Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

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Project Title: Clean Water and Renewable Energy from Beet Processing Wastewater and Manure

Project number: 125-E

The research addendum should be complete, but does not necessarily need to be long. Each project should include the following information:

1. **Abstract** - Summarize the research and its essential qualities including a clear statement on the purpose of the research.

The long term goal of this project is to promote and establish sustainable agriculture in Minnesota (and in US as well) by recycling agricultural production wastes into renewable products, thus protecting the environment and conserving natural resources. This goal will be accomplished by developing a novel system to co-treat swine manure with sugar beet processing wastewater (molasses) to produce biohydrogen, biomethane, a slow release fertilizer (struvite), and a derivative combustion engine fuel called "biohythane" that is more environmentally friendly than methane during combustion. Specific objectives include 1) determining the optimal operating values of swine manure to molasses ratio, hydraulic retention time (HRT), and pH for an anaerobic sequencing batch reactor (ASBR) biohydrogen fermenter to maximize biohydrogen production; 2) determining the optimal operating values of organic loading rate, pH and HRT for an ASBR biomethane digester receiving the effluent from the biohydrogen fermenter to maximize biomethane generation and for the effectiveness in COD reduction; 3) developing an absorption reactor to remove CO_2 from the biogases from either the biohydrogen fermenter or biomethane digester using alkaline chemicals as an absorbent; 4) developing a process to recover nitrogen and phosphorus in the digestate by forming struvite; and 5) based on the lab-scale setup, building and evaluating an integrated system consisting of a biohydrogen fermenter, a UASB biomethane digester, a CO_2 removal reactor, and a struvite precipitator to co-treat swine manure and sugar waste molasses. The proposed project addresses the issues raised in E(5) in the 2014 funding priorities.

2. **Background** - Provide the basic information and other relevant work that are the context for this research.

Minnesota is ranked #2 in hog production and #1 in sugar beet production in the nation, which generate about 11 million tons of pig manure and over 1 million tons of sugar processing wastes annually. Up to this date, there are no cost-effective methods available to deal with (or beneficially use) these waste streams environmentally and resourcefully other than land application that lead to polluting issues in many places in the state due to the buildup of nutrients (nitrogen and phosphorus) in the soil receiving these two wastes such as surface and ground water pollution due to nutrients runoff and/or leaching from overloaded soils. In early 2012, Minnesota became the nation's first test site for a novel federal program designed to stem the flow of agricultural pollution to water resources (http://phys.org/news/2012-01-strategy-farm-runoff-minnesota.html). Needless to say, this sounds an alarm that immediate actions must be taken to treat these wastes. However, treatment alone is not only expensive but fails to recover the resource values of both waste streams. Therefore, new technologies need to be developed to accomplish the dual goals of treatment and reuse and the proposed research project will fill the blank and provide the following benefits to the Minnesota agricultural industries and natural resources conservation. Potential commercial implementation sites in Minnesota could be located in the fastest growing hog production counties including Martin, Blue Earth, Waseca, Cottonwood, and Brown, which are relatively close to a sugar processing facility in Renville County.

- Reduce/eliminate the impact of land application of over 1 million tons of sugar processing wastewater that threatens water resource
- Reduce/eliminate the impact of land application of over 11 million tons of pig manure that increases surface and groundwater pollution
- Save $10 million for sugar beet processers annually for wastewater treatment
- Generate bio-electricity of 143 million kWh ($9.72 million) yearly from the two waste materials
- Reduce greenhouse gas (CO_2) emission by 57% when combustion engines are run on “biohythane” produced from biohydrogen and biomethane (equivalent to 27 million gallons of diesel that can be produced annually)
- Produce 15,560 tons of ammonia/phosphate fertilizer (struvite) annually ($5 mil value)

3. **Hypothesis** - State the premise or propositions set forth to explain and achieve the described outcome of the research.

The hypothesis of this project is that the proposed treatment system (Figure 1) will successfully treat two waste streams in one process and increase bioenergy production and generate a valuable fertilizer at the same time. Recent reports from other researchers have primarily focused on single substrates such as tofu processing wastewater, rice winery wastewater, starch manufacturing wastewater, potato processing wastewater, beer processing wastewater, pineapple wastes, etc., for biohydrogen production. These practices were not considered economical from the perspective of commercialization due to addition of synthetic medium containing high sugar content. Therefore, co-fermentation of two waste streams with complementary nutrients to produce biohydrogen is a novel idea which has only debuted in the scientific literature recently based on our previous work. The two wastes, pig manure and sugar beet processing wastewater (containing molasses), are a perfect mix for biohydrogen fermentationbecause one has all the nutrients but sugar for biological activities, while the other has residual sugar which is an ideal carbon source highly needed in biological processes such as fermentation and anaerobic digestion. Our early study showed that using swine manure alone was found to be ineffective in biohydrogen fermentation, and a sugar source, such as glucose, needed to be added at 10g/L to the culture media due to the lack of sufficient sugars in the manure for the fermentative bacteria. Following that line, a trial study was conducted using sugar beet processing waste molasses to replace glucose in the fermenter liquid. The amount of molasses added was determined based on its sucrose content to match the amount of glucose used in the early study, i.e., 10 g/L. Preliminary results showed an improvement in maximum biogas production rate as opposed to that from the glucose study. This finding clearly indicates that replacing glucose with a sugar containing wastewater as a carbon source is as effective, if not more, in biohydrogen fermentation. Similarly, fermenting molasses alone without swine manure was also found to produce little biohydrogen due to the lack of all the other nutrients, which evinced the importance and necessity of using co-substrates.

For methane production, the separated two-stage process proposed herein, acidogenesis/ acetogenesis followed by methanogenesis can increase methane yield by 22% as opposed to the one-stage process (digestion alone) due to separate control of the fermentation conditions, accompanied by an increase in total energy efficiency from 51% to 65% according to literature reports.

For the chemical scrubber study, CO_2 in the biogas will be transferred from the gas to the gas/liquid interface, and then to the bulk of the liquid phase, where reactions take place following two steps: 1) $CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O$ and 2) $CO_2 + CO_3^{2-} + H_2O \rightarrow 2HCO_3^-$. All that needs for this process to happen is the close contact of the absorbent with the gases to be removed in the biogas. To date, application of chemical absorption in biogas cleaning is quite rare, even more so in the agricultural sector. As such, the proposed work will be among the first attempt to investigate the technical feasibility of this concept and its application by developing a lab-scale (followed by a pilot scale), packed bed column reactor using NaOH as the absorbent to capture CO_2 in the biogases from both the biohydrogen fermenter and the biomethane digester.

The last component in the treatment system is the struvite precipitation. As a matter of fact, the effluent resulting from anaerobic digestion is a better candidate for struvite precipitation than raw wastes because, to form struvite, the ions, Mg^{2+}, NH_4^+, and PO_4^+, must be available in an alkaline solution and in sufficient concentration to provide an adequate precipitation potential. However, in the raw wastewater from animal feeding operations, there is always a shortage in aqueous Mg^{2+} and reactive phosphorus (PO_4^+), making the struvite precipitation process unable to proceed since the product of the ion concentrations is below the struvite solubility product. After anaerobic digestion, the amounts of these two ions in the digested swine manure can be increased considerably, potentially leading to high removal rates for both ammonium and phosphorus from agricultural wastewaters, which constitutes the basis for the proposed technique. Based on the above analysis, it can be concluded that the hypothesis of this research proposal is practically and theoretically achievable upon completion of the project.

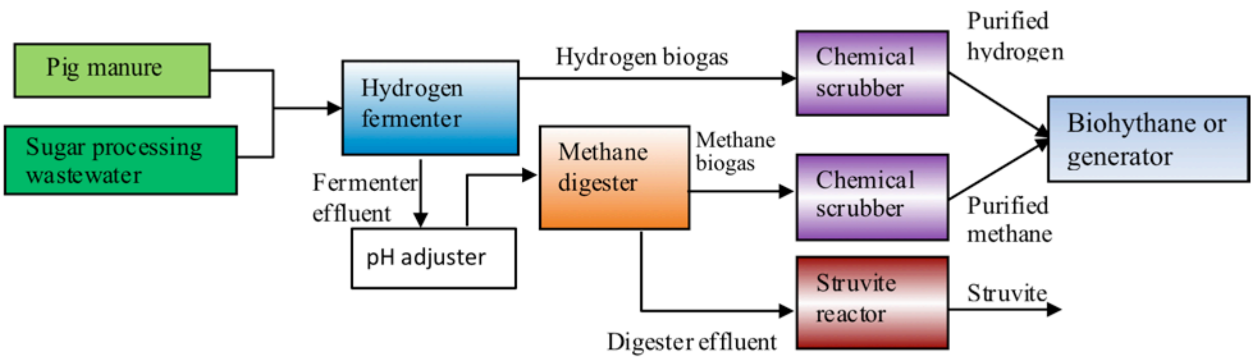

Figure 1. The flowchart of the proposed treatment system

4. **Methodology** - Describe the methodology to be employed to carry out the proposed research. Including descriptions of the sample design(s), if applicable.

Methods for specific objective 1 (biohydrogen fermenter)

Experimental apparatus: A lab-scale anaerobic sequencing batch reactor (ASBR) system will be built with reference to the ones developed in the early work at the University of Minnesota Southern Research and Outreach Center (SROC) at Waseca (Figure 2). The reactor body will be a polyethylene jar, 4 L in total volume with a working volume of 2 L. The reactor will be placed on a hot plate stirrer that maintains the temperature (37°C) of the mixed liquor content in the reactor and the complete-mix condition will be achieved by using a peristaltic pump circulating the liquid through the reactor assisted by an internal magnetic stirrer. Mixing also reduces biohydrogen inhibition on the bacteria generating biohydrogen. The pH inside the reactor will be controlled by a pH controller that adjusts the liquid pH by turning on and off two peristaltic pumps that add either hydroxide (supplied as 1.0*M* NaOH) or acid (supplied as 1.0*M* HCl) to the reactor. The prepared influent (mixture of manure/sugar waste molasses) as substrate will be stored in a 20 L influent tank with a mixer, and the fermented liquid discharged into an effluent tank of the same size. The influent and effluent flows will be regulated by peristaltic pumps in order to adjust the hydraulic retention time (HRT). A computer system will be used including a programmable control module (Campsci CR1000) with its software (Campsci PC400) installed on the computer capable of repeating a programed cycle operation in a time sequence. The reactor will be seeded with a volume of 400 mL heat-treated biohydrogen-producing sludge obtained from a local wastewater treatment plant at Waseca, MN. The heat treatment by boiling the sludge at 100°C for 15min is to inactivate thermal-susceptible methane-producing species and other non-biohydrogen-producing bacteria in the sludge.

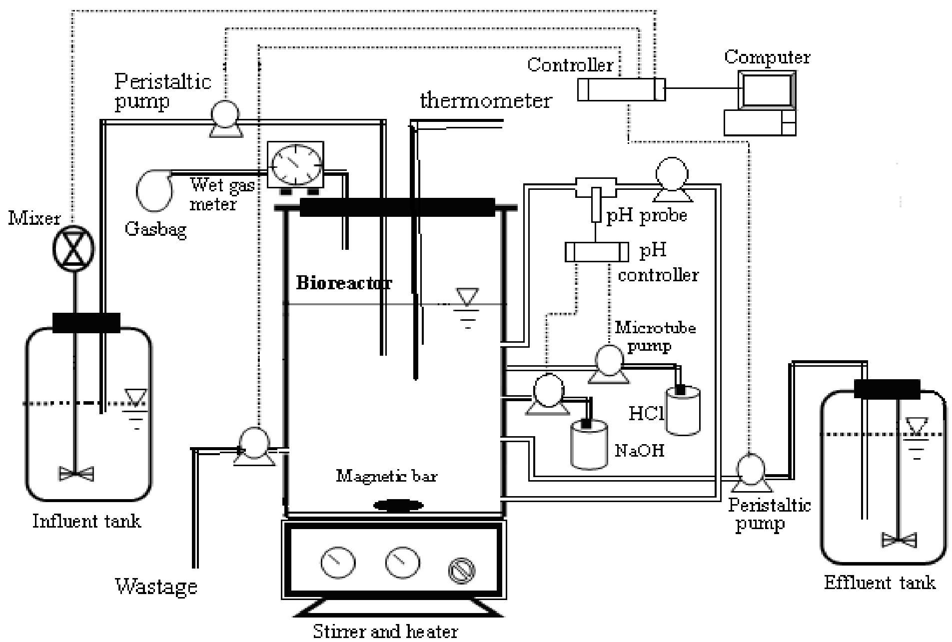

Figure 2. Schematic of the biohydrogen fermenter

Experimental design: The ASBR will be run on 4-h cycles with 2.5 min each for feeding and discharging, 30 min for settling, and 205 min for reaction. Our previous research results indicated that pH played a key role in fermentative biohydrogen production and different substrates might have different optimal pH values. The same can probably be said of hydraulic retention time (HRT) as well. Therefore, in this study, an extended range of pH (4.5, 5.0, 5.5, 6.0, and 6.5) and HRT (6, 12, 18, 24, and 30) will be investigated to determine the optimal combinations corresponding to high biohydrogen production. The amount of sugar waste molasses added to swine manure (the mixing ratio) will also be a variable in this study. Based on our trial results, five ratios of manure to molasses will be tested initially and more may be added during the experiment if needed. These five ratios will be represented by varying manure total solids content (TS) while keeping the sugar concentration in the mixed substrate constant, i.e., 10 g/L (calculated based on the sucrose content in the molasses). The five manure TS contents to be tested include 0.25, 0.5, 0.75, 1.0, and 1.25% (corresponding to TS to molasses ratios of 0.25, 0.5, 0.75, 1.0, and 1.25, given 10 g/L = 1%). The manure TS range selected is typical for the liquid swine manure from pit recharge and/or flushing systems. This experimental plan will constitute a factorial design of 5x5x5, leading to a total of 125 test runs in order to investigate all the combinations, which cannot be completed in the proposed project period, i.e., 3 years, given that a minimum of three weeks are needed for one test run. To avoid a complete three-level factorial design without losing statistical significance, a central composite design (CCD) coupled with response surface methodology will be adopted to build a second order (quadratic) model for the response variable. The full factorial CCD experimental design will thus be applied to the following three variables: (i) pH, X_1; (ii) HRT, X_2 (hr), and (iii) manure TS, X_3 (%).

The range and the levels of variables employed in this study are listed in Table 1. The center values (zero level) chosen for the experimental design will be pH=5.5, HRT=18 h, and TS=0.75%, with which an optimal process condition for a response variable can be determined in the neighborhood.

Table 1. Experimental range and levels of the independent variables

Variables		Code and real values				
		-2	-1	0	1	2
X_1	pH	4.5	5.0	5.5	6	6.5
X_2	HRT (h)	6	12	18	24	30
X_3	TS (%)	0.25	0.5	0.75	12.0	1.25

As mentioned early, a second order quadratic model will be used to fit the data set of responses with the experimental parameters. To evaluate the effects of pH (x_1), HRT (x_2) and TS (x_3) on the model response of biohydrogen production, the corresponding results will be subject to regression analysis by the least square approach to generate the quadratic equation (Eq. 2) using statistical software.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

Where *Y* is the predicted response (i.e., biogas production rate (BPR), biohydrogen production rate (HPR), biohydrogen content (HC), and biohydrogen yield (HY)); x_1, x_2 and x_3 are coded independent variables, pH, HRT and TS; β_0 is the offset term; β_1, β_2 and β_3 are linear coefficients; β_{11}, β_{22} and β_{33} are the quadratic coefficients; and β_{12}, β_{13} and β_{23} are the interaction coefficients.

After the values of coefficients are determined, the regression equation will be examined using the statistical *F* test and the optimum values of pH, HRT and TS for the biohydrogen ASBR system for any response *Y* can be obtained by solving the quadratic regression equation.

Sampling and analysis: Gas and liquid samples will be collected at respective sampling ports. The amount of gas produced will be recorded daily using a wet gas test meter and the gas will be released continuously to keep a low biohydrogen partial pressure in the headspace. Gas sampling will be conducted every other day and gas analysis will include biohydrogen, biomethane, and CO_2 using a gas chromatography (Varian 3800). The liquid samples will be taken at the same time intervals as in gas sampling after at least one HRT under stable conditions for analysis of chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS), total suspended solids (TSS), volatile suspended solids (VSS), volatile fatty acids (VFAs), sugar content, and total Kjeldahl nitrogen (TKN) following the Standard Methods.

Methods for specific objective 2 (biomethane digester)

Experimental apparatus: The characterized effluent from the biohydrogen fermenter will be the influent for the biomethane digester. Considering the HRT used for the biohydrogen fermenter and the slow growth of methanogens, a much larger ASBR reactor (16 L with working volume of 12 L) than the biohydrogen fermenter will be built. Similar to the biohydrogen fermenter, this digester will also be equipped with all the necessary controls and pumps so that it can run automatically without significant human intervention. The temperature will be controlled at the same temperature as for the biohydrogen fermenter (37^{o}C)Before entering the biomethane digester, the pH of the influent (biohydrogen fermenter effluent) will be adjusted to around 7, and the liquid pH inside the digester will also be controlled at 7, using NaOH (1.0 *M*). Additional mixing to the digester content may be provided by a pre-installed mechanic mixer if needed.

Experimental design: The ASBR digester will first be seeded with sludge from an existing anaerobic digester treating municipal wastewater at Waseca. When up and running, the ASBR in this stage will be operated as a one-stage digester under controlled conditions of temperature (37^{o}C) and pH (7.0). Four HRTs (2, 4, 6, and 8 days) and four organic loadings (5, 10, 15, and 20 kgVS/m^3) will be examined to determine the best combination for biomethane generation. These organic loadings are selected with reference to the published information for a two-stage hydrogen and methane production system using household solid waste. Please note that choosing organic loading as an independent variable is aimed at determining the maximum throughput capacity of the digester. When the HRT is short while organic loading is high, the system is defined as being able to achieve high treatment capacity. The organic loading may be adjusted during the experiment according to the organic strength in the biohydrogen fermenter effluent. The solids retention time (SRT) will be kept at 15 days in all runs. This 4x4 factorial experimental design will need a total of sixteen experiments to complete the project.

Sampling and analysis: Both biogas and liquid samples will be collected from the respective sampling (discharge) ports once a day at the same time for a period of two HRTs after the biomethane digester has entered into steady-state operation (the number of samples collected represents replications). Biogas samples will be analyzed for biomethane and CO_2 using the same gas chromatography as in biohydrogen analysis. Total and daily biogas volumes produced will be recorded continuously using a wet gas test meter. Parameters such as COD, BOD, TS, TVS, TSS, VSS, VFAs, and TKN for the liquid samples will be analyzed following the Standard Methods. Statistical t and F tests (ANOVA) will be used to determine the difference between the data collected at a significance level of $\alpha \leq 0.05$.

Methods for specific objective 3 (chemical scrubber)

Experimental apparatus: The biogas cleaning unit (a packed bed reactor) will be fabricated from an acrylic column, 4 in. (10 cm) in diameter, 4.0 ft. (122 cm) in height, which will be equipped with pumps and gauges to move the absorbent and biogas through the system at desired flow rates (Figure 4). The absorption column will be randomly packed with a packing material (known by the trade name “plastic bioball”) to a height of 3 ft. to facilitate gas/liquid interaction (this spherically shaped material has a high surface area to volume ratio of 1895 m^2/m^3), which is ideal for applications where efficient gas/liquid interaction is needed. The biogas purification process will take place in the packed bed section where the gas will be continuously fed from the bottom while the absorbent will be sprayed from the top of the reactor, creating a counter current flow to improve the gas/liquid contact. The absorbent will be circulated between the reactor and a reservoir (20 L liquid volume) by means of a pump. The liquid flow rate will be controlled by a liquid flow meter to maintain a smooth liquid film, while the biogas flow rate will be controlled using a gas flow meter to achieve the best gas flow rate in removing CO_2. The pH of the absorbent reservoir will be monitored and recorded using a pH meter with a controller to determine the time elapsed between absorbent replacements when the pollutants approach saturation. At the beginning of a typical run, a certain amount of absorbent will be placed in the reactor to prime the packed bed. NaOH (0.1 M) solution will be used as the absorbent for this study. The reason for selecting NaOH in preference to other alkaline liquid lies in its relatively low cost and availability in bulk volumes, thus avoiding the process of regeneration used for other chemicals that may release the captured CO_2 back to the atmosphere.

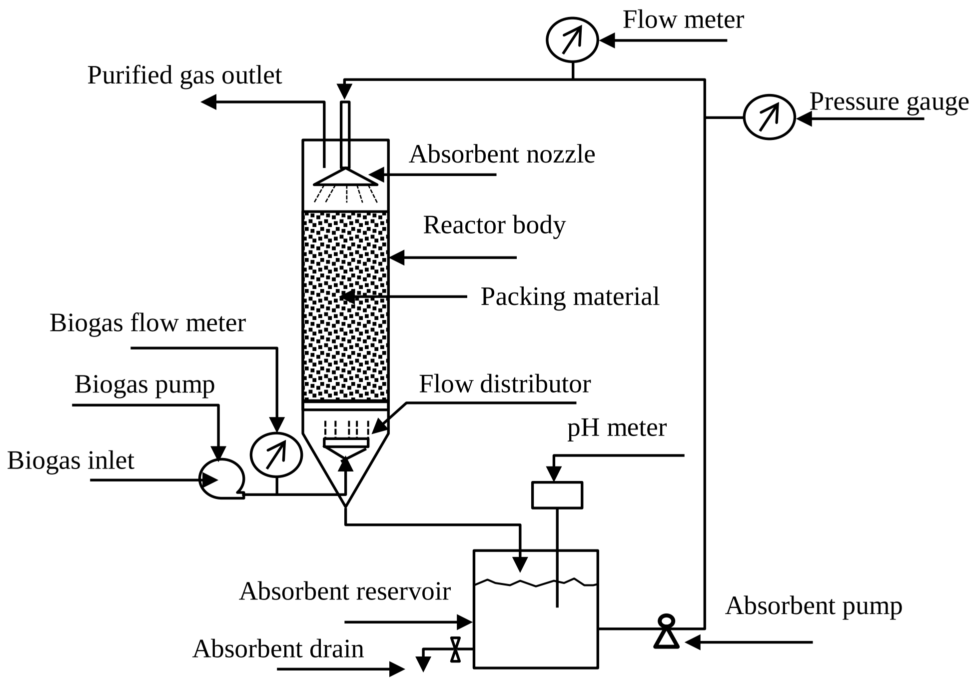

Figure 3. Schematic of the biogas cleaning reactor

Experimental design: The experiment will be carried out in two phases, starting with a batch phase followed by a continuous phase. Since the ratio of gas to liquid flow rate is a critical factor affecting the performance of CO_2 removal in this type of reactor, the batch study will investigate the effect of different gas/liquid flow rate ratios. Due to the paucity of information available in literature in this area, the liquid flow rate will be used as a reference for determination of the gas flow rate, and will be obtained by slowly adjusting the flow rate to a level when a smooth liquid film coming out of the absorbent spaying nozzle is observed (a few more nozzles of different size may be tested to accommodate potential different gas flow rates). With the liquid flow rate determined, five ratios of gas to liquid flow rate (0.6, 0.8, 1.0, 1.2, and 1.4, more if needed) will then be examined for the packed bed reactor with respect to its performance in removing CO_2 and H_2S from the biogas. For each gas/liquid flow rate ratio in the batch study, the reactor system will be run continuously until the absorbent in the reservoir reaches complete saturation as indicated by the cease of pH drop that is continuously monitored and recorded during the experiment. Each batch test will repeat three times to find the best gas/liquid flow rate ratio and the time curve of each ratio showing the course of reaching saturation. This information will be used in the second phase of the experiment.

In the second phase, the reactor will be evaluated on removing CO_2 in continuous operation, in which a fraction of the original volume of the absorbent in the reservoir will be replaced at certain time intervals to maintain high absorption rates. With reference to the data from the batch study, four percentages of the total volume in the NaOH reservoir and four replacing time intervals (e.g., % saturation time) will be determined and examined via a 4x4 factorial experimental design to find the best combination of these two variables in achieving a good removal efficiency for the treated biogas, while keeping the replacement of absorbent at a reasonable frequency.

Sampling and analysis: For all the experimental runs, gas samples from the inlet and outlet of the reactor will be collected every 5 minutes in 1 L Tedlar bags for analysis of CO_2, H_2, and CH_4. A gas chromatograph (Varian 3800) will be employed for analysis of the gases. In the meantime, the pH in the absorbent reservoir will be continuously monitored and recorded. For batch tests, the liquid in the reservoir will be sampled at the end of test and the amount of CO_2 absorbed will be determined based on measuring HCO_3^- concentration in the sample using the Hach method 8010 (Hach Co., Loveland, Colo.), which will be corroborated by mass balance analysis for gaseous CO_2 between the inlet and outlet gas streams. For the continuous tests, liquid samples of 10 mL each from the reservoir will be collected every 5 minutes for the same analysis. The lost liquid volume will be replenished during absorbent replacement. The H_2S reduction will be determined by the difference between the measurements of inlet and outlet gas samples. Statistical *t* and *F* tests (ANOVA) will be used to determine the difference between the data collected at a significance level of $\alpha \leq 0.05$.

Methods for specific objective 4 (struvite precipitator)

Experimental apparatus: The effluent from the biomethane digester will first be analyzed for pH, TSS, TP, reactive PO_4^{3-}, TN, NH_4^+, soluble Mg^{2+}, and Ca^{2+} to adjust accordingly the $PO_4^+/Mg^{2+}/NH_4^+$ ratio in the influent for the struvite precipitator. The precipitator will be fabricated from an acrylic column, 10 in. (25.4 cm) in diameter and 3.5 ft. (106.7 cm) in height (working height: 3 ft.), with a cone shaped bottom to collect settled struvite (Figure 4). The reactor will consist of a center section (5 in. in diameter, working as draft-tube type) and a peripheral section. The influent will be continuously added into the draft tube. Aeration will be used as a means to increase the liquid pH in the column and agitation. If insufficient, either NaOH or $Mg(OH)_2$ (or MgO) will be added to the liquid to raise pH, but use of the latter will depend on the level of Mg^{2+} in the solution. The pH in the reaction section will be controlled automatically by a pH controller. The generated struvite will be separated from the effluent in the peripheral section outside the draft tube by gravity separation and settle to the bottom of the precipitator for removal. The recovered product will then be dewatered from the small amount of solution, which is lost when the crystals are removed, by natural drying. The precipitator is aimed at continuous production of struvite from anaerobically treated effluent from the biomethane digester.

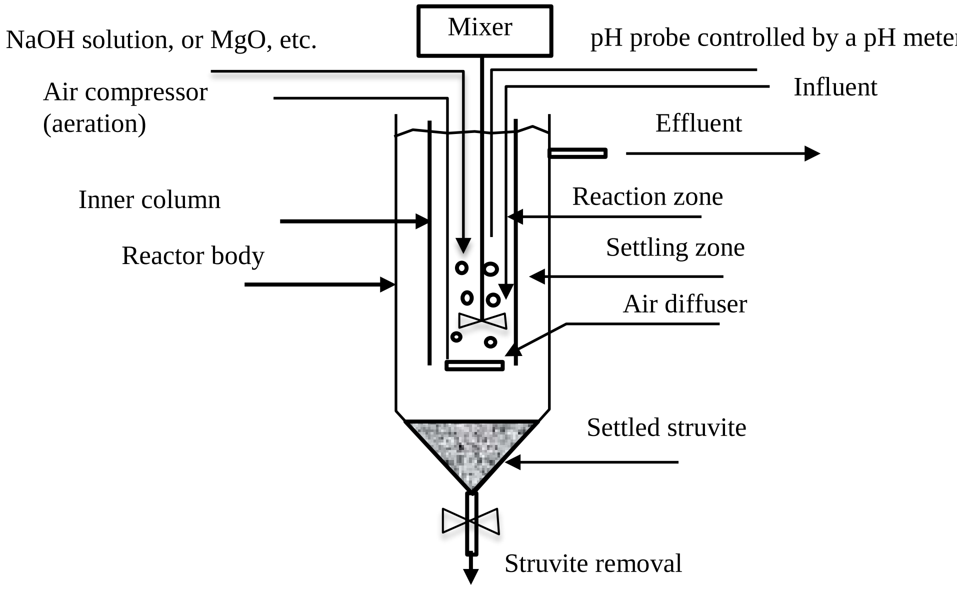

Figure 4. Schematic of the struvite precipitator to be used in the experiment

Experimental design: Since pH and the molar ratio of $Mg^{2+}/PO_4^{+}/NH_4^{+}$ in the liquid play a decisive role in struvite precipitation, a 4x4 factorial experimental design will be adopted to examine their interacting effect on the process performance. The four levels of $PO_4^{+}/Mg^{2+}/NH_4^{+}$ ratio to be tested are 1/1/1, 1.2/1/1, 1.4/1/1, and 1.6/1/1. These values are chosen based on past research results that a ratio of PO_4^{+}/Mg^{2+} should be greater than 1/1 to obtain struvite formation in swine wastewater. And the four values of pH to be tested are 8.7, 9.0, 9.3, and 9.6 based on the pH range reported in the literature (from 9.0 to 9.5) that produced good struvite precipitation for different wastewaters. The molar ratios of the ions in the influent will be adjusted to the experimental values before it enters the struvite precipitator, and further adjustment during the experiment can be made by adding MgO or $Mg(OH)_2$, if needed. Several airflow rates such as 5, 10, 15, and 20 L/min will be tried first to determine an appropriate aeration rate to be used in the experimental runs, and once started, aeration will be provided continuously. An influent flow rate of 2 L/min (may be adjusted if needed) will be employed to feed the struvite precipitator, leading to an HRT of 23 min. The struvite settled at the bottom of the precipitator will be removed periodically, depending upon quantity. The test will be repeated three times for each combination of the molar ratio of ions and pH and each test will be considered complete after 10 HRTs.

Sampling and analysis: Effluent samples from the struvite precipitator will be collected two times a day for analysis of concentrations of soluble forms of Mg^{2+}, PO_4^{+}, and NH_4^{+} for each experiment. The concentrations of other parameters in the samples will also be analyzed including pH, TS, TSS, TP, TN, COD, and VFAs. Reactive phosphorus, PO_4^{+}, will be analyzed using an ion chromatograph, while Mg^{2+} will be determined using the Hach calmagite colormetric method (Method 8030, Hach Co., Loveland, Colo.). NH_4^{+} will also be measured with a Hach test using the Nessler Method (Method 8038). The same methods presented early for analyzing wastewater chemical constituents will be applied to the rest of parameters here. The solid struvite product in triplicate for each treatment will be analyzed for N (as TKN) using the standard digestion and distillation method while the TP content will be determined by the X-ray Diffraction analysis. After each run, all the produced struvite will be dried and weighed for productivity comparison. Statistical *t* and *F* tests (ANOVA) will be used to determine the difference between the data collected at a significance level of $\alpha \leq 0.05$.

Methods for specific objective 5 (the integrated system)

Experimental apparatus: With all the component units of the proposed system for co-treating swine manure and sugar waste molasses developed and tested with their respective optimum operating conditions determined, the entire treatment system according to Figure 1 will be assembled for evaluation. The size of the each component will be determined based on their throughput capacities when integrated. The size change for the chemical scrubbers may also be achieved by increasing the feeding gas flow rate, which can be determined by preliminary trials. A centralized and integrated computer control system with software will be constructed to coordinately operate the treatment system in terms of controlling pH, temperature, influent and effluent flow rates, gas flow rates, pumps, mixers, and data logging according to the design for each individual unit.

Experimental design: The evaluation will be carried out in three aspects. First, the performance of the system in treating two wastewaters in reducing organic pollutants will be examined in terms of removals of COD, TN, TP, and solids and the throughput capacity. Second, the net energy recovery of the system will be evaluated according to energy produced less consumed. The energy consumed will include electricity used for heating and running all the component units, including the chemicals used (equivalent). The energy gained will include the energy contained in the final products such as the cleaned biohydrogen, biomethane, and struvite (equivalent). Also, the GHG (CO_2) removed through the treatment process will be considered a gain as opposed to the same amount of energy needed to remove it (1.36 kg CO_2 equiv./kWh). Third, the costs of constructing and operating the pilot-scale treatment system will be calculated based on which the potential revenues (values of different products) and the initial capital investment can be estimated.

Sampling and analysis: Liquid samples in triplicate of 100 mL each will be collected daily before (raw) and after the system treatment (from the struvite precipitator) at the same time for analysis of COD, TN, ammonium nitrogen, TP, dissolved phosphorus, TS, TSS, VSS, and pH according to the standard methods. Gas samples in triplicate will also be collected daily from the outlet of chemical scrubbers to determine the content of biohydrogen, biomethane, and CO_2 using gas chromatography. Total gas volumes produced from the biohydrogen and biomethane reactors will be continuously recorded using wet gas test meters throughout the experiment. The amount of struvite produced will be dried and measured every two days (or longer depending upon the volume produced each day). Statistical *t* and *F* tests (ANOVA) will be used to determine the time for the system to reach steady state and the system stability based on the difference between the data collected on consecutive days at a significance level of $\alpha \leq 0.05$.

5. **Results and Deliverables** - Describe in detail the expected outcomes of each of the results and deliverables.

Expected outcomes for specific objective 1 (biohydrogen fermenter)

It is expected that upon completion of specific objective 1, the optimal running parameters in terms of pH, HRT, and manure TS for the biohydrogen ASBR will be determined and the ASBR system will be successfully established to produce biohydrogen continuously and efficiently. Information obtained can be used to design the scale-up biohydrogen reactor in the pilot-scale system.

Deliverables include detailed data concerning the design and performance of the biohydrogen reactor in relation to the optimum values in the ratio of manure to sugar molasses, pH, and HRT. The scale-up reactor will be designed. A LCCMR progress report will be submitted.

Expected outcomes for specific objective 2 (biomethane digester)
The outcome of specific objective 2 will be the determination of the optimal combination of HRT and organic loading for the ASBR so that it can effectively treat biohydrogen fermenter effluent to produce biogas containing high methane content.

Deliverables include detailed performance information of the biomethane digester in biogas production under optimal operating conditions. The scale-up digester will be designed.

Expected outcomes for specific objective 3 (chemical scrubber)
A novel biogas-cleaning reactor will be developed and tested with its performance information obtained. The cleaned biohydrogen and biomethane can be used to produce a better combustion engine fuel, biohythane, to reduce CO_2 emissions.

Deliverables include detailed information related to the design, operation, and performance of the chemical scrubber in cleaning the biogas from both the biohydrogen fermenter and biomethane digester. A larger scrubber for the pilot-scale system will be designed and constructed. Information on production of "biohythane" using the cleaned biohydrogen and biomethane (biohythane is a mixture of 10% hydrogen, 60% methane, and 30% CO_2) and its tests in combustion engines will also be obtained. A LCCMR progress report will be submitted

Expected outcomes for specific objective 4 (struvite reactor)
The feasibility of using struvite formation to remove phosphorus and ammonium from the second stage digester effluent will be determined and information of developing a functional struvite precipitator will be obtained and can be used for scale-up applications.

Deliverables include detailed data describing the developed struvite reactor in terms of design, operation, and performance, based on which information on the scale-up reactor will also be generated and the reactor designed and constructed.

Expected outcomes for specific objective 5 (integrated system evaluation)
A complete, integrated treatment system to co-treat swine manure with sugar beet processing waste molasses to produce high quality biohydrogen, biomethane, and a slow release fertilizer will be successfully established and evaluated for performance, energy recovery, and costs. The technology will arrive at the stage of estimating capital cost and output for farm use.

Deliverables include all information related to the performance of the integrated system in treating the combined wastewater to produce biohydrogen, biomethane, and struvite. A lab-scale integrated treatment and energy production system will be running for demonstration. Educational activities such as workshops, seminars, and field trips will be organized and made available to all interested parties. A final project report to LCCMR will be submitted.

6. **Timetable** - Layout the proposed times for completing the proposed research including proposed dates for individual results and deliverables.

The timeline (3 years) and deliverables of the project are presented below.

Objectives/Tasks	Year 1				Year 2				Year 3			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Objective 1	***	***	***	***								
Objective 2			***	***	***	***						
Objective 3					***	***	***	***				
Objective 4							***	***	***	***		
Objective 5									***	***	***	***
Data processing			*	***			*	***			*	***
Refereed publication				**				**				**
Non-refereed publication				**				**				**
Demonstration												*
Reporting				*				*				*

* represents a period of one month.

7. **Budget** - Update the budget sheet from the original proposal based on the amount of funding recommended. Additional details can be added to the budget sheet to more fully describe the budget (The budget sheet is expandable so that additional information can be provided). Additional narrative on the budget can also be provided to more fully explain how the funds will be spent. The “Other Funding” section of the budget sheet should also be updated and include sufficient detail so that the source and amount of contribution is clear.

Please refer to the updated budget excel file for the details of budget and its narrative.

8. **Credentials** - Provide brief background of the principal investigators and cooperators who will carry out the proposed research and selected publications (targeted/abbreviated resumes are acceptable).

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<u>EDUCATION</u>

2006- 2009: **Ph.D.** in Bioproducts and Biosystems Engineering, University of Minnesota
2004- 2006: **M.S.** in Biosystems and Agricultural Engineering, University of Minnesota

2000- 2004: **B.S.** in Environmental Engineering, Wuhan University of Science and Technology, China

EMPLOYMENT HISTORY

2013- present: **Research Associate,** Department of Bioproducts and Biosystems Engineering, University of Minnesota

2012- 2013: **Post-doctoral research associate**, Department of Bioproducts and Biosystems Engineering, University of Minnesota

2010- 2012: **Post-doctoral research associat**e, Department of Chemical Engineering, Tsinghua University, China

2009- 2010: **Post-doctoral research associate**, Department of Bioproducts and Biosystems Engineering, University of Minnesota

2004- 2009: **Research Assistant**, Department of Bioproducts and Biosystems Engineering, University of Minnesota

AREAS OF EXPERTISE

Renewable Energy Engineering and Technologies

- Bioenergy and biohydrogen, nutrient cycling to produce value-added products, and bioconversion and bioremediation
- Biological reactor engineering, design, development, evaluation, and operation
- Bioenergy and bioresource recovery and conservation using renewable bio-based and organic waste materials
- Synthetic microbial consortia construction for simultaneous saccharification and fermentation/ microbial fuel cell from low-grade biomass/wastewater

Waste Management and Environmental Engineering

- Biological wastewater treatment systems and technologies including bioreactors, anaerobic digestion, fermentation, and aeration with emphasis on evaluation, research, development, improvement, and application of various treatment techniques
- Physical and chemical processes for animal manure and organic waste treatment and nutrient management
- Aeration for liquid manure odor control
- Environmental microbiology and biochemistry

RESEARCH PROJECTS

Renewable Energy Engineering

- **Synthetic ecology on hierarchical carbon electrodes for clean energy generation from biomass:** investigation of a concept, namely Synthetic Ecology, on a solid surface to achieve the synthesis of biohydrogen from waste biomass and in-situ conversion of it to electricity through co-growing microorganisms on a hierarchical carbon electrode

- **Simultaneous saccharification and fermentation of corn stover for hydrogen production by indigenous microbial consortia:** establishment of microbial consortia of cellulolytic bacteria with cellulosome and hydrogen-producing strains; study of synergic effect and metabolic network of each microbial consortium for hydrogen production; development of lab-scale mesophilic/thermophilic processes of hydrogen production by simultaneous saccharification and fermentation of corn stover

- **Biological hydrogen production from molasses with swine manure as a nitrogen source:** various pretreatment of seed sludge; process control of hydrogen production with and without swine manure supplement; statistical optimization of system operation; effect of carbon to nitrogen ratio on the hydrogen production; effect of swine manure supplement ratio on the hydrogen yield and generation rate

- **Biofuel cell development:** innovative biohydrogen-based biofuel cells for highly efficient and clean electricity generation using mixed wastewater feedstock
- **Biological hydrogen production from swine manure in anaerobic sequencing batch reactor system:** lab-scale anaerobic sequencing batch reactor construction; fermentative anaerobic sludge characterization; fermentation reactor process control; effect of pH, hydraulic retention time (HRT) and substrate concentration on hydrogen production; statistical and kinetic modeling of fermentative hydrogen production process; hydrogen-producing bacteria community analysis
  Dissertation: Fermentative hydrogen production from liquid swine manure with glucose supplement using an anaerobic sequencing batch reactor
- **Co-digestion of swine manure with crop residues:** Building and operation of anaerobic digester; process control of anaerobic digestion; enhancement of methane production by adding three different crop residues include wheat straw, oat straw and corn stalks; effect of carbon to nitrogen ratio on the methane productivity
- **Co-digestion of dairy wastewater with wasted milk for methane generation:** effect of milk content and carbon to nitrogen ratio on the methane production rate and yield in batch mode; removal of organic content in the digestion process
- **Lactic acid production with dairy manure wastewater as substrate:** development and evaluation of nutrient recovery and utilization from dairy manure; conversion of manure nitrogen into biomass and $_L$-lactic acid by *Rhizopus oryzae* under batch fermentation to evaluate the performance of the crude protein in dairy manure as a nitrogen source and develop a low-cost culture medium for lactic acid production

Environmental Engineering

Treatment of milking center wastewater by a sequencing batch reactor (SBR):
lab scale SBR reactor set-up; milking center wastewater characterization; aerobic bioreactor process control; aerobic sludge characteristics; real-time monitoring of process indicator parameters (dissolved oxygen, pH, oxidation-reduction potential (ORP)); effect of operational conditions on wastewater treatment efficiency which includes removal of solids, organic loading, grease, nitrogen and phosphorus; farm-scale SBR construction and application to a dairy farm for milking center wastewater treatment

- **Odor control of an open swine manure lagoon using surface aeration:** design of novel aerator modules with significantly improved oxygen transfer efficiency; farm-scale trial of a surface aeration system using the developed aerator module for odor emission control (reduction of BOD_5)
- **Biological and chemical treatment of swine manure for phosphorus removal:** investigation of the dynamic responses of different phosphorus fractions to the aeration treatment of liquid swine manure, including the phosphorus contained in biomass (represented by the cell DNA/RNA/poly-P), lipid, and protein, in both soluble and insoluble and organic and inorganic forms
- **Treatment of restaurant wastewater using a combined bioreactor system consisting of an upflow anaerobic sludge blanket (UASB) + an aerobic biofilm (AF):** startup, stability and anti-shock test of UASB and AF respectively; characterization of restaurant wastewater; operation and process control of the combined process; COD, nitrogen and phosphorus removal efficiency; investigation of anti-shock ability of the combined process
-

GRANT WRITING EXPERIENCE

- "Development of a novel technique to produce granular fertilizer from liquid swine manure and reduce water pollution", MN Legislature Rapid Agricultural Response Fund, 2014 (100% contribution)
- "A system to co-treat sweet corn processing wastewater and pig manure to produce bioenergy and reduce water pollution", MnDrive Postdoctoral Fellows, University of Minnesota, 2014 (funded, 100% contribution)
- "Develop Technologies That Co-Treat Swine Manure And Sugar Processing Wastewater To Produce Valuable Products And Reduce Water Pollution", NIFA/USDA, 2013 (80% contribution)
- "Lactic acid fermentation using dairy manure as the sole carbon and nitrogen source", Minnesota Legislative Initiative for Renewable Energy and the Environment (IREE), 2010 (funded, 60% contribution).
- "A System to Co-Ferment Sweet Corn Processing Wastewater with Liquid Swine Manure for Biohydrogen, Methane, and Algae Production", Minnesota Corn Grower Association, 2010 (80% contribution)
- "Biohydrogen-based biofuel cells: highly efficient and clean electricity generation using mixed wastewater feedstocks - a rural development project", Minnesota Legislative Initiative for Renewable Energy and the Environment (IREE), 2009 (funded, 50% contribution)
- "Co-digesting the wasted milk from dairy operations with cattle slurry to reduce water pollution", MN Legislature Rapid Agricultural Response Fund, 2009 (funded, 65% contribution)
- "Wet scrubbers for the recovery of NH3 emission from animal feeding operations for fertilizer", USDA/CSREES/NRI Air Quality Competitive Grant Program, 2008 (funded, 30% contribution)
- "Investigating the potential of enhancing CH4 productivity by co-digesting swine manure with crop residues as an external carbon source", MN Legislature Rapid Agricultural Response Fund, 2006 (funded, 25% contribution)

PUBLICATIONS

Refereed Journal Publications (selected)

1. **Wu, X.**, H. Lin, J. Zhu. 2013. Optimization of continuous hydrogen production from co-fermenting molasses with liquid swine manure in an anaerobic sequencing batch reactor. *Bioresource Technology* 136:351-359.
2. **Wu, X**., J. Zhu, C. Miller. 2013. Kinetics study of fermentative hydrogen production from liquid swine manure supplemented with glucose under controlled pH. *J. of Environ Sci. Health Part B* 48(6):477-485.
3. Lin, H.; **X. Wu**; C. Miller; J. Zhu. 2013. Improved performance of microbial fuel cells enriched with natural microbial inocula and treated by electrical current. *Biomass and Bioenergy* 54:170-180.
4. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. Miller. 2013. In vitro enzymatic conversion of γ-aminobutyric acid immobilization of glutamate decarboxylase with bacterial cellulose membrane (BCM) and non-linear model establishment. *Enzyme and Microbial Technology* 52(4-5):258-264.
5. Wang, L.; Z. Liu; T. Wang; **X. Wu**; C. Zhang; Qunhui Wang; X. Xing. 2013. Optimization of culture conditions for Clostridium cellulolyticum. *Shengwu Gongcheng Xuebao/Chinese Journal of Biotechnology* 29(3):392-402.

6. Liu, Z; C. Zhang; Y. Lu; **X. Wu**; L. Wang; L. Wang; B. Han; X. Xing. 2013. States and challenges for high-value biohythane production from waste biomass by dark fermentation technology. *Bioresource Technology* 135:292-303.
7. **Wu, X.**, C. Dong, W. Yao, J. Zhu. 2011. Anaerobic digestion of dairy manure influenced by the wasted milk from milking operations. *Journal of Dairy Science* 94(8): 3778-3786.
8. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. Miller. 2011. Enhanced Production of Glutamate Decarboxylase by Batch, Fed-Batch, and Repeated Batch Cultivations of Escherichia coli. *ASABE Biological Engineering Transactions* 4(4): 169-182.
9. Yao, W., **X. Wu**, J. Zhu, B. Sun. 2011. Comprehensive evaluation and selection of the potential complex medium for industrial glutamate decarboxylase (GAD) production by Escherichia coli. *Int. J. Agric. Biol. Eng.* 4(2): 74-82
10. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. Miller. 2011. System establishment of ATPS for one-step purification of glutamate decarboxylase from *E. coli* after cell disruption. *Applied Biochemistry and Biotechnology* 164(8):1339-1349.
11. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. Miller. 2011. Bacterial cellulose membrane - a new support carrier for immobilization of yeast for ethanol fermentation. *Process Biochemistry* 46(10): 2054-2058.
12. **Wu, X.**, J. Zhu. 2010. The effect of milk co-digested with dairy manure on biogas production and COD removal in batch processes. *J. of Environ Sci. Health Part A* 45(12):1543 - 1549.
13. **Wu, X**., J. Zhu, W. Yao. 2010. Effect of pH on continuous biohydrogen production from liquid swine manure in an anaerobic sequencing batch reactor. *International Journal of Hydrogen Energy*. 35(13):6592-6599.
14. **Wu, X.**, W. Yao, J. Zhu, C. Miller. 2010. Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology* 101(11):4042–4047.
15. Li, Y., J. Zhu, **X. Wu**, C. Miller, L. Wang, C. Dong. 2010. The effect of pH on continuous biohydrogen production from swine wastewater supplemented with glucose. *Applied Biochemistry and Biotechnology* 162(5): 1286-1296.
16. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. F. Miller. 2010. Utilization of protein extract from dairy manure as a nitrogen source by *Rhizopus oryzae* NRRL-395 for L-lactic acid production. *Bioresource Technology* 101(11): 4132–4138.
17. **Wu, X**., J. Zhu, C. Dong, C. Miller, Y. Li, L.Wang, W. Yao. 2009. Continuous biohydrogen production from liquid swine manure supplemented with glucose using an anaerobic sequencing batch reactor. *International Journal of Hydrogen Energy* 34(16): 6636 - 6645.
18. Yao, W., **X. Wu**, J. Zhu, B. Sun, C. F. Miller. 2009. L-lactic acid fermentation by *Rhizopus oryzae* using dairy manure as a nitrogen source. *Transactions of ASABE* 52(6): 2047-2054.
19. Zhu, J., Y. Li, **X. Wu**, C. Miller, P. Chen, and R. Ruan. 2009. Swine manure fermentation for hydrogen production. *Bioresource Technology* 100(22):5472-5477.
20. Zhu, J., **X. Wu**, C. Miller, F. Yu, P. Chen, and R. Ruan. 2007. Biohydrogen production through fermentation using liquid swine manure as substrate. *J. Environ. Sci. Health, Part B* 42 (4): 393-401.

JUN ZHU
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EDUCATION

Ph.D. in Agricultural Engineering - University of Illinois at Urbana-Champaign, 1995
M.S. in Civil Engineering - Zhejiang University, Hangzhou, China, 1985
B.S. in Civil Engineering, Zhejiang University, Hangzhou, China, 1982

EMPLOYMENT HISTORY

Professor with tenure (55% research, 15% teaching, 30% administration, Nov 2013 to present), Department of Biological and Agricultural Engineering, University of Arkansas
Professor with tenure (80% research, 20% extension, May 2009-Oct 2013), Department of Bioproducts and Biosystems Engineering, Southern Research and Outreach Center, University of Minnesota
Associate Professor with tenure (80% research, 20% extension, May 2004 – April 2009), Department of Bioproducts and Biosystems Engineering, Southern Research and Outreach Center, University of Minnesota
Assistant Professor (Tenure track, 80% research, 20% extension, February 1999 – April 2004), Department of Biosystems and Agricultural Engineering, Southern Research and Outreach Center, University of Minnesota
Research Associate (October 1997 – January 1999), Department of Biosystems and Agricultural Engineering, University of Minnesota
Post-Doctoral Associate (August 1995 – September 1997), Department of Agricultural and Biosystems Engineering, Iowa State University
Instructor (January 1997 – May 1997), Department of Agricultural and Biosystems Engineering, Iowa State University, Taught an Agricultural Systems Technology course titled “Waste Management Systems for Livestock Production” to undergraduate and graduate students (AST 475)
Research and Teaching Assistant (January 1991 – July 1995), Department of Agricultural Engineering, University of Illinois at Urbana-Champaign
Civil Engineer (1985-1990), the Building Design and Research Institute, Hangzhou, China

AREAS OF EXPERTISE

Renewable energy, waste management, and environmental engineering

- Biological systems, bioreactors, and physical and chemical processes for animal manure and organic waste treatment and nutrient management including evaluation, research, development, improvement, and application of various treatment techniques
- Bioresource recovery, bioenergy and biohydrogen, nutrient cycling to produce value-added products, and bioconversion and bioremediation
- Odor control techniques and applications; livestock manure odorous compounds determination; odor dispersion modeling
- Environmental microbiology and biochemistry

Corrosion engineering in industrial and agricultural facilities

- Corrosion mechanisms of metal products in industrial and agricultural facilities
- Environment control and management related to metal corrosion problems
- Applicable knowledge and hands-on experience in analyzing corrosion products and microbial colonization using energy dispersed X-ray scanning electron microscopy and X-ray photoelectron spectroscopy
- Principles and mechanisms of metal corrosion caused by microbes

Civil engineering

- Structural design for buildings, foundations, and bridges

- Applicable knowledge and hands-on experience in instrumentation and data acquisition for wood, concrete, and steel structural tests
- Finite element analysis; fluid, soil, and structural mechanics

ADVISING GRADUATE STUDENTS AND POSTDOCTORAL ASSOCIATES

Postdoctoral associates supervised

- Dr. Jianping Sun (2010 – 2011), current position: Associate Professor in the Department of Environmental Engineering, Zhejiang Shuren University, Hangzhou, China
- Dr. Xiao Wu (2009-2010, 2012 – 2013), Southern Research and Outreach Center at Waseca, University of Minnesota, USA
- Dr. Zhijian Zhang (2002 – 2004), current position: Associate Professor in the Department of Environmental Engineering, Zhejiang University, Hangzhou, China
- Dr. Pius M. Ndegwa (1999-2001), current position: Associate Professor in the Biological Systems Engineering Department at the Washington State University, USA
- Dr. Ancheng Luo (1999 – 2001), current position: Professor in the Department of Soil Science and Natural Resources, Zhejiang University, Hangzhou, China

Graduate students advising and co-advising

MS student

- Wu, Xiao, Biosystems and Agricultural Engineering Department, University of Minnesota (2006, advisor); Thesis title: Treatment of milking center wastewater using a sequencing batch reactor.
- Li, Yecong, Bioproducts and Biosystems Engineering, University of Minnesota (2007, advisor); Thesis title: Hydrogen production from liquid swine manure in dark fermentation.
- Hennessy, Kevin, Biosystems and Agricultural Engineering Department, University of Minnesota (2009, co-advisor); Thesis title: Developing a continuous system for the hydrothermal processing of wet biomass slurries.
- Sundareshwar, Ananth, Bioproducts and Biosystems Engineering, University of Minnesota (2008, co-advisor); Thesis title: Purification of digester gas using a pressure swing absorber.
- Yao, Wanying, Bioproducts and Biosystems Engineering, University of Minnesota (2009, advisor); Thesis title: Bio-converting the nutrients in dairy manure for $_{L}$-lactic acid production by *Rhizopus oryzae* NRRL 395.

PhD student

- Fei, Yu, Bioproducts and Biosystems Engineering Department, University of Minnesota (2007, co-advisor); Thesis title: Renewable energy from corn wastes by thermo-chemical conversion. Current position: Assistant Professor, Biological and Agricultural Engineering Department, Mississippi State University.

- Wu, Xiao, Bioproducts and Biosystems Engineering Department, University of Minnesota (2009, advisor); Thesis title: Fermentative hydrogen production from liquid swine manure supplemented with glucose using an anaerobic sequencing batch reactor. Current position: Research Associate in the Bioproducts and Biosystems Engineering Department, University of Minnesota.
- Dong, Chunying, Bioproducts and Biosystems Engineering Department, University of Minnesota (2009, advisor); Thesis title: Study on surface aeration to reduce odor generation potential. Current position: Associate Professor, Zhejiang University of Commerce, Hangzhou, China.
- Wang, Liang, Bioproducts and Biosystems Engineering Department, University of Minnesota (2009, advisor); Thesis title: A two-step fed sequencing batch reactor combined with pre-nitritation for treating swine wastewater. Current position: Associate Professor, Advanced Research Institute, Chinese Academy of Science Shanghai Branch, Shanghai, China.
- Li, Yecong, Bioproducts and Biosystems Engineering Department, University of Minnesota (2011, co-advisor); Thesis title: Cultivation of algae on municipal wastewater as potential feedstock for biodiesel production.
- Yao, Wanying, Bioproducts and Biosystems Engineering Department, University of Minnesota (2011, advisor); Thesis title: Glutamate decarboxylase production from *Escherichia coli* and in-vitro conversion of γ-amino-butyric acid (GABA). Current position: Research Assistant Professor, Biological and Agricultural Engineering Department, University of Kentucky.
- Han, Zhiying, jointly advising with the Department of Environmental Engineering, Zhejiang University, China (2010, co-advisor); Current position: Assistant Professor, Academy of Water Environment Research, Zhejiang University, Hangzhou, China.
- Lin, Hongjian, Bioproducts and Biosystems Engineering Department, University of Minnesota (August 2013, advisor); Current position: Post-doctoral Associate, Department of Bioproducts and Biosystems Engineering, University of Minnesota.

RESEARCH ACTIVITIES

Funded research proposals ($3,148,000)

Publications (only refereed publications are included)

Book chapters	1
Refereed journal publications:	112
Non-refereed publications (in the following subcategories):	124
Popular press and newsletters:	11

Book Chapter (1)

†Yao, W. J. Zhu, B. Sun, Chapter: One-step purification of glutamate decarboxylase from E. coli using aqueous two phase system. In: *Methods in Molecular Biology*. Editor Nikos Labrou. Springer Humana Press, New York, NY, USA. Accepted and in revision process.

Refereed Publications (112)

(†indicates that the author is either my postdoc, or graduate student, or visiting scholar)
*ASAE – American Society of Agricultural Engineers (the name was changed to “American Society of Agricultural and Biological Engineers” – ASABE – since 2006)

1. [†]Wu, X., H. Lin., J. Zhu. 2013. Optimization of continuous hydrogen production from molasses co-fermenting with liquid swine manure in an anaerobic sequencing batch reactor. *Bioresource Technology* 136: 351-359.
2. [†]Zhao, J., Z. Meng, X. Meng, J. Zhu, X. Yan, S. Ren. 2013. Technological conditions for treatment of high concentration swine manure by SBR. *Chinese J. of Environ. Eng.* 7(12): 4854-4860.
3. [†]Meng, X., Z. Meng, J. Zhao, J. Zhu, X. Yan, J. Liu. 2013. Effect of influent concentrations of ammoniacal nitrogen on SBR treatment for swine wastewater. *J. Agro-Environment Science* 32(8): 1656-1663.
4. Wang, S., X. Liang, G. Liu, H. Li, X. Liu, F. Fan, W. Xia, P. Wang, Y. Ye, L. Li, Z. Liu, J. Zhu. 2013. Phosphorus loss potential and phosphatase activities in paddy soils. *Plant Soil Environ.*59: 530-536.
5. [†]Yao, W., X. Wu, J. Zhu, B. Sun, C. Miller. 2013. In vitro enzymatic conversion of γ-aminobutyric acid immobilization of glutamate decarboxylase with bacterial cellulose membrane (BCM) and non-linear model establishment. *Enzyme and microbial technology* 52(4-5): 258-64.
6. [†]Lin H., X. Wu, C. Miller, J. Zhu. 2013. Improved performance of microbial fuel cells enriched with natural microbial inocula and treated by electrical current. *Biomass & Bioenergy* 54: 170-180.
7. [†]Wu, X., J. Zhu, C. Miller. 2013. Kinetics study of fermentative hydrogen production from liquid swine manure supplemented with glucose under controlled pH. *J. of Environ Sci. Health Part B* 48(6): 477-485.
8. Wang Z., S. Li, J. Zhu, Z. Zhang[†]. 2013. Phosphorus partitioning between sediment and water in the riparian wetland in response to the hydrological regimes. *Chemosphere* 90(8): 2288-2296.
9. Long, D., X. Tang, K. Cai, G. Chen, L. Chen, D. Duan, J. Zhu, Y. Chen. 2013. Cr(VI) reduction by a potent novel alkaliphilic halotolerant strain Pseudochrobactrum saccharolyticum LY10. *Journal of Hazardous Materials* 256-257: 24-32.
10. Hu B., M. Min, W. Zhou, Z. Du, P. Chen, J. Zhu, R. Ruan. 2012. Enhanced mixotrophic growth of microalga Chlorella sp. on pretreated swine manure for simultaneous biofuel feedstock production and nutrient removal. *Bioresource Technology* 126: 71-79.
11. [†]Sun, J., J. Zhu, W. Li. 2012. L-(+) lacic acid production by *Rhizopus Oryzae* using pretreated dairy manure as carbon and nitrogen source. *Biomass and Bioenergy* 47: 442-450.
12. [†]Sun, J., W. Li, P. Zheng, J. Zhu. 2012. Toxicity evaluation of antibiotics in piggery wastewater by luminescent bacteria. *Polish Journal of Environmental Studies* 21(3): 741-747.
13. [†]Dong, C., J. Zhu, X. Wu, C. Miller. 2012. Aeration efficiency influenced by venturi aerator arrangement, liquid flow rate, and depth of diffusing pipes. *Environmental Technology* 33(11): 1289-1298.
14. [†]Zhang Z., H. Wang, J. Zhu, S. Suneethi, J. Zheng. 2012. Swine manure vermicomposting via housefly larvae (Musca domestica): The dynamics of biochemical and microbial features. *Bioresource Technology* 118: 563-571.
15. Chen, X., K. Wendell, J. Zhu, J. Li, X. Yu, Z. Zhang[†]. 2012. Synthesis of nano-zeolite from coal fly ash and its potential for nutrient sequestration from anaerobically digested swine wastewater. *Bioresource Technology* 110: 79-85. (3)
16. Rahman, S., D. Lin, and J. Zhu. 2012. Greenhouse gas (GHG) emissions from mechanically ventilated deep pit swine gestation operation. *J. Civil Environ. Eng.* 2(1): 1-7. doi:10.4172/2165-784X.1000104. (3)
17. Li, H, Z. Zhang[†], H. Wang, X. Xu, Y. Lin, J. Zhang, J. Zhu. 2011. Biological phosphorus removal performance and relevant microorganism characteristics of activated sludge in

municipal wastewater treatment plants, China. *Water Environment Research* 83(7):594-602. (1)
18. †Li, Y., Y. Chen, P. Chen, M. Min, W. Zhou, B. Martinez, J. Zhu, R. Ruan. 2011. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology* 102(8):5138-5144. (24)
19. †Yao, W., X. Wu, J. Zhu, B. Sun, C. Miller. 2011. Enhanced Production of Glutamate Decarboxylase by Batch, Fed-Batch, and Repeated Batch Cultivations of *Escherichia coli*. *ASABE Biological Engineering Transactions* 4(4): 169-182.
20. †Yao, W., X. Wu, J. Zhu, B. Sun. 2011. Comprehensive evaluation and selection of the potential complex medium for industrial glutamate decarboxylase (GAD) production by *Escherichia coli*. *Int. J. Agric. Biol. Eng.* 4(2): 74-82.
21. †Yao, W., X. Wu, J. Zhu, B. Sun, C. F. Miller. 2011. System establishment of ATPS for one-step purification of glutamate decarboxylase from *E. coli* after cell disruption. *Applied Biochemistry and Biotechnology* 164(8):1339-1349.
22. †Yao, W., X. Wu, J. Zhu, B. Sun, Y. Zhang, C. F. Miller. 2011. Bacterial cellulose membrane - a new support carrier for immobilization of yeast for ethanol fermentation. *Process Biochemistry* 46(10): 2054-2058. (5)
23. †Wu, X., C. Dong, W. Yao, J. Zhu. 2011. Anaerobic digestion of dairy manure influenced by the wasted milk from milking operations. *Journal of Dairy Science* 94(8): 3778-3786. (1)
24. †Han Z., J. Zhu, Y. Ding, W. Wu, Y. Chen, and R. Zhang. 2011. Effect of feeding strategy on the performance of sequencing batch reactor with dual anoxic feedings for swine wastewater treatment. *Water Environment Research* 83(7): 643-649. (0)
25. †Zhang, Z., H. Li, J. Zhu, W. Liu, X. Xu. 2011. Improvement strategy on enhanced biological phosphorus removal for municipal wastewater treatment plants: Full-scale operating parameters, sludge activities, and microbial features. *Bioresource Technology* 102(7): 4646-4653. (4)
26. †Wang, L., J. Zhu, C. Miller. 2011. The stability of accumulating nitrite from swine wastewater in a sequencing batch reactor. *Appl. Biochem. Biotechnol*. 163(3): 362-372. (2)
27. †Wu, X., W. Yao, J. Zhu, C. Miller. 2010. Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology* 101(11):4042-4047. (37)
28. †Wu, X., J. Zhu. 2010. The effect of milk co-digested with dairy manure on biogas production and COD removal in batch processes. *J. Environ. Sci. Health, Part A* 45(12): 1543-1549. (0)
29. †Wang, L., J. Zhu. 2010. Shortcut nitrification and denitrification of swine wastewater in a sequencing batch reactor system. *Trans ASABE* 53(3): 813-818. (1)
30. †Li, Y., J. Zhu, X. Wu, C. Miller, L. Wang. 2010. The Effect of pH on continuous biohydrogen production from swine wastewater supplemented with glucose. *Applied Biochemistry and Biotechnology* 162(5): 1286-1296. (4)
31. Mukhtar, S., M. S. Borhan, S. Rahman, J. Zhu. 2010. Evaluation of a field-scale surface aeration system in an anaerobic poultry lagoon. *Applied Engineering in Agriculture* 26(2): 307-318.
32. †Wu, X., W. Yao, J. Zhu. 2010. Effect of pH on continuous biohydrogen production from liquid swine manure with glucose supplement using an anaerobic sequencing batch reactor. *International Journal of Hydrogen Energy* 35(13): 6592-6599. (7)
33. †Wu, X., W. Yao, J. Zhu, C. Miller. 2010. Biological and chemical phosphorus fractionalization in swine manure under aeration. *J. Environ. Sci. Health, Part B* 45(4): 293 – 299. (0)
34. †Yao, W., X. Wu, J. Zhu, B. Sun, C. F. Miller. 2010. Utilization of protein extract from dairy manure as a nitrogen source by *Rhizopus oryzae* NRRL-395 for L-lactic acid production. *Bioresource Technology* 101(11): 4132-4138. (4)

35. [†]Wang, L., Y. Li, P. Chen, M. Min, Y. Chen, J. Zhu, R. R. Ruan. 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technology* 101(8): 2623-2628. (66)
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9. **Dissemination and Use** – Describe how the findings of the research will be disseminated and describe the expected audience and potential use.

Successful publication and dissemination of project findings will be a key component of this proposal to maximize its impact, which will be achieved by sharing information with not only the scientific community but also the general public on a timely basis. In order for this to happen, the research outcomes will be presented in both technical and non-technical formats, including refereed journal publications for pundits and other outlets for lay people, aiming at distributing the information of this project not just in Minnesota but across the nation, and the world as well. Quantitatively, starting from the end of first year of the project, at least one manuscript will be generated and submitted for possible publication in refereed journals annually on the findings gained from the project. Concomitantly, two to three non-refereed publications will also be generated and published in trade magazines such as *The National Hog Farmer*, *Pork,* and *The Sugarbeet Grower Magazine*, or presented at technical symposia and professional conferences. In addition, a project *Newsletter* will be developed providing up-to-date information on the status of the project and made available to LCCMR commission members as well as all other concerned parties. The target audience of the outcome of this project include, but not limited to, hog and sugar beet processors, agricultural engineers and consultants, state regulatory agencies, renewable energy industries, and the general public, within the state and across the country. A special field day for people in the concerned industries and the stakeholders will be organized at the end of the project to demonstrate the complete system for co-treating swine manure and sugar processing waste molasses. In the meantime, talks will be initiated to those interested in adopting the newly developed technology on their farms or plants to benefit their production and protect the environment. Finally, under the same token, the obtained information will potentially be included in the teaching materials for two courses, BBE4713/5713, Biological Process Engineering; BBE4733/5733, Renewable Energy Technologies, to educate our future scientists/engineers in a long run.