Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.	
Project Number:	USB # 1630-512-5211
Project Title:	Aquaculture Critical Control Points
Organization:	Auburn University
Principal Investigator Name:	Terry Hanson, Jesse Chappell, Daniel Wells, David Blersch
Report Period:	Final Report covering Jan. 1 through Dec. 31 2016
Project Status:	

The **OBJECTIVES** of this project were:

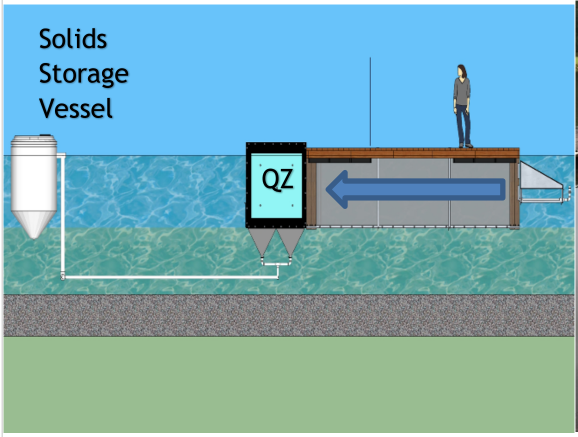
Objective 1 - Enhancement of Solids Collection and Removal: The rising cost of fish feed demands we pay more attention to the efficiency of its use but also the capture and re-use of all possible forms of nutrients passing into and through the In-Pond Raceway System (IPRS) pond. This project explored development of warmwater fish diets which seek both efficiency in assimilation into fish biomass and at the same time functionally enhance solids collection by coagulating fish fecal material. We conferred with Dr. Rick Barrows to structure an extruded, floating ration which contained the binder - guar gum - to improve solids capture and removal.

Objective 2 - The Production Base: We deployed mechanical solids removal devices to Floating IPRS systems (FLIPRS) and to a Ground based IPRS system (GRIPRS) located at the E.W. Shell Fisheries Station, Auburn University at Auburn Alabama. These units were stocked with hybrid catfish (channel catfish female x blue catfish male). Preliminary projects have demonstrated desirable feed efficiency and production traits in densely stocked IPRS production systems. Until now, the fish excretion has typically been left in the pond environment to be assimilated by naturally occurring biological and chemical processes. Adding the nutrient re-capturing devices to the IPRS raceway cells should remove significant quantities of settleable fish waste solids and result in improved water quality (and the increased fish production levels). Nutrient removal data were collected, entered into a database and used to analyze nutrient collection (quantity, quality, volatile organic compounds (VOCs) and NPK levels).

Objective 3 - Value Added Products from Solid Waste Utilization: Solid fish waste contains valuable nutrients, specifically carbon, nitrogen, phosphorus and potassium. We investigated downstream processes for transformation and release of those elements for use in further production of valuable products from the initial soybean based fish feed. By employing well-known processes of anaerobic digestion and horticultural crop production, we re-tasked captured nutrients from the solid waste stream. With these technologies, we attempted to produce biogas for energy, high quality fertilizer for plant production, and valuable plant material (vegetables and/or ornamentals). It is thought that the resulting products could improve the economics of fish production systems fed soybean based feeds and therefore increase sales of soybeans.

The **RESULTS** for each objective were:

Results from Objective 1: We developed an in-pond raceway system (IPRS) solids re-capture apparatus and employed two types with both FLIPRS (floating) and GRIPRS (ground-based) in-pond raceway systems. Data was collected that characterize the nutrient "waste" by-product collected (see results from Objective 3a and 3b below) and its quantity (see results from Objective 1 above). We equipped eight floating IPRS systems with harvesting gear and solids holding vessels in the ponds adjacent to the test IPRS raceway units. Solids removal ports and plumbing were installed as well. A semi-solids pump was installed and solids collection and removal occurred. The pump lifted the water and solids slurry from the collection vessels onto truck mounted tanks for transport to the next test arena (settling tanks, Objective 3) where characterization of the nutrient solid "waste" by-product and evaluation of its relative quantity and value with and without guar gum as a binder was conducted. The solids collection devices functioned as designed but were not able to collect solids products at as high a rate as we planned due to length of the quiescent zone and water flow rate.



Results from Objective 2: Fish waste solids from this project were recovered at a low rate, with recovery less than 1% by weight of amount of feed supplied. No difference in recovery rates were seen between experiment (guar at 0.03% inclusion rate) and control (non-guar) feed types. We believe the length of the quiescent zone (QZ) and relative rate of water exchange (flow) to the settling rate for solids hampered collection of higher percentages of solids. We will continue to evaluate solids collection in subsequent trials using a longer QZ more likely to allow for significant levels (%) of solids collection. Solids content in recovered slurry remained fairly dilute after 24 hours of settling, with no significant differences observed between experimental and control feed conditions. Volatile solids fraction was consistently high, however, for both experiment and control feed conditions. Both solids recovery rate and volatile solids fraction decreased throughout the season, likely related to temperature effects as well as size (weight) of fish in the systems. Methane yield potential was highly variable for all trials for both experimental and control feed conditions, and generally was moderate compared to other waste types. Methane yield potential was higher for control (non-guar) than for experimental (guar) conditions, although this difference was not seen as significant because of inter-trial variation, likely due to seasonality effects. Ultimate methane yield generally declined throughout the sampling season for both the experiment and control feed types, again suggesting a temperature effect on quality for digestion.

Results from Objective 3 - Value Added Products from Solid Waste Utilization: Solid fish waste contains valuable nutrients, specifically carbon, nitrogen, phosphorus and potassium. We investigated downstream processes for transformation and release of those elements for use in further production of valuable products from the initial soybean based fish feed by employing the processes of anaerobic digestion and horticultural crop production. In the laboratory, we investigated the methods to produce biogas for energy, high quality fertilizer for plant production, and valuable plant material (vegetables and/or ornamentals). Results from the investigations show preliminary promise of using fish waste solids as a value stream, but technical aspects of solids separation and concentration still exist and were not overcome in this research.

Results from Objective 3a: Determining fertilizer value of catfish waste collected from an IPRS, determining differences due to guar gum feed additive, and utilize various waste streams for production of high-value horticultural crops.

We constructed on-land waste settling tanks and developed a protocol for moving waste from IPRS to settling tanks in Q1 and Q2 of the project. Dissolved nutrients in the waste stream were not collected from IPRS, so we focused on utilizing latent nutrients in fish waste solids.

The first waste stream that was of horticultural interest was what we referred to as “tea”. The tea was the liquid portion that was separated from the slurry we pulled from each IPRS. We loaded the slurry into cone-bottom settling tanks and allowed solid and liquid portions to separate for 24 hr. We then siphoned off the liquid portion and saved the solids for later use. A preliminary assessment of fertilizer value of collected IPRS waste was conducted in Q3. Nutrient analyses revealed low macronutrient levels in waste supernatant or “tea”. However, high levels of available iron (avg. 4%), an essential micronutrient, were present. We designed an experiment to isolate the effect of the high-iron tea on plant growth. Two species, Solanum lycopersicum ‘Celebrity’ tomato and Petunia x hybrida ‘Mambo Purple’ petunia were grown in 1.6-L containers, filled with 80:20 peat:perlite substrate, and amended with two rates of dolomitic limestone (4 lbs. yd.-3 and 8 lbs. yd.-3). Limestone rate was varied in order to test pH effects on nutrient availability within the substrate. Two fertilizer treatments (1. Complete micronutrient solution; 2. Micronutrient solution without iron 3. IPRS tea) were combined with two liming rates to yield a 3 x 2 factorial experiment. The experiment was arranged as a completely randomized design for both tomato and petunia. Both species grew best with the high liming rate. However, iron deficiency was not induced, even in plants that were not fertilized with iron. Therefore, the effect of IPRS tea on iron uptake was not elucidated. The iron taken up by plants that were fertilized with nutrient solution not containing iron may have come from municipal water or from the substrate itself, with the latter being more likely. Although iron is a plant-essential nutrient it is required in very low quantities. In addition, iron fertilizers are abundant and relatively inexpensive. Therefore, we determined that the tea produced in this trial had no fertilizer value.

The second nutrient source of interest was the solid manure that settled in the collection tanks. Animal manures have been used for centuries as fertilizer amendments in crop production. In recent years, fish wastes have been soil-applied to field-grown crops and utilized as components in soilless substrates. Since application of solid wastes is a well-established method, we decided to investigate the liquid digestate from anaerobic digestion of the solids as a fertilizer amendment for high-value greenhouse crops.

In Q3, digestion of the solids was delayed due to cool weather. In Q4, we digested appreciable amounts of solids, but quality control was questionable due to escaped gases. Therefore, no plant trials were conducted. We did perform a manure nutrient analysis to determine fertilizer value of lab-scale digestate. We anticipated high concentrations of plant macronutrients, especially N. No difference in any essential nutrient was caused by guar feed additive (data not shown).

Total N was 153 ppm, which is within an acceptable range for liquid fertilizers for greenhouse crops; however, the majority (140 ppm) of the total N was ammoniacal-N (Table 1). This is problematic because the form of N required in highest quantities by greenhouse crops is nitrate-N. There was no detectable nitrate-N in the digestate. Nitrification would need to be performed on this digestate to convert ammoniacal-N to a nitrate-N form before it could be successfully used as a fertilizer.

Available P averaged 24 ppm, which is within an acceptable range for liquid fertilizers, however K was very low (37 ppm). Greenhouse crops typically require K concentrations in excess of 200 ppm for optimal growth. Calcium was also low and Mg was absent. However, the primary problem with this digestate was the exceedingly high Na content. Sodium is not a plant-essential nutrient and typically causes physiological problems in concentrations higher than 75 ppm. The Na content of the digestate was nearly 19x too high for horticultural crop production.

The excessive sodium must have originated in the original feed. We did not add sodium during the digestion process. The solid manure collected from IPRS may still have application as a fertilizer source, but high Na levels may be problematic. It is possible that when soil-applied the solids do not have Na levels that exceed the crops tolerance. In a soilless substrate with typically low cation exchange capacities, excessive Na levels cause rapid crop damage. We concentrated latent nutrients from the solids during the digestion process, which likely compounded the Na problem.

It is possible that another type of feed with lower Na would yield more desirable fertilizer waste.

Table 1. Fertilizer analysis of solid manure captured from IPRS

Total N	NH_4-N	NO_3-N	P	K	Ca	Mg	Na
			Ppm				
153	140	0	24	37	18	ND	1398

Results from Objective 3b: Analyze recaptured solid waste in the laboratory for its ability to be digested and composted for release of nutrients and biogas.

Fish waste solids and volume: Weekly collection of fish waste occurred from 8/11/16 to 11/3/16. Fish feed was alternated by week between Guar Experiment (n=6) and Non-Guar Control (n=7) soy feed variants. Fish waste slurry was collected from raceways and settled for 24 hours, and settled slurry was decanted and analyzed. Solids analysis revealed for settled slurry an overall mean total solids (TS) content of 38.4 g/L and an overall volatile solids content of 31.7 g/L, with a mean volatile solids (VS) percentage of 82%. The high VS content is promising for downstream utilization, but the rather low TS content shows the difficulty in separating solids from liquid streams. Further solids analysis revealed no difference between solids recovery concentration or volatile solids content for experiments (guar) and controls (non-guar) (Table 2), suggesting that guar additions to feed did not appreciably increase the solids settling rates.

Table 2. Solids content characterization of fish waste solids from experiment and control collection replicates. Values represent Mean ± Standard Deviation.

Parameter	Guar (Experiment)	Non-Guar (Control)
Total Solids Conc. (g/L)	39.6 ± 13.7	37.3 ± 18.9
Volatile Solids Content (%)	82 ± 42	81 ± 37

Additionally, it was observed that feeding rate and temperature were shown to have the greatest effect on both TS and VS contents. A general decline in mean solids concentration (Figure 1) and a slight decline in mean volatile solids fraction (Figure 2) was observed. This suggested seasonal aspects to solids quantity and quality, and was most likely influenced by average temperature of water in the fish ponds.

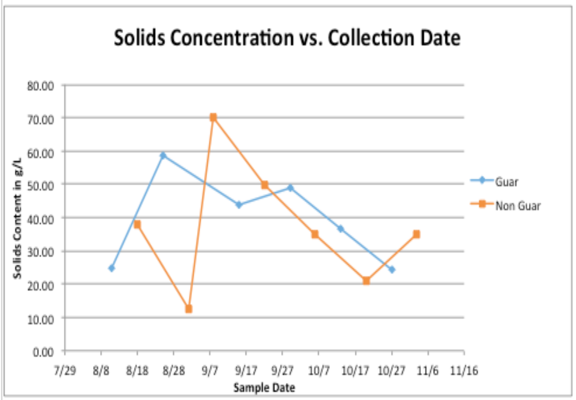


Figure 2. Mean solids concentration of fish waste vs. collection date for experiments (Guar) and control (non-Guar) groups.

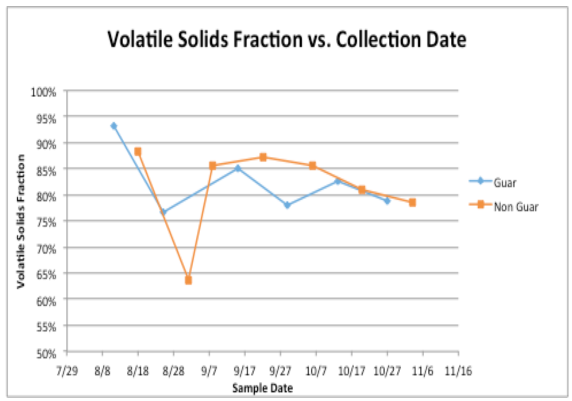


Figure 1. Volatile solids fraction of fish waste vs. collection date for experiments (Guar) and control (non-Guar) groups.

The solids data also allowed the estimation of the efficiency of the solids collection device and system. Solids recovery rate, as a function of soy feed, was low (Table 3), and generally less than 1% of amount of feed into the system. No appreciable difference was observed between experiments and controls, again suggesting that guar addition had no appreciable benefit on solids settling capabilities.

Table 3. Mean feed rate and mean solids recovery rate for solids collection events from experiment (Guar) and control (non-Guar) collection replicates. Values represent Mean ± Standard Deviation.

Parameter	Guar (Experiment)	Non-Guar (Control)
Feed in (lb)	2753 ± 166	2511 ± 214
Total solids collected (lb)	13.8 ± 3.9	10.3 ± 8.3

Fish solids quality for biomethane: With the completion of waste collection, further steps were made on waste analysis. Most notably, biomethane potential (BMP) laboratory tests of 41 days at

35 deg. C in length were started November 23, 2016. Waste was inoculated with anaerobic sludge provided by the wastewater treatment plant in Columbus, Georgia. A substrate to inoculum ratio of 0.5 volatile solids was selected after a brief literature review. For all replicates, gas production occurred and leveled out within the 41 days of the BMP test (Figures 3 an 4), suggesting that time length is sufficient for complete digestion of fish waste.

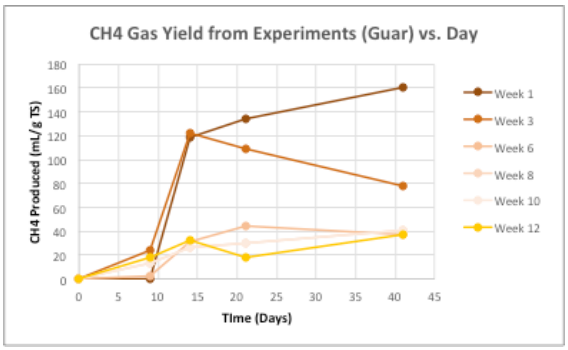


Figure 3. Methane evolution vs. Day during the BMP testing for experiments (Guar) for different weeks of collection.

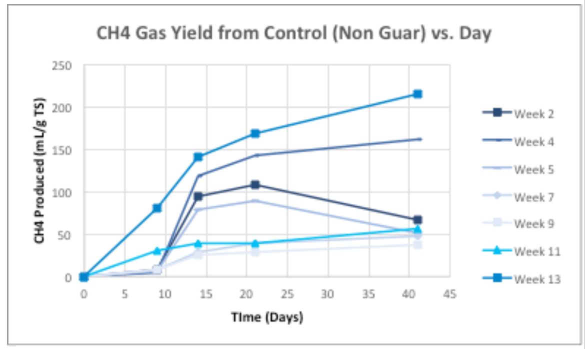


Figure 4. Methane evolution vs. Day during the BMP testing for controls (non-Guar) for different weeks of collection

The mean gas yield vs. day during the BMP test shows that guar had an effect on gas yield, generally suppressing gas yield compared to non-guar (Figure 5). The difference in ultimate gas yield for guar vs. non-guar was not significant, due to noise in the measurement signal caused by variations in BMP measurements (Figure 6). The mean gas yield for guar and non-guar was 75.6±52.5 mL/g TS and 102.4±65.6 mL/g TS, respectively. To compare, typical results for liquid dairy manure yield 240 mL/g TS (King et al. 2011).

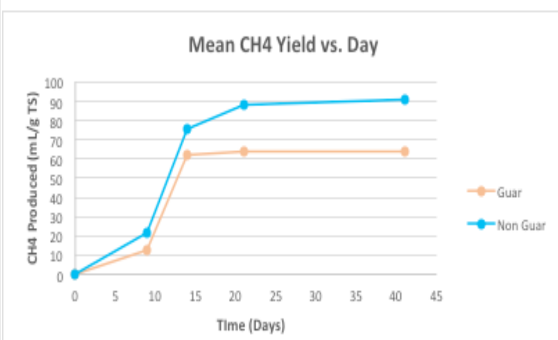


Figure 6. Mean gas yield vs. day for BMP testing for all experiments (Guar) and controls (non-guar).

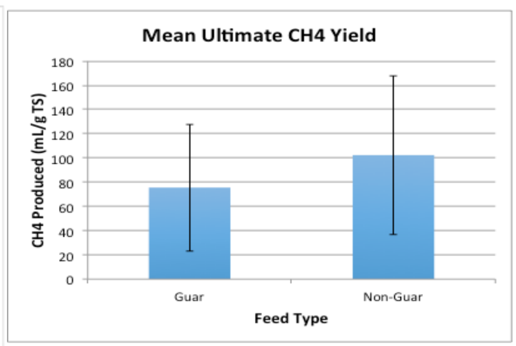


Figure 5. Mean ultimate gas yield for guar and non-guar feed, as a result of 41-day BMP tests. Error bars represent standard deviation.

The mean ultimate methane gas yield was observed to be a function of date of collection (Figure 7). Gas yield decreased for each successive collection date for both guar and non-guar feed conditions, suggesting a strong seasonality factor to the digestibility of the collected waste solids. No significant differences were seen between guar and non-guar feed conditions, except at the last sampling, where an increase in total gas produced was observed for the non-guar condition. This may be the result of changing solids chemistry due to temperature changes; more investigation is necessary to determine the cause, however.

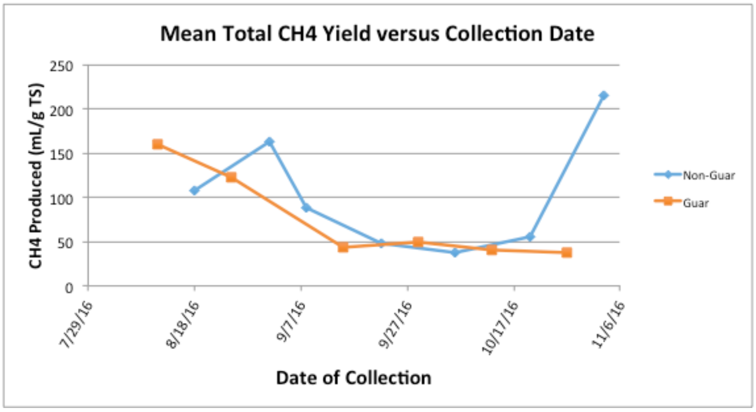


Figure 7. Mean ultimate methane gas production vs. date of collection for experiment (guar) and control (non-guar) fish waste.

Pilot Scale testing: Pilot-scale reactors were built and digestion tests on solids run in December. Failure of the reactors, however, due to containment breach prevented the collection of gas, and results for gas collection were inconclusive. Solids digestion was evident, however, due to pressure increases in the reactor containment. Samples of digestate have been taken and stored for future analysis for nutrient and elemental content.

Overall, these results suggest that guar has no measurable effect on waste collection in the current raceway configuration. Collected waste remains difficult to concentrate to desired solids ratio; volatile solids ratio for fish waste is generally favorable; seasonal effects are strong determinants of process efficiency; and ultimate methane production potential for fish waste solids, regardless of the presence of guar, is of a moderate level to warrant continued investigation as a biomethane source for bioenergy.

References:

Barrows, R. 2014 (?). "The effect of feed ingredients and feeding regimen on fecal output of rainbow trout and impact of soy products in a serial reuse system." Final Report USB project # 1365-512-5697.

King, A., Zomalloa, C., Schmidt, D., Hu, B. 2011. Bio-methane potential (BMP) of sieved dairy manure with condensed distillers solubles (CDS) and cheese whey. Project report, Agricultural Utilization Research Institute, University of Minnesota.