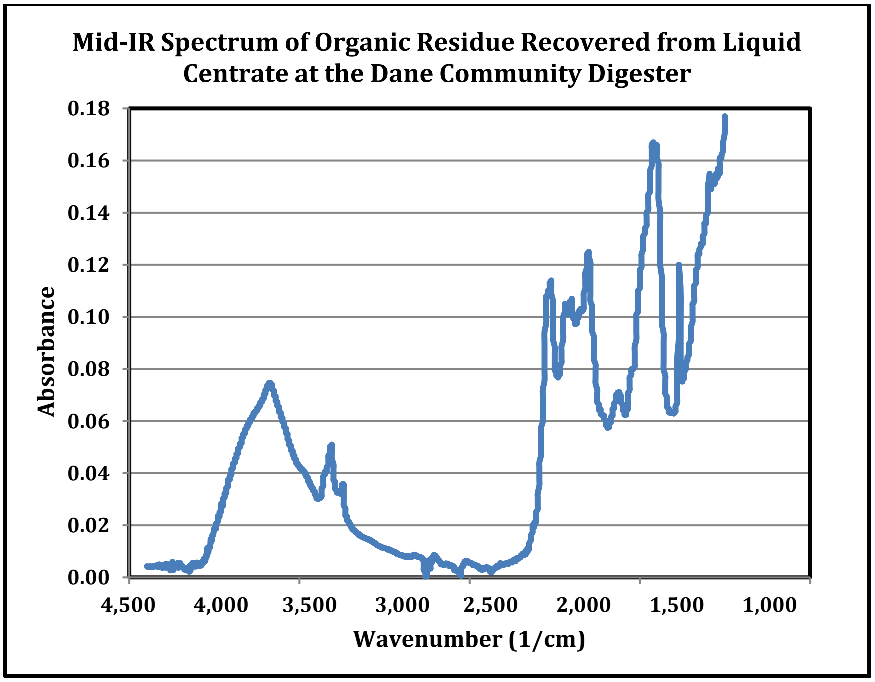
This BMP analysis should be viewed as providing only preliminary results. It demonstrated that samples of centrate from the Dane Community Digester have varying capacities for generating additional volumes of CH_4, even after a 30-day anaerobic digestion of these materials inside the digester tanks. The centrate (or at least the 1/31/2013 and 2/15/2013 samples) clearly have enough organic material to fuel this production. The availability of organics is supported by COD values collected in this BMP analysis. More specific measures of organics in the form of Total Solids supports the availability of these materials in centrate for metabolic processing to CH_4. Measures of more-readily available organics as Volatile Total Solids also support availability of metabolic fuel, though averages for this measurement are perhaps greater than would be reasonably expected. CH_4 production by centrate samples is lower than that by the digestate control, as expected, because the digestate is un-separated and contains a greater supply of solids that can be broken down to acetate over the course of the 25-day BMP incubation.

Identification of casein-rich residue in centrate Throughout the first part of our data collection, there occurred an ongoing realization that substantial quantities of organic compounds were passed along to both the liquid centrate and fiber-solids fractions. This awareness helped trigger the decision to conduct mid-infrared spectrometric analysis of one particular residue that blocked attempts to culture the unicellular planktonic cyanobacterium *Synechococcus* sp. Several attempts had been made to directly test the efficacy of diluted, autoclaved centrate as a growth medium for this alga under laboratory conditions. The prepared centrate was recognized as being substantially clouded by suspended particulates, to the extent of blocking required light transmittance. Consequently, a description of these particulate materials was pursued by first centrifuging 500-mL samples on a high-speed, refrigerated lab centrifuge at 60,000 rpm for 20 minutes. Centrifugation of six separate samples showed the recovered pellet of oily residue to constitute from 25 to 34% of centrate mass. The residue was subjected to mid-infrared analysis on a Thermo-Nicolet 380 Fourier transform spectrometer that was equipped with a standard KBr beam splitter and a Pike MIRacle™ ATR (attenuated total reflection) accessory. FTIR-ATR operates in the mid-IR region (500 to 4000 cm^{-1}) and utilizes peaks that represent assorted chemical bonding patterns (including hydrocarbon stretching and bending, ester carbonyls, and carbon-carbon double bonds) to identify chemical compounds.

A spectrum of the recovered residue is shown in **Figure 19**. The spectrum's pattern of peak positions and intensities were used to enter a library of standard spectra, yielding a positive identification (greater than 99% probability) as the phosphoprotein casein. This protein, commonly isolated industrially as a byproduct of milk-processing, is hydrophobic in its tertiary structure, which explains why it is not susceptible to metabolic breakdown in the liquid medium of anaerobic-digester tanks. The casein molecules pass through the digester system intact, forming the light-blocking micelles that were suspended in the separated liquid fraction of centrate. Identification of such an indigestible substrate in the digester's output was not necessarily surprising, although the plant operators were quick to block acceptance of food-processing wastes from the company later identified as the protein's source. This small project of organics identification is considered to represent the degree of monitoring that will become necessary in future to assure smooth operation of community digesters.

As projects make more-frequent use of industrial substrates to supplement CH_4 production, it will be necessary to guard against acceptance of indigestible materials that simply "take up space" in the tanks, away from potential energy-rich substances that could boost productivities substantially.

Figure 19. Mid-infrared spectrum (un-flattened) of residue isolated from centrate samples at the Dane Community Digester. Peak positions and intensities were utilized to secure a positive identification (99% statistical probability) as the hydrophobic milk protein casein.



Summary

A series of chemical and physical analyses were conducted on replicate samples of materials associated with varied stages of anaerobic digestion at the Dane Community Digester, including raw-manure (providing 90% of the system's feedstock), substrates (food-processing and other industrial wastes that formed ≤ 10% of feedstocks), digestate (the remaining slurry pumped from digester tanks at the end of a 30-day period of digestion), centrate (the liquid fraction derived from centrifugal separation of digestate), and fiber-solids (the solids fraction of fibrous and particulate materials that derives from centrifugal separation of digestate).

Analyses demonstrated increasing values for pH and alkalinities throughout the anaerobic digestion process, and these increases carried over to affect the chemistry of the liquid centrate and fiber-solids fractions. Concentrations of the macronutrients N, P, K, and S were measured on each of the process materials. Average concentrations of N, P, and K showed no change in the availability of these elements inside the closed system of digestion tanks, between the input of feedstocks and the collection of digestate output. Some evidence attests to an expected change in form of nitrogen compounds (from NO_3-N to NH_4-N) under the anaerobic conditions inside digester tanks. Clear evidence points to decreased concentrations of S recovered in the digestate output, however, likely owing to volatilization of H_2S gas from the liquid slurry into the tanks' overlying gas space. Some slight concentration of all macronutrients in the digestate probably derived from an average 9.5% loss of mass, as volatilized CO_2 and CH_4, from the liquid slurry to the overlying gas space of digester tanks. A clear separation of macronutrient concentrations was evident for N, P, and S between the liquid centrate and fiber-solids fractions generated by centrifugal SLS of digestate. For these three macronutrients, markedly higher concentrations were measured on the solids fraction than in liquid centrate, as would be expected by the elements' lowered solubilities and tendencies for strong particulate bindings. Potassium, however, has a higher solubility in comparison, and was associated with a more even distribution between the liquid and solids fractions.

It was possible to assemble an N:P:K ratio of (2.5):(1):(3) for the raw-manure feedstock contributed by participating dairies. This ratio falls well within the range of published values for dairy-cattle manures. The calculated N:P:K ratio for liquid centrate was (4):(1):(5). The ratio for fiber-solids was demonstrated as (2.5):(2.5):(1). A supplemental study of micronutrient concentrations in centrate showed the liquid to contain significant levels of the elements Cl, Cu, Fe, Mn, and Zn, indicating potential for development of renewable fertilizers.

Measurements of organic concentrations illustrate a consistent consumption of these compounds between introduction of feedstock inputs and collection of digestate output. A set of several analyses described increasingly specific classifications of organic compounds. The category of volatile acids showed the greatest decline (72%) by stage of digestate output, illustrating that these compounds were most readily consumed by saprophytic bacteria and methanogenic Archaeans in the closed system of digester tanks. Analyses of organics also showed a residual portion of these compounds leaving the digester tanks at time of digestate collection. These residual organics migrate into the liquid and solid fractions generated by digestate SLS, with the majority of concentration collected by the dried fiber-solids. A supplemental measure of BMP on samples of centrate showed residual capacity for additional methane production by this liquid fraction of manure derivative and suggested some levels on incomplete digestion at the Dane Community Digester. An analysis using mid-infrared spectroscopy identified a particular residue in centrate as the phosphoprotein casein. This hydrophobic protein entered the digester system for a limited period of time as a supplemental food-processing substrate, and was apparently not susceptible to the anaerobic-digestion process.

Notes and References

(1) Leudtke, B., R. Michitsch, and A. Razvi. 2010. Use of compost teas as a nutrient amendment for plant growth in a re-circulating hydroponic system. Student project report, Univ. of Wisconsin System Solid-Waste Research Program. Stevens Point, Wisconsin, pp.16.

(2) Wright, P. 2001. Overview of anaerobic digestion systems for dairy farms. Natural Resource, Agriculture, and Engineering Service (NRAES-143), March, 2001.

(3) Peters, J., S.M. Combs, B. Hoskins, J. Jarman, J.L. Kovar, M.E. Watson, A.M. Wolf, and N. Wolf. 2003. Recommended Methods of Manure Analysis. Madison, WI; Board of Regents of the University of Wisconsin System.

(4) Holstege, D., P. Price, R.O. Miller, and D. Meyer. 2010. University of California, Davis, California. California Analytical Methods Manual for Dairy General Order Compliance –Nutrient Management Plan Constituents. University of California, Davis Analytical Laboratory.

(5) Kane, P.F. 1998. AOAC Method 978.02. Nitrogen (total) in Fertilizers. In: Official Methods of Analysis of AOAC International. 16th ed., P. Cunniff (ed.), Gaithersburg, MD; AOAC International.

(6) Adapted from *Standard Methods for the Examination of Water and Wastewater*, Method 4500-NH3F.

(7) Huang, C.L, and E.E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun. Soil Sci. Plant Anal., 16: 943-958.

(8) USEPA. 1986. Method 3050. Acid Digestion of Sediments, Sludges, and Soils. Test Methods for Evaluating Solid Waste. Volume 1A: 3rd Edition. EPA/SW-846. National Technical Information Service. Springfield, VA.

(9) Anderson G. H. and Yang G. (1992) Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. Water Env. Research, 64(1): 53-59.

(10) Cooke, G.W. 1978. Fertilizing for Maximum Yield. Second Edition. Granada Publishing, Ltd. London.

(11) Clear Horizons Dane, LLC. 2013. Dane Community Digester, 2012 Annual Report. May 1, 2013. 8 pp.

(12) Michigan State University Board of Regents. 2010. Michigan On-Farm Anaerobic Digester Operator Handbook. 97 pp. East Lansing (MI).

(13) MidWest Plan Service. 2004. Manure Characteristics. Pub. 18 Section 1, Second Edition. Iowa State Univ., Ames.

(14) Gunaseelan, V.N. 1997. Anaerobic digestion of biomass for methane production: a review. Biomass and Bioenergy, 13: 83-114.

5. Culture of Freshwater Filamentous Algae and Duckweed

Background

Analyses of digester process materials identified varied physical/chemical attributes of the two manure-derivatives (centrate and fiber-solids) that are available from the Dane Community Digester for nutrient-medium preparations. A concurrent task was incorporating these preparations into an effective plan for growing and harvesting two types of aquatic plants (free-floating duckweed and assemblages of attached filamentous algae). We sought to integrate these aquatic cultures into the treatment of stormwater runoff at a dairy farm immediately adjacent to the digester system.

We had committed to utilizing "algal-troughs" (1, 2) for cultures of attached filamentous algae both inside the greenhouse facility throughout the year and outside the greenhouse, in open air, for growth during warmer months. The duckweed cultures were planned from the beginning to utilize a livestock watering tank inside the greenhouse year-around and an excavated pond outside during warmer months. For both indoor and outdoor culture systems, initial plans involved diluting prepared nutrient media into a continuous flow of freshwater. This flow was planned to pass initially through the algal-troughs in the form of a generated wave surge, then into a quiescent tank supporting growth by duckweed, and then to exit the culture systems directly for collection in a large-capacity sediment basin. While passing through the culture systems, the media would be subjected to a degree of nutrient-clearance as a result of the plants' ongoing metabolic sequestrations. Inside the sediment basin, the flow would undergo evaporation, some degree of ground infiltration, and occasional overflow during major ("25-year") storm events. Any overflow from the sediment basin would be directed into a constructed wetland for further biological treatment, ongoing evaporation/infiltration, and occasional overflow to a nearby intermittent stream.

After we had submitted our plans for approval by the State of Wisconsin Department of Natural Resources (WDNR), however, we learned that it was not possible to design our culture and stormwater-management systems for any "direct" release of manure derivatives (no matter how diluted or biologically processed) into the environment. Instead, we were guided to modify our plans so that all water drained from either the algal-troughs or the duckweed-culture systems would be transferred not to the sediment basin directly, but into a buried, concrete cistern (1,600 gallons capacity). Once collected in the cistern, the partially processed media could be irrigated onto an adjacent alfalfa field, which drained *via* culvert into the sediment basin. From this field, any excess nutrients from the manure-derivatives could drain during storm events into the sediment basin and, ultimately, the constructed wetland. So long as a manure-derivative was land-applied under the farm's Nutrient Management Plan, it would not be considered direct release to the environment.

The most important aspect of this newly encountered restriction was the requirement to abandon plans for continuous-flow culture systems. System designs were re-formulated to organize the production of filamentous algae and duckweed in semi-batch cultures. These aquatic plants were cultured for short periods (3-days), during which the media was continuously re-circulated and subjected to wave generation. At the end of each 3-day period, the culture systems were drained, flushed, and refilled with fresh preparations of manure-derivative nutrients. The filamentous algae were grown by attachment to multiple layers of polypropylene screening, so they remained in place inside the algal-troughs during the draining and refilling procedure. The free-floating duckweed was collected by scoop-net and held in buckets during preparations of fresh media. The partially cleared media from the greenhouse systems was flushed to the outdoor duckweed pond, where it was further processed during warm-weather months, periodically transferred to the concrete cistern, and ultimately land-applied to the alfalfa field. The outdoor algal trough was drained to the outdoor duckweed pond in similar fashion.

The restriction of utilizing only re-circulating media presented numerous obstacles to our efforts to describe and optimize these aquatic-plant productivities. This interference was compounded substantially by problems of greenhouse-temperature control. Although it was not possible to optimize our culture systems during this study, we were able to identify limited periods throughout the calendar year in which substantial rates of growth were documented. Focus upon these periods of successful culture then allowed us to identify the factors that most directly support culture of aquatic plants using manure derivatives from an anaerobic digester system.

Findings

Establishment of culture systems. A total of five systems were established for the culture of filamentous algae and duckweed: 1) a **large greenhouse trough** for the indoor culture of attached filamentous algae, 2) a **small greenhouse trough** for algal culture, 3) a livestock-watering tank inside the greenhouse to serve as an **indoor duckweed pond**, 4) a large **outdoor algal trough** for open-air culture of filamentous algae, and 5) an excavated **outdoor duckweed pond** for open-air culture of this floating aquatic plant.

The **large greenhouse trough** consisted of a shallow wooden tray (23.75-foot long, 3.8-foot wide, 1-foot deep) that was covered by a black sheet of HDPE (60 mils) to block water leakage. The head of the trough was fitted with a 11.7-gallon-capacity (44.25-L) stainless-steel wavebucket designed for gravity-triggered tipping once full-volume is reached through water input. The tipping bucket provided wave action to reduce diffusional resistance within the assemblage of algal filaments. The top surface of the trough was fitted with a three-layer "sandwich" of black polyethylene screening material (3 x 4 mm mesh; InterNet, Minneapolis, MN) divided into three 2.8-m^2 squares. These squares were placed end-to-end along the length of the tray surface to provide a medium for attachment and upright growth by algae. An 8.43-m^2 area of the trough surface was covered by such screening. The foot of the trough was fitted with two standpipes that collected water, which traveled along the trough in the form of a wave-surge. Overflow of water through the standpipes was directed towards a 300-gallon capacity open-air reservoir tank of black polypropylene. Water collected in this reservoir tank was continuously drawn by an external transfer pump (3-phase, adjustable, 25 gpm) for return to the wavebucket at the head of the trough. The total-system volume of this trough was 350 gallons (1,326.5 L). Pump rate was adjusted to provide one tip of the wavebucket every 30 sec, to achieve an overall recirculation rate of 88.5 L/min (23.4 gallons/min). A collection of 12 tubes, each fitted with an 18-W array of red/blue LED's, was assembled to provide artificial illumination (216 watts total) to the lower 2.8-m^2 section of the trough during various periods of the calendar year. This set of LED's was positioned 0.5 m above the surface of the screens, which allowed partial transfer of ambient light to underlying screens. When in use, the lights were operated by a timer set to a 12-h photoperiod.

The **small greenhouse trough** consisted of a shallow, black polypropylene livestock feed/water trough (10-foot long, 2-foot wide, 0.8-foot deep) that was fitted along the bottom with a sheet of drilled Plexiglas™ so as to provide a flat bottom for support of a single rectangle of multi-layered polypropylene screening. The total area of three-layer screening was 1.6 m^2. The head of this trough was fitted with a 2.16-gallon-capacity (8.0-L) Plexiglas™ wavebucket of a similar design described above. Water emptied from the wavebucket traveled across the screening, as described above, and was collected by bulkhead-fittings that supported variable height of standpipes. Water from the wavebucket also was able to pass vertically through the screening and drilled Plexiglas ™ and into the underlying "deadspace", where it was drained *via* flexible tubing. All water was collected into a 150-gallon capacity open-air reservoir tank of black polypropylene, as described above. Water collected in this reservoir tank was continuously drawn by a submersible recirculation pump (10 gpm) for return to the wavebucket. The total-system volume of this smaller trough was 200 gallons (758 L). Pump rate was adjusted by gate-valve to provide one tip of the wavebucket every 20 sec, to achieve an overall recirculation rate of 24-L/min (6.33-gallons/min). No artificial illumination was assembled for this smaller trough, which relied entirely on ambient sunlight for culture of the filamentous algae.

The **indoor duckweed pond** consisted of a galvanized-steel, 144-gallon capacity watering trough (6-foot long, 2-feet wide, 2-feet high). The water in this container supported surface-growth by floating duckweed. The tank was quiescent, with water slowly but continually recirculated by outflow from a small submersible pump. No artificial illumination was provided for this duckweed pond.

The **outdoor algal trough** was partially excavated into land immediately to the south of the greenhouse. The head of this trough (27-foot long, 9-foot wide) was fitted with two stainless-steel wavebuckets of the design described above for the large indoor trough. The two wavebuckets filled independently from the same stream of recirculating water, providing a combined capacity for the two independent tippings of 23.4 gallons (88.5 L). The area covered by the single rectangle of multi-layered polypropylene screening (described above) on the surface of this trough was 22.6 m^2. Water was collected by direct flow into an excavated trough (6-foot long, 9-foot wide, 1.5-foot deep) at the foot of the trough, where it was recirculated by a submersible pump (25 gpm) to the two wavebuckets. Pump rate was adjusted by gate-valve to provide one tip for each wavebucket every 30 sec, to achieve an overall recirculation rate of 75-L/min (20-gallons/min). The total-system volume of this outdoor algal trough was 400 gallons (2,653 L). The entire area covered by the trough surface, wavebucket area, and collection trough was lined by a layer of black HDPE (60 mils) to prevent passage of growth media.

The **outdoor duckweed pond** was formed by a 30,000 gallon-capacity excavation (oval shape, *ca*. 80-foot long, 30-foot wide) immediately to the south of the greenhouse. The total surface area of this excavated pond was 223 m^2 (2,400 ft^2). The entire surface area of this excavation was lined by a layer of black HDPE (60 mils) to block passage of growth media. No mechanical mixing or recirculation of the water in this pond was provided, though some stirring of the water was provided by wind action.

Development of on-site management strategies. Our analyses of anaerobic-digestion process materials had highlighted compositions of liquid centrate and fiber-solids, both generated by centrifugal separation of digestate slurry. Large masses of these materials are produced at the digester site daily. The liquid centrate is returned to contributing dairies for lagoon-storage and later periodic field applications. The fiber-solids are subjected to drying by heat-transfer before export to operations outside the watershed, where they are used for animal bedding and soil-amendment agents. Because many of the soluble nutrients in digestate precipitate into the solids fraction during centrifugation, export of fiber-solids provides economically viable means for removing such agents, particularly phosphate, from the local environment. Significant nutrient concentrations remain in the liquid state, however, and the centrate fraction appears also to receive the majority of volatile acids passed through the digestate slurry (relevant values for volatile-acid concentrations in the fiber-solids fraction are not available for comparison, however). Both centrate and fiber-solids could ostensibly provide nutrients for aquacultural applications, but are considered by the DNR to constitute manure-derivatives. Such classification impacted the manner in which these materials could be used for growing aquatic plants. Because direct release of manure derivatives to the environment is prohibited, continuous-culture systems become impractical. To limit the total volume of polluted water that eventually requires field application, a system of semi-batch culture was formulated, incorporating some degree of media recirculation. We anticipated that certain problems would accompany this change in culture plans. Our previous chemical/physical analyses had demonstrated that high concentrations of both minerals and organic compounds are passed along to centrate and fiber-solids. Consequently, it was understood that recirculation of even highly diluted preparations could pose risks in semi-batch culture by promoting the growth of competing heterotrophic bacteria.

A general strategy was developed to test preparations of the two manure-derivatives for utility in both establishment and ongoing culture of filamentous algae and duckweed. We tested direct dilutions of the liquid centrate, utilizing our previous determinations of macronutrient-concentration ratios. To test fiber-solids, varied masses of these materials were first soaked in freshwater, which produced solutions of compost-tea water with differing concentrations of nutrients. By measuring certain of these concentrations, appropriate subsequent dilutions were identified and selected for use in our study. The centrate and fiber-solids preparations were then employed to establish the algae and duckweed in culture. If growth of starting cultures proceeded adequately, the starting concentrations of nutrients were maintained by returning set volumes of preparations to the culture systems on a daily basis.

The culture systems organized for this study were "seeded" with either filamentous algae or duckweed collected from a small urban stream (Pheasant Branch Creek) in Middleton, WI. Collections of these aquatic plants could be easily made at this site from about April through October, and it was possible to collect small but persistent stands of filamentous algae from beneath the ice throughout winter months. The assemblage of filamentous algae collected most commonly comprised species of *Klebsormidium*, *Mougeotia* and *Ulothrix* (tentatively identified as *U*. *zonata* (Weber & Mohr) Kütz. These three algae were collected and cultured (when successful) in an assemblage of roughly equal proportions. When examined microscopically, however, there was observed an abundance of both epiphytic LRGT's and cyanophytic bacteria. During one period of greenhouse culture from late-January to early-March, it was evident that this seed stock supported a bloom by a species of *Cladophora*. Collected masses of algae were introduced to the algal troughs in this study once or twice each week during periods of culture establishment. Once the assemblages were determined to persist, collections were made for re-introduction to culture systems every 2-3 weeks. It was evident that these algae could easily persist within the plumbing, pumps, and other components of water recirculation between routine cultures, harvests, and system flushings. The species of duckweed most commonly collected was tentatively identified as *Lemna minor* (L.). Little effort was necessary to maintain growth by this duckweed in our system ponds. In the outdoor pond, an initial seeding of the plant supported an apparently permanent growth, which was undoubtedly augmented by periodic introductions of new material *via* waterfowl and other vectors. In the indoor duckweed pond, established specimens grew well during the prime spring and fall culture periods (see discussion below), but began to show susceptibility to competition by cyanophytic filaments as temperatures and light intensities increased during warmer months. A replenishment of plants was made to the indoor duckweed pond each fall.

Significant problems in environmental control were encountered during greenhouse cultures, which led us to identify seasonal restrictions of our operations. The two indoor algal troughs and duckweed pond were installed inside a greenhouse (80-foot long, 30-foot wide) of "hoop-house" design. We encountered significant problems of temperature control, because there was limited capacity for ventilation incorporated into the structure's design. Consequently, temperatures in the greenhouse reached intolerable levels for plant culture from roughly the first of May to the middle of October. During this period, air temperatures could reach 120-130 °F on a daily basis, and the temperatures of recirculating growth media could easily rise above 80 °F. Heat captured by large areas of black polymers in the algal-culture systems contributed to these high temperatures. Temperature control during winter months (roughly mid-October to the first of May) was not an issue, as the greenhouse was kept warm (*ca*. 60 °F) through capture and transfer of the digester system's excess gen-set heat. This problem of temperature control was very likely exasperated by the intensity of ambient sunlight entering the greenhouse structure, although it was not possible to separate these two factors through simple observations.

A graphical illustration of these impacts is provided in **Figures 20, 21, and 22**. Figure 21 shows average daily temperatures and average daily high temperatures to rise above 50 °F and 60 °F, respectively, by May 1st at Waunakee, WI. This level of seasonal increase is taken as the beginning of the period during which no indoor plant culturing was possible. Figure 22 illustrates increases in both maximal solar flux (to about 1,000 watts/m^2) and daylength (to about 14 hours) by May 1st. These factors of ambient temperature and light do not return to acceptable levels until roughly mid-October. Based on this information, we were able to identify a few basic periods of greenhouse culture (Figure 20) that were possible during non-summer months: 1) the period from the beginning of the calendar year until roughly the first of May, when culture of filamentous algae is successful under LED lighting, with no interference by elevated temperatures, 2) the period from about the middle of March to roughly the first of May, when culture of filamentous algae is successful to a lesser degree without supplemental LED lighting, relying entirely upon ambient sunlight, 3) the period from the first of May to roughly the middle of October, during which no culture of filamentous algae is possible inside the greenhouse due to the (likely) combined interferences of heat and elevated light intensities, 4) the period from about mid-October to mid-November when very low levels of algal biomass may be produced under ambient sunlight, and 5) the period from mid-October through May 1st of the following year when acceptable levels of algal biomass are produced under LED lighting with no interference by elevated temperatures.

Figure 20. Major periods of filamentous-algae culture in greenhouse troughs throughout the calendar year. Periods of successful growth with and without supplemental LED lighting are noted, along with a 22-week period during which no culture was possible due to elevated temperatures and light intensities.

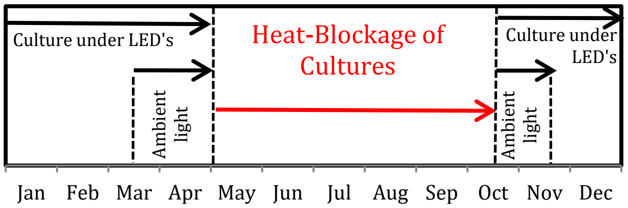


Figure 21. Daily temperatures and daily high-temperatures, averaged and compiled by month, at Waunakee, WI (site of Dane Community Digester).

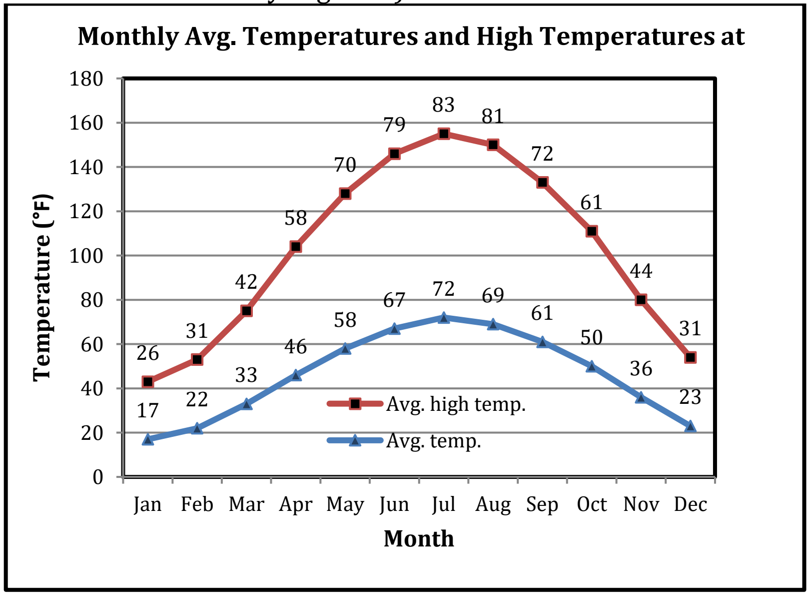
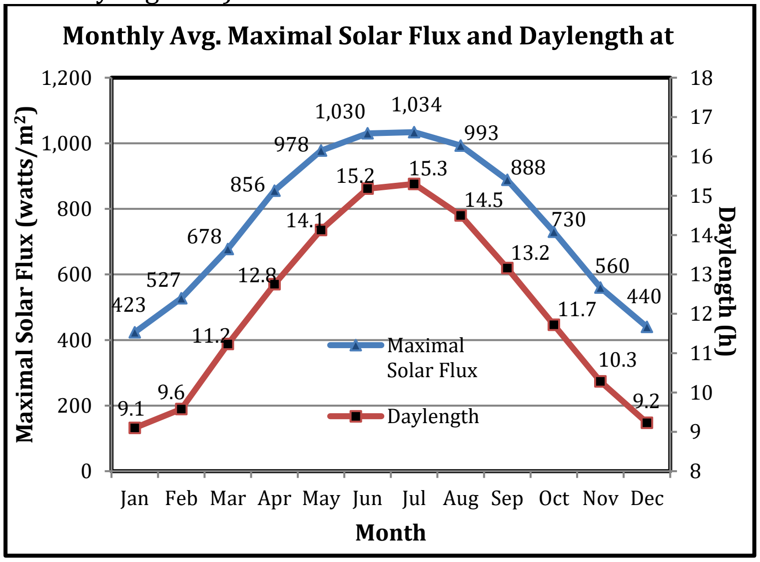


Figure 22. Daily maximal solar flux and daylength, averaged and compiled by month, at Waunakee, WI (site of Dane Community Digester).



Early work showed that problems of elevated temperatures and light intensities were in fact accompanied by the expected interference of organic compounds in prepared growth media. High concentrations of these organics were demonstrated to promote the growth of competing planktonic microbes, which led us eventually to restrict the potential lengths of semi-batch cultures.

Our previous physical/chemical analyses of process materials had demonstrated significant transfer of volatile acids from output digestate to the separated centrate fraction. Though no measures of volatile acids in fiber solids were conducted here, we assumed that at least some concentration of these readily metabolized compounds was also present in these materials. We identified the presence of these compounds during culture of aquatic plants on the basis of substantial blooms by planktonic microbes. These blooms were observed to be promoted by high temperatures and light intensities past a certain point in calendar spring, and were of such intensity as to promote total blockage of aquatic-plant cultures inside the greenhouse during summer months. We assume that most of these microbes were photoheterotrophs, life-forms that use light as an energy source and organic compounds as a carbon source. Common references to this biological lifestyle include purple and green photosynthetic bacteria (3). In principle, such photoheterotrophs could have entered our culture systems through the seedings of either filamentous algae or duckweed. However, we ultimately recognized that a more-likely vector was the introduction of our prepared growth medium. Such introduction was previously unanticipated, because it was assumed (somewhat naively) that these organisms would not survive extended periods of anaerobic digestion. We now know that this was an invalid assumption.

The photoheterotrophic plankton interfered with growth of filamentous algae by directly competing for space in the column of recirculating water and for energy transmitted through sunlight. As growth by these single-celled photoheterotrophs increased, they settled as epiphytes upon the algal filaments, blocked light transmission, and quickly decreased potential for algal primary productivity.

Our work in establishing filamentous algae and duckweed into culture led to development of a system whereby starting nutrient concentrations might be maintained at near-constant levels through return of set volumes of media to troughs or tanks on a daily basis. Our recognition of competition by photoheterotrophic forms led us to limit this period of nutrient replenishment (semi-batch culture) to three days maximum. At this point, all cultures inside the greenhouse were suspended to allow removal of processed media, flushing of the system with clean water, and re-introduction of a fresh preparation of growth media.

These seasonal and daily restrictions to the greenhouse culture of filamentous algae were never applied to cultures in the outdoor algal troughs. We did establish filamentous algae cultures in these troughs in the manner described for greenhouse systems, with the benefit that temperatures had moderated by the August start-up date. The lack of a ‘greenhouse effect’, combined with an in-ground system, and occasional rain events, made for a longer cycle (and reduced labor). This also corresponded with rapid declines in both daylength and maximal solar flux (see Fig. 22). Consequently, the algae production was never completely optimized in this system, though a sort of dynamic equilibrium of production was observed by late September. Leaf contamination was also a minor issue, due to the proximity to deciduous trees and time of year.

The greenhouse-culture restrictions were never applied to outdoor culture of duckweed. For this culture system, successful biomass production was achieved entirely through methods of trial-and-error. Once the excavated pond was filled with freshwater in June, 2012, seed collections of duckweed were introduced along with a 300-gallon volume of undiluted centrate. In the quiescent waters of the pond, this volume of centrate was able to maintain immiscibility and settle to the bottom of the water column undisturbed. Anaerobic decomposition of the centrate probably commenced at this point, based on observations of continuous gas bubbling to the pond surface. Growth by the introduced duckweed was probably stimulated by this process, because the pond surface was covered by the culture within just a few weeks' time. No effective competition by photoheterotrophic forms was observed during culture in this outdoor pond. No negative impacts by environmental factors (elevated temperatures or maximal solar fluxes) were noted.

Development of nutrient-media preparations. Test volumes of pre-diluted compost-tea water were prepared by soaking masses of oven-dried fiber-solids in freshwater. Soakings were conducted by first packing cylindrical milk filters with 80-g lots of the fiber-solids and then submersing these packed filters in 20 L freshwater for periods of 100 h. pH readings and concentration measures for certain nutrients that were solubilized during these soakings were performed by our laboratory, utilizing ion-selective-electrode and polarographic-meter technologies. Five volumes of tea water were prepared in this way, and average measures of physical and chemical values are presented in **Table 7**. Similar measurements were conducted periodically on tea-water volumes throughout the course of this study, demonstrating a high degree of consistency between individual preparations. Due to limited resources, the parameters summarized in Table 7 were the only ones accessible during this phase of our study.

Table 7. Average pH values and average concentrations of NH_4-N, NO_3-N, and PO_4^{3-} measured on five preparations of compost-tea water. Tea water was prepared by soaking oven-dried samples of fiber-solids collected at the Dane Community Digester.

pH	NH_4-N (mg/L)	NO_3-N (mg/L)	PO_4^{3-} (mg/L)
7.54	2.92	3.13	15.0

Replicate measures of a much broader selection of centrate nutrients were provided by an outside laboratory throughout the period of study. These measurements were earlier presented in **Table 1 (Section 4)**, and average concentrations of Total-N, NH_4-N, and Total-P are re-posted below in **Table 8** in units of (mg/L).

Table 8. Average concentrations (mg/L) of Total-N, NH_4-N, NO_3-N, and Total-P for replicate analyses of centrate from the Dane Community Digester. Values were earlier presented (Table 1, Section 4) in units of (lbs/1000 gallons).

Total-N (mg/L)	NH_4-N (mg/L)	NO_3-N	Total-P (mg/L)
2,459.0	1,310.2	16.0	336.53

A 2002 study by Wilkie and Mulbry (4) utilized preparations of digestate slurry to culture assemblages of freshwater filamentous algae. Although several differences occur between these authors' experimental design and our own, we wish to replicate as closely as possible their identification of nutrient-loading rates provided to their algal cultures. In the Wilkie and Mulbry study, loading rates were expressed in terms of three different nutrients found in their digestate preparations. Average concentrations of these nutrients are presented below, in Table 9.

Table 9. Average concentrations of Total-N, NH_4-N, and Total-P measured on preparations of digestate slurry in a 2002 study by Wilkie and Mulbry.

Total-N (mg/L)	NH_4-N (mg/L)	Total-P (mg/L)
2,370	1,620	240.0

In order to maximize our study's correspondence to the Wilkie and Mulbry work, we choose to base the nutrient loading rates for our centrate and compost-tea water preparations on the 2002 study's values for Total-N and NH4-N concentrations, respectively.

Tables 10 and 11 present our calculated loading rates for different nutrients, across culture series pursued in the course of this study. Between the beginning and end of our culture activity, a variety of media formulations were employed, depending on compiled concentration averages updated by the most-recent process-material analyses. The nutrient that contributed to loading rate calculations varied between use of either centrate or compost-tea water in media formulations, because all analyses of tea water were performed with a limited variety of ion-selective-electrode probes in our own laboratory. The calculation of these nutrient-loading rates allows conversion of algal-biomass production values (Table 12, below) and algal-tissue compositional values (Tables 13 and 14, below) to final nutrient-clearance rates (Table 15, below), which directly measure the efficiency of algal primary productivities.

Table 10. Average Total-N, NH_4-N, and Total-P loading rates associate with fiber-solids-derived growth media (*via* preparation of compost-tea solutions) used for indoor culture of filamentous algae and duckweed in the current study.

Preparations by centrate dilution			
Culture series	**Total-N loading rate**	**NH_4-N loading rate**	**Total-P loading rate**
0.036 L centrate/day to large indoor algal trough (2 cultures without LED's, Mar-Apr, 2012)	0.841 g Total-N/m^2/day	0.538 g NH_4-N/m^2/day	0.138 g Total-P/m^2/day
0.050 L centrate/day to large indoor algal trough (2 cultures without LED's, Oct-Nov, 2012)	1.40 g Total-N/m^2/day	0.746 g NH_4-N/m^2/day	0.192 g Total-P/m^2/day
0.030 L centrate/day to small indoor algal trough (1 culture, without LED's, Oct, 2012)	2.11 g Total-N/m^2/day	1.12 g NH_4-N/m^2/day	0.288 g Total-P/m^2/day

Table 11. Average NH_4-N, NO_3-N, and PO_4^{3-} loading rates associate with centrate-derived growth media used for indoor culture of filamentous algae and duckweed in the current study.

Preparations by dilution of compost-tea water (derived by soaking fiber-solids in freshwater)			
Culture series	**NH_4-N loading rate**	**NO_3-N loading rate**	**PO_4^{3-} loading rate**
16 L tea-water/day to large indoor algal trough (3 cultures, all using LED's, Feb-Apr, 2013)	0.532 g NH_4-N/m^2/day	0.570 g NO_3-N/m^2/day	2.74 g PO_4^{3-} /m^2/day
9 L tea-water/day to small indoor algal trough (3 cultures, all without LED's, Feb-Apr, 2013)	2.76 g NH_4-N/m^2/day	2.96 g NO_3-N/m^2/day	14.19 g PO_4^{3-} /m^2/day

<u>Culture of filamentous algae and duckweed</u>. Cultures of filamentous algae inside the greenhouse were pursued under the restrictions identified in the first half of this study. That is, it was recognized that greenhouse culture would be limited to spring and fall months of the calendar year and that the total period for each culture would be restricted to a total of 9 days (three consecutive 3-day cultures, with complete flushing of the culture systems between each 3-day culture period) to combat eventual blooms by single-celled, photoheterotrophic organisms.

A general progression of culture activity found us first employing direct dilutions of centrate during the spring and fall periods of 2012, as we began recognizing the necessity of system flushings to avoid plankton blooms. The chemical composition and physical qualities of collected centrate appeared to shift substantially between culture attempts, which probably relates to the still-tentative operating protocols employed by system operators during this time period. More specifically, the wide variety of food-processing and other industrial wastes that were employed as digestion substrates at this time were suspected to have a large impact upon our ability to employ direct dilutions of centrate as growth media. The large number of aborted attempts to establish recognizable algal production prompted us to turn attention towards use of compost-tea water for preparation of growth media and towards use of supplemental illumination. As mentioned previously, however, there were frequent indications that soluble organics (likely volatile acids) were transferred in significant quantities to fiber-solids and, as might be expected, to the compost-tea water generated by fiber-solids soakings. Consequently, we never achieved a system which guaranteed replication of successful algal-filament cultures. When circumstances of centrate or fiber-solids compositions turned in our favor, we could attain moderate levels of growth on our screens. However, these instances were limited in number, and spread across a long period of this study.

When respectable growth was achieved, the third 3-day period of culture was terminated by stopping recirculation in the trough and removing the screening squares to a “harvest” platform for scraping with a plastic blade. The thick slurry of scraped algal filaments was collected into portions of previously rinsed nylon fabric, squeezed to remove excess moisture, and returned to our lab for drying at 40 °C for 24 h in a forced air dryer. Following this drying, the mass of harvested algae was weighed to units of (g dry wt. algal biomass). Following harvests, scraped screens were returned to the greenhouse troughs to re-establish filament growth during a subsequent attempt at culture.

Recorded rates of algal-production from our successful cultures are compiled in **Table 12** across culture series, which indicates the general period of culture attempts, the use or non-use of supplemental LED lighting, the specific volume and type of prepared nutrient media employed in culture, and the total number of successful cultures achieved. As shown by the average, minimal, and maximal values for each series, there was little relative variation in production rates over the course of our study. We have considered the possibility that our moderate levels of production reflect, to some extent, the necessity of shortened periods of culture under the parameters encountered in this study.

As mentioned earlier, the higher-volume productivity of filamentous algae was not achieved in the outdoor trough, due to our hot summer/late start in commissioning this system. Culture of duckweed in the greenhouse tank followed the same restrictions of seasonal culture periods and need for system flushings. Growth by duckweed in this system was blocked entirely by elevated temperatures and light intensities during summer months. During spring months, high levels of duckweed productivity could be observed visually, but the plant’s free-floating habit meant it was impossible to measure growth on an aerial basis without significantly depleting the population. Production was estimated by attempting to remove biomass from one corner of the tank in a replicable manner, at the end of a 9-day culture period (three consecutive 3-day cultures, with complete flushing of the tank between each 3-day culture period). Prepared nutrient media was introduced to the duckweed tank on the same schedule followed for the small indoor trough. Collected biomass was dried and weighed in the same manner as employed for the filamentous algae. A set of 10 duckweed-biomass harvests of this sort during the spring of 2013 provided an estimated rate of 0.009 g dry wt. duckweed biomass/m^2/day. Roughly similar levels of productivity were estimated for the outdoor duckweed pond throughout the spring and summer growing season.

Table 12. Average, maximal, and minimal values for algal-production rates on two indoor troughs across series of successful cultures.

Culture series	Average, maximal, and minimal values for algal-production rates (g dry wt. algal biomass/m^2/day)
0.036 L centrate/day to large indoor algal trough (2 cultures, without LED's, Oct-Nov, 2012)	**4.50 g dry wt./m^2/day** Min = 4.5 Max = 4.5
0.050 L centrate/day to large indoor algal trough (2 cultures without LED's, Oct-Nov, 2012)	**4.65 g dry wt./m^2/day** Min = 4.6 Max = 4.7
0.030 L centrate/day to small indoor algal trough (1 culture, without LED's, Oct, 2012)	**4.6 g dry wt./m^2/day**
16 L tea-water/day to large indoor algal trough (3 cultures, all using LED's, Feb-Apr, 2013)	**5.27 g dry wt./m^2/day** Min = 5.0 Max = 5.8
9 L tea-water/day to small indoor algal trough (3 cultures, all without LED's, Feb-Apr, 2013)	**4.8 g dry wt./m^2/day** Min = 4.6 Max = 5.0

Analysis of algal biomass. Two separate analyses of algal-biomass composition were conducted during this study. The first analysis subjected dried algal filaments to Total X-Ray Fluorescence Spectroscopy ("TXRF"). TXRF enables detection of extremely low levels of elements from Sodium to Uranium in the periodic table (excluding Niobium to Technetium). TXRF operates on a principle of detecting all fluorescence emitted as a result of an X-ray beam reflecting at a very slight angle to the sample preparation. TXRF is based on internal standardization, and thus is calibrated through addition of an element not present in the sample material. In these studies, gallium was found not present in any samples, and therefore was chosen as the internal standard. Analyses of centrate were performed using a Bruker S2 PICOFOX spectrometer. Many of the elements that may be examined by TXRF are recognized as micronutrients for plant metabolism. In addition, total concentrations of the macronutrients P, S, and K are potentially detectable by this analysis. Results from TXRF analysis of dried algal filaments are compiled in **Table 13**, which shows significant accumulation of the micronutrients Cu, Cl, Fe, Mn, and Zn. In addition, notable sequestration of both P and K are indicated by these values.

A second analysis took the form of the routine "manure" analyses compiled within Table 1 (Section 4) for anaerobic-digester process materials. Results from this series of measure are compiled in **Table 14**. The TXRF measures of P and K in dried algal-biomass may be compared for consistency with those detected by "manure" analyses. TXRF-derived values of 3,866 mg/kg for P and 6,508 mg/kg for K are notably close to the wet-chemical measures of 4, 690 mg/kg for P and 5,940 mg/kg for K. In the current study, such consistency supports the reliable use of algal-tissue compositions for calculation of nutrient-clearance rates by algae under culture in our greenhouse systems (see below).

Table 13. Compilation of values from TXRF spectroscopic analysis of dried algal-biomass samples. Data include concentrations of both macronutrients (P and K) and micronutrients (Cu, Cl, Fe, Mn, and Zn) important to plant growth.

Element	Concentrations in dried algal biomass (mg/kg)	Element	Concentrations in dried algal biomass (mg/kg)
P	3865.95	Ni	n.d.
S	n.d.	Cu	7.39
Na	n.d.	Zn	28.95
Mg	4,140.26	Ga	418.04
Si	34,776.93	As	5.72
K	6,507.77	Se	1.11
Cl	1,043.14	Br	55.37
Ca	123,568.43	Rb	9.86
Ti	152.85	Sr	121.61
Cr	13.11	Ba	n.d.
Mn	1,389.85	Pb	n.d.
Fe	1,687.61		

Table 14. Compositional analyses of dried algal biomass harvested from greenhouse troughs in the current study. Measurements of this sort are usually applied to dairy-cattle manure and agricultural residues.

Physical/chemical measurement	Averages detected on dried algal biomass
Total-N (mg/kg)	18,475
Total-P (mg/kg)	4,690
Total-K (mg/kg)	5,940
Total-S (mg/kg)	6,550
NH_4-N (mg/kg)	490
NO_3-N (mg/kg)	45
pH	7.5
Ash content, %	63.26
Acid-detergent fiber content (%)	31.30
Neutral-detergent fiber content (%)	33.55
Lignin (%)	6.55
Moisture (%)	2.25
Dry Matter (%)	97.95

Estimate C:N ratio	11:1

Calculation of nutrient-clearance rates. The nutrient-clearance rates compiled in **Table 15** are calculated from a cluster of measurements related to productivity by cultures of filamentous algae, including loading rates for specific nutrients, averages of algal-production rates that were computed on an aerial basis, and compositional values for the biomass harvested in order to calculate such algal-production rates. A nutrient-clearance rate provides a direct measure of a plant's photosynthetic efficiency, but can only be compared directly to rates calculated for the same form of nutrient. At the present time, it is not possible to compare our findings for NH_4-N, PO_4^{3-}, and NO_3-N to those calculated on other assemblages of filamentous algae.

Table 15. Nutrient-clearance rates calculated on PO_4^{3-}, NH_4-N, and NO_3-N for filamentous algae cultured indoors on algal troughs in the current study.

Nutrient	Nutrient-clearance rates
PO_4^{3-}	21.11-27.20 mg PO_4^{3-}/m^2/day
NH_4-N	2.21-2.84 mg NH_4-N/m^2/day
NO_3-N	0.00041-0.00052 mg NO_3/m^2/day

Summary

Attached filamentous algae and free-floating duckweed were grown inside and immediately adjacent to a greenhouse, to investigate the utility of AD by-products as nutrient sources for aquaculture. Two sizes of algal troughs were constructed to support semi-batch culture of the benthic algae, which were subjected to continuous re-circulation of growth media during 9-day periods of culture. High concentrations of organic compounds are likely to have been transferred to the liquid centrate and fiber-solids fractions used for preparation of nutrient media in this study. These organic compounds undoubtedly migrated into the nutrient media prepared from these manure derivatives, and adversely affected the productivities of algae grown upon these media. Rapid blooms of unicellular, photoheterotrophic forms were supported by uptake of volatile acids, allowing these organisms to outcompete attached filamentous algae for resources of light energy and space within the water column.

Contamination by high concentrations of organic compounds required an overall shortening of culture periods for these algae and intermittent flushings of the culture systems to control growth of competing microbes. In addition, problems of heat control inside the greenhouse during warmer months limited our studies to early spring and late fall throughout the calendar year.

Consequently, our project was not able to optimize culture systems in support of these algae. A limited number of cultures were carried to completion throughout the study period, though harvests of biomass from these cultures demonstrated moderately successful rates of biomass production (4.5-5.8 g dry wt algal biomass/m^2/day). Nutrient compositions of dried algal biomass showed reasonably high rates of mineral sequestration, and calculated rates of nutrient-clearance may yet compare favorably to other published values.

Notes and References

(1) Adey, W.H., and J.M. Hackney. 1989. The composition and production of tropical marine algal turf in laboratory and field experiments. In: Adey, W.H. (Ed.), The Biology, Ecology, and Mariculture of *Mithrax spinosissimus* Utilizing Cultured Algal Turfs. The Mariculture Institute, Washington, D.C.
(2) Adey, W.H., C. Luckett, and K. Jensen. 1993. Phosphorus removal from natural waters using controlled algal production. Restor. Ecol. 1 (1), 29-39.
(3) Madigan, M.T., J.M. Martinko, and J. Parker. 2003. Brock Biology of Microorganisms. 10th Ed. Pearson Education, Inc., Upper Saddle River (NJ).
(4) Wilkie, A.C., and W.W. Mulbry. 2002. Recovery of dairy manure nutrients by benthic freshwater algae. Bioresource Technology, 84: 81-91.

6. Practical Applications of Algal-filament Production

6-1. Filaments as renewable fertilizer
6-2. Lipid analysis of scum/scum layers

Potential of algal filaments in production of renewable fertilizer. A small project undertaken by our laboratory investigated the potential of dried algal filaments as a matrix in production of renewable fertilizer. A description of this project and our findings constituted a poster presented at the 2011 Algal Biomass Summit in Minneapolis, MN. A .pdf copy of this poster is included in Appendix 6.

A summary of this poster would focus upon production of filamentous algae at our culture facility and use of dried algal filaments in preparation of soil amendments. The dried filaments were used to soak up liquid centrate produced at the Dane Community Digester. Then, both plain and (dried) centrate-soaked filaments were ground and used as a portion of feedstock in vermicomposting studies. These studies showed that centrate-soaked algal filaments could not be consumed by the worms (in fact, such filaments drove worms from the colonies). The castings produced by feeding worm plain algal filaments or ground corn stover (control) were collected by sieving of the worms' bedding.

Plain algal filaments, centrate-soaked algal filaments, ground corn stover, worm castings produced on plain algal filaments, and worm castings produced on ground corn stover were each used directly or in 1:1 mixes with commercially prepared topsoil in order to test ability to support soybean germination/early-growth. Results showed that centrate-soaked algal filaments and both forms of worm castings hindered germination and early growth rather dramatically. Plain algal filaments, either used directly or as a mix with topsoil, support germination and early growth of soybeans. Plain algal filaments used directly (100% algal filaments) appears to promote early stages of soybean growth, possibly due to easy release of sequestered nutrients.

Chemical and physical analyses of centrate-soaked algal filaments show high absorption of nutrients into this biomass. Centrate-soaked algal filaments (or algal filaments soaked in other high-nutrient-concentration liquids) could easily find use in raising soil nutrient levels if cut sharply with other amendment materials.

The (C:N) ratio of plain algal filaments suggests the efficacy of this materials could be improved significantly if mixed with biochar or some other form of condensed carbon.

Identification of lipid production by photoheterotrophs. A finding of ongoing interest to our laboratory is recognition that many of the organisms growing on manure-derivatives in our culture systems are capable of quality lipid production.

Our greenhouse algal culture systems maintain a continuous recirculation of media across flat surfaces of screening, which are fully exposed to high intensities of sunlight and daily surges of heat. Such culture parameters are not necessarily conducive to producing filamentous-algae biomass, but they have been found to promote growth of scum-producing organisms. These scum-producers are single-celled microbes, most-likely placed in a category of photoheterotrophic organisms, *i.e*. life forms that use organic molecules as a carbon supply, but sunlight as an energy source. Because photoheterotrophs can directly consume organic compounds, they thrive in media prepared from anaerobic-digester by-products ("manure-derivatives"). Their exploitation of such an environment can often interfere with production of filamentous algae (they can cloud liquid media, blocking transmission of sunlight, or settle directly on the surface of filaments, initiating decomposition of the alga's cell wall). Their ultimate success is recognized by a massing of cells into scum layers that spread across exposed surfaces.

Our work with the algal-culture systems led us to recognize a continuous presence of these photoheterotrophs. Scum-layers in areas receiving high levels of solar radiation would often develop a slick, almost metallic sheen (characteristic of growth by green- or purple-sulfur bacteria). From time to time, such scum could attain large mass and cover significant surface area in our troughs.

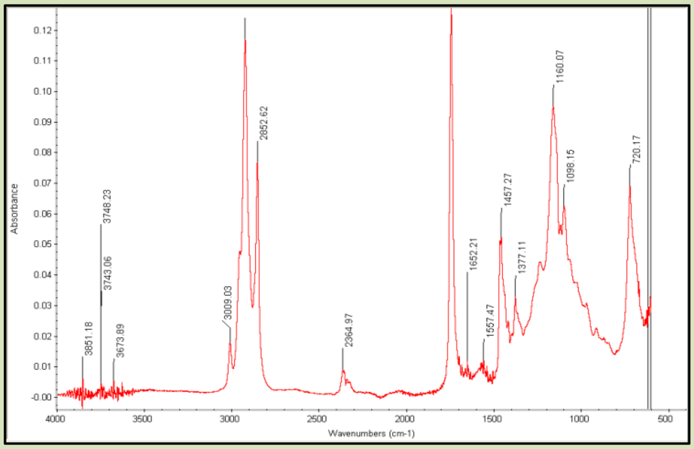
On a few separate occasions, we collected scrapings of these scum layers, once they had attained substantial thickness. To begin investigating the organic composition of the layers, a very simple extraction of non-dried scum, using hexane as solvent, was performed in a separation funnel. The extraction from this exercise was dried and the residue was collected for mid-infrared analysis on a Thermo-Nicolet 380 Fourier transform spectrometer that was equipped with a standard KBr beam splitter and a Pike MIRacle™ ATR (attenuated total reflection) accessory. FTIR-ATR operates in the mid-IR region (500 to 4000 cm^{-1}) and utilizes peaks that represent assorted chemical bonding patterns (including hydrocarbon stretching and bending, ester carbonyls, and carbon-carbon double bonds) to identify chemical compounds.

A mid-IR spectrum of the recovered residue is shown in **Figure 23** (top). The sharp peaks of this plot immediately suggested detection of long-chain fatty acids that display high levels of unsaturation. For an immediate comparison, a sample of commercially prepared vegetable oil was scanned (Figure 23 bottom). The two spectra were statistically compared on the basis of peak positions and heights (full widths at half-maximum) and were matched with significance at greater than 99% probability. In such comparison, it may be recognized that the photoheterotroph-derived residue is only a little less refined that the commercial vegetable oil, and shows slight presence, or "contamination", of salts.

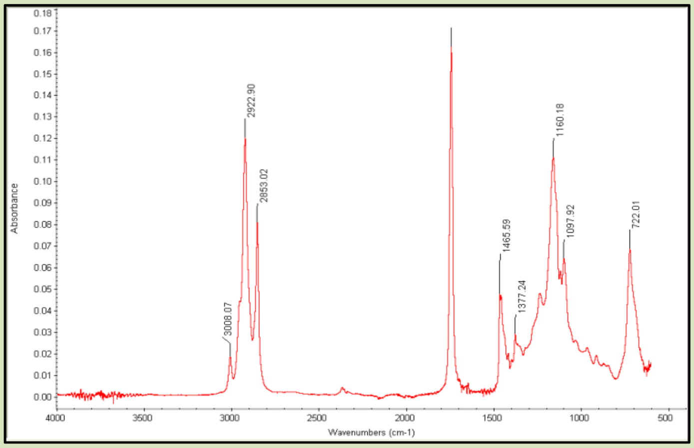
This rather simple analysis was able to confirm lipid production by the photoheterotrophs, and the relatively small amount of scum used in extraction suggests a high rate of production. Rather than occurring as mere nuisance organisms, such photoheterotrophs appear to be manufacturing an energy-rich substance that is easily harvested from our algal-culture systems. The commercial potential of this material awaits further investigation.

Figure 23. Mid-infrared spectra of residue extracted from mass of photoheterotrophic organisms (top) and commercially prepared vegetable oil (bottom). Peak heights and positions of both spectra show close statistical match and confirm the presence of long-chain fatty acids.

Residue from photoheterotroph-produced scum layer



Wesson™ vegetable oil



7. **Balance of Systems** (Cistern, alfalfa field, sediment basin, constructed wetland, and culvert/standpipe connections)

Measures of performance. Original plans for the **Lower-Components** system intended utility in receipt and processing of manure-derivatives that had been partially cleared of nutrients by aquatic plants. As mentioned, clarification by the W-DNR directed us to store all manure-derivatives, whether or not partially processed, until use in fertigation of a nearby alfalfa field. This directive forced us to view the features that constitute the Lower-Components system as having a sole purpose of treating stormwater runoff from fields and adjacent dairy-operations yards. When viewed in this light, the Lower-Components system consists of four major features: 1) the alfalfa field, 2) a ditch/culvert structure that collects runoff from the field and uphill operations yards, 3) the excavated sediment basin that holds this uphill runoff, and 4) the constructed wetland that receives intermittent overflow from the sediment basin.

Our intent through the course of this study was to routinely measure concentrations of ammonium, orthophosphate, and other chemical parameters in water samples that might be collected in the ditch/culvert structure, the sediment basin, or the constructed wetland. Such measurements would provide some understanding of the degree to which nutrients are carried by runoff and the extent to which the sediment basin and constructed wetland can process this runoff biologically.

Of course the spring/summer of 2012 encompassed a period of extreme drought in Dane County, consequently the constructed wetland (established in the fall of 2011) was unable to gather and hold enough water to maintain growth by plantings of emergent vegetation. The drought also impacted runoff that would otherwise have collected in the sediment basin, and construction of this feature had previously demonstrated that much of the basin's underlayment consisted of sandy soil. Thus, neither the sediment basin nor the constructed wetland ever held enough water to take meaningful measurements during 2012. There were nearly no major storm events during the entire period of this study generating consequential levels of runoff.

On two separate occasions, to calibrate our abilities in phosphate measurement, we examined water samples from puddles in the culvert and a rock crevice in the drainage ditch. The four measurements of orthophosphate in these samples showed an average of 29.0 mg PO_4^{3-}/L in the ditch water and 6.7 mg PO_4^{3-}/L in the culvert. Although this might suggest that some degree of biological processing was occurring during flow of water from the operations yard to the collection culvert, such possibility is miniscule. The water samples that we collected were not suited for the colorimetric analysis we performed and, unfortunately, provided no useful insights. We are hopeful that a return to an extended period of seasonal precipitation in Southern Wisconsin will provide opportunity for valid testing of this system's performance in future.

* Note: In fact, a normative period of more seasonal precipitation (in 2013) has provided such an opportunity for valid testing of field runoff. A colorimetric measure of P in field runoff (from culvert) just at the end of the study period (Q3, 2013), measured 3 mg/l.

** Additional Note: Our farmer-partner/hosts the Maiers, have been able to incorporate the Lower Component features into the engineering of their White Gold Dairy's new calf barn drainage water plan. (CIG project partner, Resource Engineering Associates, was the project engineer for this new structure.)

Cistern

The cistern was a late addition to the project, added after it was deemed that any processed media (centrate or manure-solids tea water used in the algae troughs or duckweed/harvestable bog) from the Upper Component areas was 'manure derivate' and could not be directly discharged into the Lower Components or natural areas in or around the project site.

Buried 1500-1600 gal. concrete cistern

Alfalfa (Irrigation) Field

The Maier Brothers, White Gold Dairy, is home to a 1,200 head milking herd, and hundreds of calves/heifers. They grow more than ½ of their own rations, including corn, corn fiber (silage), protein (soy), and carbohydrate (alfalfa).

Perennial alfalfa field adjacent to balance of project site

Sediment Basin

Sediment Basin

Per Wisconsin Dept. of Natural Resources Conservation Practice Standard #1064;
A sediment control device constructed with an engineered outlet, formed by excavation or embankment to intercept sediment-laden runoff and retain the sediment.

Constructed Wetland

Per NRCS Code 656, the definition of an Artificial Wetland is; *An artificial ecosystem with hydrophytic vegetation for water treatment.* The purpose for construction should be,

1) 'For treatment of wastewater and *contaminated runoff*, from agricultural processing, livestock, and aquaculture facilities or
2) For improving the quality of storm water runoff or other water flows lacking specific water quality discharge criteria.

Constructed wetland (Q2, 2012) as drought conditions began drying the basin.

A list of aquatic species planted in the wetland is included in the next section of this report. 5,000 individual plants were hand-laid with help from eager volunteers (many of whom escaped their classrooms at Waunakee High School for several hours as a reward.)

8. Actual Costs & Economic Analysis

- System Capital Cost 'CapEx'
- System Operating Costs 'OpEx'
- Economics of using aquatic plants & algae to sequester/recycle nutrients

Resources & Proximity to Anaerobic Digester (AD) Operations

Basic resources required for year-round, high-yield propagation of algae and other high-rate, aquatic species in the Upper Midwest include, at a minimum, indoor 'conditioned' space. It was thought that both indoor and outdoor spaces would help reduce both initial CapEx and continuous operating costs. Even in the northern climate found in Dane County (43 degrees N latitude) it is possible to achieve excellent yields 4 mos. out of the year outdoors. Additionally, cooling is required to maintain plant health indoors during the warmest months. However, on balance, costs per pound of P sequestered did not turn out to be less expensive in the projects outdoor troughs.

With daylight diminishing rapidly after September 21st, and length of day dropping to < 9 hours by Dec. 21st, both heated space and artificial lighting are required to maintain continuous plant propagation/nutrient uptake 12 mos./yr. Year-round temperatures must be sufficiently warm (ideally 55F-80F) to promote on-going, rapid growth. There must be enough photonic energy, preferably from sunlight, for sustained photosynthesis. Indoors, during low daylight months, we utilized both conventional 'grow lights' (lower CapEx) and LEDs (lower OpEx). Of course even artificial LED sources (blue/red, shown in photo to right) with their low CapEx are costly when compared to natural light, so their use must be minimized. Under the best circumstances daylight is maximally exploited; to accomplish this, accommodations must be made for more oblique sun angles and motorized lamp winches (on timers) that are then employed to raise lamps when natural light is available.

Relatively flat topography will reduce upfront grading and site-prep costs, as will higher-clay soils, that improve liquid retention and conform better to standards for handling and storage of 'manure derivatives'. Finally, with proximity to a large AD operation, including combined heat & power (CHP) generation, comes lower-cost utilities, and potential access to CO_2. In this study we were able to take full advantage of the CHP system's excess heat, however the CO2 was more challenging. Our large indoor trough system was designed to sparge exhaust directly from the genset engine 'stack', and charge the water with CO_2, rather than directly effect the ambient greenhouse air (photo); however this process could not in the end be executed when the 2 inch-thick stack pipe cover (atop the gen-set) could not be safely/physically penetrated.

Production Economics

In 2009, Benemann reported that the lowest costs for producing *Spirulina* were \$5,000/tonne. His team additionally estimated overall [outdoor] pond production costs for 25% useable algal triglycerides of at least \$40,000/ac (\$98,840/ha) for an annual yield of 1,500 gal (5,680 L) of oil. Not surprisingly, since propagating for lipids was not a project objective, our cost estimate for full-scale PANR processing falls in between these numbers.

As described in Chapter 5, our best production rate was 5.8 g (dry weight) algae/m^2/day. This equates to just over 2 kg./yr. (5.8 g x 365 = 2,117 g). With the analysis described in Sections 4 & 5 above, using conventional IPC (wet chemistry), our domestic average 'pounds/ton' of nitrogen and phosphorus in the filamentous algae, was found to be 36.95 and 9.38 pounds respectively. Less than 0.5% by weight then, is P. Using these numbers, we derived the following footprint/pound of the macro-nutrient P sequestered into plant tissue:

System Capital (Construction) Costs

The 'Phyto-Aquatic Nutrient Recycling' (PANR) system was constructed immediately adjacent to the Dane Community Digester (a \$13M complex producing biogas and facilitating manure management for three large dairies). As previously mentioned, this location is in the Yahara River Watershed (leading to the Rock/Mississippi Rivers), approx. 13 miles northwest of the University of Wisconsin, which made it convenient to contractors, and the sourcing of project materials and supplies.

Project construction and execution costs for the system are broken down into three primary components;

1) Upper conditioned space (greenhouse)
    A) Greenhouse
    B) Indoor troughs
    C) Propagation tanks, drying racks, LED/grow lights, hydroponic rails, etc.
2) Upper component features (outdoor)
    A) Harvestable bog (duckweed pond)
    B) Outdoor troughs
    C) Cistern/irrigation field(s)

3) Lower component features (outdoor)
    A) Field Drainage/culvert
    B) Sediment Basin/standpipe
    C) Engineered Wetland/headwater feed feature

In each case, the costs to build are further broken down into equipment & CapEx labor, and supplies & OpEx labor.

1) Upper Indoor 'Conditioned' Space

Capital Costs (CapEx)

FEATURE	COST
A) 2400 s.f. Greenhouse w/ utilities (Cap Ex)	$62,275 ($25.95/s.f.)
B) Raised Troughs (Cap Ex)	
Large 3'10" x 23' 8" = 91 s.f. (growing surface)	$3,500 ($38.46/s.f.)
Small 2'0" x 8.5' = 17 s.f.	$1,314 ($77.27/s.f.)
C) Other; propagation tanks, drying racks, lights, etc.* (*Electric cords, hoses, gloves, tools, small pumps)	$3,750 ($1.56/s.f.)

Using the lower number for raised trough space (larger trough), and assuming 30% of greenhouse needed for circulation and propagation, the price/ft^2 of indoor algae growing surface area is reflected in the table below;

CAPEX	Cost/gross s.f.	/usable s.f.	Floorspace	TOTALS
Greenhouse	$25.95	$37.07	2,400 sf	$62,775
Trough	$38.46		1,680 sf (70%)	$64,613
Other	$1.56	$2.23	1,680 sf	$3,746
Gross Cost				$131,134
Cost/usable s.f.		131,134/1,680		$78/s.f.

	Conversion to sf	P/ft^2/day	Production lbs/sf	Indoor CapEx
P uptake/sf/day	.0223 g/m^2/10.75	.0021 g/sf/day		
Annually	(x 365)	0.76 g/s.f.	.00167 lbs/sf	$45,805/lb
In greenhouse	(x 1,680 s.f.)	1,277 g/yr.	2.8 lbs/yr	
Cost/pound P	$131,134/2.8 lbs			$46,834/lb
[grams/pound]	[454 g/lb]			
10 yr amortization	$46,834/lb/10 yrs			**$4,683/lb of P**

2) Upper Outdoor Component Features

A) Duckweed Pond (Harvestable Bog)
B) Algae Trough
C) Cistern

	FEATURE		COST
A)	30,000 gal./2,400 s.f. Bog	(Cap Ex)	$9,475 ($3.95/s.f.)
B)	2 x in-ground algae troughs	(Cap Ex)	$8,750
	8’0” x 30’0” = 240 s.f. (growing surface)		($36.46/s.f.)
C)	Buried Cistern/1,500 gal. storage tank*		$3,850
	* 10% attributed to cost of outdoor trough CapEx		

CAPEX	Cost/s.f.	Cost/s.f. + storage	TOTALS
Bog+ 90% of storage	$3.95	$5.39	$9,475
Troughs+10% storage	$36.46	$38.06	$8,750
Cistern (storage)			$3,850
Gross Cost			$22,075
Cost/usable s.f.		$22,075/2,640 s.f.	$8.36/s.f.
Cost/sf algae outdoor surface		$9,135/240 s.f.	**$38.06/sf**

(Upper) outdoor processing costs are broken down into harvestable (duckweed) bog, the algae-growing troughs, and required holding tank (buried storage cistern). Since nutrient processing (uptake) values for duckweed rely on secondary values that vary widely (0.03—2.8% dry wt.*) and the rapid, bulk propagation benefits likely inure more from potential starch/fermentation value (also not an objective here), combining features in this context would require excessive calculus. A simpler number for the purposes of economic evaluation of this project then, includes only the trough (algae) processing. [* Data originally collected by Landolt and Kandeler, *Biosystematic investigations in the family of duckweeds (Lemnaceae)* vol 4, 1987 (Phytochemistry, physiology application, bibliography), vol. 2 of *The Family of Lemnaceae- a monographic study*.]

	Peak conversion	P uptake/day	Production	Outdoor CapEx
P Uptake/day	.0223 g/m^2/10.75	0.0021 g/ft^2/day	(454 g/lb)	
Annually (4 mos/yr)	(x 120 days)	0.252 g/ft^2	.00055 lbs/ft^2	
Per 240 sf			.132 lbs./yr	
Cost/pound P				$69,200/lb

Ft^2 required/lb of P	(1/.00055)		1,824 ft^2	
10 yr amortization				**$6,920/lb of P**

As the above numbers demonstrate, while production on a per acre basis is very close to the best terrestrial crops (#2 field corn for example; 200 bu/ac corn x 56 lbs/bu = 11,200 lbs with 14% moisture content), the cost of production is many times greater (again using corn, the annual price is still <$1,000/ac). Additionally, algae is reported to be many times faster growing under ideal conditions, though this result was not found over one outdoor growing season.

Upper Component Operating Costs (**OpEx**)

Project utilities were readily available vis-a-vis the 2 x 1 megawatt (CHP) 'gensets', located less 100 meters from the greenhouse. Their circulating heat-transfer liquid runs first to an adjacent operations trailer, then underground to many of the features of the community digester, including all three of the primary digester vessels, and finally to the PANR-CIG project greenhouse. Fresh water was also provided from the community digester well, in addition to a direct line supplying 'centrate' liquid.

Heating and electric costs for the entire project averaged $692/mo. over 12 mos., while labor averaged 10 hours/week (approx.. 12 hrs/wk summer, 8 hrs/wk winter). Using a conservative $15.05/hr. wage (Iowa Extension, 2012 average) reflecting 'farm hand' pay in the Upper Midwest, the additional OpEx for propagation, harvesting, drying, and handling plants is reflected in the table below, for indoor (year 'round) and outdoor (6 mos.) production. Some cleaning time is also included. Actual costs then are;

Utilities	Summer Labor	Indoor Labor	Supplies/Consumables	Summer TOTAL	TOTAL
$692/mo.	$783/mo.	$522/mo.	$150/mo.	$1,625/mo.	$1,364/mo.
	($261 outdoor)				
$8,304/yr		$30,000/yr.	$1,800		$40,100/yr.

				Annual labor	
				$40,100/2.93 lbs P	**$13,686/lb P**

Since costs for the indoor growing area were extrapolated to 1,680 s.f., labor must also be estimated for tending to the larger growing area (from only 113 s.f.), a simple multiplier then would be 1,680 sf/113 sf = 14.87, though this would give no credit for economies of scale. A more reasonable assumption then, might be that one fulltime employee, earning at the aforementioned hourly rate, would be earning just over $30,000/yr., or $2,500/mo. An est. of summer only labor required for operation of 1,824 ft^2 (7.6 x the space actually operated) might reasonably be expected to take two people x 4 mos./yr.

Overall costs per pound then, for P sequestration is;

		Indoor/pound of P	Outdoor/lb P
CapEx	(10 yr. amort.)	$4,683	$6,920
Indoor OpEx	($30,000/yr labor)	$13,686	
Outdoor OpEx	($30,000/12 x 4 mos)		$10,000
		$18,369/lb P	**$16,920/lb P**

3) Engineering & Lower Component Features

A) Field Drainage & Culvert
B) Sediment Basin
C) Wetland

Engineering costs and the civil engineering value associated with the project were well over $100,000. The billed amount, **$94,752**. This included time spent on planning, front-end design, construction drawings, permitting, surveying, supervision, reporting, construction interface, and project management assistance. Much of this has been attributed to the cost of the Lower Components, though a great deal of time was donated to the project's Upper Components, greenhouse and farmer/producer relations. Especially important is the need for expertise in manure handling, jurisdictional codes, and livestock producer understanding/relations.

The lower features of the PANR site are by far the largest physically, encompassing nearly 2.5 acres (>100,000 s.f.), including the slopes of the sediment basin and earthen bulwark between the basin and wetlands. Including the alfalfa field the site is approximately 7.5 acres. Capital costs for this portion of the study were overwhelmingly associated with the labor for engineering, excavation, and grading, while planting the wetlands was the second greatest expense, limited mostly to the cost of the plants ($2.50/each x 5,000), since labor was donated by Waunakee High School (agriculture class) students, and wetlands professional Susan Priebe, of Aquaterra Systems. The cost of the five acre alfalfa field, where Upper PANR-processed 'manure derivative' irrigation occurred (only twice over the course of the project), is not included in this economic analysis as it was not rented for the project. (Indeed very little *runoff* data could even be collected from the edge of this field during the summer 2012 drought, though a cement catchment basin was installed at the lip of the culvert leading to the sediment basin. – lower left in left photo.)

A) Field Drainage & Culvert

For most of the project period (2011-2012 seasons) the irrigation field was planted in alfalfa (see photo below), however during the 2013 season, the White Gold Dairy planted this field in corn. Again, including this ‘connecting’ feature within the project was the direct result of the project team’s interaction with the WDNR, and state (federal) policy prohibiting the addition of even a trace amount of P from directly entering an ‘impaired’ waterway vis a vis a ‘point-source’ dairy, digester, or other manure-handling/livestock facility.

Although tracking nutrient uptake was not reliable for the reasons previously cited, a cost of construction was derived as follows for the entirety of the Lower Project Features;

Feature	Grading/Excavating cost	Engineering	Total
Field drainage	$3,000 (5% of project total)	$9,000	$12,000
Sediment Basin	$24,000 (40%)	$18,000	$42,000
Wetland	$18,000 (30%)	$18,000	$36,000
LOWER COMPONENT TOTAL	$45,000	$45,000	$90,000

B) Sediment Basin

The largest physical feature of the project was the sediment basin, which took project partner/vendor Snyder Excavating several days to dig out. It was outfitted with a concrete standpipe and boulder-lined spillway for storm-event overflow to the wetland. As noted in the table above, an estimated cost of $24,000 was attributed to this component, of the total excavation cost for all of the lower features.

C) Wetland

The wetland was planted with 5,000 aquatic, emergent plants, supplied by project partner AquaTerra Systems. (List provided below.) The cost was $12,500, or $2.50 per plant. These were a complementary assemblage of native species, as selected by our project partner, Ms. Susan Priebe, to thrive in the local environment, under the conditions expected.

AQUATERRA
STORMWATER MANAGEMENT

Plant List

for

Artificial Wetland – Waunakee, WI

Latin Name	Common Name
Acorus calamas	Sweet flag
Carrex sp.	3 species - emergent
Juncus effuses	Soft rush
Sagittaria latifolia	Duck potatoe weed
Scirpus acutus	Hardstem bulrush
Scirpus americanus	Olneys bulrush
Scirpus cyperimus	Wool grass
Scripus validus	Soft stem bulrush
Sparganium eurycarpum	Giant burreed

Dated: June 4, 2011

Susan Priebe, Owner

2494 Charmany Way, McFarland, WI 53558 email: susan@aquaterrastormwatermanagement.com
- phone: 608-556-0570

9. Education & Outreach

Education, outreach, and just 'spreading the word' with regard to both novel and traditional nutrient management was a primary objective within the deliverables of this CIG-project. To that end, events began already during the first quarter of the project, and have continued throughout;

Courses, Lectures & Events	Dates
Dane County Dairy Breakfast	6/8/13
BBI – Biomass Show (Mpls., MN)	4/10/13
MATC/CERET Nutrient Mgmt Class	11/10-11/12
Project Open House	10/20/12
Fitchburg Green Thursday	10/4/12
SWCS (Ft. Worth, TX)	7/24/12
MATC Engineering Class	1-5/12
NASECA Presentation	2/1/12
Biocycle Conference (St. Louis, MO)	11/2/11
Algal Biomass Summit (Minneapolis, MN)	10/25-27/11
MATC/CERET AD Class	10/22-23/11
Dane County Executives Presentation	12/10/10
MATC/CERET AD Class (+Scholarships)	10/13-14/10

Our educational partner for the duration of the project was Madison College (formerly Madison Area Technical College). Through their Certificate in Education for Renewable Energy Technology (CERET) program, we were able to reach out to nearly 100 students beginning with the first month of the project and continuing indefinitely (currently through fall semester, 2013). 16 hour, weekend short-courses allowed for a 50-50 mix of classroom and field study, with visits to several Southern Wisconsin digesters, nearby municipal WWTF, and landfills with biogas capture. Additionally, we offered 10 x $100 scholarships over the period to offset the cost of tuition.

The CIG-PANR project was a destination in all but the first session (taught Nov. of 2010, construction had not yet commenced). Courses focused on both the process of anaerobic digestion and nutrient management concepts and practices. Other, past CIG recipients including Dr. Richard Cooke, Univ. of Illinois, lectured to students. The following is the course bulletin description for one of the weekend short-courses offered in the fall of 2012;

10-484-164 **Instructor:** **Tony Hartmann**	**Biomass Systems, Nutrient Management, and Recycling** Excess nutrients, particularly nitrogen (N) and phosphorus (P), are the major pollutants in lakes and estuaries and the second leading source of pollution in rivers according to the U.S. EPA, yet all living flora and fauna need nutrients to grow and thrive. This course will discuss how to balance the inputs and outputs within a healthy ecosystem. In this weekend short-course, we will explore conventional practices, next-generation solutions, and practical ways to recycle these major agricultural inputs. Our focus will be mostly 'on-farm' with field trips as well as guest speakers from academia, agriculture, public and private sectors. This course is relevant for everyone who has a stake in the U.S. food-chain.

The course included a handbook (cover shown in Appendix 4), and additional handouts covering lecture material/programs like the Dane County ‘Adaptive Management’ effort -- lecturer, Dr. Kathy Lake, of Madison Municipal Sewerage District (MMSD).

Special thanks in the creation of these courses over the past three years to;

- Dr. Ken Walz & Barb Anderegg, MATC/CERET
- Lee Elver & Bob Pofahl, PE, Resource Engineering Associates
- Greg Sorge, Waukesha Engine Corp.
- Gary Radloff, Wisc. Bioenergy Initiative
- Peter Taglia, Clean Wisconsin
- John Baldus, Wisc. Office of Energy Independence
- Karl Crave (Jeff Janzen), Clear Horizons/Pieper Power
- Charlie Crave, Crave Bros. Dairy & Cheese Plant
- Kathy Lake, Madison Metro’ Sewerage District (MMSD)
- Paul Nehm, MMSD
- Troy Statz, Statz Family Dairy
- Mark Torresani, Cornerstone Engineering
- Kevin Shelley, U.W. Extension
- Dr. Emily Read, U. of Wisconsin - Dept. of Limnology
- Keith Strade & Monte Lamer, Clear Horizons/Pieper Power
- Steve Stumbras & Sandy Syburg, Purple Cow Organics
- Dr. Richard Clarke, U. of Ill. – Dept. of Agricultural & Biological Eng.
- Rick Eilertson, City of Fitchburg – Environmental Engineering

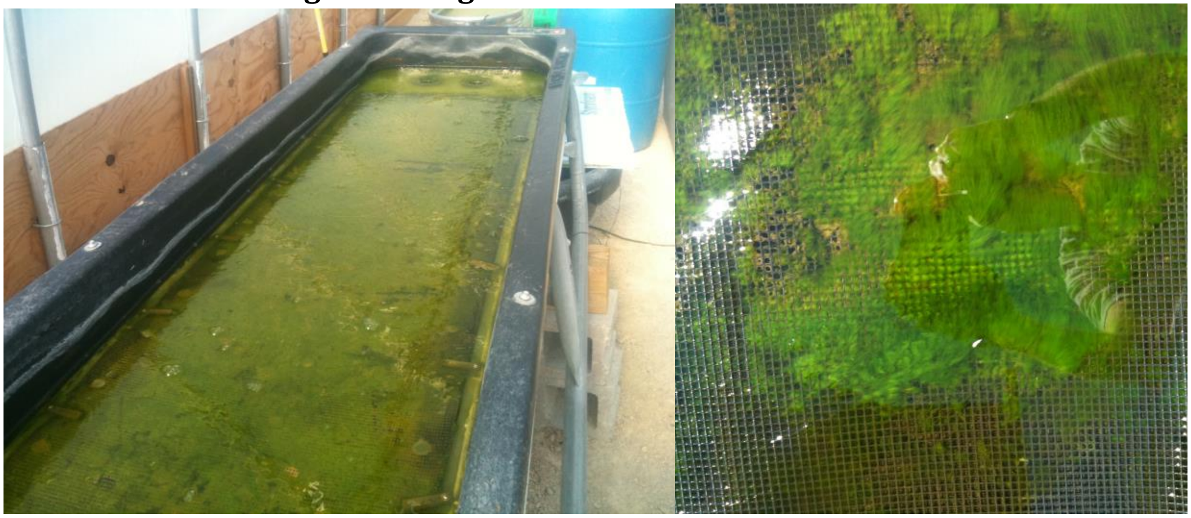
Additional Exposure: Special events like the World Dairy Expo and project open house in 2012, and the Dane County Dairy Breakfast in 2013, further exposed the project to thousands of farmers, educators, researchers, technology developers, politicians, children, and just curious citizens, from rural and urban backgrounds.

10. Fact Sheet & Recommendations

Phyto-Aquatic Nutrient Recycling ©

Great Lakes Ag Energy (GLAE) & Resource Engineering Associates (REA) introduce their novel *PANR* (Phyto-Aquatic Nutrient Recycling) system to the Upper Midwest. The technology integrates aqua-cultural wastewater treatment, aka High Rate Algae Pond (HRAP) systems, with anaerobic digesters and other practical wastewater bio-treatment technologies. The *PANR System* combines advances in algae bioreactors and other harvestable water features, with retention ponds and engineered or natural wetland habitat, to sequester and recycle nutrients on-site. This in turn, enables broader use of digester technology, promotes energy efficiency, and improves wastewater and 'non-point source' cleanup & handling.

Growing Macro-algae:



Using anaerobic digester technology for managing & recovering farm-utilized nutrients (nitrogen, phosphorus, potassium) is limited at present to mineralizing the elements and isolating some portion with the solids fraction of the post-digested material. The balance is left in liquid form, mostly as inorganic phosphate -- containing from 200 to as much as 1,000 mg/liter (ppm) phosphorus. Traditional livestock management practice returns nutrient-rich manure to crop fields *via* land spreading. However, large farms (esp. dairies) now face restrictions on phosphorus application. More and more this requires producers to undertake nutrient removal through expensive liquid transport outside of the watershed. **Production of algae and other biomass provides a means of converting macro-nutrients like phosphate** to water-insoluble organic compounds for a variety of uses, including: minerals for livestock rations, controlled-release fertilizers, low-lignin bio-fiber, potting mix, and even animal bedding. To finish the phosphorus sequestration after algal harvests, the culture liquid is passed onto further phyto-treatment within secondary, harvestable water features, settlement ponds, and constructed wetlands.

*** GLAE & REA** have teamed up to co-market the PANR integrated system to dairy and livestock operators, in addition to promoting these solutions by teaming with colleges, USDA-NRCS, and ag' extension networks. This material is based upon work supported by the Natural Resources Conservation Service, U.S. Dept. of Agriculture, under #69-3A75-10-161.

**** FOR IMPLEMENTATION OR FEASIBILITY STUDY PLEASE CONTACT:***

Tony Hartmann, 608-215-4446 thartmann@greatlakesagenergy.com
OR Carl Chenoweth, 608-444-9893 cchenoweth@reaeng.com

USDA NRCS
United States Department of Agriculture
Natural Resources Conservation Service

The above fact sheet/marketing brochure was produced for distribution to producers and parties interested in nutrient management issues.

Recommendations

1) **More Study;** especially of digestate (before and after separation) from different types of digesters will improve digester viability and farmer/producer results from land-spreading/irrigating with the manure fractions.
2) **Algae ID;** Identify algae strains for different tasks; algae for more than just nutrient sequestration; prospecting for the most prolific/best mixed culture consortium (to optimize nutrient uptake, or improve product for use as feed/food/fertilizer.)
3) **Duckweed Sugars;** *Lemna Minor* (duckweed) has been found to contain as much as 35% starch, and 35% protein. While our production was relatively low (.009 g/m2/day), it is possible to optimize duckweed even in 'sub-optimal' climates -- production rates as high as 40 tons/acre were shown in Germany (Landolt, E., & Kandeler, R. 1987), which beats #2 field corn -- at best 5 tons/acre in Wisconsin (73% starch).
4) **Digester Operator Training;** digester owners are often compelled to produce more biogas in order to reap the economic returns from methane/kW sales. To do this they mix in potent substrates, which can be both a benefit on the one hand, and complication on the other. Operators need to get more education and training, specifically with regard to organic substrates (other than manure.)
5) **Greenhouse Selection/Construction;** The high ceiling, hoop house design is less expensive to build, but must be more sophisticated for better temperature control, especially during the hottest part of the year.
6) **Lab Facilities;** incubation chambers (cabinets) for comparison and propagation of wild cultures would improve selection, and productivity.

Appendices

1) Resource Engineering Associates Design Report-1058 (with Appendices)
2) Wis-DNR Sediment Basin Code #1064
3) NRCS Constructed Wetland Code #656
4) MATC – Biomass Systems, Nutrient Management and Recycling Course Handbook
5) Wave Bucket Prototypes & Designs
6) 2012 Biomass Conference Poster

1) Resource Engineering Associates Design Report (1058)

Design Report

CIG Pilot Project
Town of Vienna
Dane County, Wisconsin

November 4, 2011

Prepared By:

Resource Engineering Associates, Inc.
3510 Parmenter Street, Suite 100
Middleton, Wisconsin 53562

TABLE OF CONTENTS

~~Robert J. Pofahl, P.E.~~

1. INTRODUCTION

1.1 Key Information

I. Site Owner:
Jerry Maier
6200 Maier Road
Waunakee, WI 53597

II. Site Location:
Dane County, Wisconsin
Town of Vienna (T9N, R9E)
SW ¼ NW ¼ Section 31

III. Site Contact:
Lee Elver
Resource Engineering Associates, Inc.
Telephone: 608-831-5522 ext. 22

IV. Wisconsin Department of Natural Resources
Mark Cain
Agricultural Runoff Mgmt. Specialist
3911Fish Hatchery Road
Fitchburg, WI 53711
Telephone: 608-275-3252
mark.cain@wisconsin.gov

V. Engineer
Resource Engineering Associates, Inc (REA)
3510 Parmenter Street, Suite 100
Middleton, WI 53562
Telephone: 608-831-5522
Fax: 608-831-6564
www.reaeng.com

1.2 Background Information

This design plan and report is for construction of a geomembrane lined manure storage basin located at 6200 Maier Road, Waunakee, Wisconsin. The 60 mil HDPE lined manure storage basin is a component of the Conservation Innovation Grant (CIG) research project being conducted by Resource Engineering Associates, Inc. (REA). The research is planned to include piping centrate from the community digester located at 6200 Maier Road to dilution tanks located within a proposed greenhouse. Within these tanks, centrate is planned to be diluted with freshwater to varying extents to achieve a total phosphorus concentration of about 150-200 ppm. Following dilution, the centrate is planned to be used to culture freshwater plants at two locations, inside and adjacent to the greenhouse. The culture inside the greenhouse will operate year round while the culture outside the greenhouse will operate during warm weather months, approximately April-October. Once the diluted centrate treated in the indoor bog reaches a concentration of 1-3 ppm, it is planned to be pumped to the Wieser Concrete underground holding tank located outside the greenhouse. Once the diluted centrate treated outdoors reaches a concentration of 30 ppm, it is planned to be emptied into an adjacent geomembrane lined manure storage basin, which is planned to act as a harvestable bog. Once the water in the bog has been lowered to 1-3 ppm, it is planned to be pumped to the underground holding tank. The holding tank is planned to provide approximately two weeks of storage duration. The liquid is planned to be loaded into tanker trucks and emptied into the larger manure storage facility at White Gold Dairy during the winter months and is planned to be used as irrigation for the adjacent alfalfa field during summer months. Please see **Appendix D** for a facility operations plan.

1.3 Proposed Facility Layout

The CIG Pilot Project expansion is planned to include construction of a research greenhouse, a gravel pad for outdoor research, a geomembrane lined manure storage basin, and an underground Wieser Concrete holding tank. Centrate from the community digester is planned to be piped into holding tanks within the greenhouse. During the winter months, waste from the greenhouse is planned to be transferred (gravity) to the underground holding tank via 4” PVC pipe. During the summer months, waste from the greenhouse is planned to be transferred (gravity) to the geomembrane lined manure storage basin via 6” PVC pipe. A 6” PVC overflow pipe is planned from the geomembrane lined manure storage basin to the underground holding tank. The planned geomembrane lined manure storage basin is approximately 2,000 cu. Ft. The holding tank is planned to be a Wieser Concrete WLP1600-P precast concrete tank. The holding tank is planned as the end point for waste in this system.

The proposed greenhouse (80’x30’) is planned to be located south of the community digester on the west side of the access road. The outdoor treatment troughs, the geomembrane lined manure storage basin (2,000 cu. Ft.) and the underground holding tank (1,600 gal) are planned to be located south of the greenhouse.

1.4 Erosion Control Plan

For this facility expansion construction project, approximately 20,500 sq. ft. is planned to be disturbed. An erosion control plan has been developed and is planned to include the following practices.

- Use of existing and proposed gravel access roads as a mud tracking pad
- Silt fence down gradient from disturbed areas
- Erosion matting for 3:1 cut/fill slopes
- Seeding and mulching of remaining disturbed areas
- Manure storage basin to be used as a sediment trap during construction

2. WASTE STORAGE FACILITY

2.1 Management Assessment

The purpose of the Management Assessment as identified in NRCS Code 313 is to determine options for manure and waste handling and disposal. The assessment is performed to explore option and to determine the purpose of storage components, available resources, manure disposal schemes, and waste characterization. Issues included are as follows:

Source and Volume of Waste: Approximately 100 gallons of liquid manure effluent from the community digester is planned to be pumped to the holding tank within the greenhouse.

Land Base: White Gold Dairy has an alfalfa field approximately 4.5 acres in size available for liquid application during the summer months. During the winter months, liquid is planned to be pumped from the underground holding tank into tanker trucks and emptied into the larger manure storage basin located at White Gold Dairy. Manure from the larger manure storage basin at White Gold Dairy is spread according to the farms nutrient management plan prepared by others.

Planned Storage Period: The facility is planned to draw approximately 100 gallons per day from the community digester. The Wieser Concrete WLP1600-P holds approximately 1600 gallons and is planned to be emptied weekly. The tank provides approximately two weeks of storage.

Waste Handling & Transfer: Liquid will be field applied during the summer months for irrigation.

Facility Waste Removal Methods: Liquid accumulated in the storage basin will be mixed and pumped as a liquid and will be land applied.

Storage Basin Liner: The manure storage basin is planned to be lined with 60 mil HDPE liner. Three (3) feet of soils directly below the liner are planned to have a $P_{200} > 20\%$. The manure storage basin is designed as a geomembrane lined basin in accordance with NRCS 313 Table 3 specifications.

Access and Safety: The manure storage basin is not a confined space and is planned to be approximately three feet deep. Warning signs are planned to be placed around the perimeter of the basin.

Labor & Equipment Needs: The farm currently has labor and manure-handling equipment to load spreaders, transport, and field apply manure.

Odor, Aesthetics & Animal Health: The research greenhouse site and manure storage basin is located approximately 1,300 ft away from the nearest neighbor in an agricultural setting. The facility is located away from property lines. Due to the overland distance, odor emissions from the basin are not anticipated to be a nuisance.

Expansion Considerations: Additional expansion is not being considered at this time.

2.2 Site Assessment

The purpose of the site assessment is to evaluate physical characteristics that may influence location, construction, maintenance, and environmental integrity of the waste storage facility.

Location: The building locations, land elevations (topography), public road access, soil test excavation locations, nearby property lines, and other existing features are identified on the attached plan set. The project benchmark (CP #11) is a site-specific elevation of 976.55’ as shown on the drawings.

Test Excavation Logs &Test Data: Soil test excavation locations are as identified on the attached plan set. One soil test boring was advanced on October 31, 2011 by Snyder’s Excavation & Paving, LLC extending approximately 9 feet below existing site grade. REA, Inc. personnel logged the soil and a copy of the soil log is presented in the plan set and in **Appendix B**. Two soil samples representative of soil, which is planned to be placed for the geomembrane lined manure storage basin were selected for laboratory analysis of P_{200}, visual classification, and Atterberg limits. As reported by CGC, Inc. Geotechnical Laboratory the soil sample results are described below:

Sample #	Depth	Location	P_{200}%	USCS
South	2’	South of Greenhouse	33.1%	SM
North	2’	North of Greenhouse	85.8%	CL

A copy of the CGC, Inc. laboratory analytical results is presented in **Appendix B**.

Soil Survey – Dane County: Based on review of the Dane County Soil Survey database, two soil map units are located in the footprint of the planned research greenhouse, geomembrane lined manure storage basin, and underground holding tank. The primary soil unit is identified as Kidder loam (KdC2) with 6 to 12% slopes, eroded. The secondary soil unit is identified as

Kidder soils, 20 to 35 percent slopes, eroded. A copy of Dane County Soil Survey data is presented in **Appendix B**.

Bedrock Indicators: As summarized in the soil test excavation logs, evidence of bedrock was encountered at the terminus of the test boring advanced on October 31, 2011 (to 9' below site grade).

Borrow Areas: Lean clay and fine to medium sand with some silt excavated from the site is planned to be used as the below the geomembrane lined manure storage basin.

Saturation Indicators & Lenses: As summarized in the soil excavation log, soil encountered throughout the bore hole generally consisted of light brown sand (SM) with cobbles. Evidence of wet and or saturated conditions was not observed in the boreholes.

Based on review of the Waunakee, Wisconsin 7.5 minute USGS topographic quadrangle map, the approximate site elevation is 970 feet above mean sea level (msl).

Potential Discharge Impacts: The basin is below ground, thus potential discharge would likely be from overflow. Overspills, if they were to occur from an unpredictable catastrophic event, would discharge down the basin embankment and would drain into the sediment basin to the south of the manure basin. Equipment should be available to construct an embankment to contain a spill in an emergency.

2.3 Flood Plain Considerations

The manure storage basin is not believed to be in a 100-year flood plain. It is located approximately 275' east of an intermittent stream. The intermittent stream eventually flows into the Six Mile Creek approximately 1.5 miles south of the proposed manure storage basin.

2.4 Location Criteria

The proposed manure storage basin top of embankment is planned at elevation 980.0', with the manure storage basin top of floor elevation at 977.0'. These elevations are based on the topographic survey by REA, Inc. using a benchmark (CP #11) datum of 976.55'. The site benchmark is described as a nail driven flush with the ground off the northwest corner of the bunker silos.

Water Table: Evidence of regional ground water table was not identified in the soil test borings, which extended 9 feet below site grade.

Bedrock: Bedrock was encountered approximately 9' below grade at the terminus of the excavation.

2.4.1 Bedrock & Saturation Separation Criteria

Specific criteria outlined in NRCS 313 – Table 3 for geomembrane lined manure storage facilities specifying 60 mil High Density Polyethylene (HDPE) include separation distances as follows:

Soils (directly below liner)

% Fines	> 20%	>20%
Plasticity Index (PI)	>7%	---
Thickness	>1.5 ft	>3 ft
Compaction of Placed Material	WI Spec 202	WI Spec 202

Separation Distances

Well Distance	>250 ft	>250 ft
Sinkholes	>400 ft	>400 ft
Saturation (V.A.10)	>3 ft	>4 ft
Bedrock	>3 ft	>3 ft

A copy of NRCS 313 is attached in this report in **Appendix C**.

2.5 Erosion Control and Storm Water Drainage Management

2.5.1 Erosion Control Practices

An erosion control and storm water management plan have been developed and submitted to the Dane County LCD and approved.

2.5.2 Storm Water Drainage

Upon final grading, runoff from the north side of the facility will be directed west and south along the west side of the proposed site. Storm water will rejoin the drainage area's existing historic flow path and discharge to the southwest. Storm water runoff from the impervious pads will flow into the geomembrane lined manure storage basin. Storm water in the basin overflows through a 6" pipe to an underground concrete holding tank. The holding tank has a 1,600 gallon capacity and is planned to be pumped weekly.

2.6 Safety Considerations

Safety items as summarized in the NRCS Waste Storage Facility Code 313 and local ordinances should be inspected monthly and be maintained and repaired to operate efficiently.

2.6.1 Ventilation

Do not enter a confined space, such as the proposed underground holding tank, manholes, or transfer pipes without a health and safety plan ventilation and monitoring. Additional ventilation requirements are not proposed for the geomembrane lined manure storage basin.

2.7 Transfer Pipe (Gravity)

A gravity transfer system is proposed to transfer waste from the community digester to the greenhouse and from the greenhouse to the geomembrane lined manure storage basin and underground holding tank.

Liquid Tight: All waste transfer piping is designed to be liquid tight. Piping is planned to be bedded according to NRCS Wisconsin Construction Specification 15, Plastic Pipe Conduits and backfilled according to NRCS Wisconsin Construction Specification 204, Earthfill for Waste Storage Facilities.

Materials: Gravity pipe transferring waste from the greenhouse to the geomembrane lined manure storage basin and underground holding tank is planned to be SCH 40 pipe in 4" and 6" diameters.

Pipe Penetrations: Pipe penetrations for the gravity pipes in the geomembrane lined manure storage basin and underground holding tank are planned to be watertight. The connection with the underground holding tank is planned to be a PSX boot type connector. The connection with the geomembrane lined manure storage basin is planned to be in accordance with the liner manufacturers specifications.

Bedrock: Bedrock was encountered at the terminus of the soil boring test excavation at approximately 9' below site grade.

Frost Protection: The gravity piping from the greenhouse to the underground holding tank is planned to have a minimum of 4 feet of soil cover for frost protection. If 4 feet of soil cover is not achieved, insulation is planned to be added around the pipe. The gravity piping from the greenhouse to the geomembrane lined manure storage basin and from the basin to the underground holding tank is not planned to be operated during the winter months. That piping is planned to have as much cover as the grading allows.

3. CONSTRUCTION SCHEDULE

Construction of the proposed manure storage basin and placement of the concrete holding tank is planned to begin in the fall of 2011 upon approval of the plans. Construction is expected to be complete within two months after commencement, unless inclement weather or contracting scheduling delays progress. The entire storage/treatment system is planned to be fully operational in the spring of 2012.

- Begin construction – November 2011
- Complete construction activities – January 2011

The construction site will be seeded and mulched as grading is complete.

4. OPERATION & MAINTENANCE

4.1 Operations Plan

An operations plan for the research greenhouse and manure storage basin and transfer system is presented in **Appendix D**.

4.2 Requirements for Emptying Storage Facility

Waste removed from the proposed underground holding tank is planned to be removed and field applied for irrigation purposes during the summer months. During the winter months, the waste removed is planned to be emptied into the larger manure storage basin at White Gold Dairy.

4.3 Inspecting and Maintaining Storage Facility

The underground holding tank is planned to be emptied weekly and either spread on an adjacent alfalfa field or emptied into the larger manure storage basin at White Gold Dairy. A copy of a site maintenance plan is attached in **Appendix E**.

4.4 Pre-Construction Meeting

A pre-construction meeting should be performed prior to construction to clarify relative project design and plan features. A copy of a pre-construction form is attached in **Appendix E**.

APPENDIX A

SITE MAPS

APPENDIX B

OCTOBER 31, 2011 SOIL TEST EXCAVATION LOG
&
SOIL TEST LABORATORY ANALYSIS – CGC, INC.
&
SOIL SURVEY MAP

APPENDIX C

NRCS CODE 313, WASTE STORAGE FACILITY, JUNE 2009

APPENDIX D

FACILITY OPERATIONS PLAN

OPERATIONS PLAN

Centrate (liquid manure effluent) from the community digester will be piped (intermittently) to dilution tanks within the greenhouse. Within these tanks, centrate will be diluted with freshwater to varying extents (usually about 1:2, to achieve a total phosphorus concentration of about 150-200 ppm). Following dilution, the centrate will be used to culture freshwater plants at two locations on the greenhouse platform: in 1) culture troughs **indoors of the greenhouse**, and in 2) culture troughs outdoors, **immediately adjacent to the greenhouse**.

Culture troughs inside the greenhouse. Once an appropriate volume of centrate has been prepared by dilution, all inflow to the dilution tanks will cease and the tanks will be utilized, in effect, as holding tanks for ongoing circulation by pumping. The diluted centrate will be pumped from individual tanks to individual culture troughs, each 10 feet long by 4 feet wide, designed to hold approximately 4 inches' depth of water. These troughs inside the greenhouse will be supported on adjustable scaffolding to a height of about 4 feet, and will be tilted very slightly from head to foot to assist flow. There will be three culture troughs in year-around operation within the greenhouse. One of the three will be managed by ambient sunlight, a second will be managed by supplemental overhead high-pressure-sodium lighting, and the third will be managed by supplemental overhead LED lights. Each trough will incorporate a gravity-triggered wave bucket at its head. Diluted centrate will be pumped from the holding tank to flow into each trough's wave bucket, to generate a periodic wave surge that sweeps over the bottom of the trough for its entire length. Surges of diluted centrate will empty from the bottom end of the trough through short standpipes into a small holding box running across the width of the trough. Diluted centrate accumulating in the holding box will be pumped continuously back to the holding tank (originally operated as a dilution tank).

The bottom of each culture trough will be covered by adjacent, removable squares of screening. These squares of screening will be composed of about three layers of polypropylene screening to form a porous substratum for growth by attached filamentous algae. Small clusters of these algae will be collected from the farm's manure pit and seeded into each trough at the beginning of our study period. Under active management, the algae will establish growth on the screens and sequester phosphorus and other nutrients from the diluted centrate as it flows continuously over the screens. The algae will require periodic harvesting to maintain their high growth rates. Such harvests will be accomplished by scraping the biomass off of the screens every 3-4 days. Because these algal filaments are attached to porous substrates by basal attachment cells, they will re-establish their growth on the screens quickly following each harvest.

The phosphorus concentration will be monitored for each recirculating volume of diluted centrate until a total phosphorus concentration of about 30 ppm is achieved. At that point, the volume of diluted centrate will be pumped to a pond within the greenhouse known as a harvestable bog. This bog will be approximately 2 feet wide by 8 feet long, designed to hold about 12 inches' depth of liquid. It will receive both ambient sunlight and supplemental overhead high-pressure sodium lighting. We plan to grow floating duckweed within this harvestable bog. Duckweed will sequester phosphorus and other nutrients from the diluted centrate as it grows within this bog. Once the diluted centrate treated in each culture trough has been pooled into this harvestable bog, the total phosphorus concentration will be monitored until it has reached a concentration of 1-3 ppm. At this point, the entire volume of diluted centrate will be pumped outdoors of the greenhouse, into a concrete cistern (Wieser Concrete WLP1600-P) having a volume of about 1600 gallons. Water will be stored in this outdoor cistern for approximately 2 weeks' duration. The cistern will be emptied by pumping into a tanker truck. During warm months, the phosphorus-cleared water will be transported to the top of the alfalfa field for irrigation. During cold months, the water will be transported down the hill and emptied into the farmer's manure pit.

Culture troughs outside the greenhouse. For the culture troughs immediately outside the greenhouse, centrate will be diluted into a tank located within the greenhouse. This tank will then serve as a holding tank for diluted centrate being pumped to a set of culture troughs outdoors, on the greenhouse pad. These troughs will be plastic-lined and inserted partially into the ground. They will be built to incorporate a slight tilt. They will be constructed so as to form a set of four parallel troughs, each 4 feet wide by 30 feet in length. Again, these troughs will be designed to hold about 4 inches of constantly recirculating water. An individual wave bucket will be situated at the head of each trough, to generate wave surge as described above. There will be a holding box at the far end of the troughs to collect the diluted centrate for continuous pumping back to the holding tank in the greenhouse. The outdoor troughs will receive no lighting other than ambient sunlight, and will probably be operated from late April through October each year. Once again, when the total phosphorus concentration is measured to have dropped to about 30 ppm, the trough water will be emptied into an adjacent harvestable bog. The harvestable bog is planned to be the geomembrane lined manure storage basin underlain with three (3) feet of soil with $P_{200} > 20\%$. Again, we will grow duckweed in this bog for periodic harvesting of biomass. The bog will, like the outdoor algal-culture troughs, be operated from late April through October of each year. The water in the bog will also be monitored for total phosphorus concentration, and once the phosphorus has been lowered to 1-3 ppm, it will be pumped into the outdoor cistern for storage, prior to transport to the top of the alfalfa field for irrigation.

APPENDIX E

PRE-CONSTRUCTION MEETING FORM
MAINTENANCE PLAN

PRE-CONSTRUCTION MEETING

A meeting shall be scheduled before construction to discuss construction schedules, plan details, construction specifications, and known site limitations. The meeting should include the following:

	Invited	Attended	Not Attended
❖ Owner ____*Jerry Maier*________	☐	☐	☐
❖ County LCD__*Craig McCallum*__	☐	☐	☐
❖ Engineer__*REA, Inc.*___________	☐	☐	☐
❖ Building Contractor___________	☐	☐	☐
❖ Excavator____________________	☐	☐	☐
❖ Concrete Contractor___________	☐	☐	☐
❖ Liner Contractor______________	☐	☐	☐
❖ NRCS_______________________	☐	☐	☐

Items of discussion should include:

- Review plans & specifications
- Locate and observe benchmark
- Determine how the basin location will be staked
- Discuss the concrete testing requirements

Other items discussed:

☐

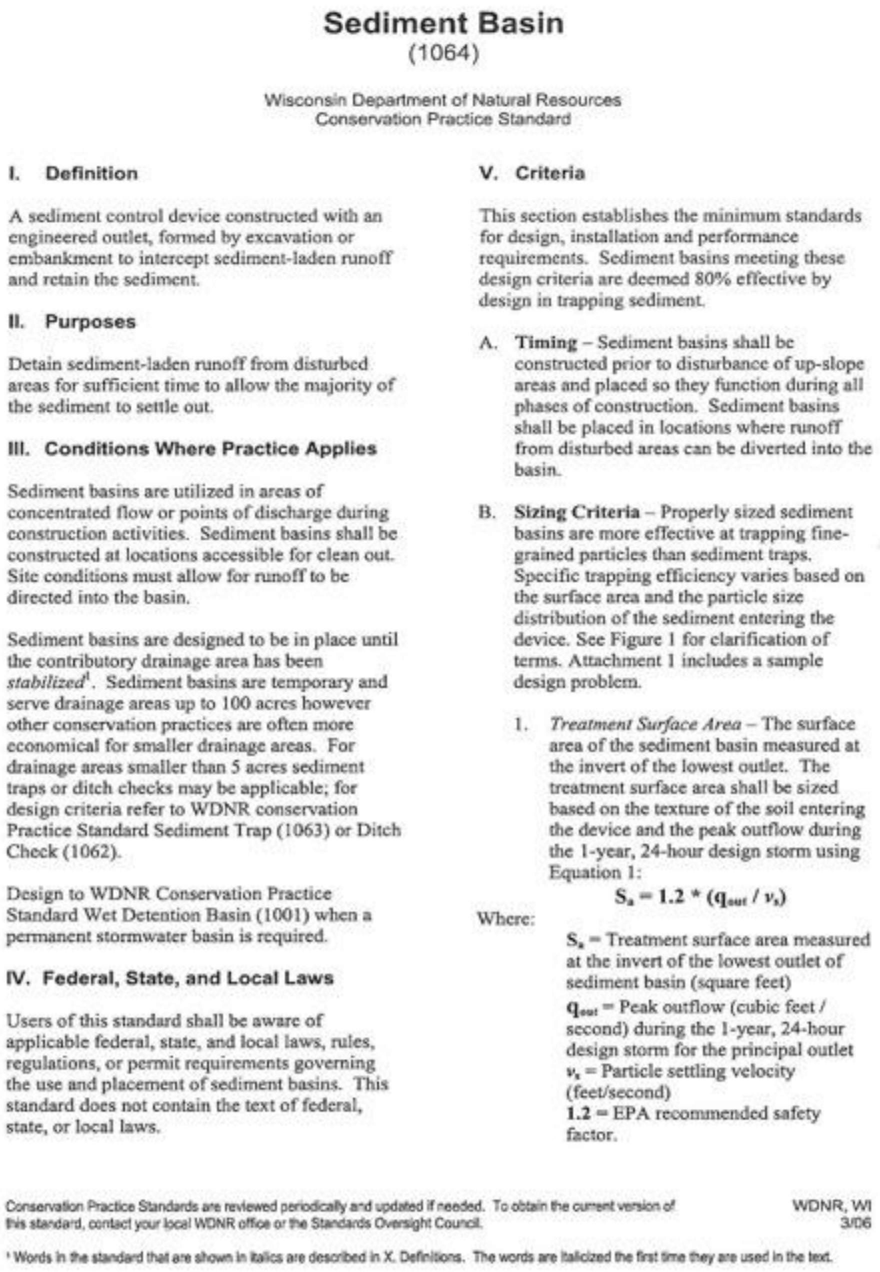
☐

Comments:

MAINTENANCE PLAN

- ☐ Check inlets, outlets, rodent guards, and repair as needed.
- ☐ Limit traffic around the basin walls to design loads/limits.
- ☐ Maintain safety fences, rails, and gates.
- ☐ Maintain vegetative cover, riprap, geotextile, and repair/reseed as necessary to maintain a stable embankment. Observe the embankment top for evidence of slippage.
- ☐ Mow embankment side slopes and reseed to maintain vigorous plant growth. Eliminate the growth of woody plants on embankment side slopes.
- ☐ Periodically remove sediment or soil that may deposit in the waterway to maintain design capacity.
- ☐ Maintain roof gutters and down spouts or splash pads for proper support and operation.
- ☐ Concrete areas damaged by weather or equipment should be repaired by grouting or caulking as appropriate.
- ☐ Bank erosion or rodent holes should be repaired as soon as possible.
- ☐ Damage to the geomembrane liner should be repaired per manufacturer recommendations as soon as possible.

2) Wis-DNR Sediment Basin Code #1064



Sediment Basin

(1064)

Wisconsin Department of Natural Resources
Conservation Practice Standard

I. Definition

A sediment control device constructed with an engineered outlet, formed by excavation or embankment to intercept sediment-laden runoff and retain the sediment.

II. Purposes

Detain sediment-laden runoff from disturbed areas for sufficient time to allow the majority of the sediment to settle out.

III. Conditions Where Practice Applies

Sediment basins are utilized in areas of concentrated flow or points of discharge during construction activities. Sediment basins shall be constructed at locations accessible for clean out. Site conditions must allow for runoff to be directed into the basin.

Sediment basins are designed to be in place until the contributory drainage area has been *stabilized*[1]. Sediment basins are temporary and serve drainage areas up to 100 acres however other conservation practices are often more economical for smaller drainage areas. For drainage areas smaller than 5 acres sediment traps or ditch checks may be applicable; for design criteria refer to WDNR conservation Practice Standard Sediment Trap (1063) or Ditch Check (1062).

Design to WDNR Conservation Practice Standard Wet Detention Basin (1001) when a permanent stormwater basin is required.

IV. Federal, State, and Local Laws

Users of this standard shall be aware of applicable federal, state, and local laws, rules, regulations, or permit requirements governing the use and placement of sediment basins. This standard does not contain the text of federal, state, or local laws.

V. Criteria

This section establishes the minimum standards for design, installation and performance requirements. Sediment basins meeting these design criteria are deemed 80% effective by design in trapping sediment.

A. **Timing** – Sediment basins shall be constructed prior to disturbance of up-slope areas and placed so they function during all phases of construction. Sediment basins shall be placed in locations where runoff from disturbed areas can be diverted into the basin.

B. **Sizing Criteria** – Properly sized sediment basins are more effective at trapping fine-grained particles than sediment traps. Specific trapping efficiency varies based on the surface area and the particle size distribution of the sediment entering the device. See Figure 1 for clarification of terms. Attachment 1 includes a sample design problem.

1. *Treatment Surface Area* – The surface area of the sediment basin measured at the invert of the lowest outlet. The treatment surface area shall be sized based on the texture of the soil entering the device and the peak outflow during the 1-year, 24-hour design storm using Equation 1:

$$\mathbf{S_a = 1.2 * (q_{out} / \mathit{v}_s)}$$

Where:

$\mathbf{S_a}$ = Treatment surface area measured at the invert of the lowest outlet of sediment basin (square feet)
$\mathbf{q_{out}}$ = Peak outflow (cubic feet / second) during the 1-year, 24-hour design storm for the principal outlet
v_s = Particle settling velocity (feet/second)
1.2 = EPA recommended safety factor.

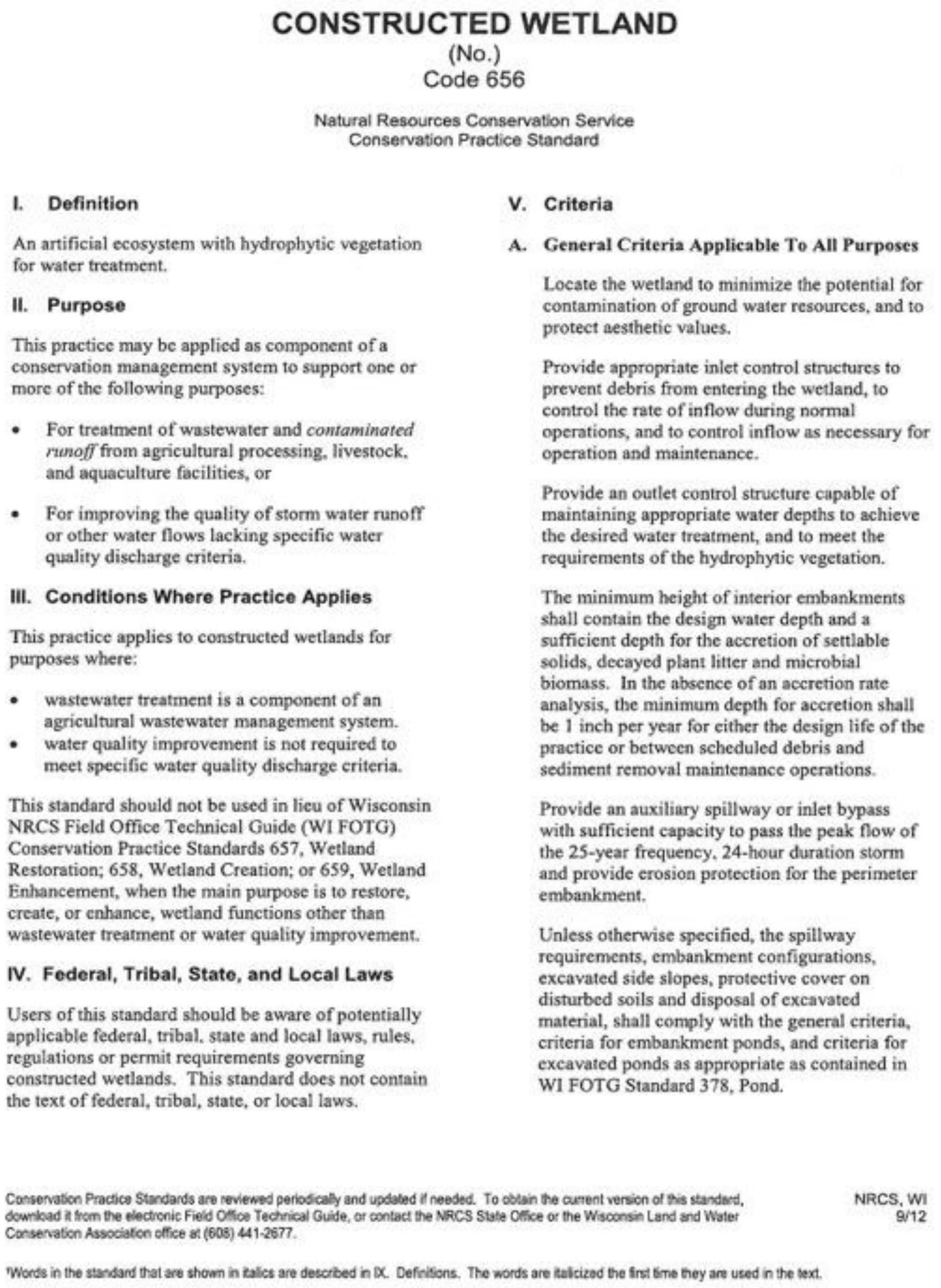
Conservation Practice Standards are reviewed periodically and updated if needed. To obtain the current version of this standard, contact your local WDNR office or the Standards Oversight Council.

WDNR, WI
3/06

---

[1] Words in the standard that are shown in italics are described in X. Definitions. The words are italicized the first time they are used in the text.

3) NRCS Constructed Wetland Code #656



CONSTRUCTED WETLAND

(No.)
Code 656

Natural Resources Conservation Service
Conservation Practice Standard

I. Definition

An artificial ecosystem with hydrophytic vegetation for water treatment.

II. Purpose

This practice may be applied as component of a conservation management system to support one or more of the following purposes:

- For treatment of wastewater and *contaminated runoff* from agricultural processing, livestock, and aquaculture facilities, or
- For improving the quality of storm water runoff or other water flows lacking specific water quality discharge criteria.

III. Conditions Where Practice Applies

This practice applies to constructed wetlands for purposes where:

- wastewater treatment is a component of an agricultural wastewater management system.
- water quality improvement is not required to meet specific water quality discharge criteria.

This standard should not be used in lieu of Wisconsin NRCS Field Office Technical Guide (WI FOTG) Conservation Practice Standards 657, Wetland Restoration; 658, Wetland Creation; or 659, Wetland Enhancement, when the main purpose is to restore, create, or enhance, wetland functions other than wastewater treatment or water quality improvement.

IV. Federal, Tribal, State, and Local Laws

Users of this standard should be aware of potentially applicable federal, tribal, state and local laws, rules, regulations or permit requirements governing constructed wetlands. This standard does not contain the text of federal, tribal, state, or local laws.

V. Criteria

A. General Criteria Applicable To All Purposes

Locate the wetland to minimize the potential for contamination of ground water resources, and to protect aesthetic values.

Provide appropriate inlet control structures to prevent debris from entering the wetland, to control the rate of inflow during normal operations, and to control inflow as necessary for operation and maintenance.

Provide an outlet control structure capable of maintaining appropriate water depths to achieve the desired water treatment, and to meet the requirements of the hydrophytic vegetation.

The minimum height of interior embankments shall contain the design water depth and a sufficient depth for the accretion of settlable solids, decayed plant litter and microbial biomass. In the absence of an accretion rate analysis, the minimum depth for accretion shall be 1 inch per year for either the design life of the practice or between scheduled debris and sediment removal maintenance operations.

Provide an auxiliary spillway or inlet bypass with sufficient capacity to pass the peak flow of the 25-year frequency, 24-hour duration storm and provide erosion protection for the perimeter embankment.

Unless otherwise specified, the spillway requirements, embankment configurations, excavated side slopes, protective cover on disturbed soils and disposal of excavated material, shall comply with the general criteria, criteria for embankment ponds, and criteria for excavated ponds as appropriate as contained in WI FOTG Standard 378, Pond.

Conservation Practice Standards are reviewed periodically and updated if needed. To obtain the current version of this standard, download it from the electronic Field Office Technical Guide, or contact the NRCS State Office or the Wisconsin Land and Water Conservation Association office at (608) 441-2677.

NRCS, WI
9/12

---

[1] Words in the standard that are shown in italics are described in IX. Definitions. The words are italicized the first time they are used in the text.

4) MATC – Biomass Systems, Nutrient Management and Recycling Course Handbook

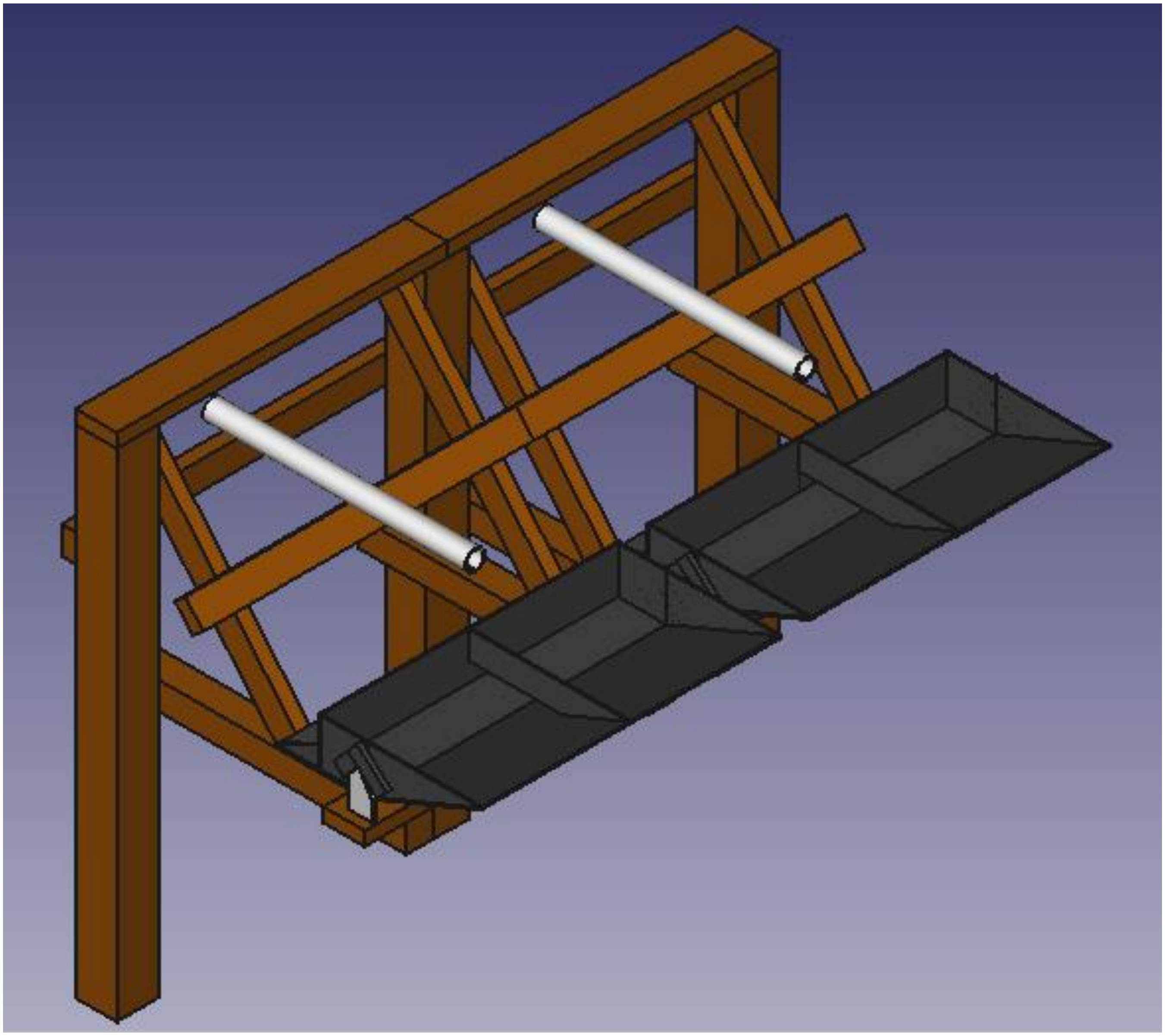
Course Handbook:
Biomass Systems, Nutrient Management and Recycling
Madison College -- Catalog #10484164
November 10-11, 2012

Phyto-Aquatic Nutrient Recycling – Dane Community Digester

Instructor contact: Tony Hartmann, 608-215-4446 (cell)

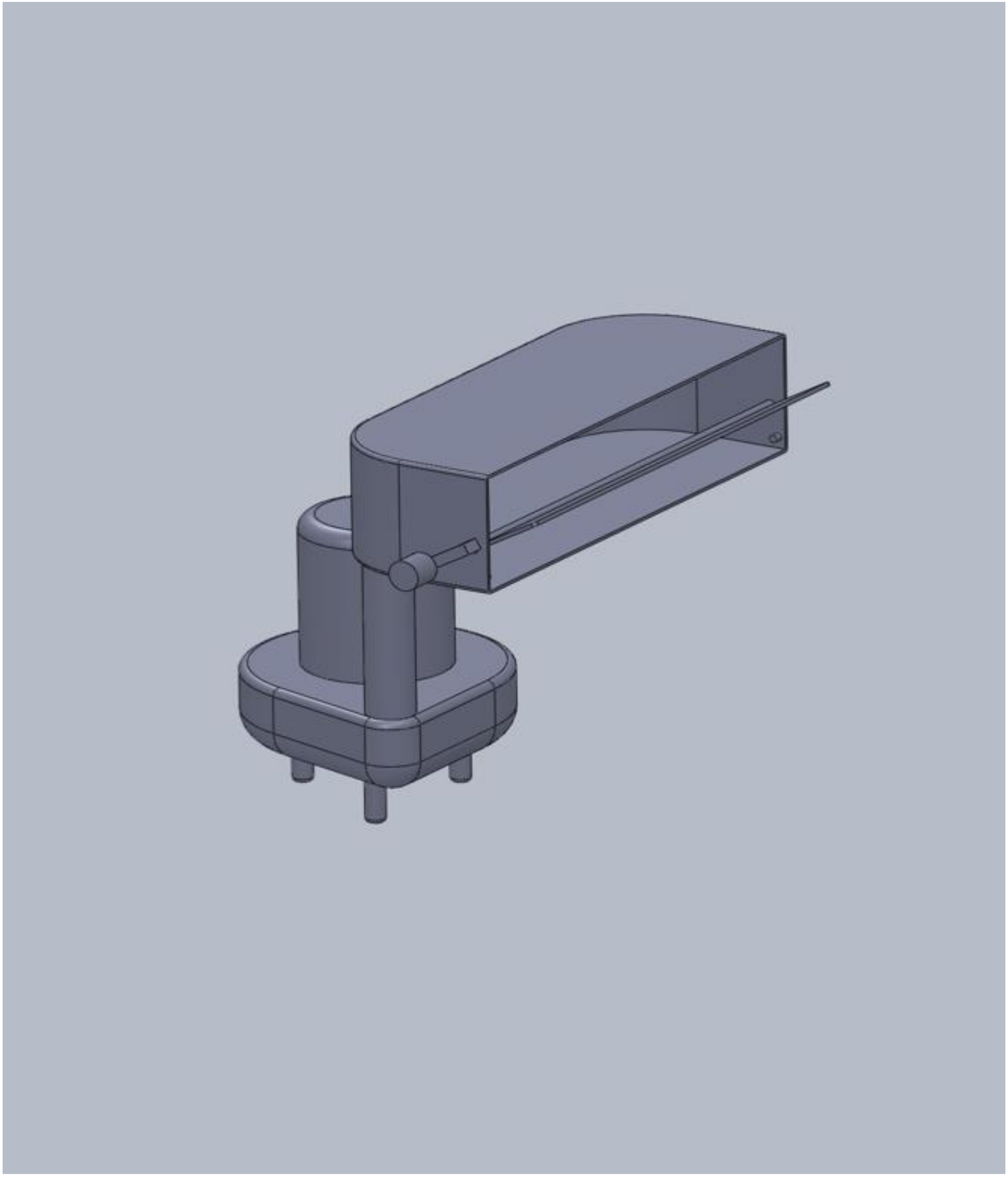
## 5)	Wave Bucket Prototypes & Designs

Outdoor trough buckets – As built (10 gallon each)

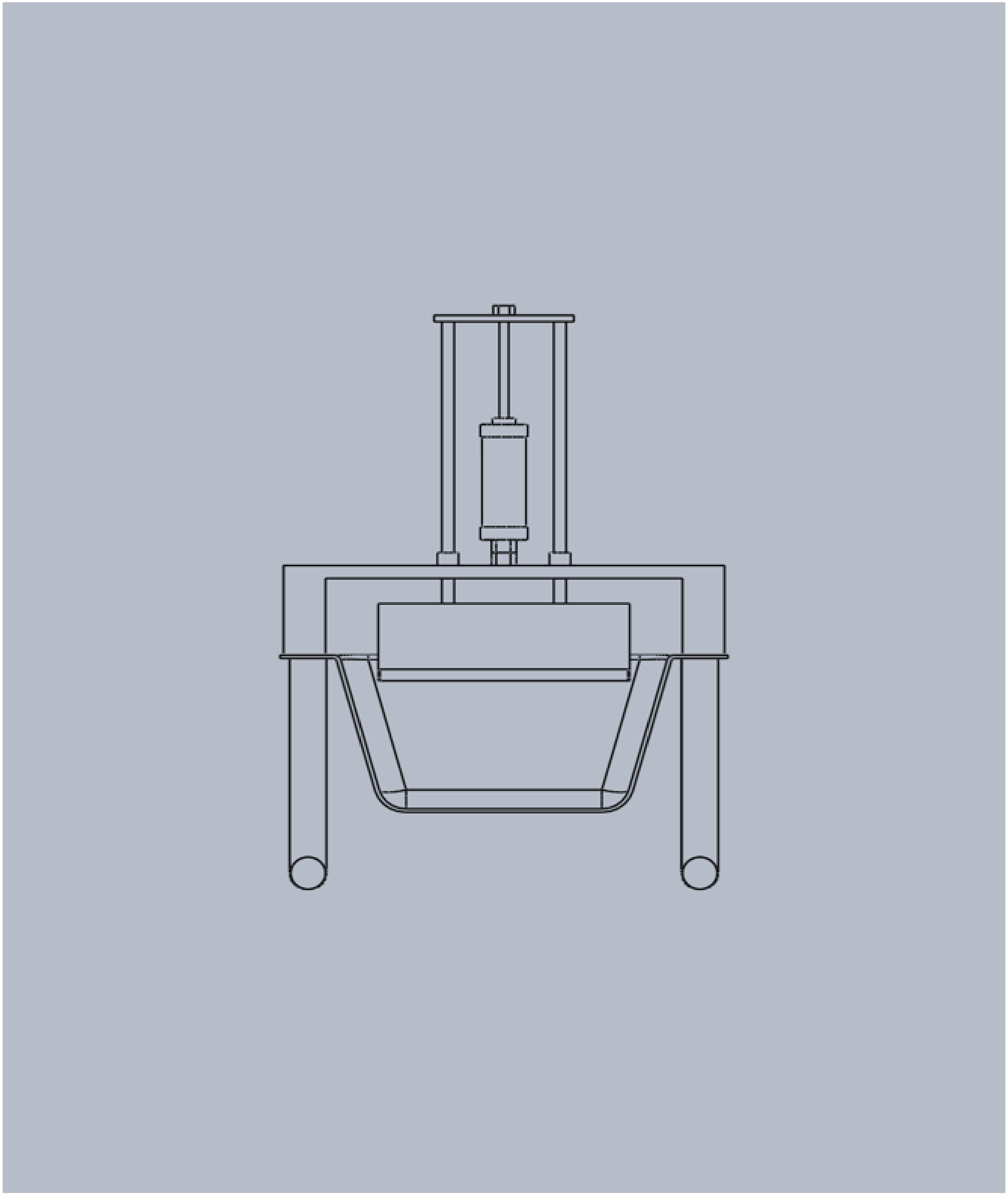


Indoor (Small trough) Concept Prototypes

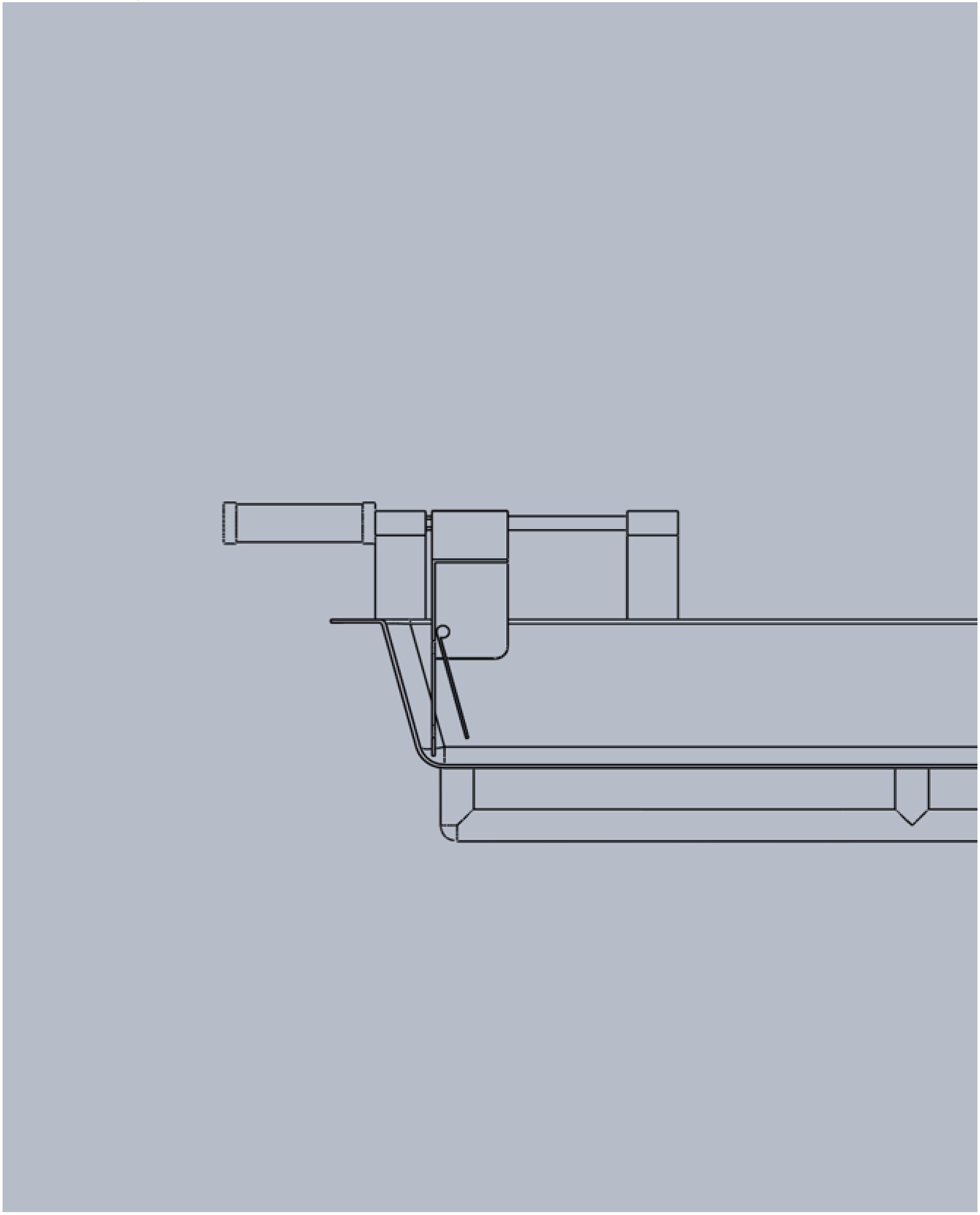
A) “Sump” Wave



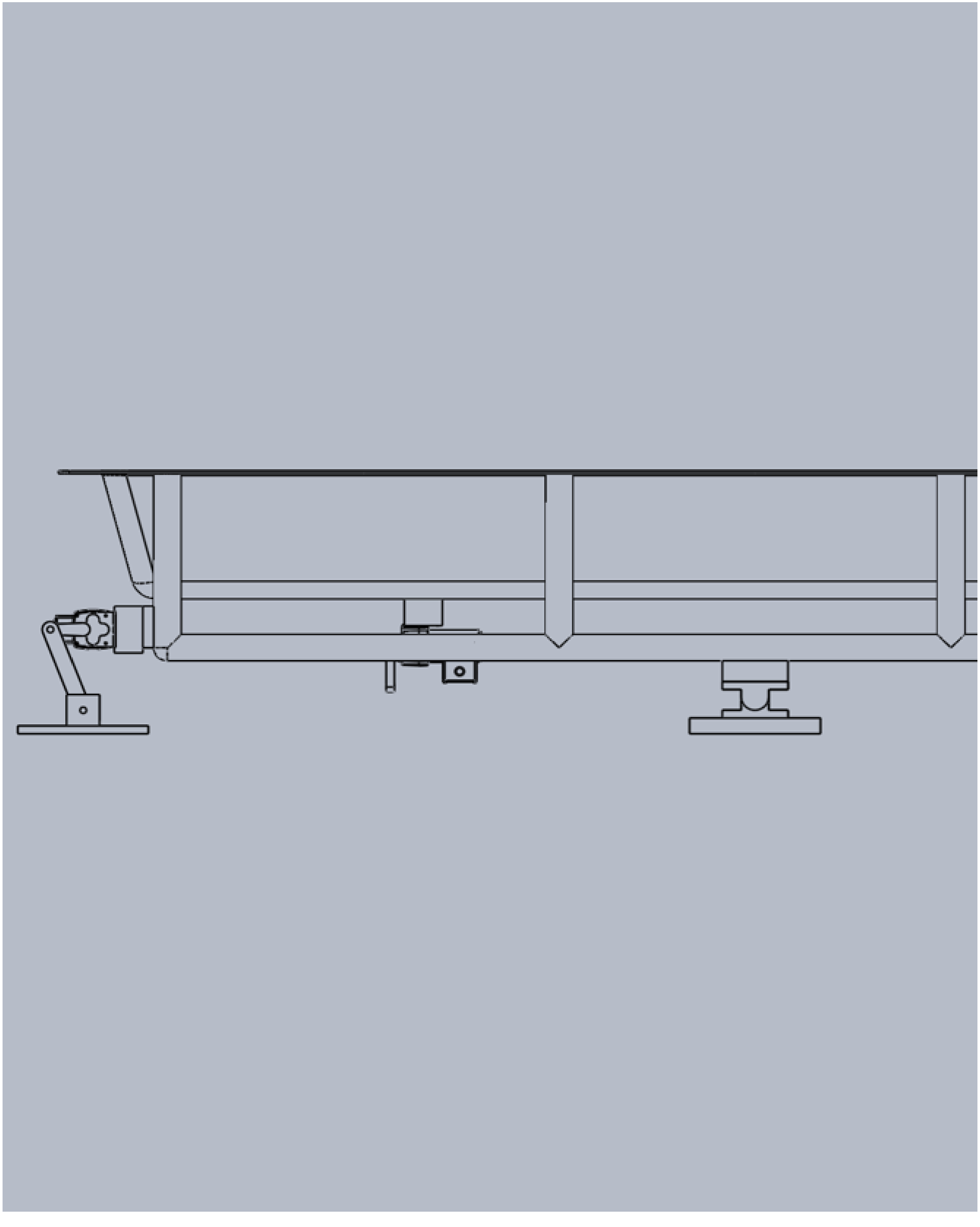
B) “Piston” Wave



C) Wave “Surger”



D) "Tilt" Wave



6) 2012 Biomass Conference Poster

Absorbance of methane-digester centrate by processed freshwater benthic-algal filaments; application to development of renewable soil amendments.

John M. Hackney, Great Lakes Ag Energy, LLC (Middleton, WI); **Mike Beauchaine**, Bruker AXS, Inc. (Madison, WI); **H. Tony Hartmann**, Great Lakes Ag Energy, LLC (Middleton, WI)

Background

- At a Wisconsin community digester (**Figure 1**), a liquid nutrient solution known as centrate is continuously separated by centrifugation from fibrous solids found in the slurry remaining after anaerobic digestion of dairy-cattle manure.
- The solids may be used as a potting-soil mix or, more commonly, are returned to the farmer as animal bedding. The largely mineralized nutrients in the centrate are also returned to the farmer for storage until they are applied to crop fields after spring thaw. Centrate is a valuable form of fertilizer, but the liquid is susceptible to storm runoffs and is often produced in volumes that surpass those allowed for field spreading under a farm's nutrient management plan. Dairymen often take on considerable expense to store centrate (**Figure 2**) or to tranport it long distances for donation to willing neighbors.
- To study alternative uses of centrate, the solution was utilized as a medium for culture of attached filamentous algae in open-air troughs. These algae were harvested and then processed in various ways to create different forms of soil amendments. Processing included vermicomposting of the filaments for production of worm-castings. Chemical compositions of the amendments were analyzed by application of various wet-chemistry analyses and a spectroscopic technique for detecting minor elements important to early stages of plant growth. In a two-week study under controlled parameters, preparations of pure amendments and 1:1 amendment mixtures were tested for impact upon soybean germination and early growth. Results were analyzed to consider the potential of centrate-cultured algae as an agent for delivering digester nutrients to crop fields and as a component in future production of renewable fertilizers.

Figure 1. Complete-mix anaerobic digester tanks at a Wisconsin community digester. Tanks are employed in the anaerobic digestion of dairy-cattle manure and wastes fats and oils from local businesses. Digestion produces methane gas, which is combusted on-site for generation of electricity.

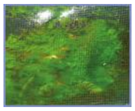
Figure 2. Centrate-storage lagoon of a dairy utilizing anaerobic digestion of cattle manure. Highly concentrated nutrients in centrate may be land-spread as fertilizer, but large volumes create storage and nutrient-management problems. Lagoons of several acres surface area are common on large dairy farms with on-site digesters.

Production of Algal Filaments

- Centrate liquid was collected directly from the digester site after having been centrifugally separated from digestate solids. Varied dilutions of this solution were continuously recirculated through multiple open-air troughs (**Figure 3**) with submerged layers of 25-mm mesh polypropylene screening that supported attachment and growth by a multi-species assemblage of freshwater filamentous algae. Dilutions were introduced at the head of each trough through a gravity-triggered wave bucket, which generated periodic surges of the medium and thereby promoted high rates of algal productivity. Depth of medium within each trough averaged *ca.* 8 cm.
- Samples of algae collected from area waterways were used to seed each trough weekly, promoting development of an assemblage with stable composition early in the study period (**Figure 4**). The assemblage comprised both single- and multi-cellular forms, but was generally dominated by filamenous species such as *Cladophora*, *Rhizoclonium*, *Ulothrix*, *Oedogonium*, *Microspora*, *Spirogyra*, and *Oscillatoria*.
- Concentrations of total phosphorus in the re-circulating medium were monitored 2-3 times each week throughout the study period. When measurements indicated clearance to below *ca.* 30 ppm total phosphorus, the systems were drained and supplied with with fresh preparations of diluted centrate.
- Trough screens were harvested by manual scraping approximately twice each week, from time of assemblage establishment in early May through September, 2011. Production within troughs averaged *ca.* 18-20 g dry weight biomass/m^2/day over the course of the study.

Figure 4 (right). Filamentous algae growing on 25-mm polypropylene screening submerged at bottom of an open-air trough. After harvest, attachment cells in screen recesses promote rapid regrowth of assemblage, contributing to relatively high levels of trough productivities.

Figure 3 (left). Troughs used in production of freshwater filamentous algae. Troughs supported continuous recirculation of diluted centrate across submerged screens, supporting growth by attached filaments. Biomass was harvested periodically by manual scraping of screens.



Biomass Processing

- Immediately following harvest from troughs, algal filaments were freshwater-rinsed and spread on mesh netting for air drying. One portion of this dried biomass was then compiled and stored under freezing for use as feedstock in vermicomposting tests. To examine utility of dried filamentous material as an agent of nutrient transfer in field applications, a second portion of dried filaments was soaked in non-diluted centrate for 30 min. before oven-drying.
- Biomass was processed for soil-amendment tests. Portions of both dried, non-soaked filaments and dried, centrate-soaked filaments were ground separately in a food processor. These materials then were employed either directly as single soil amendments or were mixed with equal parts commercial topsoil for use as mixed soil amendments.

- Because this investigation focused on potential of algal filaments as absorbent materials for agricultural applications, shredded corn stover was selected as a control for tests of chemical composition, performance as feedstock for vermicomposting, and utility as a soil-amendment agent.
- Worm-castings produced by employing plain algae or shredded corn stover as feedstocks in the vermicomposting tests were collected by sifting through a sequence of wire-mesh sieves down to a 2-mm^2 grid. The highly pure fractions of isolated castings were oven-dried for tests of performance as soil-amendment agents.

Vermicomposting Study

- Mats of previously frozen algal filaments and portions of shredded corn stover were employed as feedstocks for cultures of the worm *Eisenia fetida* ("red wigglers"; see **Figure 5**). Worm bins were established with shredded newspaper as bedding, and a small amount of sand was added as grist for the worms' gizzards. Feedstocks were spread on top of the bedding, and bins were covered loosely with opaque plastic film to retain moisture. Bin lids were fitted with squares of screening to allow adequate air circulation. Worm cultures were supplied with fresh supplies of feedstocks as needed, and the bins were thoroughly sprayed with distilled water daily. Duration of the vermicomposting study was 120 days.
- Anecdotal observations demonstrated that worms consumed the algal filaments more quickly than the shredded corn stover, and reproduced sooner on a diet of filamentous algae than one one of corn stover. These results probably reflect greated ease for consumption and digestion of the filamentous-algal materials.
- Differences in the physical properties of corn-stover- and algal-filament-derived castings were observed. Castings associated with corn-stover feedstock were larger, more-firm, and dark-colored, whilst those produced on filaments were smaller, softer, and light-brown in color light-brown. Both types of castings were oven-dried at 30° C before use in soil-amendment tests.
- Attempts to feed worms centrate-soaked algae failed miserably! Presentation of the ground form of this material drove worms outside of the bin within 12 hours, where the worms perished in a desiccated state. These results are most-likely associated with the high concentration of salts found in centrate and in filaments that have absorbed centrate.

Figure 5. Portion of filamentous-algal mat used as feedstock in cultures of Red Wigglers. Worms have infested this biomass, and close examination demonstrates clear presence of castings along the perimeter. Castings were collected for study by sifting through wire-mesh sieves.

"Manure Analyses" of Biomass

- Samples of liquid centrate, plain (non-soaked) algae, centrate-soaked algae, and shredded corn stover were subjected to a battery of tests traditionally associated with analysis of manure composition. Test results are collated in **Table 1**.
- Results demonstrate that plain algae has a higher P:K ratio (0.8) than that typical of dairy manure (0.5), indicating an enrichment of P in the non-processed filaments. Algal biomass is enriched substantially with N, P, K, and S when soaked in liquid centrate, and displays a notably high ash-content value in both the plain or soaked states.
- The fiber content of the algal filaments appears to increase slightly after soaking in centrate, for reasons that are not immediately apparent. Both plain and soaked filaments have notably lower fiber contents than corn stover.
- Centrate-soaked algae is considerably more basic than either plain algae or corn stover, consistent with anecdotal observations of high salt concentrations in the vermicomposting study.
- Algal filaments appear to absorb N as NO_3 very effectively when soaked in digester centrate. By comparison, far less N in the form of NH_4 was transfered to filaments.
- Both forms of filaments displayed C:N ratio estimates (11:1) far below that for corn stover (117:1). This suggest that the efficacy of algal filaments as a soil-amendment agent might be enhanced by combination with biochar or some other form of condensed carbon.

	Centrate	Plain Algae	Centrate-Soaked Algae	Corn stover
Total N (lbs./ton)	**17.43**	**36.95**	**41.52**	**9.23**
Total P (lbs./ton)	**7.73**	**9.38**	**12.68**	**1.57**
Total K (lbs./ton)	**13.01**	**11.88**	**32.94**	**21.13**
Total S (lbs./ton)	**1.39**	**13.1**	**16.91**	**4.52**
NH4-N (lbs./ton)	**2.18**	**0.98**	**1.35**	**0.79**
NO3-N (lbs./ton)	**0.0045**	**0.09**	**0.14**	**0.09**
pH	**n.a.**	**7.5**	**8.6**	**7.1**
Ash content	**n.a.**	**63.26%**	**58.71%**	**5.31%**
Acid-detergent fiber content	**n.a.**	**31.30%**	**41.21%**	**58.06%**
Neutral-detergent fiber content	**n.a.**	**33.55%**	**41.42%**	**80.87%**
Lignin	**n.a.**	**6.55%**	**8.74%**	**9.17%**
Moisture	**97.7%**	**2.25%**	**3.90%**	**1.81%**
Dry matter	**2.30%**	**97.95%**	**96.10%**	**98.19%**
Estimated C:N ratio	**n.a.**	**11:1**	**11:1**	**117:1**

Table 1. Compositional analyses of biomasses employed in current study. Such tests are associated traditionally with analyses of dairy-cattle manure and agricultural residues.

Spectroscopic Analysis of Biomass

- Samples of worm castings from both algal-filament and corn-stover feedstocks were limited in volumes required for wet-chemical analyses of chemical compositions. However, these samples and those of the plain and centrate-soaked filaments, the pure centrate, and the shredded corn stover were each subjected to Total X-Ray Fluorescence Spectroscopy ("TXRF"), which enables detection of extremely low levels of elements from Sodium to Uranium in the periodic table (excluding Niobium to Technetium). Inclusion of P, S, and K in this range of elements allows direct comparison to levels detected in samples subjected to manure analyses.
- TXRF operates on a principle of detecting all fluorescence emitted as a result of an X-ray beam reflecting at a very slight angle to the sample preparation. TXRF is based on internal standardization, and thus is calibrated through addition of an element not present in the sample material. In these studies, gallium was found not present in any samples, and therefore was chosen as the internal standard. Analyses were performed using a Bruker S2 PICOFOX spectrometer.

- Results (**Table 2**) show levels of total P, S, and K as generally consistent with those found by wet-chemical manure analyses. Values for Mg, Si, and Mn suggest these elements are both present in filaments at markedly higher concentrations than in the other biomass samples. Ca shows higher values in both plain and centrate-soaked algal samples than in the other biomasses, though it is suggested that this element is effectively concentrated through worm digestion.

Element	Concentrations in **Plain Algae**, (mg/kg)	Concentrations in **Centrate**, (mg/L)	Concentrations in **Centrate-Soaked Algae**, (mg/kg)	Concentrations in **Corn Stover**, (mg/kg)	Concentrations in **Plain-Algae Worm Castings**, (mg/kg)	Concentrations in **Corn-Stover Worm Castings**, (mg/kg)
P	3,865.95	81.6	2,434.085	403.891	1,354.724	807.467
S	n.d.	182.219	8,072.117	601.629	2,722.604	1,699.956
Mg	4,140.26	n.d.	n.d.	n.d.	n.d.	n.d.
Si	34,776.93	n.d.	n.d.	n.d.	n.d.	n.d.
K	6,507.57	2,388.697	28,593.496	3,760.904	1,682.682	4,433.695
Cl	1,043.14	598.403	7,821.799	n.d.	353.597	187.87
Ca	123,568.43	391.11	135,555.807	1,521.051	77,214.616	18,849.551
Ti	152.85	1.462	221.208	n.d.	152.198	429.784
Cr	13.11	0.075	23.765	2.462	3.975	6.35
Mn	1,389.85	6.989	1,304.706	20.276	307.051	77.623
Fe	1,687.61	43.345	2,739.328	72.313	934.999	2,768.134
Ni	n.d.	0.098	55.887	1.318	n.d.	4.755
Cu	7.39	10.274	21.397	2.23	49.585	8.867
Zn	28.95	17.621	189.918	11.525	19.958	26.317
Ga	418.04	10.0	349.65	621.118	310.559	341.297
As	5.72	n.d.	5.568	n.d.	n.d.	n.d.
Se	1.11	0.082	0.339	n.d.	1.471	0.666
Br	55.37	0.874	45.198	0.674	26.423	7.188
Rb	9.86	0.851	9.003	n.d.	n.d.	9.505
Sr	121.61	2.007	245.181	12.832	100.991	59.509
Ba	n.d.	0.822	n.d.	36.092	133.307	143.583
Pb	n.d.	n.d.	2.688	n.d.	n.d.	n.d.

Table 2. Compilation of values from TXRF spectroscopic analysis of biomass samples. Data includes concentrations of both macro- and micronutrients important to plant growth.

Germination Studies

- Preparations of pure amendments and 1:1 amendment mixtures were tested in a two-week study to show their impacts upon soybean germination and early growth. 250 mL of soil-amendment preparations (listed in **Table 3**) filled eleven pots, each planted with ten seeds from this season's local soybean harvest. Controlled parameters (20 °C, 12:12 L:D, 10 mL distilled water/dish/day) were provided in an enclosed germination chamber.
- Time of first seed germinations, final germination success rate, and average heights of foliage were monitored and recorded throughout the study (**Table 3** and **Figure 6**).
- Observations found test amendment preparations to cluster about results of **Early Germination/Fast Growth** (100% plain algae, 100% topsoil, and 1:1 mixtures of both plain algae and corn stover with topsoil), **Early Germination/Slow Growth** (100% corn stover), **Later Germination/Slow Growth** (1:1 mixtures of both algal and corn-stover worm castings with topsoil and of 100% centrate-soaked algae with topsoil), and **Germination Failure** (100% centrate-soaked algae, 100% corn-stover worm castings, and 100% algal worm castings).

	Days to Germination	Final germination success rate	Avg. foliage height at 10 days
Single-Amendment Tests			
Topsoil	2	100%	25.5 cm
Plain algae	2	100%	30.5 cm
Centrate-soaked algae	n/a	n/a	n/a
Corn stover	2	100%	10 cm
Plain-algae worm castings	n/a	n/a	n/a
Corn-stover worm castings	n/a	n/a	n/a
Mixed-Amendments Tests			
1:1 plain-algae:topsoil	2	100%	25.5 cm
1:1 centrate-soaked-algae:topsoil	5	90%	5 cm
1:1 corn-stover:topsoil	2	100%	20 cm
1:1 plain-algae-worm-castings:topsoil	4	80%	15 cm
1:1 corn-stover-worm-castings:topsoil	5	100%	15 cm

Table 3. Germination and early-growth characteristics of soybeans cultured in various amendment tests.

Figure 6. Photo showing results of soybean germination and early growth after two weeks of the study.

Conclusions

- The use of soil-amendment mixtures at a high proportion of 1:1, or 50%, emphasized factors that either hinder or promote soybean germination and early growth. Anecdotal results suggest no single agent promotes germination above topsoil rates, but both corn stover and plain algae support the process equally with topsoil.
- Centrate-soaked algae appears clearly to hinder soybean germination, even when this amendment agent is cut 50% with topsoil. Centrate-soaked algae might eventually contribute its elevated levels of nutrients to promoting plant growth, but this was not evidenced for soybeans during the period of this study.
- Both types of worm castings appeared to hinder germinations. When cut 50% with topsoil, hindrance was reduced to some extent.
- Whilst 100% preparations of both plain algae and corn stover might promote germination equally to topsoil, only plain algae showed promotion of early growth. Germination probably responds most directly to a moist environment. Pure oven-dried worm castings don't hold moisture well, and thus hindered germination. Provision of nutrients to support early growth probably depends on available concentrations. Plain algae might release enough sequestered nutrients during a plant's early growth to support rates equal to those for topsoil. Centrate-soaked algae has high nutrient concentrations that raise salt levels and draw moisture away from seeds. This material could only prove effective as an amendment agent if cut sharply with other materials.