2014 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Clean Water and Renewable Energy from Beet Processing Wastewater and Manure
PROJECT MANAGER: Shaobo Deng
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 08f

APPROPRIATION AMOUNT: $400,000
AMOUNT SPENT: $396,193
AMOUNT REMAINING: $3,807

Overall Project Outcomes and Results

This project has developed a novel treatment system to co-treat swine manure with sugar beet processing wastewater and produce biohydrogen, biomethane, and a slow release fertilizer (struvite). Overall outcomes and results can be summarized in four parts:

1) A continuous biohydrogen and biomethane production process, taking swine manure with sugar beet processing wastewater as feedstock, has been investigated in a two-stage anaerobic sequencing batch reactor (ASBR) system. Three significant operational parameters (pH, hydraulic retention time (HRT), and total solids level (TS) of the swine manure) were identified and the optimal condition was determined to be pH 5.3, HRT 16.5h, and TS 0.78% for the biohydrogen stage with highest yield at 1.52 mol-H_2/mol glucose, while pH 6.9 and HRT 51h was determined for the highest biomethane yield of 0.21 g-CH_4/g-COD-feed.

2) A wet-scrubbing biogas purification system for cleaning both biohydrogen and biomethane gases was investigated with optimal gas flow rate at 0.84L/min and 1.32L/min, with 0.3M and 0.2M NaOH solution, 6 min and 9 min replacing interval, respectively, to achieve complete CO_2 removal;

3) A struvite precipitation reactor system with pH 9.0, Mg^{2+}/PO_4^{3-} molar ratio at 1.5, air flow at 2 LPM for 30 mins determined for efficient struvite formation of 650 mg per liter upstream waste;

4) An integrated system including the above three units has achieved a daily production of 7.8L/d H_2, 28.3L/d CH_4, and 1.95g/d struvite, with removal efficiency of TS, COD, TN and TP at 49.6, 76.5%, 65.8% and 76.8%, respectively, with a negative net energy gain due to the small system size and relatively high running cost.

A pilot scale system with 1000-gallon daily loading was estimated to have a positive energy gain of 630 kWh/d and the payback period for its capital and running cost will be nearly 2 years not considering maintenance cost.

Project Results Use and Dissemination

Results produced from this project has been incorporated in teaching material for courses: BBE 4733/5733 – Renewable Energy Technologies at Department of Bioproducts and Biosystems Engineering, University of Minnesota, lectured by Xiao Wu, 2015-2016; BE 461 – Bioprocessing Engineering at Department of Biological Engineering, University of Idaho, lectured by Xiao Wu, 2017.

An oral presentation has been made at ASABE 110th Annual International Meeting, with paper#: #1701057, at Spokane, Washington. July 16-19, 2017. Presenters: Wu, S., S. Deng, J. Zhu. Title: Hydrogen and methane production from swine manure and sugar beet wastewater by a two-step ASBR system.

Dissemination of this project will continue with publishing refereed and non-refereed articles, talking to people in the concerned industries and the stakeholders, developing teaching materials in college and graduate levels, demonstration of the complete system for co-treating swine manure and sugar processing waste molasses and education of stake holders and general public, etc.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan Final Report

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Date of Report: August 14, 2017

Final Report

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2017

PROJECT TITLE: Clean Water and Renewable Energy from Beet Processing Wastewater and Manure

Project Manager: Shaobo Deng

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Location: Waseca, Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$400,000**
	Amount Spent:	**$396,193**
	Balance:	**$3,807**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 08f

Appropriation Language:
$400,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota for the Southern Research and Outreach Center in Waseca to research the co-fermentation of sugar beet processing wastewater and swine manure for hydrogen and methane production and to install and evaluate a pilot-scale system. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Clean Water and Renewable Energy from Beet Processing Wastewater and Manure

II. PROJECT STATEMENT:

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 these waste streams environmentally and resourcefully other than land application, which, over the years, is linked to environmental issues in many places in the state due to the buildup of nutrients (nitrogen and phosphorus) in the soil receiving these two wastes, increasing the potential of surface and ground water pollution caused by 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. In fact, the two wastes, i.e., sugar beet processing wastewater (containing molasses) and pig manure, have complementary nutrients, one having residual sugar which is an ideal carbon source highly needed in biological processes such as fermentation and anaerobic digestion, while the other having all the other nutrients but sugar for biological activities. Therefore, this proposal is aimed at using these two waste streams with complementary nutrients to produce bioenergy and reduce their negative impact on water resources. There are two sugar beet processing companies in MN (American Crystal Sugar Company and Southern Minnesota Sugar Cooperative) that produce all the sugars (the former has three facilities located in East Grand Forks, Moorhead, and Crookston, while the latter has one facility located in Renville, MN). There are also a total of around 4700 hog farms across the state but concentrated in the southern counties in Minnesota (see the attached concentration map). The proposed research project will address the environmental issues and produce bioenergy as indicated below.

- Land application of over 1 million tons of sugar processing wastewater threatens water resource
- Land application of over 11 million tons of pig manure increases surface and groundwater pollution
- $10 million can otherwise be saved for sugar beet processers annually for wastewater treatment
- Bio-electricity of 143 million kWh ($9.72 million) can be produced yearly from the two waste materials (at a ratio of 5 (pig manure) to 1 (sugarbeet wastewater))
- An alternative use of the produced hydrogen and methane is to produce “biohythane”, which is a better combustion engine fuel that can cut down on greenhouse gas emission by 57% when used in combustion engines (equivalent to 27 million gallons of diesel that can be produced annually); biohythane is a mixture of 10% hydrogen, 60% methane, and 30% carbon dioxide, which can be used for combustion engines on the farm, such as tractors, etc.
- 15,560 tons of ammonia/phosphate fertilizer (struvite) will also be produced annually ($5 mil value)

The proposed system flow chart is shown in figure 1 in the appendix. The specific objectives of this proposal will include 1) determining the optimal operating values of swine manure to molasses ratio, hydraulic retention time (HRT), and pH for a biohydrogen fermenter to maximize biohydrogen production; 2) determining the optimal operating values of organic loading rate, pH and HRT for an anaerobic sequencing batch reactor (ASBR) biomethane digester receiving the effluent from the biohydrogen fermenter to maximize biomethane generation and for the effectiveness in chemical oxygen demand (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 results of 1)-4), building and evaluating an integrated system consisting of a biohydrogen fermenter, a ASBR biomethane digester, a CO_2 removal reactor, and a struvite precipitator to co-treat swine manure and sugar waste molasses and harvest the biogases and fertilizer.

III. PROJECT STATUS UPDATES:

Project Status as of January 1, 2015:

The work accomplished so far has been focused on the Activity 1 and followed the schedule as planned. A software-controlled bench-scale hydrogen fermenter was constructed and successfully started up and operated for continuous hydrogen production from co-fermentation of waste molasses and swine manure wastewater. Two significant operational parameters (pH and HRT) for the biohydrogen reactor system, each tested for five levels, have been optimized in terms of hydrogen production rate (HPR), hydrogen content of the biogas (HC), and hydrogen yield (HY). The third significant operational parameter, solid content of the substrate (TS%), will be tested and optimized for the hydrogen production process as the next step. After that the optimal combined condition for hydrogen production will be obtained using a central composite design (CCD) coupled with response surface methodology. As the second part of Activity 1, construction of the methane digester is underway and the experimental protocol for methane production from the effluent of hydrogen fermenter is under development. It is expected that rest of the Activity 1, and Activity 2 and 3 of the project will stay on schedule and proceed as planned.

Project Status as of July 1, 2015:

The work accomplished during the past 6 months has been focused on the Outcome 2 and 3 of Activity 1 and followed the schedule as planned. The continuous hydrogen production from co-fermentation of waste molasses and swine manure wastewater was successfully conducted and compared at 20 different operational conditions to optimize the H2 fermentation process. Three significant operational parameters (pH, HRT and total solids rate in substrate (TS%)) for the biohydrogen reactor system, each tested for five levels, have been optimized in terms of hydrogen production rate (HPR), hydrogen content of the biogas (HC), and hydrogen yield (HY), using a central composite design (CCD) coupled with response surface methodology. As the second part of Activity 1, construction of the methane digester has been finished and the methane production reactor has been started up with the effluent of hydrogen fermenter as substrate. For the next step, the optimal condition for continuous biomethane production will be determined and the experimental protocol for biogas purification of Activity 2 is under development. It is expected that rest of the Activity 1, and Activity 2 and 3 of the project will stay on schedule and proceed as planned.

Project Status as of January 1, 2016:

The work accomplished during the past 6 months has been focused on the Outcome 4 of Activity 1, Optimal condition for continuous biomethane production, and the Outcome 1 of Activity 2, Biogas cleaning reactor setup, followed the schedule as planned. First, the continuous biomethane production from the discharge effluent from the hydrogen fermentation of waste molasses and swine manure wastewater using an ASBR reactor was successfully conducted and the methane production performance was compared at 13 different operational conditions to optimize the biomethane production process. Two significant operational parameters (pH, HRT) for the ASBR digester system, each tested for five levels, have been optimized in terms of methane production rate (MPR), methane content of the biogas (MC), and methane yield (MY), using a central composite design (CCD) coupled with response surface methodology. Second, As the third part of Activity 1, construction of the chemical scrubber for biogas cleaning has been finished and tested with synthetic biogas composing hydrogen and carbon dioxide. For the next step, the operation and optimal condition for continuous biogas cleaning will be determined and the experimental protocol for struvite production of Activity 2 will be established. Activity 1 has been finished upon finishing this report. It is expected that rest of the Activity 2 and 3 of the project will stay on schedule and proceed as planned.

Project Status as of July 1, 2016:

The work accomplished during the past 6 months has been focused on the Outcome 2 and 3 of Activity 2, i.e., optimal conditions for CO_2 removal from biogas to get pure H_2 gas, and setup of struvite precipitator as the last treatment step in the system. The activities are on schedule as planned. First, the synthetic gas mixture simulating biogas from hydrogen fermenter was successfully purified by the wet gas scrubber with 0.2 M NaOH solution, tested with 1000 psi liquid pump pressure and various biogas flow rate to achieve five different ratios of gas to liquid flow rate and find the best value for CO_2 removal. Two significant operational parameters (NaOH concentration and changing time) in the continuous gas cleaning phase, each tested for five levels, have been

optimized in terms of CO_2 removal rate, using a central composite design (CCD) coupled with response surface methodology. Second, construction of the struvite precipitator has been finished and tested with effluent from digester. For the next step, the operation and optimal condition for continuous biomethane cleaning as well as for struvite production will be determined.

Amendment Request (08/01/2016):

We are requesting a change in Project Manager due to Dr. Xiao Wu's departure from the University of Minnesota (taking a faculty position with the University of Idaho effective August, 2016). Because of project's importance and the significant impact on the Minnesota agriculture, economy, and environment upon its completion, we are requesting your approval of changing the Project Manager for this proposal and proposing Mr. Shaobo Deng to replace Dr. Wu and take full responsibility in execution of the project in its entirety, effective August 15th, 2016. That being said, Dr. Wu will continue to provide oversight to the project as an external co-PM and assist with reporting. In addition, Dr. Forrest Izuno, the department head at SROC, will join the team as a co-PM to ensure that the project will continue to be carried out as planned and completed fully and on time.

Mr. Shaobo Deng is a research fellow/engineer who has been working on this project since the very beginning. In the last two years, Shaobo was involved from setting up experimental apparatus to working with producers to conducting research and to reporting. He not only executed the planned works, but also brought and developed innovative process for the system. With 14 years of working experience in renewable energy and environmental protection in the University of Minnesota, Mr. Deng possesses the expertise and technical know-how to continue this project to its conclusion. He currently holds a non-tenure track, Professional and Academic faculty position in the University of Minnesota Southern Research & Outreach Center at Waseca, MN, the site for the originally proposed project. According to the University of Minnesota policy, a P&A faculty member is qualified to serve as an independent Principal Investigator on grant applications, which means that Mr. Deng can technically assume the PM's position for this project. With our commitment to the project and constant collaboration in the remaining time for the project, we truly believe that the project will be completed successfully. As we understand, this change of PM will not incur any budgetary and/or contact changes for this project.

Amendment approved: [08/18/2016]

Project Status as of March 24, 2017:

The work accomplished during the past 6 months has been focused on the Outcome 2 and 4 of Activity 2, i.e., optimal conditions for CO_2 removal from biogas to get pure CH_4 gas, and optimization of struvite precipitation from the digester effluent as the last treatment step in the system. First, the synthetic gas mixture simulating biogas from methane digester was successfully purified by the wet gas scrubber with 0.2 M NaOH solution, tested with 600 psi liquid pump pressure and various biogas flow rate to achieve five different ratios of gas to liquid flow rate and find the best value for CO_2 removal. The best purification capacity of the NaOH scrubber is 78 liters of biogas per hour with a flow rate NaOH solution of 4.4 liters per hour. Two significant operational parameters (NaOH concentration and changing time) in the continuous gas cleaning phase, each tested for five levels, have been optimized in terms of CO_2 removal rate, using a central composite design (CCD) coupled with response surface methodology. Optimization of struvite precipitation from digester effluent has been conducted in batch experiments. The optimal condition of operational parameters of pH, air flow rate, mixing time, and Mg^{2+}/PO_4^{3-} molar ratio has been determined as 9.0, 0.6 L/min, 1 hour, and 1.5/1, respectively. Continuous production of struvite will be investigated in the next step and struvite accumulation on the surface of stainless steel, wood and rubber, respectively will be evaluated to achieve the most effective harvest of struvite.

Amendment Request (05/23/2017):

We are proposing several budget shifts between activities and budget categories. Below is the justification and plans for the budget change:

1. The use of university vehicles and funds for in-state traveling throughout the project has resulted in savings on Travel expenses. Savings on Traveling for activity 1, 2 and 3 ($3000+$2000+$800=$5800) are shifted to the personnel budget and will be spent on two new hires to accomplish the research work of activity 2 and 3 on time.

2. Savings on Sample analysis due to the in-lab analysis available are shifted to Personnel and Purchasing parts and component. Savings on Sample analysis in the Equipment/Tools/ Supplies category for Activity 2 is $18000-$2500=$15500, in which $4200 is shifted to personnel and $11300 is moved to the sub-category of Purchasing parts and component for Activity 3. Savings of $9000-$1000= $8000 on Sample analysis in the Equipment/Tools/ Supplies category for Activity 3 is also shifted to sub-category of Purchasing parts and component for Activity 3, and will be dedicated for building the proposed integrated treatment system. The extra budget needed for purchasing parts and components for Activity 3 is because of the upgrading of the bioreactor and control system, which will ensure more reproducible operation, and higher system stability and productivity. A total of $10,000 is shifted into Personnel and $19,300 is shifted to Purchasing parts and component for Activity 3.

3. The total budget of $400,000 for the overall project remains unchanged.

Amendment Approved: [06/01/2017]

Overall Project Outcomes and Results:

This project has developed a novel treatment system to co-treat swine manure with sugar beet processing wastewater and produce biohydrogen, biomethane, and a slow release fertilizer (struvite). Overall outcomes and results can be summarized in four parts: 1) A continuous biohydrogen and biomethane production process, taking swine manure with sugar beet processing wastewater as feedstock, has been investigated in a two-stage anaerobic sequencing batch reactor (ASBR) system. Three significant operational parameters (pH, hydraulic retention time (HRT), and total solids level (TS) of the swine manure) were identified and the optimal condition was determined to be pH 5.3, HRT 16.5h, and TS 0.78% for biohydrogen stage with highest yield at 1.52 mol-H_2/mol glucose, while pH 6.9 and HRT 51h was determined for the highest biomethane yield of 0.21 g-CH_4/g-COD-feed. 2) A wet-scrubbing biogas purification system for cleaning both biohydrogen and biomethane gases was investigated with optimal gas flow rate at 0.84L/min and 1.32L/min, with 0.3M and 0.2M NaOH solution, 6 min and 9 min replacing interval, respectively, to achieve complete CO_2 removal; 3) A struvite precipitation reactor system with pH 9.0, Mg^{2+}/PO_4^{3-} molar ratio at 1.5, air flow at 2 LPM for 30 mins determined for efficient struvite formation of 650 mg per liter upstream waste; 4) An integrated system including the above three units has achieved a daily production of 7.8L/d H_2, 28.3L/d CH_4, and 1.95g/d struvite, with removal efficiency of TS, COD, TN and TP at 49.6, 76.5%, 65.8% and 76.8%, respectively, with a negative net energy gain due to the small system size and relatively high running cost. A pilot scale system with 1000-gallon daily loading was estimated to have a positive energy gain of 630 kWh/d and the payback period for its capital and running cost will be nearly 2 years not considering maintenance cost.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Biohydrogen and biomethane production processes development
Description: Anaerobic sequencing batch reactor (ASBR) systems for biohydrogen and biomethane production from the two waste streams will be built at the University of Minnesota Southern Research and Outreach Center (SROC) at Waseca. The reactor size for hydrogen generation will be 4 L with a working volume of 2 L, which will

be completely mixed with pH controlled. The influent and effluent flows will be regulated in order to adjust the hydraulic retention time (HRT, h). Mixing, pH controller and pumps will be controlled by a programmable control module with its software capable of repeating a programed cycle operation in a time sequence. 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. The ASBR setup for biomethane production will be similar to that of the hydrogen-producing reactor, only with larger reactor size of 10L with a working volume of 5L. The effluent from biohydrogen reactor will be used as influent for biomethane reactor. Reactor systems will be startup and operated for stable and continuous operation.

The reactors will be seeded with biohydrogen/methane-producing sludge obtained from a local wastewater treatment plant/anaerobic digester. Gas and liquid samples will be collected at respective sampling ports. The amount of gas produced will be recorded using a wet gas meter. Gas sampling will be conducted every day and gas analysis will include biohydrogen, biomethane, and CO_2 using a gas chromatography. The liquid samples will be taken at the same time intervals as in gas sampling 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.

Key operational parameters for hydrogen production (pH, HRT, substrate TS level) and methane production (pH, HRT), each with 5 levels will be tested to determine the optimal combination conditions for a particular substrate. Optimum values of pH, HRT and TS level for the ASBR system in terms of biogas production rate (L/d/L), hydrogen/methane production rate (L/d/L), hydrogen/methane content (%) and hydrogen/methane yield (L/g major substrate) will be obtained using a central composite design (CCD) coupled with response surface methodology. Second order (quadratic) models for each response variable will be established for predicting the response value for different operational conditions.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 172,375
Amount Spent: $ 172,735
Balance: $ 0

Activity Completion Date: Dec. 31, 2015

Outcome	Completion Date	Budget
1. ASBR reactor setup for biohydrogen production finished	Oct. 1, 2014	$30,013
2. Optimal condition for continuous biohydrogen production reported	June 30, 2015	$50,242
3. ASBR reactor setup for biomethane production finished	June 30, 2015	$50,001
4. Optimal condition for continuous biomethane production reported	Dec. 31, 2015	$45,479

Activity Status as of January 1, 2015:

Outcome 1. ASBR reactor setup for biohydrogen production

A lab-scale anaerobic sequencing batch reactor (ASBR) system has been built at the University of Minnesota Southern Research and Outreach Center (SROC) at Waseca. The reactor body was a polyethylene jar, 4L in total volume with a working volume of 2L. The reactor was wrapped with foam foil insulation and 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 was achieved by using a peristaltic pump circulating the liquid through the reactor assisted by an internal magnetic stirrer. Mixing also reduced biohydrogen inhibition on the bacteria generating biohydrogen. The pH inside the reactor was controlled by a pH controller that adjusts the liquid pH by turning on and off two peristaltic pumps that add either hydroxide (supplied as 1.0M NaOH) or acid (supplied as 1.0N HCl) to the reactor. The prepared influent (mixture of manure/sugar waste molasses) as substrate was 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 were regulated by peristaltic pumps in order to adjust the hydraulic retention time (HRT, h), which was calculated as working volume divided by volume of content goes into/out from the reactor per hour. A computer was used including a programmable control module (Campsci CR1000) with its software (Campsci PC400) installed capable of repeating a programed cycle operation in a time sequence.

The reactor was 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 was to inactivate thermal-susceptible methane-producing species and other non-biohydrogen-producing bacteria in the sludge. The ASBR was running on 4-h cycles with about 1 min each for feeding and discharging, 30 min for settling, and 208 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, after reaching high performance stability of the reactor system, an extended range of pH (4.5, 5.0, 5.5, 6.0, and 6.5) and HRT (8, 12, 16, 20, and 24h) was investigated to determine the optimal combinations corresponding to high biohydrogen production.

Gas and liquid samples have been collected at respective sampling ports. The amount of gas produced was recorded daily using a wet gas test meter and the gas was released continuously to keep a low biohydrogen partial pressure in the headspace. Gas sampling was conducted every day and content of hydrogen, biomethane, and CO_2 in the biogas was analyzed using a gas chromatography (Varian 3800). The liquid samples were 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.

After several trials, our system achieved highly stable hydrogen production after a short startup period of 12 days indicated by variation of hydrogen production rate within 5% at HRT of 24h. Operation at progressively decreasing HRTs from 24 to 8h gave rise to an increasing biogas production rate from 15.2-34.4L/d, while good linear relationships were observed between both total biogas and hydrogen production rates correlated to HRT. The maximum hydrogen yield of 1.63 mol-H_2/mol-glucose-feed occurred at HRT of 16h, with carbohydrates utilization efficiency over 98%, considerable hydrogen production rate up to 3.6 L/d/L and hydrogen percentage of off-gas up to 43% (i.e., a CO_2/H_2 ratio of 1.2) with the absence of CH_4 production throughout the whole course of experiment at a pH of 5.5 strongly validated the feasibility of the fermentative H_2 production from liquid swine manure using an ASBR system. Ethanol as well as acetic, butyric acids were produced in the system accompanying the hydrogen production, with acetic acid being the dominant one, which contributed to 56-58% of the total soluble metabolite production, indicative of an acetic acid fermentation system, and acetate-to-butyrate ratio was found to be closely related to hydrogen yield.

PH level influenced every aspect of the ASBR performance for hydrogen production. ASBR operation at five pHs ranging from 4.5 to 6.5 (4.5, 5.0, 5.5, 6.0, 6.5) showed distinct dynamic profiles of both biogas production and the changes of H_2 and CH_4 percentage in the biogas during a running period of 22 days. The H_2 content in biogas, H_2 production rate and H_2 yield were all pH-dependent, in the range of 5.1-36.9%, 0.18-2.25 L/d/L and 0.12-1.60 mol-H_2/mol-glucose, respectively, and maximum values for all three responses were simultaneously achieved at pH 5.5. Methanogens appeared to be significantly activated at pH of 5.5 or higher since significant CH_4 evolution and concurrent reduction in H_2 production was observed at pH 5.0 and 6.0. Acetate, propionate, butyrate, and ethanol were main aqueous products in all pH tests and their distribution was influenced by pH.

Later, the amount of sugar waste molasses added to swine manure (the mixing ratio) will also be a variable in this study. Five ratios of manure to molasses (0.25, 0.5, 0.75, 1.0, and 1.25%) will be tested based on our initial test. 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 manure TS range selected is typical for the liquid swine manure from pit recharge and/or flushing systems. To avoid a complete three-level factorial design (5x5x5 test runs, at least 3 weeks per run) 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, X1; (ii) HRT, X2 (h), and (iii)

manure TS, X3 (%). 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 can be obtained by solving the quadratic regression equation. It is expected that upon completion of Activity 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.

Activity Status as of July 1, 2015:

Outcome 2. Optimal condition determination for continuous biohydrogen production

With the data from the central composite design (CCD) experiments, the quadratic model built in the Design Expert statistical software was able to fit all the response variables in our experiments. The model fitting results, including ANOVA analyses of the fitted equations were obtained with respect to biogas production rate (BRP), hydrogen production rate (HPR), hydrogen content in biogas (HC), and hydrogen yield (HY).

Quadratic modeling results for BPR, HPR, HC, and HY, respectively, were obtained based on CCD design in terms of three variables: (i) pH, X1; (ii) HRT=hydraulic retention time, X2 (h), and (iii) manure TS= total solids content in the diluted swine manure without sugar wastewater molasses, X3 (%). All the coefficients for different responses were determined by regressions of the experimental data performed by the statistical software. Results showed that the maximum BPR, HC, HPR and HY are not quite consistently achieved at one optimal conditions of the three variables. BPR, HC, HPR and HY of 32.21 L/d, 30.51%, 2.23 L/d/L and 1.57 mol-H2/mol-sugar were estimated at the optimal pH, HRT, and TS of 5.55, 15.78 h, and 0.71% for BPR; 5.22, 12.04, and 0.69 for HC; 5.32, 15.62, and 0.78% for HPR; and 5.36, 17.56, and 0.74% for HY, respectively. The BPR, HC, and HY were 31.9 L/d, 29.33%, and 1.52 L/g sugar at the overall optimum condition of pH=5.32, HRT =15.62 h, and manure TS=0.78%. The performance of the biohydrogen process was affected more by pH than by HRT. Model validation indicated good linear relationships of the predicted and tested results for all the parameters.

Outcome 3. ASBR reactor setup and startup for biomethane production

A lab-scale anaerobic sequencing batch reactor (ASBR) system has been built at the University of Minnesota Southern Research and Outreach Center (SROC) at Waseca for biomethane production with the effluent from hydrogen fermentation as substrate. The reactor body was a polyethylene jar, 8L in total volume with a working volume of 5L. Same as the hydrogen fermentation, the methane reactor was wrapped with foam foil insulation and placed on a hot plate stirrer that maintains the temperature 35-37°C) of the mixed liquor content in the reactor and the complete-mix condition was achieved by using a peristaltic pump circulating the liquid through the reactor assisted by an internal magnetic stirrer. Mixing also reduced methane inhibition on the bacteria generating methane. The pH inside the reactor was controlled by a pH controller that adjusts the liquid pH by turning on and off two peristaltic pumps that add either hydroxide (supplied as 1.0M NaOH) or acid (supplied as 1.0N HCl) to the reactor. The effluent from hydrogen fermentation was stored in a clarification bottle, and the supernatant was taken into the second-step methane reactor as influent. The digested effluent after methane production was stored for next-step struvite extraction. The influent and effluent flows were regulated by peristaltic pumps in order to adjust the hydraulic retention time (HRT, h), which was calculated as working volume divided by volume of content goes into/out from the reactor per hour. A computer was used including a programmable control module (Campsci CR1000) with its software (Campsci PC400) installed capable of repeating a programed cycle operation in a time sequence.

The methanogenic microflora was the anaerobic sludge obtained from a local wastewater treatment plant at Owatonna, MN. The seed sludge was acclimatized by incubating with acidic effluent of hydrogen fermentation at anaerobic conditions at 30 °C for 7 days prior the usage. The reactor was seeded with a volume of 2L acclimated seed sludge. The methane gas was observed to be produced in a 7-day initial startup in batch mode, and then reactor started to run continuously on 12-h cycles with about 1 min each for feeding and discharging, 120 min for settling, and 598 min for reaction. The initial HRT was then kept at 60h. Gas and liquid samples have been collected at respective sampling ports. The amount of gas produced was recorded daily using a wet gas test meter and the gas was released continuously to keep a low biomethane partial pressure in the headspace.

Gas sampling was conducted every day and content of hydrogen, methane, and CO_2 in the biogas was analyzed using a gas chromatography (Varian 3800). The liquid samples were 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, total nitrogen (TN), total phosphorus (TP) following the Standard Methods.

A 35-day startup period with relatively low and fluctuating biogas volume rate at 1-3 L/d was observed. In the whole process there was no hydrogen production at all, which indicated that our experimental setup had successfully inhibited the hydrogen-producing bacteria from the influent. After 35 days, the methane-producing system started an exponential phase with fast-increasing biogas production rate from 2.7L/d to 7.5L/d in 6 days. The CH_4 content in the biogas was in the range of 75.1-83.3% (CH_4/CO_2 ratio of 3.0-5.0). The maximum methane yield by the time of report was 0.2g-CH_4/g-COD-feed, with COD utilization efficiency over 50%. COD value in the effluent from CH_4 production reactor system is 5.1g/L. The CH_4 production rate was up to 1.14 L/d/L with the absence of H_2 production throughout the whole course of experiment at a pH of 6.8-7.0 strongly validated the feasibility of the methane production from the H_2 fermentation effluent using an ASBR system.

Studies in literature indicated that pH and HRT played a key role in digestive biomethane production and each type of substrates might have different optimal pH and HRT values. Therefore, for the next step, after reaching high performance stability of the reactor system, an extended range of pH (6.0, 6.5, 7.0, 7.5, and 8.0) and HRT (60, 48, 36, 24, and 12h, the range may be adjusted according to the results) will be investigated to determine the optimal combinations corresponding to high biomethane production. The volatile acid concentration in the influent will also be considered as an influential operating parameter for system optimization.

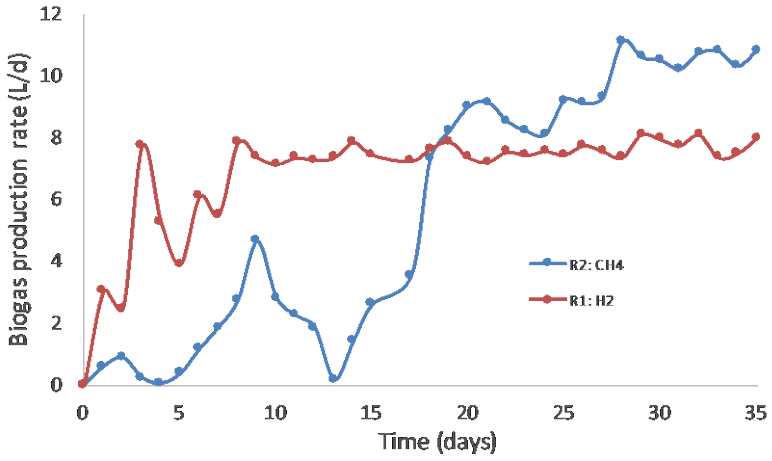
Activity Status as of January 1, 2016:
Outcome 4. Optimal condition determination for continuous biomethane production.
The working volume of the biomethane digester was set to 6L, and the digester was running to consume the whole effluent from the hydrogen fermenter, with the pH of the hydrogen reactor controlled ≥ 5.0 with the real pH varies between 5.0-5.6, which is the optimal pH range for hydrogen gas generation determined by last section. The following experiments were conducted to optimize the operational conditions for the two-stage operation of both hydrogen and methane reactors, in terms of biomethane productivity. With the data from the central composite design (CCD) experiments, the quadratic model built in the Design Expert statistical software was able to fit all the response variables in our experiments. The model fitting results, including ANOVA analyses of the fitted equations were obtained with respect to biogas production rate (BRP), methane production rate (MPR), methane content in biogas (MC), and methane yield (MY).

Quadratic modeling results for BPR, HPR, HC, and HY, respectively, were obtained based on CCD design in terms of two variables: (i) pH, X1, at 5 levels at a range of 6.5-8.5; (ii) HRT=hydraulic retention time, X2 (h), at 5 levels at a range of 72-24h. Total COD in the substrate of the methane digester is dependent on the HRT of the hydrogen fermenter, which is kept 1/3 of the HRT of methane digester. Therefore, the starting COD or organic loading rate was not taken as one of the independent variables. All the coefficients for different responses were determined by regressions of the experimental data performed by the statistical software. Results showed that the maximum BPR, MC, MPR and MY are achieved at different optimal conditions of the two variables. Maximum BPR of 48.37 L/d was estimated at the optimal pH of 6.9 and HRT of 41.5h; MC, MPR and MY of 85.32%, 5.74 L/d/L and 0.29 g-CH_4/g-COD-feed were estimated at the optimal pH and HRT of 7.58 and 47.34h for MC; 7.12 and 48.2h MPR; 7.26 and 55.6h for MY, respectively. The performance of the biomethane process was affected significantly by HRT and pH. Model validation indicated good linear relationships of the predicted and tested results for all the parameters. The setup of two-stage reactor system for biohydrogen and biomethane production is shown in the picture below.

Activity Status as of July 1, 2016:
Finished.

Activity Status as of January 1, 2017:
Finished

Final Report Summary:
Overall, successful biohydrogen and biomethane production is achieved by co-fermenting swine manure with sugar beet processing wastewater in a well-designed two-stage anaerobic sequencing batch reactor (ASBR) system composed of influent pump, effluent pump, mixing by circulating the liquid through a t-shaped connector facilitating real time pH monitoring and control. The operation of the ASBR system is controlled by a programmable control module (Campsci CR1000) with a software (PC400) for repeating fill-react-settle-draw cycles and realizing a continuous production of biohydrogen gas. The figure below shows system performance in the startup period over 35 days for the two-stages, respectively. H_2 production reactor reaches steady state within a week, while CH_4 reactor needs about one month to arrive steady state of biogas production. This matches the fact that growth rate of H_2-producers is 4-5 time higher than methanogens.



Results showed that pH, hydraulic retention time (HRT), and swine manure to molasses ratio (represented by TS %, total solids level) are the significant operating parameters for the biohydrogen productivity, while pH and HRT are for the biomethane productivity. Two stages are connected by using a working volume of 6 L for biomethane and 2 L for biohydrogen to make sure the HRT for biomethane is three times that of biohydrogen production. The optimal operating conditions for yield of biogases as well as COD and solids removal are summarized in the table below.

Optimized condition for yield	R1: H2 production	R2: CH4 production
pH	5.3	6.9
HRT (h)	16.5	51
TS%	0.78%	
Yield of product	1.52 mol-H_2/mol glucose	0.21 g-CH_4/g-COD-feed
COD removal	30-40%	70-80%
Total solids removal	60-70%	30-40%

ACTIVITY 2: Gas cleaning and struvite recovery processes development

Description: The chemical scrubber (gas cleaning reactor) will be fabricated from an acrylic column (10 cm in diameter, 122 cm in height) equipped with pumps and gauges to move the absorbent and biogas through the system at desired flow rates. The absorption column will be packed with a packing material (named "plastic bioball") to a height of 100 cm to facilitate gas/liquid interaction, which is ideal for applications where efficient gas/liquid interaction is needed. 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 for the biogas purification process. The absorbent will be circulated between the reactor and a reservoir (20 L liquid volume) by means of a pump. The flow rate of both liquid and biogas will be controlled to achieve effective CO_2 removal. The pH of the absorbent reservoir will be monitored and recorded to determine the time elapsed between absorbent replacements when the pollutants approach saturation. NaOH (0.1 M) solution will be used as the absorbent for this study, due to relatively low cost and availability in bulk volumes.

The experiment will be carried out in two phases, starting with a batch phase to investigate the effect of different gas/liquid flow rate ratios, followed by a continuous phase to evaluate CO_2 removal. The best liquid flow rate 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 (e.g., 0.6, 0.8, 1.0, 1.2, and 1.4) will then be examined with respect to its performance in removing CO_2 and H_2S from the biogas to determine the optimal ratio. In continuous operation, percentages of the total volume in the NaOH reservoir and 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.

The struvite precipitator will be built in cone shape, 25.4 cm in diameter and 106.7 cm in height (working height: 90 cm), with a bottom to collect settled struvite. It will consist of a center section (5 in. in diameter, working as draft-tube type) and a peripheral section. The effluent from biomethane reactor will be continuously added into the draft tube. 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 struvite will then be dewatered from the small amount of solution, which is lost when the crystals are removed, by natural drying. 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.

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. pH and the molar ratio of $Mg^{2+}/PO_4^{3-}/NH_4^+$ 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 and optimal combination of ion ratio and pH will be determined.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 131,424
Amount Spent: $ 131,424
Balance: $ 0

Activity Completion Date: March 31, 2017

Outcome	Completion Date	Budget
1. Biogas cleaning reactor setup finished	Dec. 31, 2015	$45,479
2. Optimal condition for CO_2/H_2S removal reported	June 30, 2016	$32,158
3. Struvite precipitator setup finished	June 30, 2016	$45,479
4. Optimal condition for struvite production reported	March. 31, 2017	$25,808

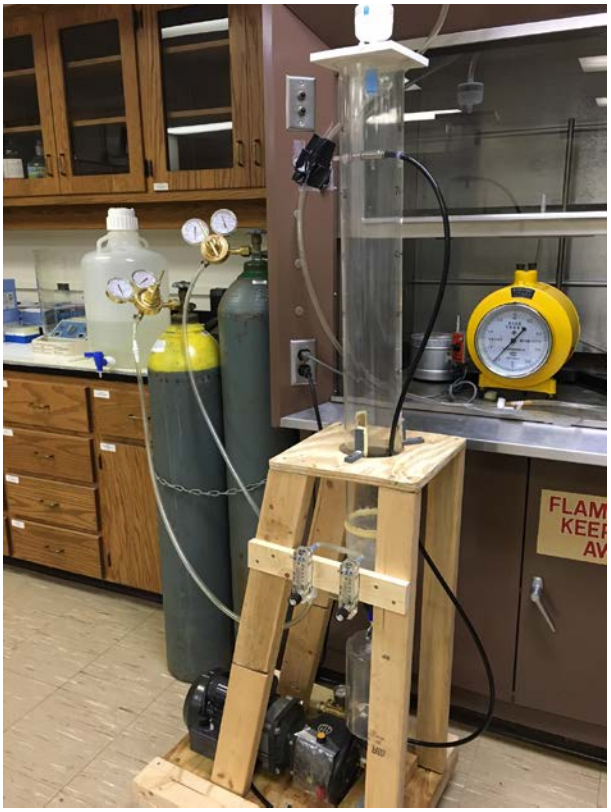
Activity Status as of January 1, 2015:
Not started.

Activity Status as of July 1, 2015:
Not started.

Activity Status as of January 1, 2016:
Outcome 1: Biogas cleaning reactor setup and feasibility test.
The gas cleaning reactor (chemical scrubber) was fabricated from an acrylic column (10 cm in diameter, 100 cm in height) equipped with a high pressure pump and gauges (0-2000 psi) which is to pump the absorbent solution through spraying nozzles of two available sizes: 0.008 mm and 0.012 mm, so that the absorbent can be sprayed into fine mist and flow down the column at designed flow rates. The absorbent (0.1~0.5M NaOH solution) can be circulated between the reactor and a reservoir (2 L liquid volume), or flow through the reactor uncirculated, by means of the high pressure pump. An air pump is used to transport biogas through the system at certain flow rates regulated by acrylic flow meters. The biogas is continuously fed from the bottom while the absorbent is sprayed from the top of the reactor, creating a counter current flow to improve the gas/liquid contact for the biogas purification process. The reactor can be packed with the “plastic bioball” material to certain heights for facilitating gas/liquid interaction. The experimental setup of the biogas cleaning reactor is shown in the picture below. Effective CO_2 removal has been achieved by controlling the flow rate of both liquid and synthetic biogas. Pure CO_2 gas (tested with 99% gas cylinder) flow at 0.5 was completely absorbed by spraying 0.5 M NaOH at 64 ml/min, without packing materials added, which verified the feasibility of total removal of CO_2 by the NaOH scrubber.

The optimization of the biogas cleaning process for both hydrogen and methane, respectively, will be carried out for the next step in two phases. It will be starting with a batch phase to investigate the effect of different gas/liquid flow rate ratios and filling height of the packing material, followed by a continuous phase to evaluate CO_2 removal, using synthetic biogas to simulate the composition of biogases from the reactors. The best liquid flow rate 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 each of the three liquid flow rates determined, five ratios of gas to liquid flow rate (e.g., 0.5, 1.0, 1.5, 2.0, and 2.5) will then be examined with respect to its performance in removing CO_2 and H_2S from the biogas to determine the optimal ratio. Effect of packing materials at different heights from 0 (without the bioball) to 80 cm will be tested for facilitating gas/liquid interaction in the batch mode. In continuous operation, starting concentration of the NaOH solution (mol/L) in the reservoir and replacing time

intervals (e.g., % saturation time) will be determined and examined via factorial experimental design or CCD 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.



Activity Status as of July 1, 2016:

Outcome 2: Optimal conditions to achieve completely CO_2 removal for biogas

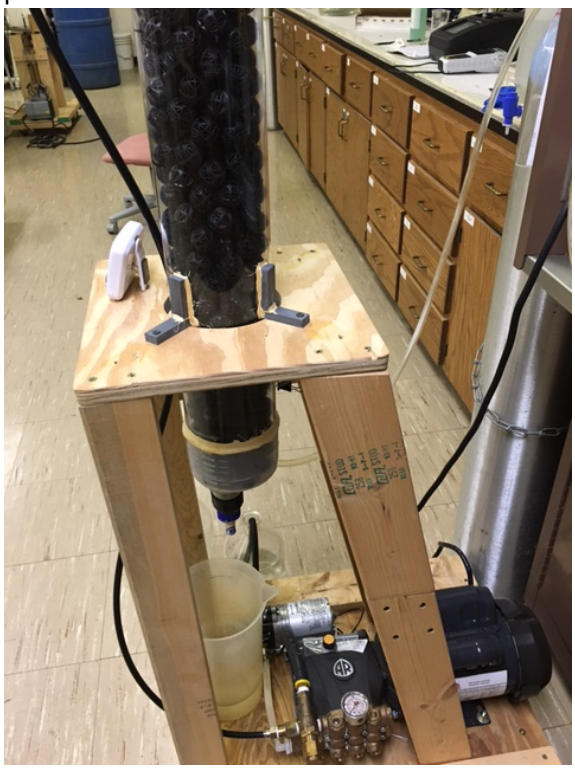
A synthetic gas mixture of 30% H_2 and 70% CO_2 was used to represent the biogas produced from the hydrogen fermenter for purification. In the batch experiments to investigate the effect of different gas/liquid flow rate ratios and filling height of the packing material for CO_2 removal, the gas flow rate is regulated by an acrylic flowmeter at a range of 0-1 liter per minute (LPM). The 0.08 mm nozzle provides a liquid flow rate at 0.09 LPM at the maximum pump pressure of 1200 psi, which didn't achieve a good CO_2 removal even with the lowest gas flow rate. Therefore, a 0.12mm nozzle was used for the experiments to determine an optimal ratio of gas/liquid flow rate. The pump pressure was set at 1000psi to create a smooth liquid film coming out of the absorbent (0.2M NaOH). A diaphragm pump is added in line to help stabilize the flow from the high pressure pump. At the pressure of 1000psi, the liquid flow rate is 0.14 LPM. The bioballs as packing material was used to fill the column and 80cm was found to be the best filling height to allow the spray spread completely from the nozzle as well as a longest liquid/gas contact time.

Five ratios of gas to liquid flow rate (2, 4, 5, 6, 8) have been tested to determine the best ratio. The gas flow rate was measured by a wet gas meter as 0.28, 0.56, 0.72, 0.84, and 1.1 LPM, respectively. The CO2 removal ratio was found to be 50%, 52%, 96%, 99%, 82% at the ratio of gas/liquid flow rate of 2, 4, 5, 6, 8, respectively. 6 is found to be the best gas/liquid flow ratio for CO_2 removal of hydrogen fermenter biogas, which contains about 70% CO_2.

In the continuous operation with the best gas/liquid flow ratio of 6, five starting concentration of the NaOH solution (mol/L) in the reservoir (0.1, 0.2, 0.3, 0.4, 0.5 M) and five replacing time intervals (e.g., % saturation time, 3 min, 6 min, 9 min, 12 min, 15 min) were examined via factorial experimental design or CCD to and a combination of 0.3M NaOH as the base wash solution and 6 min replacing time interval was found to achieve

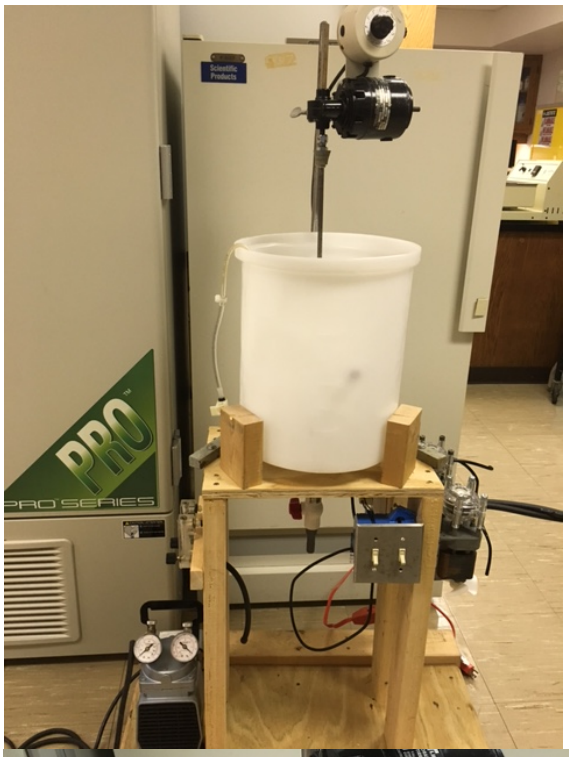
nearly 100% CO_2 removal efficiency for the treated biogas, as well as keep the replacement of absorbent at a reasonable frequency.

The optimization of the cleaning process for biogas from methane digester will be carried out in the next step. The operation of the wet scrubber setup with filling materials and the added diaphragm pump is shown in the picture below:



Outcome 3: Struvite precipitator setup and feasibility test

The struvite precipitator was built in cone shape, 27 cm in diameter and 38 cm in height (working height of 31 cm), with a cone bottom to collect settled struvite. It consisted of a center section (14.5 cm diameter, working as draft-tube type) and a peripheral section with a mixer. The effluent from biomethane reactor was collected and can be continuously added into the draft tube by a pump with certain flow rate. A pH controller is installed to monitor and control the pH in the reaction section automatically. The generated struvite could 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 struvite could then be dewatered from the small amount of solution, which is lost when the crystals are removed, by natural drying. 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 reactor setup that has been built and inside of the precipitator are show in the pictures below.



Next step, 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. pH and the molar ratio of $Mg^{2+}/PO_4^{3-}/NH_4^+$ 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 and optimal combination of ion ratio and pH will be determined.

Activity Status as of March 24, 2017:
Outcome 2: Optimal conditions to achieve completely CO_2 removal for biogas (continued)

The optimization of the cleaning process for biogas from methane digester has been conducted. Based on the typical biogas composition from the methane digester, a synthetic gas mixture of 70% CH_4 and 30% CO_2 was used to represent the produced biogas for purification by the NaOH scrubber. The 0.12mm spraying nozzle was used to determine an optimal ratio of gas/liquid flow rate. The pump pressure was set at 600psi to create a smooth liquid film coming out of the absorbent (0.2M NaOH). At the pump pressure of 600psi, the liquid flow rate is 0.072 LPM. The acrylic treating column was filled with bioballs to the height of 80cm which was found to

be the best filling height to allow the spray spread completely from the nozzle as well as a longest liquid/gas contact time in the previous experiments.

In the batch tests, five ratios of gas to liquid flow rate (6, 10, 15, 18, 23) have been tested to determine the best ratio. The gas flow rate was measured by a wet gas meter as 0.43, 0.75, 1.07, 1.32, and 1.68 LPM, respectively. The CO2 removal ratio was found to be 75.4%, 66.7%, 84.9%, 99.6%, 88.1% at the ratio of gas/liquid flow rate of 6, 10, 15, 18, 23, respectively. 18 is found to be the best gas/liquid flow ratio for CO_2 removal from the methane biogas, which contains about 30% CO_2. The final pH of the wasted NaOH solution for each gas/liquid ratio was 10.34, 10.5, 9.9, 9.7, and 8.4, as compared to the initial pH of 13.27.

In the continuous operation with the best gas/liquid flow ratio of 18, five starting concentration of the NaOH solution (mol/L) in the reservoir (0.1, 0.2, 0.3, 0.4, 0.5 M) and five replacing time intervals (e.g., % saturation time, 3 min, 6 min, 9 min, 12 min, 15 min) were examined via factorial experimental design or CCD to and a combination of 0.2M NaOH as the base wash solution and 9 min replacing time interval was found to achieve nearly 100% CO_2 removal efficiency for the treated biogas, as well as keep the replacement of absorbent at a reasonable frequency from economic perspective.

Outcome 4: Optimization of operational parameters for struvite production from the digested effluent

Effluent from biomethane digester was characterized before used for struvite precipitation. Average feedstock properties related to struvite production are displayed in the following table.

Properties	**Value**
pH	7.58 (optimal)
COD (mg/L)	3795 ± 177
NH_4^+-N (mg N/L)	273.8 ± 36.5
PO_4^{3-}Phosphate (mg P/L)	96.5 ± 13.8
Ca^{2+} (mg/L)	9.86 ± 1.64
Mg^{2+} (mg/L)	2.54 ± 0.35
Total suspended solids (mg/L)	3187 ± 155

The digestion effluent was centrifuged (8,000 rpm, 10 min) and the supernatant was used for the experiments. Batch experiments were conducted to determine optimal airflow rate, reaction time, pH and Mg^{2+}/PO_4^{3-} molar ratio for NH_4^+ and PO_4^{3-} removal. For each batch 500 mL of digestate supernatant was placed in the prepared flask and evenly mixed at 100 rpm. pH was suggested in literature as the most important factor for struvite formation. Aeration was used as a means to increase the liquid pH. Air flow rate of 0.2-2 LPM of air was tested for an hour and it was found that pH was increased from 7.5 to 8.5 with the air flow rate of 0.6 LPM and reached a plateau without going up any further. In general, the solubility of struvite increases according to increases in pH, and thus it is desirable to induce crystallization by maintaining a high pH in order to remove nitrogen and phosphorus. pH of 8-12 (set at 8, 9, 10, 11, and 12, respectively) was tested to evaluate the pH effect on nitrogen and phosphorus removal, as well as struvite formation. With an air flow rate of 0.6 LPM, pH was adjusted by adding 5M NaOH to the desired levels after $MgCl_2$ addition to increase Mg^{2+}/PO_4^{3-} molar ratio to 1/1. The removal efficiency of ammonia nitrogen increased from 21.3% to 72.6%, when pH changed from 8 to pH 12; while phosphate removal was 64.5% at pH of 8 and increased to 75.7% and was maintained at the same level at pH of 9-12.

During the struvite crystallization reaction, the mixing time was set to 10 minutes, 30 minutes, 1h, and 5h to test the influence of mixing time. Here the reaction pH was adjusted to 9, as the phosphate removal efficiency in a previous experimental result was similar in the range from 9 to 12. In this reaction, magnesium and phosphate mole ratio was 1:1. NH_4^+-N showed 39.6% removal under the condition of under 1 h, and then, it increased to 80% for 5 h reaction. The reason was considered the CO_2 stripping effect. As for PO_4^{3-}-P, the increasing reaction

time from 10 min to 24 h did not have a significant influence on the crystallization (about 76% removal). Thus, the reaction time should be kept higher than one hour to ensure satisfactory ammonia removal.

Due to the large amount of ammonium ion/ammonia was present in the wastewater making it impossible to obtain the ratio of 1:1:1 that is generally used in the process of crystallizing struvite. Thus, with a focus on the recovery of phosphorus, the optimal injection amount of Mg^{2+} was determined. The Mg^{2+}/PO_4^{3-} molar ratio was adjusted by adding $MgCl_2$ to the digested effluent feedstock, and set at five levels from 0.5 to 2.5 to evaluate the effect of Mg^{2+}/PO_4^{3-} molar ratio. 1.5 was found to the best Mg^{2+}/PO_4^{3-} molar ratio which achieved 91.2% phosphorus removal which did not increase under higher molar ratios tested. With the optimal pH, air flow rate, mixing time, and $MgCl_2$ addition determined, continuous production of struvite will be investigated in the next step. For continuous treatment of great amount of digester effluent, centrifuge of feedstock will not be economical. Since struvite will settle along with huge amounts of other suspended solids (organic matter), an accumulation device has to be designed and examined for its efficiency. For the recovery of pure struvite, struvite accumulation on the surface of stainless steel, wood and rubber, respectively will be evaluated to achieve the most effective harvest of struvite.

Final Report Summary:
Outcome 4: optimization of operational parameters for struvite production from the digested effluent (continued)
Continuous struvite production was conducted in the draft tube reactor described in Outcome 3 earlier, with a working volume of 10 L. The effluent from methane digester was settled for at least one day and only supernatant without being centrifuged is used to feed the struvite precipitator. Using optimal pH determined at 9.0, mixing time was set at 6h with a low RPM=30, and $MgCl_2$ was added based on a Mg^{2+}/PO_4^{3-} ratio of 1.5. During mixing, air flow rate was tested at 1L/min, 2L/min, 3/min, and 5L/min for the effect of pH change by air stripping. It was found that > 2L/min for 30min can raise pH to 8.5 without further increase. 2M NaOH solution is then added by a pump connected to the pH controller to maintain the pH at 9.0. Every batch of treatment stays in the reactor for 24 hours before discharging, and a new batch of influent will be pumped in, making the whole process semi-continuous. HRT of 24 hours would match the production rate of the upstream processes. Analysis shows that about 60% phosphate and 40% ammonium nitrogen removal is achieved. Part of the ammonium removal is due to the air stripping and pH raise. The lower nitrogen and phosphorus removal than in small batch might be mainly due to the inefficient solids recovery caused by an inadequate filtering/harvest process: small particles were easily lost since the retention of solids through the liquid-solid separating mechanism of the sedimentation reactor was insufficient. Nuclear material or filtration would be helpful for harvesting and recovering more struvite product and will be designed and carried out in future experiments.

In summary, biogas purification and struvite production has been finished in this Activity. The gas cleaning reactor is 10 cm in diameter, 100 cm in height and equipped with a high-pressure pump and gauges (0-2000 psi) to pump the absorbent solution through spraying nozzle of 0.012 mm. The absorbent is sprayed into fine mist and flow in counter direction of the gas flow. 0.84L/min and 1.32L/min is determined as the best gas flow rate for >99% of CO_2 removal from biohydrogen and biomethane gas, respectively. The draft tube reactor for struvite precipitation is equipped with pH controller, pumps for influent and effluent, air pump and mixer. pH of 9.0, .5 Mg^{2+}/PO_4^{3-} molar ratio at 1.5, mixing time of 6 hours with air flow at 2 LPM for 30 mins has been determined for efficient struvite formation. 650 mg struvite is produced for every liter of liquid feedstock used, with 60% phosphate removal and 43% ammonium nitrogen removal. Struvite harvest needs to be improved by adding seeds or filtration.

ACTIVITY 3: System integration and evaluation

Description: 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 resize of each component will be considered based on their throughput capacities. 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.

The evaluation of the integrated system 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 full-scale treatment system will be calculated based on which the potential revenues (values of different products) and the initial capital investment can be estimated.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 95,841
Amount Spent: $ 92,034
Balance: $ 3,807

Activity Completion Date: June 30, 2017

Outcome	Completion Date	Budget
1. Integrated system construction and assembling finished	April. 31, 2017	$32,158
2. Productivity of the integrated system evaluated and reported	June 30, 2017	$43,183

Activity Status as of January 1, 2015:
Not started.

Activity Status as of July 1, 2015:
Not started.

Activity Status as of January 1, 2016:
Not started.

Activity Status as of July 1, 2016:
Not started.

Activity Status as of March 15, 2017:
Not started.

Final Report Summary:
The evaluation of the integrated system was 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 was 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 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 is 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 has been calculated based on which the potential revenues (values of different products) and the initial capital investment has been estimated.

1) Performance of the integrated treatment system
In this activity, the entire treatment system according to Figure 1 (in appendix) has been assembled for evaluation. Each component of the system for co-treating swine manure and sugar waste molasses was adjusted and operated with its respective optimum operating condition. The working volume and retention time of influent of each unit was adjusted to handle its upstream flow. All the operation parameters for each unit are summarized in the table below, and the numbers are averaged with at least 3 runs. Starting with the biohydrogen fermentation reactor, each part of swine manure (with about 1.5-2.0% solids) is mixed with about same amount of diluted molasses of 20g/L sugar as influent for the whole system for treatment and production. The targeted carbohydrate concentration in the influent is 10 g/L.

With a 2 L working volume and optimal HRT at 16 hours, the daily throughput of the biohydrogen reactor is 3L feedstock, which is mixed swine manure and sugar-beet wastewater, producing 22.1 L H_2 biogas per day in average, with a stable hydrogen content of 30-32%. The capacity of the methane digester is matching up the biohydrogen system, with a working volume of 6L and 48h HRT, making a production of 34.5L biogas/d with a CH4 content of 79-83%. The biogases from both hydrogen fermenter and methane digester were taken by same wet-scrubber with 0.3M and 0.2M NaOH solution with 6 min and 9 min replacing interval, respectively, for biogas purification. The 8L wet-scrubber that we built has a capacity to treat 1200L/d H2 biogas or 1900L/d CH_4 biogas if running continuously with the optimal flow rate of 0.84 L/min for H2 biogas and 1.32 L/min CH_4 biogas. Therefore, the biogases were collected respectively and each biogas runs through the scrubber to be treated once a day, with a 26-30 min running time for each gas. The purity of biogas getting out of the wet-scrubber is >98%. The struvite precipitator with a 9-10 L working volume, with the capacity of 18L/d, is taking the liquid effluent from the methane digester accumulated 3 days for one batch operation, leading to an averaged

production of struvite at 1.9-2g/d. Overall production from the lab-scale system is 7.8L/d H_2, 28.3L/d CH_4, and 1.95g/d struvite.

From the wastewater treatment perspective, the treatment efficiencies were also summarized in the table in terms of removal of total solids, COD, total nitrogen and total phosphorus. The total solids removal by biohydrogen production, methane production and struvite production is 27.4%, 28%, and 3.6% respectively, resulting in an overall removal of 49.6%. As of COD, removal by biohydrogen production and methane production is 22.5% and 69.6% respectively, with an overall removal of 76.5%. Nitrogen and phosphorus each was removed by three of the four processes, hydrogen, methane, and struvite production with 27.0%, 18.4%, and 42.5% removal for nitrogen and for phosphorus, 27.2%, 21.1%, and 59.6%, respectively. The overall removal of N and P is 65.8% and 76.8%, respectively. TS, COD, TN and TP in the finally effluent is averaged at 4337 mg/L, 3090 mg/L, 137 mg/L and 36 mg/L. Despite relatively high treatment efficiencies by the integrated system, further purification for the final effluent is needed if discharging to waterbody. Aerobic treatment is a necessity for further COD, nitrogen and phosphorus removal.

Processes	*Hydrogen fermenter*	*Methane digester*	*Biogas cleaning wet scrubber*	*Struvite precipitator*
Maximum throughput capacity	6L/d of liquid with carbohydrate	6L/d of liquid with VFA	1200L/d H2 biogas 1900L/d CH4 biogas	18L liquid/d
Working volume	2L	6L	8L	9L
Optimized Retention time	16h	48h	10min for H_2 biogas 6min for CH_4 biogas	24h
Operating loading	3L/d of feedstock	3L/d of hydrogen effluent	22.1L H_2 biogas/d 34.5L CH_4 biogas/d	3L/d of methane effluent
Production	22.1L H_2 biogas/d	34.5L CH_4 biogas/d	7.8L/d H_2 and 28.3L/d CH_4	1.95g/d struvite
TS removal	27.4%	28.0%	-	3.6%
COD removal	22.5%	69.6%	-	-
Nitrogen removal	27.0%	18.4%	-	42.5%
Phosphorus removal	27.2%	21.1%	-	59.6%
Power usage	-	0.043kW x 24h/d = 1.03 kWh/d	0.18kW x (0.44h/d + 0.44h/d) = 0.16 kWh/d	0.1kw x 2h/d = 0.2 kwh/d

2) Evaluation of the net Energy gain by the integrated system

The definition of net energy gain compares the energy required to extract energy the source materials, which is the energy input for operation of the system and chemicals consumed, compared with the amount of energy associated with the energy resource produced. The energy production components in our system include the product of hydrogen gas, methane gas, struvite, and equivalent gain for COD removed (30.2g/d), N removed (0.8g/d), P removed (0.4g/d) from liquid phase and CO_2 removed from gas phase (20.5 L/d). The energy consumption elements in the system include electricity used to heat and run each component unit, and chemicals used for pH adjustment and as absorbent. Since chemicals used in the precipitator for struvite production end up in the product, i.e., struvite, they are not included in the energy balance analysis. Overall production from the lab-scale system is 7.8L/d H_2, 28.3L/d CH_4, and 1.95g/d struvite. The equivalent energy for each element is summarized in the table below, and energy gained and consumed are calculated respectively and compared. It is found that the production energy is lower than the consumed energy, thus net energy gain is about negative 0.7 kWh/d. The produced energy products covers 50% energy consumption, due to the small

system size with relatively high running cost. Scale-up systems with lower unit operating cost would be expected to result in positive and high net energy gain. Therefore, the environmental impact of the system is positive in production of value added products and removal of pollutants lessen energy input.

Energy gain items	*Energy gained (kWh/d)*	*Energy consumption items*	*Energy used (kWh/d)*
Hydrogen gas produced (39.4 kWh/kg)	0.03	Hydrogen fermenter running	-
Methane gas produced (15.4 kWh/kg)	0.31	Methane digester running	1.03
COD removal equiv. (3.8 kWh/kg COD)	0.20	Biogas-cleaning wet scrubber running	0.16
CO2 removal equiv. (3.57 kWh/kg CO_2)	0.15	Struvite precipitator running	0.2
N removal equiv. (12.5 kWh/kg N)	0.01	NaOH use for pH adjust (0.12kWh/kg)	0.001
P removal equiv. (13.6 kWh/kg N)	0.005	NaOH for wet-scrubber (0.12kWh/kg)	0.01
Total energy gain	0.675	Total energy consumption	1.400

3) Economic estimate of pilot-scale system

Base on the results from the lab-scale system operation, an economic estimate of a pilot scale system featuring 1000 gallon/d wastewater loading (scaling factor: 1260) is summarized in the table below. This pilot scale system will be able to achieve a daily production of 9800 L H_2, 35700 L CH_4, and 2500 g struvite. The cost is an estimate and can only be relatively accurately determined based on the actual costs for the parts for constructing the reactors and other devices.

The reactor sizes, operating loading, capital cost, chemical cost, and the running cost are summarized in the table below. The working volume and operating loading of the hydrogen fermenter and methane digester in pilot scale system will be 1260 folds of the lab-scale system. Two biogas cleaning wet scrubbers with 240L working volume will be able to purify the two biogases separately with 20h working and 4h idle time per day. The struvite precipitator will be 1890 L in working space to handle 3780 L/d of methane effluent with 12-hour retention time. Capital cost are estimated based on the price of pumps and large-size tanks, which could be about $50,000 for the whole system. Running cost of each component are estimated based on the size the horse power of pumps, and the total chemical cost and running cost is estimated at 213.9 kWh/d. By calculation, the energy gain for the pilot scale system will be about 1260 times of lab-scale system which is 850 kWh/d, making a daily energy gain of 630 kWh/d. Assuming the price of electricity is 12 cents per kWh, the payback period for the capital and running cost will be 662 days (1.8 years) not considering maintenance cost.

	Hydrogen fermenter	*Methane digester*	*Biogas cleaning wet scrubber*	*Struvite precipitator*
Working volume	2520 L	7560 L	2 x 240L	1890 L
Operating loading	3780 L/d of feedstock	3780 L/d of hydrogen effluent	28000 L H_2 biogas/d 43500 L CH_4 biogas/d	3780 L/d of methane effluent
Capital cost	$12500	$12500	$7500 each	$7500
Chemical cost (kWh/d)	1.26		12.6	

Running cost (kWh/d)	3.58kW*2*0.5h (infl&effl pumps) +1.76kW(mixing)*20h+1kW(heating)*20h=58.8	58.8	3.58kW*20h=71.6	3.58kW*2*0.5h (infl&effl pumps) +1.76kW(mixing)*12h=24.7

V. DISSEMINATION:

Description: 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 includes, 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 Biological Process Engineering and Renewable Energy Technologies courses, to educate our future scientists/engineers in a long run.

Status as of January 1, 2015:
A manuscript titled “Continuous hydrogen production from co-fermenting liquid swine manure with sugar processing wastewater” is under preparation and will be submitted to *International Journal of Hydrogen Energy* in February, 2015.

Status as of July 1, 2015:
Published peer-reviewed journal article:
Wu, X., J. Zhu, H. Lin. 2015. In-depth Observations of Fermentative Hydrogen Production from Liquid Swine Manure Using an Anaerobic Sequencing Batch Reactor. Journal of Integrative Agriculture. Accepted.

Manuscript under preparation:
Wu, X., S. Deng, J. Zhu, H. Lin. 2015. Two-step sequencing batch reactors for continuous hydrogen and methane production from co-fermenting liquid swine manure with sugar processing wastewater. Will be submitted to *International Journal of Hydrogen Energy* in August, 2015.

Status as of January 1, 2016**:**
Published peer-reviewed journal article:

1. Wu, X., J. Zhu, H. Lin. 2015. In-depth Observations of Fermentative Hydrogen Production from Liquid Swine Manure Using an Anaerobic Sequencing Batch Reactor. Journal of Integrative Agriculture. In press. Doi: 10.1016/S2095-3119(15)61108-X.

Submitted peer-reviewed journal article:

1. Wu, X., S. Deng, J. Zhu, H. Lin. 2016. Two-stage sequencing batch reactors for continuous hydrogen and methane production from co-fermenting liquid swine manure with sugar processing wastewater. Submitted to *Bioresource Technology.*

Manuscript under preparation:

1. Wu, X., S. Deng, J. Zhu. Base wet-scrubber for purification of biohydrogen from co-fermenting waste sugar-beet molasses and swine manure.

Results produced from this project has been incorporated in teaching material for the course BBE 4733/5733 – Renewable Energy Technologies at Department of Bioproducts and Biosystems Engineering, University of Minnesota, lectured by PI, October 2015, and will continue to be used for the same course. A poster from this project has been presented to the Advisory committee of Southern Research and Outreach Center and Regent visit in 2015, and will be presented to public in the Open House event at Southern Research and Outreach Center, Waseca, 2016.

Status as of July 1, 2016**:**

Submitted peer-reviewed journal article:

1. Wu, X., S. Deng, J. Zhu. Base wet-scrubber for purification of biohydrogen from co-fermenting waste sugar-beet molasses and swine manure. Submitted to *International Journal of Hydrogen Energy*.

Results produced from this project has been incorporated in teaching material for the course BBE 4733/5733 – Renewable Energy Technologies at Department of Bioproducts and Biosystems Engineering, University of Minnesota, lectured by PI, March 2016, and will continue to be used for this course. A poster on this project will be presented in the Open House of Southern Research and Outreach Center on September 15th, 2016.

Status as of March 24, 2017:

A poster on this project has been presented in the Open House of Southern Research and Outreach Center on September 15th, 2016. The poster has been attached with this work plan submission.

An intellectual property disclosure “Manure and sugar wastewater co-treat system for biohydrogen, biomethane and struvite fertilizer production” has been submitted to the University of Minnesota to evaluate the patent potential.

Final Report Summary:

Published peer-reviewed journal article:

Wu, X., J. Zhu, H. Lin. 2017. In-depth observations of fermentative hydrogen production from liquid swine manure using an anaerobic sequencing batch reactor. Journal of Integrative Agriculture 16(6): 1276–1285.

A poster on this project has been presented in the Open House of Southern Research and Outreach Center on September 15th, 2016. The poster has been attached with this work plan submission.

Results produced from this project has been incorporated in teaching material for courses: 1) BBE 4733/5733 – Renewable Energy Technologies at Department of Bioproducts and Biosystems Engineering, University of Minnesota, lectured by Xiao Wu, 2015-2016; 2) BE 461 – Bioprocessing Engineering at Department of Biological Engineering, University of Idaho, lectured by Xiao Wu, 2017.

An oral presentation has been made at ASABE 110th Annual International Meeting, with paper#: #1701057, at Spokane, Washington. July 16-19, 2017. Presenters: Wu, S., S. Deng, J. Zhu. Title: Hydrogen and methane production from swine manure and sugar beet wastewater by a two-step ASBR system.

On June 27th, 2017, a media report of this project was featured in a KEYC Mankato News story titled "Farm Forward: Utilizing Every Molecule of Swine Manure". The website for the full news segment can be accessed by this link: http://www.keyc.com/story/35763775/farm-forward-utilizing-every-molecule-of-swine-manure.

Dissemination of this project will continue with publishing refereed and non-refereed articles, talking to people in the concerned industries and the stakeholders, developing teaching materials in college and graduate levels, demonstration of the complete system for co-treating swine manure and sugar processing waste molasses and education of stake holders and general public, etc.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 320,000	One 100% research associate for three years: salary: $166,916; fringe: $56,084; The research associate will be the PM of this project overseeing the entire project with responsibility in all aspects including developing test protocols, conducting experiments, and preparing materials for publications and information dissemination. One 100%-time postdoc associate for two years: salary: $71,079; fringe: $15,921; The postdoc will be responsible for the execution of the project by preparing and running experiments; setting up experimental apparatuses; sampling and data analysis; preparing manuscripts and other publications; and collecting and analyzing samples and data; helping the research associate with reporting as well.
Equipment/Tools/Supplies:	$79,800	$49,300 - supplies for constructing all the reactors including hydrogen fermenter, methane digester, biogas cleaning scrubber, struvite reactor and the size-adjusted integration system with all the control systems including reactor bodies, pumps, mixers, temperature and pH controllers, etc.; $30,500 - sample analysis and data processing costs including 400 gas samples for hydrogen, methane and carbon dioxide content analysis ($60 each) and 500 liquid samples for COD, BOD, nitrogen, phosphorus, solids level and VFA tests ($60 each).
Travel Expenses in MN:	$200	$200- in-state travel from Waseca to Moorhead area to collect samples and run experiments for two people including meals and lodging during

		the three-year period (estimated $500/trip x 12 trips). Trips are covered by university funds.
TOTAL ENRTF BUDGET:	**$400,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: No capital requests are made for this proposal.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 5 FTEs

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: None

B. Other Funds: N/A

VII. PROJECT STRATEGY:

A. Project Partners:

Project Partners Not Receiving Funds:

- Kevin Hennessy, MDA, providing assistance in information dissemination activities such as workshops, seminars, field days/tours, etc.
- Minnesota Sugar Beet Processors, American Crystal Sugar and MN Southern Sugar Beet Co-op, providing sugar beet processing wastewater for the experiment and participating in demonstration of the project outcome.

B. Project Impact and Long-term Strategy:

Whether future agriculture is able to meet the demand for quality food of the ever-growing global population hinges largely on its capability of minimizing the environmental footprint of food production by maximizing the recycling of the production wastes. As stated early on, Minnesota has two major waste streams (sugar beet processing wastewater and swine manure) that are deemed infeasible to be handled by conventional treatment systems because some of them require a large land space while others incur high capital and operational costs. In addition, most conventional treatments fail to recover the values of the wastes, thus providing little benefit in building a sustainable agriculture, especially when today's agriculture is facing the quandary of finite resources with a growing consumer base. As a result, these wastes must be recycled into value-added products to the extent possible, period. This is exactly what this project will achieve. The new treatment system proposed herein will use the nutrients contained in both waste streams in a complementary way to maximize their values and benefits by producing biohydrogen, biomethane, and fertilizer. In doing so, the pollutants in both waste streams can be reduced and, at the same time, with their values recovered. Therefore, the long-term impact of this project is to promote and establish sustainable agriculture in Minnesota by developing a novel treatment system to recycle the wastes generated from two major agricultural industries in the State into renewable products, thus conserving natural resources and maintaining environmental sustainability. This strategy presented by the proposed system will change the current swine manure management infrastructure by introducing an advanced manure treatment system that not only reduces the environmental impact of swine production but also offers the producers additional revenues. The proposed system also will provide the sugar beet processing industry with an environmentally friendly, better alternative in disposing of processing wastewater. Furthermore, the new paradigm stemming from this project will educate the vast majority of swine producers and sugar beet processors and turn them into environmental stewards and advocates for environmental sustainability, which is essential for the continued economic growth in Minnesota over the long haul.

C. Spending History:

Funding Source	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13	M.L. 2013 or FY14
		N/A			

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): See attached maps.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: N/A

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2015; July 1, 2015; January 1, 2016; July 1, 2016; and January 1, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

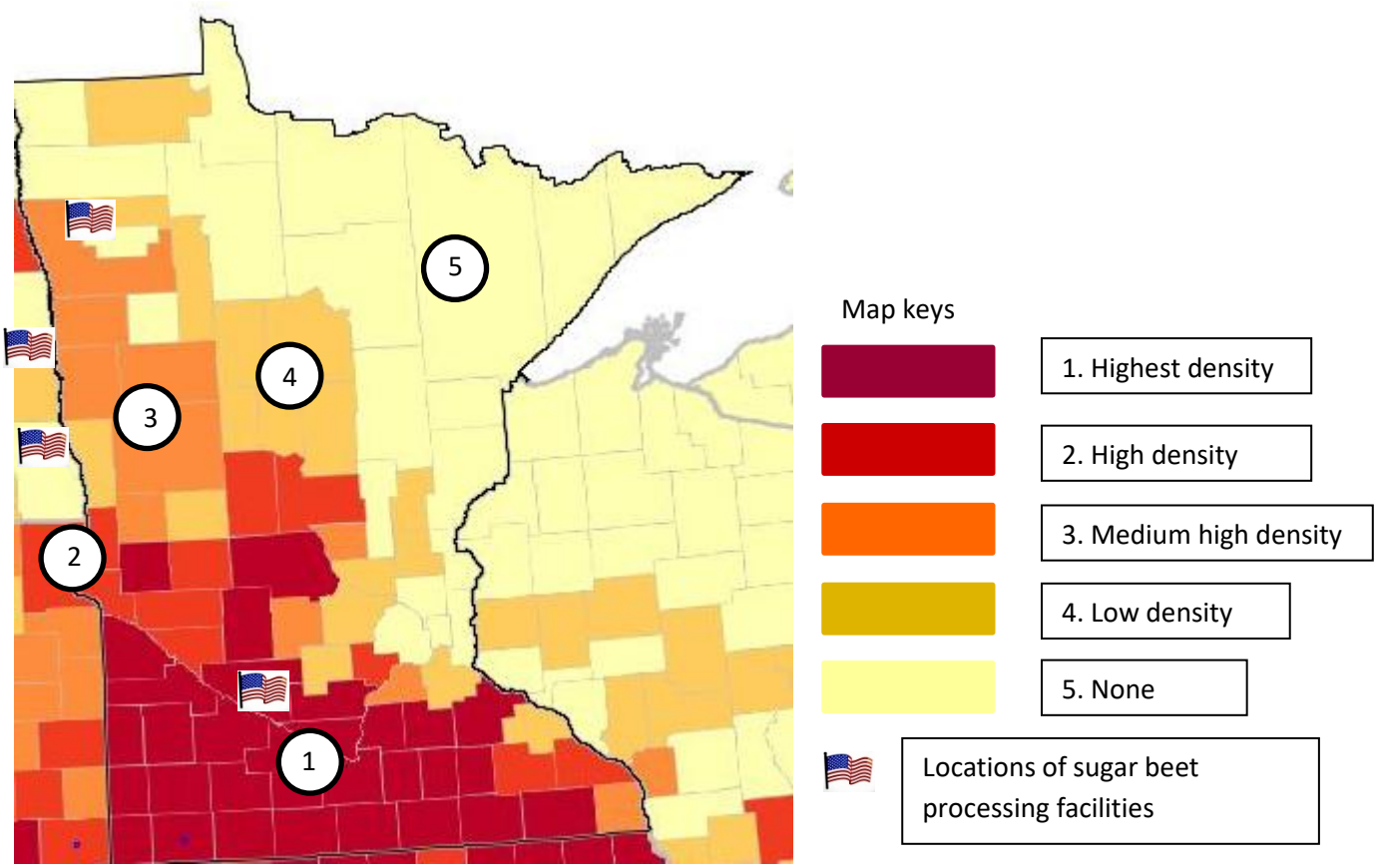
Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget

Project Title: Clean Water and Renewable Energy from Beet Processing Wastewater and Manure
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 08f
Project Manager: Shaobo Deng
Organization: University of Minnesota
M.L. 2014 ENRTF Appropriation: **$**400,000
Project Length and Completion Date: 3 Years, June 30, 2017
Date of Report: August 14th, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM											
Personnel (Wages and Benefits)	$130,735	$130,735	$0	$118,924	$118,924	$0	$70,341	$70,341	$0	$320,000	$0
Project Manager: $223,000 (75% salary, 25% benefits); one FTE for three years; The research associate will be the PM of this project overseeing the entire project with responsibility in all aspects including developing test protocols, conducting experiments, and preparing materials for publications and information dissemination.											
One 100%-time postdoc associate: $87,000 (81.7% salary, 18.3% benefits); one FTE for two years; The postdoc will be responsible for the execution of the project by preparing and running experiments; setting up experimental apparatuses; sampling and data analysis; preparing manuscripts and other publications; and collecting and analyzing samples and data; helping the research associate with reporting as well.											
Equipment/Tools/Supplies											
Purchasing parts and component for constructing all the reactors and systems including hydrogen fermenter, methane digester, biogas cleaning scrubber, struvite reactor and the size-adjusted integration system with all the control systems including reactor bodies, pumps, mixers, temperature and pH controllers, etc.: $20,000	$10,000	$10,000	$0	$6,700	$6,700	$0	$22,600	$19,753	$2,847	$39,300	$2,847
lab supplies: chemicals, tools, glasswares, gloves: $10,000	$5,000	$5,000	$0	$3,300	$3,300	$0	$1,700	$1,700	$0	$10,000	$0
sample analysis and data processing costs including 400 gas samples for hydrogen, methane and carbon dioxide content analysis ($60 each) and 500 liquid samples for COD, BOD, nitrogen, phosphorus, solids level and VFA tests ($60 each): $54,000	$27,000	$27,000	$0	$2,500	$2,500	$0	$1,000	$150	$850	$30,500	$850
Travel expenses in Minnesota	$0	$0	$0	$0	$0	$0	$200	$90	$110	$200	$110
mileage, lodging, meals for travel to and from sugar processing facilities and swine farms for substrates collection and data gathering											
COLUMN TOTAL	**$172,735**	**$172,735**	**$0**	**$131,424**	**$131,424**	**$0**	**$95,841**	**$92,034**	**$3,807**	**$400,000**	**$3,807**

Budget	310000	28340.44	39440.44
personnel spent	281659.56		
Salary P&A	203952.93		
Salary Civil Service	3471.54		
TempCasual	4882.75		
Fringe P&A	68057.78		
Fringe Civil Service	902.6		
Fringe casual	391.96		
Budget	79000	44442.12	
Supplies spent	34557.88		
Budget	5000	5000	
Service spent	0		
Budget	6000	5909.82	
Travel spent	90.18		
		83692.38	

Minnesota hog production distribution map



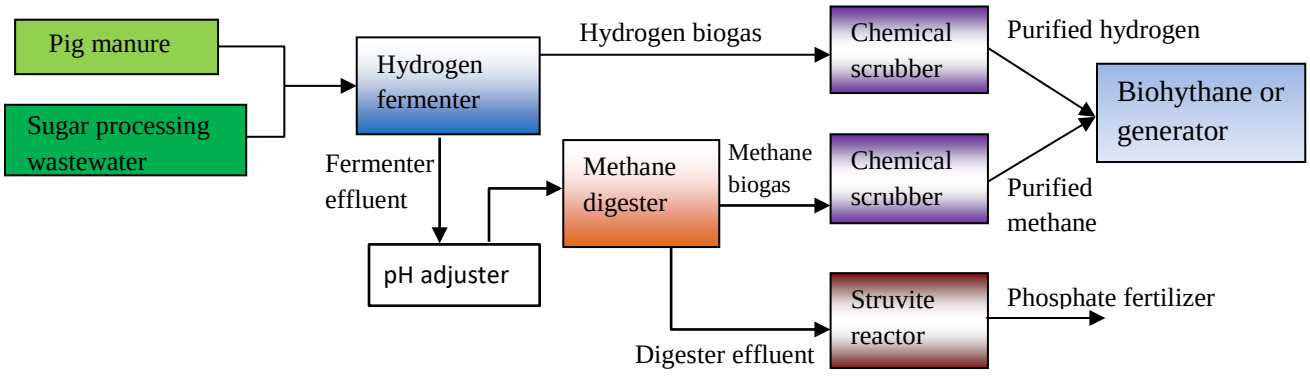


Figure 1. The schematic of the flowchart of the proposed treatment system

Journal of Integrative Agriculture 2017, 16(6): 1276–1285

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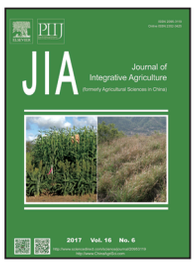

RESEARCH ARTICLE

In-depth observations of fermentative hydrogen production from liquid swine manure using an anaerobic sequencing batch reactor

Xiao Wu[1], Jun Zhu[2], Hongjian Lin[3]

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Abstract

In this study, experiments were designed to reveal in-depth information of the effect of pH and hydraulic retention time (HRT) on biohydrogen fermentation from liquid swine manure supplemented with glucose using an Anaerobic Sequencing Batch Reactor (ASBR) System. Five values of HRT (8, 12, 16, 20, and 24 h) were first tested and the best HRT determined was further studied at five pH levels (4.4, 4.7, 5.0, 5.3, and 5.6). The results showed that for HRT 24 h, there was a dividing H_2 content (around 37%) related to the total biogas production rate for the ASBR System running at pH 5.0. When the H_2 content went beyond 37%, an appreciable decline in biogas production rate was observed, implying that there might exist an H_2 content limit in the biogas. For other HRTs (8 through 20 h), an average H_2 content of 42% could be achieved. In the second experiment (HRT 12 h), the highest H_2 content (35%) in the biogas was found to be associated with pH 5.0. The upswing of pH from 5.0 to 5.6 had a significantly more impact on biogas H_2 content than the downswing of pH from 5.0 to 4.3. The results also indicated good linear relationships of biogas and H_2 production rates with HRT (r=0.9971 and 0.9967, respectively). Since the optimal ASBR operating conditions were different for the biogas/H_2 production rates and the H_2 yield, a compromised combination of the running parameters was determined to be HRT 12 h and pH 5.0 in order to achieve good biogas/H_2 productions.

Keywords: biohydrogen fermentation, swine manure, hydraulic retention time, pH values, anaerobic sequencing batch reactor

1. Introduction

Currently, hydrogen is produced exclusively from fossil fuels through energy intensive processes, which themselves are not clean technologies from the perspective of sustainability. For being used as a major energy source, hydrogen must be produced *via* sustainable means (Benemann 1996; Dunn 2002), among which biological pathways have come to the center stage due to its low energy needs and environment-friendly nature. Furthermore, biological conversion normally works with waste materials, so it can achieve both waste reduction and energy recovery. In view of these benefits, a considerable amount of research effort has been dedicated to biological production of hydrogen in the last two decades (Chen *et al*. 2008; Bičáková and Straka 2012; Zhao *et al*. 2013; Rai *et al*. 2014), among which fermentative

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hydrogen production from organic compounds (especially carbohydrates) by anaerobic bacteria is generating profound interests among researchers due to its unique advantages over other technologies (Liu *et al*. 2013). In this process, high hydrogen production rates can be achieved with an active dark-fermentative consortium without the assistance of a light source (Das and Veziroglu 2001). In addition, the majority of the substrates used for dark fermentation consist of waste materials that otherwise need to be treated before disposal, which incurs extra costs. The investigated waste streams so far for hydrogen fermentation include tofu processing wastewater (Zhu *et al*. 2002), rice winery wastewater (del Campo *et al*. 2012; Yu *et al*. 2002), starch manufacturing wastewater (Yokoi *et al*. 2002), potato processing wastewater (Yokoi *et al*. 2001), beer processing wastewater (Lay *et al*. 2005), sugar refinery wastewater (Won *et al*. 2013), sugarcane bagasse (Rai *et al*. 2014), dairy wastewater (Gadhe *et al*. 2013), cheese whey wastewater (Kargi and Uzunçar 2012), fruit juice wastewaters (Fernández *et al*. 2011), and pineapple wastes (Wang *et al*. 2006). Obviously, the fermentative pathway for hydrogen production can be an ideal vehicle to not only produce hydrogen but also reduce the volume of these wastes, thus saving the treatment costs and paving the way for building a sustainable economy.

One of the waste materials that have not been studied extensively in hydrogen fermentation is liquid swine manure, despite a few publications existing in the literature, almost all of which, however, were coming from the work conducted by the authors (Wu *et al*. 2009; Zhu *et al*. 2009). The results from these reports, in general, evidenced the feasibility of using swine manure as substrate for hydrogen fermentation, but without elaborating on some intrinsic characteristics of the fermentation process. Given the fact that swine manure contains all the necessary components for hydrogen fermentation by microorganisms such as *Clostridia* and the tremendous volume generated in the world every year, it is worthwhile to further our understanding of the process by providing in-depth information on the characteristics of the process for biogas/hydrogen production. Therefore, in this study, new information that had not been reported before related to fermenting swine manure supplemented with glucose to produce hydrogen was collected and reported using an Anaerobic Sequencing Batch Reactor (ASBR) System running on different hydraulic retention time (HRT) and pH values. Such information might provide insight on improving the hydrogen fermentation efficiency of liquid swine manure.

2. Materials and methods

2.1. Seed sludge and pretreatment

A running anaerobic digester treating dairy manure, located in St. Peter, Minnesota, USA, was the source for the seed sludge for this study. After collection, the sludge was pretreated using a prepared nutrient medium under room temperature for 24 h. The nutrient medium (1 L) contained 10 g glucose, 1.5 g KH_2PO_4, 0.5 g NH_4Cl, 0.18 g $MgCl_2 \cdot 6H_2O$, 0.05 g $FeSO_4$, 5 g polypeptone and 2 g yeast extract (Fang *et al*. 2006). The pH of the medium was also lowered from 7.1 to 5.0 with hydrochloric acid. After incubation, the sludge was boiled at 100°C for 30 min to inactivate non-H_2-producing bacterial species in the sludge.

2.2. Liquid swine manure source and preparation

The main substrate, liquid swine manure, was collected from a finishing building at the University of Minnesota Southern Research and Outreach Center at Waseca, USA. Preliminary treatments of the collected manure included dilution with tap water to a solid content of 0.5% followed by freezing in a freezer, if not placed immediately in the feeding tank. According to our preliminary trials (data not shown), swine manure alone was found to be ineffective in H_2 fermentation, and a sugar source, such as glucose, was needed in the culture media due to the lack of sufficient carbohydrates in the manure for the fermentative bacteria. To that end, to assist the growth of H_2 producing bacteria with sufficient carbohydrates, the manure in the feeding tank was supplemented with 10 g L^{-1} glucose, 500 mg L^{-1} KH_2PO_4, and 400 mg L^{-1} peptone. The characteristics of the raw liquid swine manure and the prepared substrate were presented in Table 1. The adjusted pH, which was slightly higher than 5.0, took into account the potential pH drop caused by the fresh influent fed into the reactor at the beginning of each ASBR cycle that would normally reduce the liquid pH as a result of quick production of organic acid.

2.3. Reactor setup and operation

The lab-scale ASBR System was presented in Fig. 1. A polyethylene jar, 20.3 cm in diameter and 45.0 cm in height, was employed as the bioreactor, which had a total volume of 8 L with a working volume of 4 L. The reactor was heated by a hot plate stirrer to maintain the mixed liquor temperature inside the reactor. Complete mixing of the reactor was obtained using a centrifugal water pump circulating the liquid through an outside loop where a T connector was installed with a pH probe (Cole-Parmer, USA) connected to it to simultaneously record the real-time pH. A pH controller (Cole-Parmer, USA) was used to take feedback from the probe, based on which two microtube-pumps were operated to add either base (1.0 mol L^{-1} NaOH) or acid (1.0 N HCl) to the reactor for pH adjustment. The feeding tank was a 20-L water bottle equipped with a mixer that ran for 10 s to

Table 1 The characteristics of the raw and pretreated liquid swine manure

Parameters[1)]	Values of original manure	Values of prepared substrate
pH	7.6	5.4
TS (%)	1.89	1.37
TVS (%)	1.10	1.05
TSS (%)	0.62	0.16
Ortho-P (mg P L^{-1})	174	401
TKN (mg N L^{-1})	3 421	972
BOD_5 (mg L^{-1})	4 890	9 220
COD (mg L^{-1})	13 080	13 940
VFAs (mg L^{-1})	3 547	854

[1)] TS, total solids; TVS, total volatile solids; TSS, total suspended solids; Ortho-P, ortho-phosphate; TKN, total Kjeldahl nitrogen; BOD_5, five-day biochemical oxygen demand; COD, chemical oxygen demand; VFAs, volatile fatty acids.

homogenize the content in the tank before the feeding pump started to move liquid into the reactor.

The ASBR System was operated automatically by a set program of cycle operation following a time sequence. The control of the influent and effluent movements, including all the mixers and pumps, were achieved using peristaltic pumps (Barnant Company, USA), which were controlled through a programmable data board (Campbell Scientific, USA) using the software (Campbell Scientific, USA) installed on a computer. The time for each cycle was set at 4 h, and during each cycle, the liquid was circulated continuously in all phases but the filling, settling, and withdrawal (totally about 30 min) to achieve thorough mixing and rapid diffusion of H_2. At the end of each cycle, a certain amount of reactor content (based on the HRT used) was discharged into an effluent tank. Sampling ports at different locations were installed on the bioreactor to collect both biogas and liquid samples as needed.

2.4. Experimental design and sample analysis

The experiment was carried out in two stages. First, five HRTs of 8, 12, 16, 20, and 24 h were examined for their impact on biogas/H_2 production rates and H_2 content with pH controlled at 5.0. In the second stage, the best HRT identified in the first stage was used as a fixed parameter with five varying pH values (4.4, 4.7, 5.0, 5.3, and 5.6) to investigate the pH effect on the same biogas production characteristics. For each new test, after inoculation, the reactor was first filled with the prepared liquid swine manure to the working volume of 4 L, and then sealed air-tight. To create a completely anaerobic environment, the headspace of the reactor was purged with nitrogen gas for 1 min. For tests in each stage, a break-in period of 24 h was used to operate the reactor in batch mode to establish biogas production before starting the normal fed-batch mode. For HRT experiments, the reactor started with HRT 24 h, which was then progressively reduced at 4 h increments (20, 16, 12, 8 h) by increasing the organic loading rate (i.e., hexose loading rate from 40, to 48, 60, 80, and 120 g d^{-1}). The reactor temperature was maintained at (37±1)°C for all the experiments. Evaluation of the system performance for each parameter (HRT and pH) would not start until the reactor entered the steady-state condition, which was defined as the

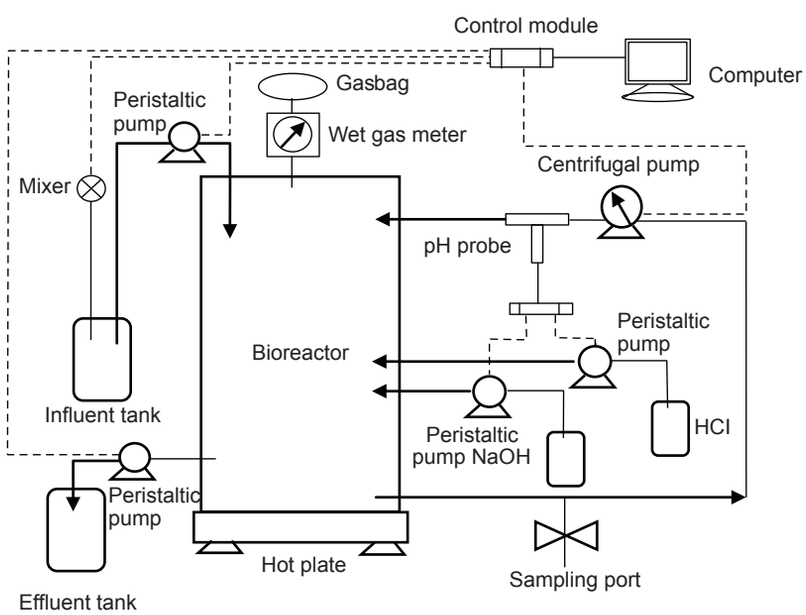

Fig. 1 Experimental set-up of Anaerobic Sequencing Batch Reactor (ASBR) System.

variations of biogas production and glucose conversion rates falling within 5% for five consecutive cycles. The volume of biogas produced was measured by a wet gas meter (GCA/Precision Scientific Inc., Chicago), and the biogas samples were analyzed using a gas chromatography (GC) (Varian 3800; Agilent Technologies, CA, USA) to determine H_2 and CO_2 content at preset time intervals. The GC had a thermal conductivity detector (TCD) installed with a Varian CP7429 column. Helium was used as the carrier gas at a flow rate of 30 mL min^{-1}. The temperatures for the oven, injector, and detector were, respectively, maintained at 50, 120 and 150°C. Each experiment (HRT or pH) lasted 3 wk after the reactor was running in steady-state.

Standard methods were used to analyze liquid samples for total solids (TS), volatile solids (VS), total suspended solids (TSS), five-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and dissolved ortho-phosphate (Ortho-P) (APHA 1998). A Foss Kjeldahl analyzer was used to analyze total Kjeldahl nitrogen (TKN). For dissolved parameters analysis (COD, ortho-P, and hexose concentration), each sample was centrifuged at 4 500 r min^{-1} for 10 min and then filtered through a paper filter (GVWP02500; Fisher, USA) with a pore size of 0.22 μm. All samples were generally analyzed promptly or stored by freezing and thawed to room temperature before analysis. Wherever applicable, the student's *t*-test was used to compare different treatments at a significance level of $\alpha<0.5$.

3. Results and discussion

3.1. Hydrogen content affected by biogas production rate

Fig. 2 presented information on the variations of biogas production rate associated with the H_2 content after the ASBR System entered the steady state under the HRT of 24 h and pH 5.0 (similar situations were not observed for the other four HRTs). The H_2 content in the biogas produced was relatively stable over a range from 32 to 37%, with the biogas production rate fluctuating between 12 and 15 L d^{-1}. However, when the H_2 content moved upwards from 37 to 41%, a drastic drop in biogas production rate occurred from about 13.8 to 3.8 L d^{-1}. This observation is interesting and appears to suggest that when everything else in terms of running conditions is unchanged, the maximum operational H_2 content in the biogas produced will not exceed 38% without causing an appreciable reduction in biogas production rate. The reason for this phenomenon is not well understood. One of the possibilities could be attributed to the rising H_2 partial pressure in the headspace as the concentration of H_2 increased in the biogas generated (Chang and Lin 2004), which has been reported by a number of past researchers (Kim *et al*. 2005; Mandal *et al*. 2006; Jin 2007). High hydrogen partial pressure can lead to accumulation of higher molecule volatile fatty acids (VFAs) than acetate, indicating a potential shift of biological pathway from production of H_2 to organic acids (Kaparaju *et al*. 2009). However, this still does not sufficiently explain the plunging reduction of biogas production rate, although H_2 production rate is part of it. It is known that, in an H_2 fermentation environment, acidogenesis is a major process for producing H_2 by acetogenic bacteria with acetate as the by-product (Mu *et al*. 2006). In the meantime, CO_2 is also produced along with H_2 as a result of anaerobic respiration. The loss of biogas production rate associated with the rise in H_2 content suggested that the production rates for both H_2 and CO_2 were severely hindered, leading to the overall reduction in biogas production rate. The scenario observed here may thus imply that there could exist an upper limit of H_2 content in the biogas with respect to the particular setups used in this experiment such as HRT, pH, and temperature. Besides, it also indicates that high biogas production rate may not guarantee a high H_2 content in the biogas, and *vice versa*. More research is thus needed to determine the intrinsic relationships between the biogas production rate and its H_2 content, as well as the underlying mechanisms.

Another observation with the ASBR System running at the HRT 24 h and pH 5.0 was related to the formation of biomass granules. The occurrence of granules was detected about 23 days after the inception of the ASBR operation including the startup period, which was much faster than reported in other studies (Lee *et al*. 2004; Zhang *et al*. 2008), indicating that the ASBR System investigated in this study was able to shorten the time for the granulation process, a critical step to develop an H_2-producing consortium responsible for effective H_2 production (Wang and Chang 2008). This was, as a matter of fact, proved by the uptick of biogas generation when the formation of granules was positively identified. This information has not been reported elsewhere in the literature.

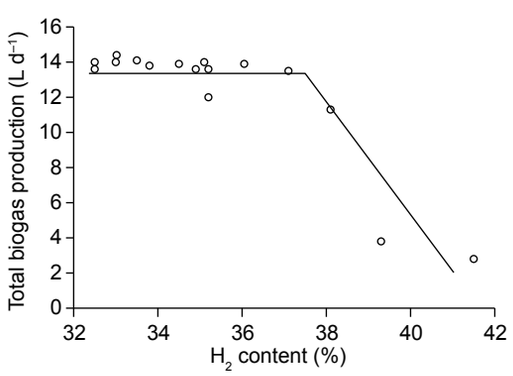

Fig. 2 H_2 content *vs*. biogas production rate at hydraulic retention time (HRT) 24 h and pH 5.0.

3.2. Effect of hydraulic retention time and pH on biogas H_2 content

The effect of HRT on the ASBR System was evaluated by progressively reducing the HRT from 24 to 8 h at 4-h intervals, which was achieved by increasing the loading rate of hexose from 40, to 48, 60, 80 and 120 g d^{-1}. At least three comments can be made about the information generated. First, the results indicated that HRT had a profound impact on the biogas H_2 content (Fig. 3). Generally speaking, for HRTs of 8, 12, 16 and 20 h, the H_2 content in biogas was similar ranging from 40 to 43%, despite that it was significantly lower for HRT 16 h as compared to the other three. As the HRT increased to 24 h, the biogas H_2 content dropped to a much lower level (35%) with a much larger variation. Thus, it may be concluded from the data obtained that the biogas H_2 contents for HRTs of 8, 12, 16 and 20 h did not appear to be significantly different for the ASBR System examined in this study, while HRT 24 h experienced a significant reduction in H_2 content.

Second, the data revealed the high conversion efficiency of sugar to H_2 of the ASBR System examined. Theoretically, if all the hexose added was converted to H_2, the H_2 content in the biogas produced would reach 67% ($C_6H_{12}O_6 \rightarrow 2CH_3COOH+4H_2+2CO_2$). In this study, the average H_2 content in biogas for HRTs from 8 to 20 h was found to be around 42% (Fig. 3), leading to an overall conversion efficiency of 63%, which was almost on par with the upper limit of typical conversion efficiencies (48–67%) of the strict anaerobic hydrogen producers, mainly *Clostridium* species, reported by Lay (2001) and Ueno *et al*. (2001). Therefore, it may be inferred that the ASBR System developed in this study could effectively produce H_2 at a nearly optimal level from swine wastewater supplemented with glucose. Further research is thus warranted to scale up the system for real applications.

Third, it was posited that the biogas produced usually contained not only hydrogen but also carbon dioxide as the other major component. The presence of CO_2 might prevent the biogas from being used in many conventional fuel cells due to the potential toxic effect of the impurities on the fuel cell electrodes which were primarily made of precious metals. Although a most recent study showed a limited effect of CO_2 on the fuel cell electrodes (del Campo *et al*. 2014), it is ideal to keep the CO_2 content in the fermentation biogas at the minimum if at all possible, which is reflected by the CO_2/H_2 ratio in the biogas. The results obtained from this study evinced a CO_2/H_2 ratio ranging from 1.33 to 1.96, which was better than those observed by other researchers (Lee *et al*. 2004; Yang *et al*. 2007). Also, previous reports showed that biogas with 70% H_2 and 30% CO_2 (CO_2/H_2 ratio=0.43) could be successfully used as fuel for proton exchange membrane (PEM) fuel cells (Mann *et al*. 2000). Therefore, the biogas produced from the ASBR tested herein has the potential to be used in PEM fuel cells only after moderate purification to further reduce the CO_2 content.

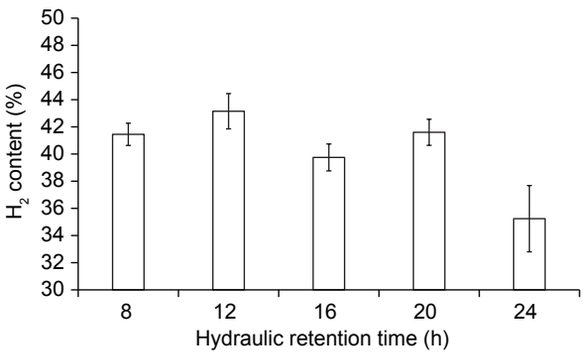

Fig. 3 H_2 content affected by hydraulic retention time (HRT) at pH 5.0. Error bars show the standard deviations of the data.

The changes of H_2 content in the biogas in relation to pH is presented in Fig. 4 (HRT 12 h was selected for this batch of tests) for the ASBR System operating in the steady state with standard error bars also provided. It appeared that the spread of the H_2 content data increased with increasing pH (longer error bars). When pH was 5 or below, the variation in H_2 content was small, but increased as the pH value went beyond 5. This indicated that the ASBR System could become unstable at higher pH values. Han *et al*. (2012) reported that at pH 4.4, the distribution of VFAs produced was reduced with butyrate being the major acid associated with H_2 production, which could be one of the reasons that narrowed the variation range in H_2 content in the biogas. On the other hand, when pH moved higher, the metabolic products of dark fermentation for H_2 production started to change from H_2 to alcohol, and it was reported that when pH reached 6.1, the alcohol production rate would transcend that of H_2, leading to large variations in H_2 content in the headspace biogas during the transition period (Jung *et al*. 2011). In addition, when pH was higher than 5.7, methanogenic reactions could gain momentum, resulting in increasing H_2 content variations due to the unstable or reduced H_2 production (Pender *et al*. 2004). These past research results might explain the decline in H_2 content as well as its increased variation observed in this study.

The data also showed that the best pH for the highest H_2 content appeared to be 5.0, which was consistent with the results obtained by Zhu *et al*. (2007) and Infantes *et al*. (2011). Thus, it may be concluded that the optimal pH for the ASBR System to achieve the highest H_2 content in the

biogas should be maintained around 5.0. Also, an interesting observation from Fig. 4 needing notice was the unequal impact that lower or higher pH values than 5.0 had on the H_2 content. Apparently, as pH decreased, the H_2 content in the biogas decreased as well, but only slightly (from around 37 to 31% according to Fig. 4). However, it was not the case if pH went to the opposite direction, i.e., increasing from 5.0 to 5.6, in which a surprisingly sharp decrease in H_2 content was seen (from 37 to 22% at pH 5.3, and to 8% at pH 5.6). As pointed out by Jung *et al*. (2011), pH has been widely accepted as having the most significant impact on dark fermentation of H_2 production among various operational parameters because of its direct effects on the hydrogenase activity, metabolic pathway, and dominant species. The fall in H_2 content in the biogas could thus be attributed to the digression of pH from its optimal values, i.e., 5.0. More interestingly, a strong inversely linear relationship between pH and H_2 content over the range from pH 5.0 to 5.6 was clearly shown in Fig. 4, with the correlation coefficient of 0.9983. This information has not been reported in the existing literature, and it emphasizes once again the importance of pH in achieving good H_2 fermentation. According to the linear equation, it appeared that the H_2 content in the biogas would arrive at zero at pH 5.78, which might not happen in real operations; however, there were reports showing that when pH was greater than 5.7, methanogenic reactions would come to dominance with the H_2-producing microflora being severely outnumbered as a result (Ting and Lee 2007). Based on the data from this study and the literature information, it may be concluded that maintaining pH below 5.3 is critical for the ASBR System experimented to achieve good H_2 production.

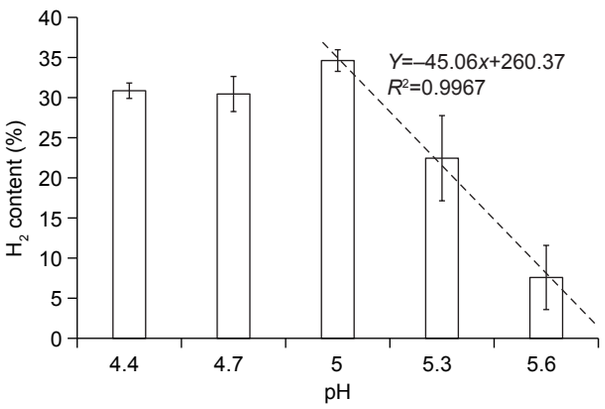

Fig. 4 The effect of pH on biogas H_2 content at hydraulic retention time (HRT) 12 h. Error bars show the standard deviations of the data. Dotted line indicates the linear relationship between hydrogen content and pH from pH ranging from 5 to 5.6.

3.3. Relationships between biogas/H_2 production rates, HRT, and H_2 yield

Fig. 5 documented the relationships of biogas and H_2 production rates as well as H_2 yield with the HRT. Apparently, two good linear relationships were observed, i.e., biogas production rate *vs*. HRT (r=0.9971) and H_2 production rate *vs*. HRT (r=0.9967). The reduction of HRT from 24 to 8 h was accompanied with the increase in biogas production rate from 15 to 34 L d^{-1}. A similar trend was seen for H_2 production rate (from 5 to 14 L d^{-1}) over the same HRT reduction period. These observations indicated that both the biogas production rate and the H_2 production rate were affected by HRT in a linear manner, and the lower the HRT, the higher these two rates would be. This is expected because the inverse relationships between biogas/H_2 production rates and HRT have been commonly encountered in biohydrogen research. Antonopoulou *et al*. (2008) identified similar characteristics of biogas/H_2 production rates *vs*. HRT, and they found that the highest production rates for biogas and H_2 occurred at HRT 4.4 d among the three HRTs tested (20, 10, and 4.4 d) for a periodic anaerobic baffled reactor treating cheese whey. Other researchers reported even much shorter HRTs (0.5–1 h) when the biogas/H_2 production rates reached the highest (Chang *et al*. 2002; Lee *et al*. 2006). Since the adjustment of HRT in this study was achieved by increasing the organic loading rate (OLR) from 40 to 120 g d^{-1} (hexose), it was thus inferred that a higher OLR (a lower HRT) was beneficial in improving biogas/H_2 production rates. However, one caveat worths to be mentioned here is that there is a limit for increasing OLR with any biohydrogen production system because it has been recognized by previous workers that too high an OLR would adversely impact H_2 production (Tawfik and El-Qelish 2012) due to the structural changes of the hydrogenic microbial community (Han *et al*. 2012). The shortest HRT used in this study (8 h) appeared to have not come across the inhibitory limit yet. And, as a matter of fact, the highest H_2 production rate of over 0.16 L h^{-1} L^{-1} obtained from this study with reduced nutrients added in the substrate was largely as good as the reported values (typically 0.1–0.3 L h^{-1} L^{-1}) in the literatures (Chang *et al*. 2002; Lee *et al*. 2006), rendering the ASBR System developed in this study more economically attractive. The results clearly suggested that HRT 8 h was the best for biogas/H_2 production.

However, there is another observation on Fig. 5 that cannot be ignored, i.e., H_2 yield. It seemed that the best HRT (8 h) for biogas/H_2 production did not coincide with the best HRT for H_2 yield (HRT 16 h). In fact, the three H_2 yields (1.58, 1.63, and 1.61 mol H_2 mol^{-1} hexose, respectively) for the middle three HRTs (12, 16, 20 h) were fairly similar,

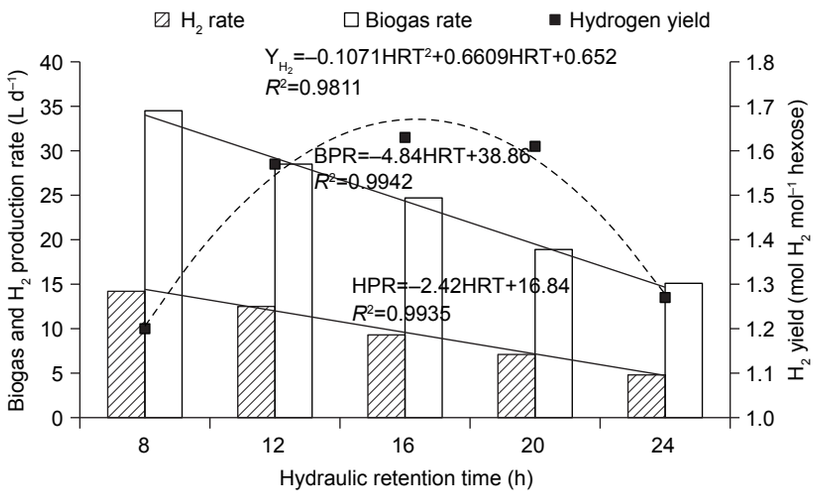

Fig. 5 The relationships between biogas production rate (BPR) and H_2 production rates (HPR), hydraulic retention time (HRT), and H_2 yield (Y_{H_2}) (pH 5.0).

and significant falls in H_2 yield were seen either when HRT went down to 8 h (1.2 mol H_2 mol^{-1} hexose) or went up to 24 h (1.26 mol H_2 mol^{-1} hexose), indicating that at these two HRTs, the ASBR System were inefficient in converting hexose to H_2. A close look at Figs. 3 and 5 might provide some answers for the low H_2 yield at HRT 24 h. According to Fig. 3, the H_2 content at HRT 24 h was low, which could be the reason for the low H_2 yield observed in Fig. 5 for the same HRT. And this certainly indicated that 24 h was not a suitable HRT for the ASBR System investigated herein for efficient H_2 production. This observation may be verified by the ratios of VFAs to soluble microbial products (SMP) because for HRT 24 h, the VFA/SMP ratio was 91% (but for the rest HRTs, it was above 95%), indicating that less H_2 was produced (Pattra *et al*. 2011). Another possible reason for the low H_2 yield and content at HRT 24 h could be the development of methanogenic activities, which weakened H_2 production (Park *et al*. 2010). For the shortest HRT, i.e., 8 h, the reason for the observed low H_2 yield needed some elaboration because comparing Figs. 3 and 5 did not yield the same inference as for HRT 24 h. Normally, shorter HRTs meant quick turnovers of the biomass in the ASBR, and for good H_2 yields, biomass concentration was considered an important factor (Argun *et al*. 2008). Although the loss of biomass during operating cycles were not quantified in this study, the washout of H_2 producing biomass to some extent was suspected for HRT 8 h when half of the reactor content was removed and refilled for each cycle. Analogous observations were reported by Chang and Lin (2004), in which they found drastic reductions in H_2 yield for HRT at both 4 and 24 h using an up-flow ASBR to treat sewage sludge. In comparison, it was interesting to note that many previous workers came to conclusions from their studies that higher H_2 yields were obtained at lower HRTs (Chang *et al*. 2002; Lee *et al*. 2004; Van Ginkel and Logan 2005), which was inconsistent with the results from this study that showed a bell shape of H_2 yield against HRT with the highest H_2 yield occurring at the center HRT (16 h) and the lower H_2 yields located at the both ends of the HRT spectrum (8 and 24 h). Reviewing their work revealed that they either employed bioreactors with high biomass retention capability (fixed bed reactors) (Chang *et al*. 2002; Lee *et al*. 2004) or reduced the organic loading rate (Van Ginkel and Logan 2005). All these strategies certainly helped improve the retention and activity of H_2-producing consortium, leading to the upkeep of high H_2 yields at lower HRTs. Therefore, it may be concluded that considering the experimental design used, the data obtained from this study relatively accurately reflected the trend of H_2 yield associated with the changes in HRT. And the best HRTs for higher H_2 yields included 12, 16, and 20 h according to Fig. 5.

Fig. 5 also revealed another interesting phenomenon that a high biogas/H_2 production rate did not occur in concurrence with a high H_2 yield, which was not uncommon in biohydrogen production because the metabolic pathway of the H_2 producers was not designed to achieve multiple optimums for products production (García-Peña *et al*. 2009). Factors, such as substrate concentration, biomass concentration, etc., all have impact on the metabolic products of H_2 producers. For instance, at low substrate concentrations, *Clostridium acetobutylicum* produces organic acids, but solvents otherwise (Argun *et al*. 2008; García-Peña *et al*. 2009). Therefore, to achieve acceptable biogas/H_2 production rates and H_2 yields at the same time, compromises are

necessary when it comes to selecting the HRT for the ASBR System. In this case, an HRT of 12 h could be a good choice for which biogas/H_2 production rates of 26 and 13 L d^{-1} and an H_2 of 1.58 mol H_2 mol^{-1} hexose can be obtained.

4. Conclusion

The results showed that for the HRT tests (pH 5.0), high biogas production rate might not guarantee a high H_2 content in the biogas, and *vice versa*. HRTs of 8, 12, 16, and 20 h generated good H_2 content (42% on average) in the biogas, while 24 h achieved much lower (35%). The ASBR System demonstrated an overall conversion efficiency of 63%. For the pH tests (HRT 12 h), the optimal pH for the ASBR System to achieve the highest H_2 content in the biogas appeared to be 5.0; however, reducing pH to below 5.0 would not affect the biogas H_2 content in the same magnitude as increasing pH to above 5.0 (31% at pH 4.4, but only 8% at pH 5.6). Good linear relationships were observed between biogas production rate and H_2 production rate *vs*. HRT (r=0.9971 and 0.9967, respectively). The optimal HRT for the ASBR System studied to achieve good biogas/H_2 production rates and H_2 yield simultaneously could be inferred to be 12 h coupled with pH 5.0.

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