2010 Project Abstract
For the Period Ending Feb 28, 2014

PROJECT TITLE: Algae for Fuels Pilot Project
PROJECT MANAGER: *Roger Ruan*
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2010, Chp. 362, Sec. 2, Subd. 7a

APPROPRIATION AMOUNT: $900,000.00

Overall Project Outcome and Results

Current biomass energy technologies have encountered economic, ecological, and policy concerns, including feed stock procurement, energy balance, carbon footprint, competition for food and fuel, water use, etc. This project was built on our existing collaborative R&D partnership, and to demonstrate an innovative photosynthetic algae production system which simultaneously produces high lipid oil for bio-fuel production, captures and recycles N and P from waste water, and sequester CO_2. The goal of the project was to develop, build, and test a pilot scale algae production system that will treat concentrated wastewater and animal facility wastewater, generate algal biomass for production of biofuels and bioproducts. More than 10 high performance algae strains have been developed for specific applications such as oil accumulation, nutrient removal, growth under low temperature and low light conditions, and accumulation of high value lipids. Growth conditions were optimized for specific applications. A pilot cultivation facility with a cultivation volume of 20,000 liters was developed and demonstrated. The microwave assisted pyrolysis was found to be an excellent conversion alternative to conventional oil extraction based biodiesel process, and the hydrothermal process is a cost effective pretreatment technology to improve dewatering of algal biomass. The LCA results indicate that our technologies, which integrate wastewater into algal cultivation, can improve the environmental performance of algal biofuels. The LCA study also suggests that utilization of multiple major waste streams in wastewater plants should be developed to maximize the economic and environmental benefits of algae based technologies. The outcomes of the project point to a great potential of algae technologies for simultaneous removal of nitrogen, phosphorus, chemical oxygen demand (COD), and other nutrients in municipal and animal wastewaters, sequestration of carbons in organic matters and flue gas, and at the same time accumulation of biomass for production of high vale biofuels and bioproducts.

Project Results Use and Dissemination

***This section NOT intended to count toward recommended 300 word length for Abstract**

The project results were disseminated in the following ways:

1) The research results were presented more than 10 times at national and international conferences.
2) The technologies developed as a result of the project were demonstrated to stakeholders 5 times.

3) Information about the project and results obtained were provided to participants of our demonstration events and on our website (http://biorefining.cfans.umn.edu).
4) More than 10 papers were published in peer-reviewed journals (See the list below).

- Du, Z.; Ma, X.; Li, Y.; Chen, P.; Liu, Y.; Lin, X.; Lei, H.; Ruan, R., Production of aromatic hydrocarbons by catalytic pyrolysis of microalgae with zeolites: catalyst screening in a pyroprobe. Bioresource Technology 2013.
- Hu, B.; Zhou, W.; Min, M.; Du, Z.; Chen, P.; Ma, X.; Liu, Y.; Lei, H.; Shi, J.; Ruan, R., Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production. Applied Energy 2013, 107, 255-263.
- Min, M.; Hu, B.; Mohr, M. J.; Shi, A.; Ding, J.; Sun, Y.; Jiang, Y.; Fu, Z.; Griffith, R.; Hussain, F.; Mu, D.; Nie, Y.; Chen, P.; Zhou, W.; Ruan, R., Swine Manure-Based Pilot-Scale Algal Biomass Production System for Fuel Production and Wastewater Treatment—a Case Study. Applied biochemistry and biotechnology 2013, 1-17.
- Wang, Z., Ma, X., Zhou, W., Min, M., Cheng, Y., Chen, P., Shi, J., Wang, Q., Liu, Y., Ruan, R. 2013. Oil Crop Biomass Residue-Based Media for Enhanced Algal Lipid Production. Applied Biochemistry and Biotechnology. 171(3): 689-703
- Zhou, W., Min, M., Hu, B., Ma, X. Liu, Y., Wan, Q., Shi, J., Chen, P and Ruan, R. 2013. Filamentous fungi assisted bio-flocculation: an efficient and low-cost technique for harvesting heterotrophic and autotrophic microalgal cells. Sep. Purif. Technol. DOI:10.1016/j.seppur.2013.01.030
- Du, Z., Hu, B., Ma, X., Cheng, Y., Liu, Y., Lin, X., Chen, P., and Ruan, R. 2012. Catalytic pyrolysis of microalgae and their three major components: carbohydrates, proteins, and lipids. Bioresource Technology. DOI: http://dx.doi.org/10.1016/j.biortech.2012.12.115
- Du, Z., Mohr, M., Ma, X., Lin, X., Liu, Y., Zhou, W., Chen, P., and Ruan, R.. 2012. Hydrothermal pretreatment of microalgae for pyrolytic bio-oil production. Bioresource Technology, 120:13-8. doi: 10.1016/j.biortech.2012.06.007
- Hu, B., Min, M., Zhou, W., Li, Y., Mohr, M., Cheng, Y., Lei, H., Liu, Y., Lin, X., Chen, P., Ruan, R. 2012. Influence of exogenous CO_2 on biomass and lipid accumulation of microalgae *Auxenochlorella protothecoides* cultivated in concentrated municipal wastewater. Applied Biochemistry and Biotechnology. 166(7):1661-73
- Zhou, W., Hu, B., Li, Y., Min, M., Chen, P and Ruan, R. 2012. Mass cultivation of microalgae on animal wastewater: a sequential two-stage cultivation process for biofuel feedstock and omega-3 rich animal feed production. Appl. Biochem. Biotechnol.168: 348-363.
- Zhou, W., Li Y, Min M, Hu B, Zhang H, Ma X, Cheng Y, Chen P, Ruan R. (2012) Growing Wastewater-born Microalga Auxenochlorella protothecoides UMN280 on Concentrated Municipal Wastewater for Simultaneous Nutrient Removal and Energy Feedstock Production. Appl Energ. 98: 433-440.
- Zhou, W., Min, M., Li, Y., Hu, B., Ma, X., Cheng, Y., Liu, Y., Chen, P, and Ruan, R. 2012. A Hetero-photoautotrophic Two-stage Cultivation process to Improve Wastewater Nutrient Removal and Enhance Algal Lipid Accumulation. Bioresour Technol. 110, 448-455.

2010 Environment and Natural Resources Trust Fund (ENRTF)
Work Program – FINAL REPORT

Date of Report: *March 18, 2014*
Final Report
Date of Work Program Approval: *June 9, 2010*
Project Completion Date: February 28, 2014

I. PROJECT TITLE: Algae for Fuels Pilot Project

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Location: *St. Paul, Ramsey County, Minnesota*

Total ENRTF Project Budget: ENRTF Appropriation $900,000.00
Minus Amount Spent: $900,000
Equal Balance: $0.00

Legal Citation: M.L. 2010, Chp. 362, Sec. 2, Subd. 7a

Appropriation Language:
$900,000 is from the trust fund to the Board of Regents of the University of Minnesota to demonstrate an innovative microalgae production system utilizing and treating sanitary wastewater to produce biofuels from algae. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

M.L. 2013, Chapter 52, Section 2, Subdivision 17
The availability of the appropriations for the following projects are extended to June 30, 2014: (11) Laws 2010, chapter 362, section 2, subdivision 7, paragraph (a), Algae for Fuels Pilot Project.

II. FINAL PROJECT SUMMARY AND RESULTS:
Current biomass energy technologies have encountered economic, ecological, and policy concerns, including feed stock procurement, energy balance, carbon footprint, competition for food and fuel, water use, etc. This project was built on our existing collaborative R&D partnership, and to demonstrate an innovative photosynthetic algae production system which simultaneously produces high lipid oil for bio-fuel production, captures and recycles N and P from waste water, and sequester CO_2. The goal of the project was to develop, build, and test a pilot scale algae production system that will treat concentrated wastewater and animal facility wastewater, generate algal biomass for production of biofuels and bioproducts. More than 10 high performance algae strains have been developed for specific applications such as oil

accumulation, nutrient removal, growth under low temperature and low light conditions, and accumulation of high value lipids. Growth conditions were optimized for specific applications. A pilot cultivation facility with a cultivation volume of 20,000 liters was developed and demonstrated. The microwave assisted pyrolysis was found to be an excellent conversion alternative to conventional oil extraction based biodiesel process, and the hydrothermal process is a cost effective pretreatment technology to improve dewatering of algal biomass. The LCA results indicate that our technologies, which integrate wastewater into algal cultivation, can improve the environmental performance of algal biofuels. The LCA study also suggests that utilization of multiple major waste streams in wastewater plants should be developed to maximize the economic and environmental benefits of algae based technologies. The outcomes of the project point to a great potential of algae technologies for simultaneous removal of nitrogen, phosphorus, chemical oxygen demand (COD), and other nutrients in municipal and animal wastewaters, sequestration of carbons in organic matters and flue gas, and at the same time accumulation of biomass for production of high vale biofuels and bioproducts.

III. PROGRESS SUMMARY AS OF:

December 31, 2010: We conducted systematic preparatory research on process parameters including growth characteristics of selected microalgae strains, culture medium conditions, nutrient consumption patterns, exogenous CO_2 and light effect, and hydraulic retention time. We examined a number of materials and design concepts for the construction of PBRs. We began our experiments on both hydrothermal liquefaction and microwave assisted pyrolysis of harvested algae and catalytic upgrading processes.

June 30, 2011: We conducted systematic strain screening and have selected several high performance mixotrophic strains suitable for wastewater environments. A new pilot demonstration facility with a volume capacity of 15,000 liters has been set up in UMN Rosemount Extension and Outreach Center and is operational. An aerobic digester was constructed to pretreat animal manure wastewater. New harvest techniques including biodegradable flocculants and air flocculation device were developed. Lab scale conversion processes including microwave assisted pyrolysis, *in situ* transesterification, and hydrothermal direct conversion are being investigated.

December 31, 2011:

- We tested pilot demonstration system set up at the Metro Wastewater Treatment Plant,
- Tested the new pilot demonstration facility set up in UMN Rosemount Extension and Outreach Center
- Developed a novel fungi assisted harvest technology
- Investigated harvest technology based on self-precipitation
- Developed a two-stage cultivation mode
- Investigated the potential of screened algae growing in a highly concentrated swine manure

June 30, 2012:

- Cultivation process development was conducted on both the lab and pilot facilities
- Sequential production of biofuel and omega-3 rich animal feed on an integrated swine wastewater-based platform was developed and studied.
- A new process to enhance production of free fatty acids from animal manure for mixotrophic growth was developed.
- A hydrothermal process was developed for the pretreatment of wet algal biomass to increase the energy density and reduce the hydrophilicity of algal feedstock.
- A new continuous hydrothermal liquefaction process and system was developed

- Preliminary life cycle analysis was conducted

December 31, 2012:

- We focused on testing of our pilot scale facilities especially the performance of Rosemount facility during winter season
- We compared algal biomass/algal lipid productivity and nutrient removal rate by using water recycle and without water recycle procedures.
- We examined the feasibility of using pretreated manure to enhance mixotrophic growth of microalga Chlorella sp.
- We tested the cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process
- We investigated the cultivation of selected algae strains for production of high protein, high omega-3 lipids and low ash aquafeed.
- We continued to conduct life cycle analysis
- A large amount of data has been generated during this period.

Amendment Request (April 18, 2013)

- We are in the final stage of the algae to fuels pilot project, e.g., finishing up the development of the pilot algal cultivation, harvesting and conversion system assembly, testing and monitoring cultivation operations, conducting life cycle analysis, integrating the systems, and preparation for demonstration. We are currently in the process of ordering a few of the finally chosen systems/modules etc. to complete the algae to fuels demonstration facility.
- We have had several staff members resigned in the past few months which has delayed the progress we were anticipating. Specific tasks affected include optimization of N and P recovery process, production process and system integration, and testing of downstream processing unit operations, which were planned for Result 2. These tasks directly affect the development and construction of the final demonstration facilities and the actual demonstration activities (Results 2 and 3). We were unable to locate additional personnel with the skills needed, and have shifted the available personnel to this project. However, we realized that we still do not have insufficient time to complete the original goals of the project. We therefore request an extension on results 2 and 3 to September 30, 2013. This will allow us to optimize the N and P recovery processes, integrate the production processes and systems, finalize the downstream processing unit operations, and put everything together to have a complete demonstration facility (Result 2). The requested extension will also give us time to conduct LCA and final demonstrations (Result 3).

Amendment Request (Approved 3/18/14)

- We would like to request extension from September to February 28 to have additional time to complete data analysis. We realize that we should have contacted you earlier and apologize for that omission due to our wrong assumption that it was part of the original extension approved.
- We would like to request rebudget of supply funds of $36,107 to be moved to personnel costs in Task 2. This was due to an error of an encumbrance being made twice and it was not recognized at the time of the last budget request. We realize that we should have contacted you earlier and apologize for that omission.
- We would like to request a rebudget of $116 in travel, $1927 in supplies to personnel costs in Task 3. Additional personnel costs were needed as we analyzed the data.

IV. OUTLINE OF PROJECT RESULTS*:*

RESULT 1: *Pilot Scale System for Production of Algae on Wastewater*

Description:
The objective of this project result is to develop, design, and construct a pilot scale algae production facility for process testing and improvement, and demonstration. This facility will consist of our proprietary multi-level continuous flow enclosed photobioreactors (PBRs) housed inside a simple structured greenhouse and will be operational about 10 out of 12 months in Minnesota weather conditions. Concentrated wastewater, diluted or undiluted, will be used as culture media. The facility will be capable of utilizing organic carbons in the wastewater and CO_2 from sludge burning. New heterotrophic algae strains capable of utilizing organic carbons without light or with minimal light have been identified, which removes limitation on building tall multi-layer PBRs. The growth of algae will simultaneously remove nitrogen, phosphorus, chemical oxygen demand (COD), and other nutrients in the wastewater, sequester carbons in organic matters and flue gas. Algae will be harvested on a continuous or semi-continuous basis. The harvested algae will be processed to produce fuels and high value materials as described in the next result. The specific activities for this result include: (1) conduct preparatory research to determine and optimize process parameters for a large pilot scale algae production system, (2) design the pilot scale algae production and harvest system, (3) construct the system, and (4) test and improve the system and process parameters.

Summary Budget Information for Result 1:

ENRTF Budget:	**$ $330,065.00**
Amount Spent:	**330,064.66**
Balance:	**$0.34**

Deliverable	**Completion Date**	**Budget**
1. Optimized process parameters necessary for the design of a large pilot scale algae production system	*12/31/2010*	$54,903
2. Production and harvest system development	*12/31/2011*	$107,828
3. System construction and installation	*06/30/2012*	$122,057
4. System testing and improvement	*12/31/2012*	$69,957

Result Completion Date: *12/31/2012*

Result Status as of December 31, 2010: A dozen of high performance microalgae strains obtained from our previous research were studied for their growth characteristics under different nutrition and light conditions and their ability to accumulate lipids and remove COD, N, and P. These strains were confirmed to be mixotrophs which are capable of growing on organic and/or inorganic carbon sources and under light and/or dark. The strains with optimal biomass and lipid yields and nutrient removal capability will be selected for further tests to determine the best strains for our pilot scale production and demonstration. We are also studying the feasibility of recycling nutrients from conversion liquids and solids. Chemical analysis shows these liquids and solids contain considerable amount of N and P. We are currently developing methods to utilize these nutrients. We evaluated different materials for PBR construction. The desirable materials should be inexpensive, mechanically strong, and easy to shape, and have reasonable light transmittance. One material has been selected for construction of our PBRs.

Result Status as of *June 30, 2011*

Strain selection and production condition development

More than 150 strains were screened in search for high performance strains suitable for municipal and animal wastewater cultivation. The production conditions for selected strains were studied. Production condition optimization for scale-up operation is under way.

Production systems development

A greenhouse based 1500-L pilot scale system was setup at the St. Paul Wastewater Treatment Plant (Metro Plant) (Fig. 1) for pilot study of algae cultivation on municipal wastewaters and two 7500-L photobioreactors were constructed inside a high tunnel greenhouse was set up at Rosemount Research and Outreach Center for pilot study and demonstration of algae cultivation on animal wastewaters (Fig 2 a and b). These systems are ready for pilot study. They will also be made available for demonstration during the Algae Biomass Organizations (ABO) 2011 annual meeting to be held in Minneapolis Oct 24-27, 2011.

Figure 1. Greenhouse-based algal production system.

(a)

(b)

Figure 2. Large scale algal production system. (a) High tunnel greenhouse. (b) PBR.

Biodegradable flocculant

We are developing a starch based flocculant for algal biomass harvest. The objectives are to produce a non-toxic flocculant that facilitates cost effective harvest of algal biomass which may be used as animal feeds or feedstock for recovery of high value compounds for nutraceutical and cosmetic industries. Currently the starch based flocculant synthesized in our lab is able to harvest 99% of algae from culture broth compared with 93% with commercial polymer CPAM flocculant. We are conducting optimization to further improve the performance and cost effectiveness of the starch based flocculant.

Harvesting System

An algal harvesting system was designed and constructed and ready for test. This system is based on the principle of air flotation. The micro-bubbles generated through pressure drop would adhere to the suspended matter causing the suspended matter to float to the surface of the water where it may then be removed by a skimming device. Figure 3 shows the harvesting system.

Figure 3. Air-flotation system.

Anaerobic Digestion System

A 1000-gallone anaerobic digester was designed and constructed. The digester was used to digest swine manure. Since animal manure could be digested to produce methane gas for generating electricity and digested animal manure still contains lot of nutrient, the digested

animal manure is suitable for growing algae. The two PBR will test the digested and undigested animal manure side by side to provide a sustainable approach for animal waste management and algal biomass production. Figure 4 shows the digestion system which includes a circulation pump, temperature control, and pressure regulator.

Figure 4. Anaerobic digestion system.

Result Status as of *December 31, 2011*

Testing of the Anaerobic Digestion System

Initially, the working volume of the AD was set at 600 gallon. An inoculation of 50 gallons dairy seed sludge and 550 gallons of raw swine manure was used to start the reactor. After a 21 day startup period was completed the reactor then entered a continuous operation mode. The AD is fed weekly with 200 gallons of fresh swine manure. Before feeding the four-hour settling time is allowed followed by the removal of 200 gallons of reactor content. The biogas is released continuously as it is created to ensure a low partial pressure in the headspace. Biogas samples are collected at the gas discharge port when necessary. Liquid samples in the reactor are collected at the sample port.

The characteristics of the raw and digested manure (after 21 days) are listed in Table 2. From the table it is apparent that decreases of 30.3%, 60.4% and 55.2% for COD, TSS and TVSS respectively are achieved. These reductions indicate that the AD is operating correctly.

Table 1. Characteristics of raw and digested swine manure.

Swine manure	Nutrients concentration (mg/L)				Solid content (g/L)	
	COD	TN	NH_3	TP	TSS	TVSS
Before digestion	9300	3150	2960	24.05	35.43	22.00
After digestion	6480	3440	3200	20.75	14.03	9.85

Testing of the Pilot System at Rosemount

In July-August 2011, the pilot system was operated using 2", 4" and 6" water depth, and harvested half of the volume three times a week. The system was inoculated with a local screened species UMNxxx and 20 times diluted swine manure as the culture medium. The productivity and nutrient removal rate were listed in table 1 in which 99% of NH3, 62% of COD and 91% of PO4-P could be removed at 2" water depth, however 6" water depth could produce best algae areal yield.

Table 2. The productivity and nutrient removal by using hog manure-based algae production system at Rosemount, MN.

Water depth (inch)	Biomass productivity (g/m^2/day)	Removal rate (%)		
		NH_3	COD	PO_4–P
2	3.4	99%	62%	91%
4	7.1	96%	25%	34%
6	18.7	86%	33%	57%

Several observations were made during the test run. It was found that there was large precipitation during the cultivation which made the estimation of the algal production difficult. The original data was based on the suspended algae solution; however, the precipitate should have a significant contribute to biomass production. It was also found the algal density in suspended solution did not vary much with different water depth which indicated higher water depth may increase the areal yield. How to collect the precipitate and harvest the algae would be a next task.

Testing of the Pilot System at the Metro Wastewater Treatment Plant

In summer 2011, the system was tested under three water depths with a hydraulic retention time (HRT) of 3 days. Based on a lab experience which indicated that using 3-day HRT could produce higher algal biomass yield, so in year 2011, we tested the system with 3-day HRT.

The algal biomass had the highest productivity at 2" water level due to very high biomass concentration at 2" (1.55 g/L). The concentration of algal biomass at 2", 4" and 6" were 1.55, 1.05, 0.64 g/L respectively, and the according biomass productivity were 29.11, 26.49, 15.82 g/(m^2 day). Nutrient removal rate in term of g/(m^2 day) were increased linearly for TP and COD due to larger volume of water per surface area were removed. Such as for COD the removal rate were 2.86, 6.51, and 9.50 g/(m^2 day) for 2", 4" and 6" water depth respectively.

Table 3. Algae biomass productivity and nutrient removal rate.

Water depth (inch)	Biomass productivity (g/m^2 day)	Removal rate (%)			
		Ammonia	TN	TP	COD

2	29.11	77.53	62.30	51.82	81.81
4	26.49	66.71	62.53	58.32	89.10
6	15.82	40.12	42.78	69.73	84.57

Developed a novel fungi-assisted harvest technology and its application in treatment concentrated wastewater

Most eukaryotic algae live freely in many terrestrial and aquatic environments. A few, however, are found only in symbiotic association with other organisms such as molluscs, flatworms, coelenterates, protozoa, and fungi (lichens) Lichens are commonly found in nature and it is proven that in the majority of lichens (Figure 1), the algae occur in a thin, well-defined layer just beneath the thallus surface, and a thin, tough cortex of fungal material is usually above the algal layer, while beneath the algal layer is a thicker layer of more loosely arranged fungal hyphae, the medulla. However, research in the area of symbiosis of pelletized fungi and algae cells and their co-effect on municipal and animal wastewater treatment is rear.

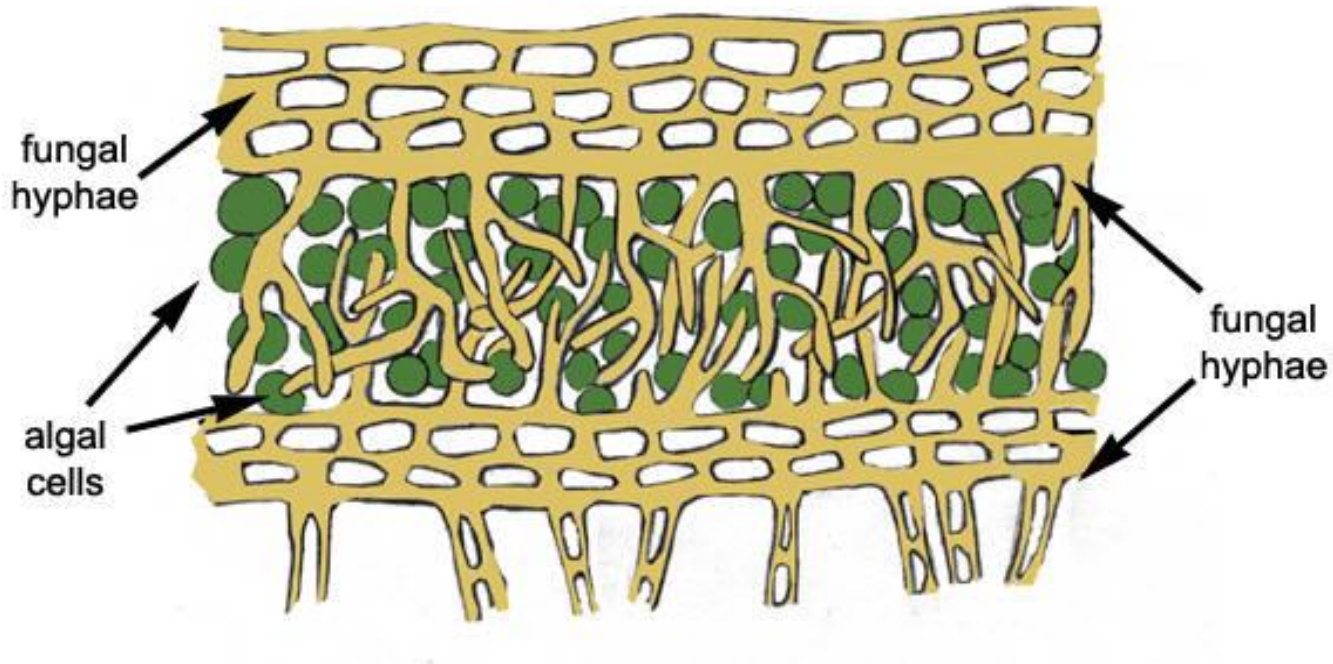


Fig.5. Structure of lichen.

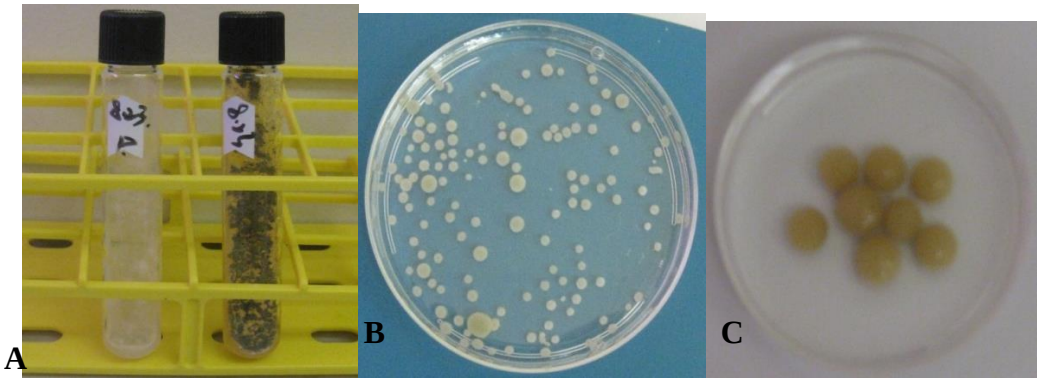


Figure 6. The newly isolated fungal species. A, fungi species *Aspergillus sp.* UMN F01 (left) and *Aspergillus sp.* UMN F02 (right); B, UMN F01 pellets; C, UMN F02 pellets

Under lab condition, two filamentous fungal species were isolated from environment, and identified as *Aspergillus sp.* UMN F01 and *Aspergillus sp.* UMN F02 by morphological analysis. It is observed that UMNF01 showed white color, while UMNF02 was in yellow-green color when grown on solid slant (Figure 6 A). As shown in Figure 6 B and 2 C, both of them showed the properties of self pelletization under mentioned conditions in section 2.3 of materials and methods after 3 days cultivation. It was also observed that the culture pH was dropped dramatically during the fungi cultivation, probably due to some unknown acid excretion. And it seems that Fungi pellets were easily formed when culture pH ranged between 5.0-6.0 and pH reached as low as 4.0, could help form large pellets (Figure 6 C).

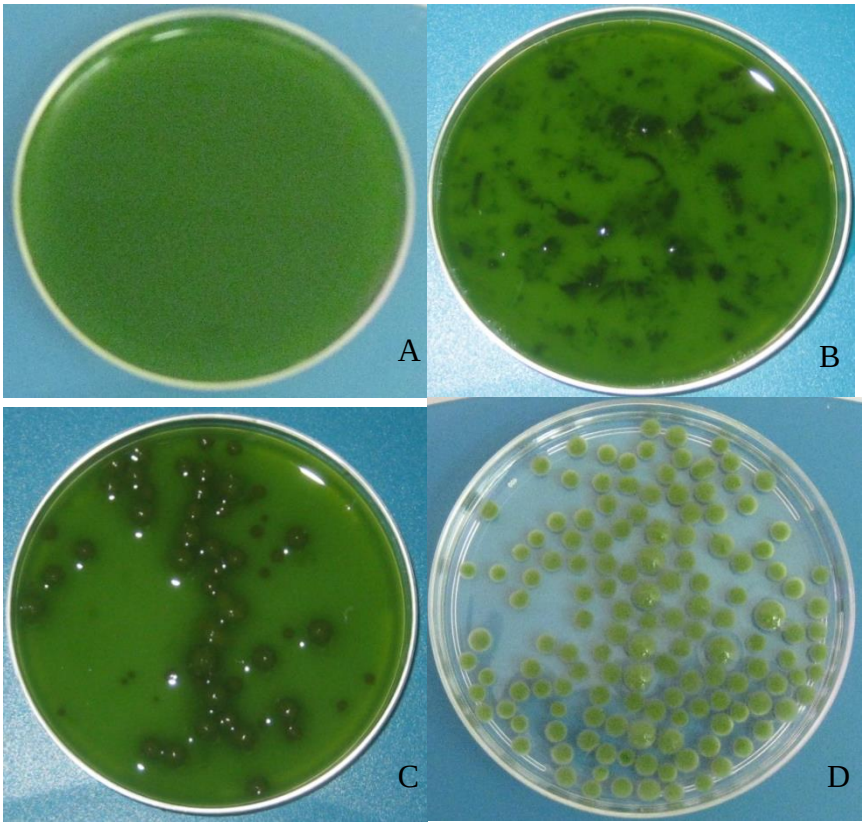


Figure 7. The process for fungi-algae pellets formation. A, algae alone; B, after adding fungi spores; C, some of algae entrapped by fungi; D, all algae entrapped by fungi.

It was observed that filamentous shape was first formed when adding the fungal spores and cultivating overnight, which then formed pellets after continuous cultivation on shakers (Figure 7).

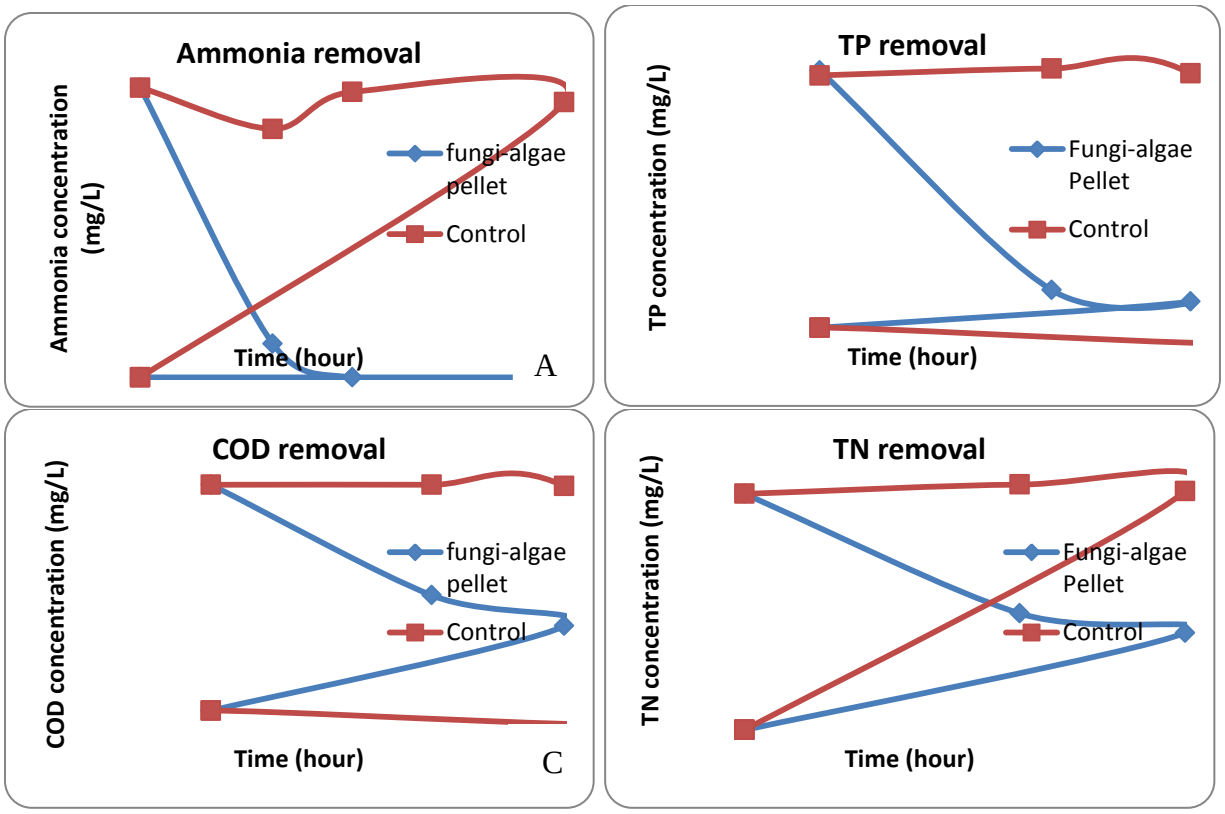
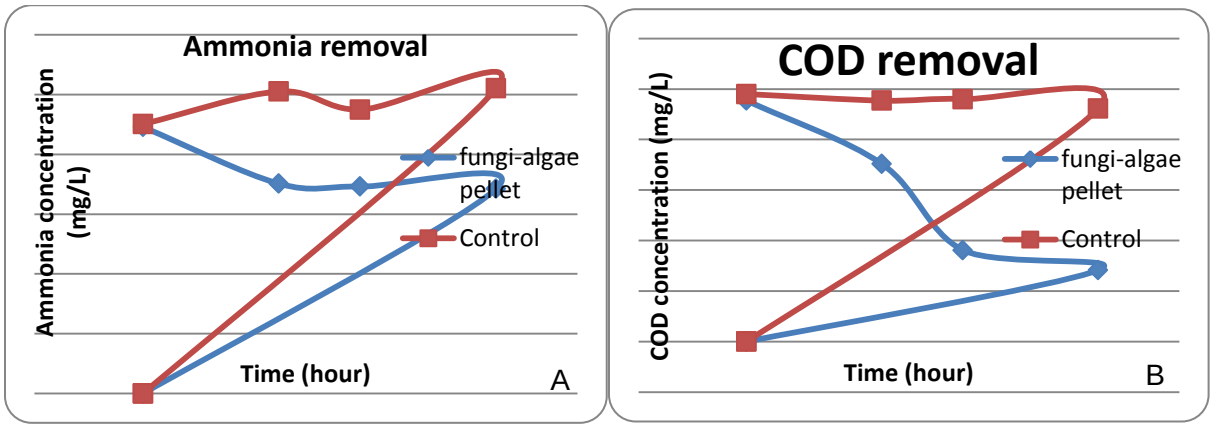


Figure 8. Centrate Nutrient removal profile in Fungi-algae culture system. A, Ammonia removal profile; B, Total phosphorus removal profile; C, COD removal profile; D, Total Nitrogen removal profile.

The results of the effect of the fungi-algae pellets on centrate wastewater treatment are shown in Figure 8. NH_4-N was removed completely in 24 hours, while it was reported that it took about 14 days to remove about 93% of NH_4-N when grown free algae cells on centrate wastewater. The total nitrogen dropped from 97.2 to 40.0 mg/L in one day. Total phosphorus was drastically reduced from 52.6 to 5.35 mg/L and the concentration of COD decreased dramatically from 1,660 to 622 mg/L in one day. These results were much better than our previous reports using algae alone for wastewater treatment. The high nutrient removal efficiency was probably due to the unique structure of such fungi-algae pellets.

D



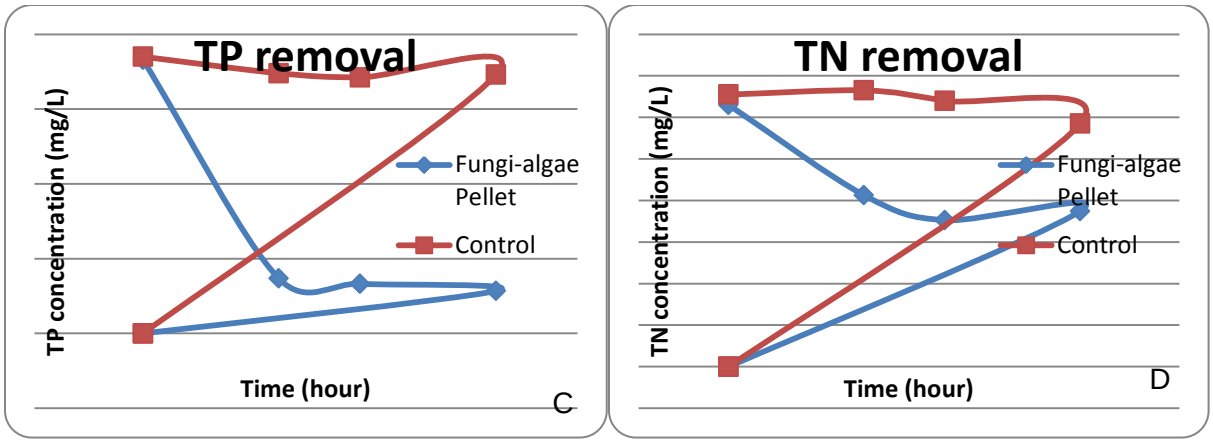


Figure 9. 20X hog manure Nutrient removal profile in Fungi-algae culture system. A, Ammonia removal profile; B, COD removal profile; C, Total phosphorus removal profile; D, Total Nitrogen removal profile.

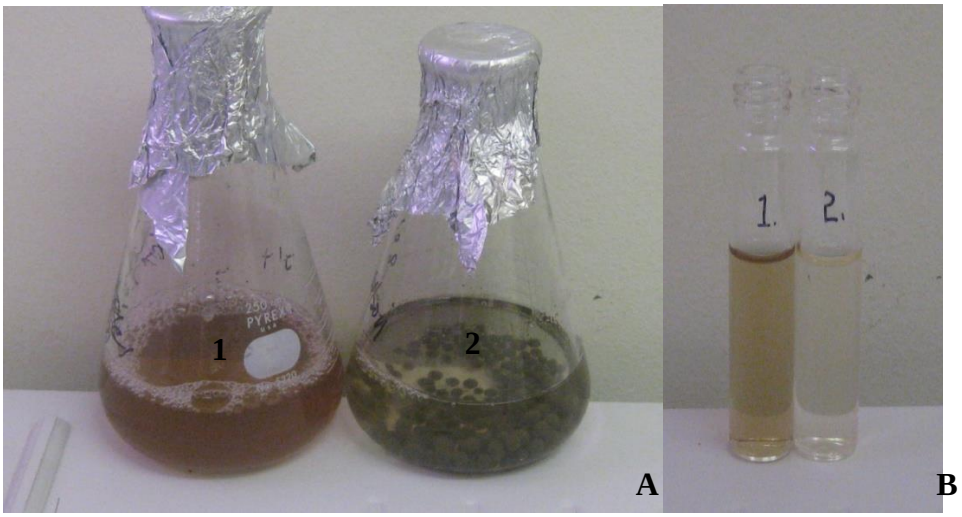


Figure 10. The color of 20X hog manure before and after treatment overnight by Fungi-algae pellets. 1, control; 2, overnight treatment (15hs).

The results of the effect of the fungi-algae pellets on 20X diluted swine manure wastewater treatment were shown in Figure 9. The NH_4-N, total phosphorus and COD dropped from 89.1 to 68.4 mg/L, 1.83 to 0.28 mg/L, and 954 to 283 mg/L, respectively in 39 hours. Total nitrogen was reduced from 126 to 70.5 mg/L in one day. All these results were much better than our earlier data and others in the literature, showed the great potential of fungi-assisted immobilized algal cell for concentrated wastewater treatment.

It is interesting to note that after only 15 hours culture in fungi-algae pellets, it was observed that the color of the swine manure become much more clear than that of initial (Figure 10), which suggested the unique absorption characteristics of such fungi-algae pellets. The unique characteristics of the fungi assisted algal immobilized cells could be further applied to treat heavy metals contaminated industrial wastewater and it was reported that some heavy metals such as Al and Cu could be used as catalysts for conversion the biomass into bio-oil by direct conversion. Thus the fungi-assisted immobilized algal cells with the absorbed metals could be

converted to refined bio-oil directly by thermochemical processes as both feedstock and catalysts.

Investigated harvest technology based on self-precipitation

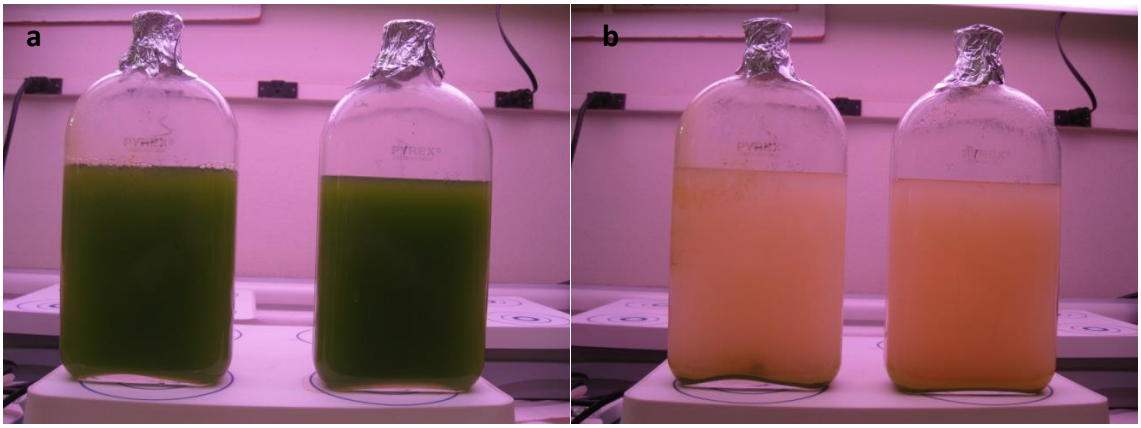


Figure 11. Microalgal strain *A. protothecoides* UMN280 self-sedimentation phenomenon observed in the static condition grown on CMW. (a) Total phoohorus removal profile; (b) Total phoohorus removal profile;

Table 4. Characteristics of Centrate for nutrient and mental iron profile.

mental ion	Initial CMW (mg/L)	after first stage of HPM treatment
Al	0.082	0.082
As	0.232	0.232
B	0.357	0.352
Ba	0.082	0.01
Be	0.001	0.001
Ca	132.67	10.904
Cd	0.011	0.011
Co	0.039	0.039
Cr	0.034	0.014
Cu	0.017	0.017
Fe	1.925	0.084
K	197.63	147.86
Li	0.017	0.013
Mg	96.209	13.141
Mn	4.01	0.172
Mo	0.095	0.117
Na	199.34	163.31
Ni	0.075	0.04
P	200.25	28.436
Pb	0.176	0.176
Rb	0.715	0.443
S	20.972	15.563
Si	23.3	19.783
Sr	0.278	0.023
Ti	0.005	0.005
V	0.02	0.016

Zn	0.115	0.016

It was observed that the microalgae strain *A. protothecoides* UMN280 grown on CMW trend to coagulate and form large colonies during the cultivation and easily to settle down by gravity at the end of experiment (Figures 11a&b). It was reported that many algae species including *scenedesmus sp.*, *micractinium sp., Actinastrum sp.*, *Pediastrum sp.*, *Dictyosphaerium sp.*, *Coelastrum sp.* that dominate wastewater treatment HRAPs often form large colonies (50-200 µm), which is in agreement with the results of this study, and suggests the great potential of using such microalgal strain in CMW based algae biofuel production and wastewater treatment. The factors that may affect the algae self-sedimentation are complex. It is well known that algae show strong negative charge on the cell surface particularly during exponential growth, thus they could coagulate with suspended particles in CMW and form large particles. Moreover, it was noted that there were still some metal ions such as Ca^{2+}, Mg^{2+} and so on present in the CMW even at the end of experiment (Table 4). These cationic ions became natural vectors to coagulate with negative algae and form large colonies, thus help to settle down Furthermore, it was reported that elevated pH also could help form large colonies between cationic metal ion and algae. Finally, there is high possibility that some residual polymers were not removed completely during CMW pretreatment process, which also contribute to algae precipitation observed in this study. The phenomena of self-aggregation observed in algae-based wastewater system could minimize the harvest cost to great extend, which will further lower the whole system cost.

Developed a two-stage cultivation mode

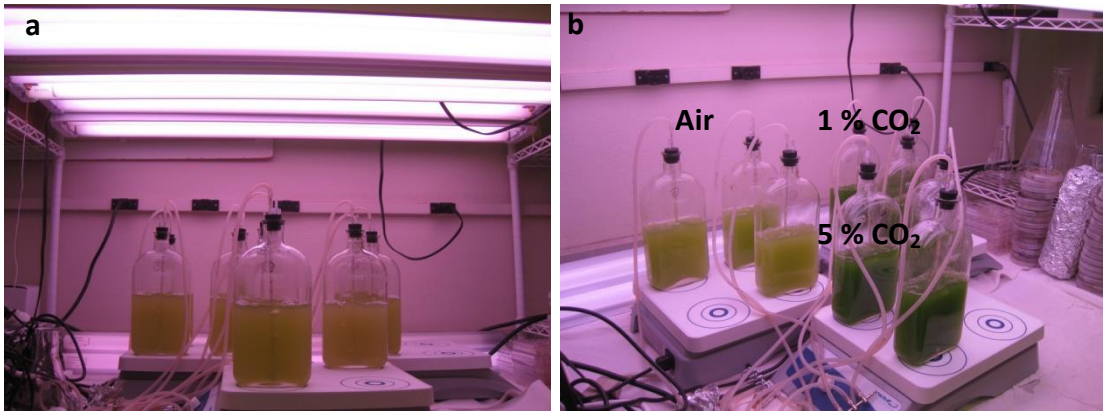


Figure 12. Experiment design of microalgal strain *A. protothecoides* UMN280 grown in the second stage of HPM. (a) initial phase of second stage of HPM; (b) stationary phage of second stage of HPM;

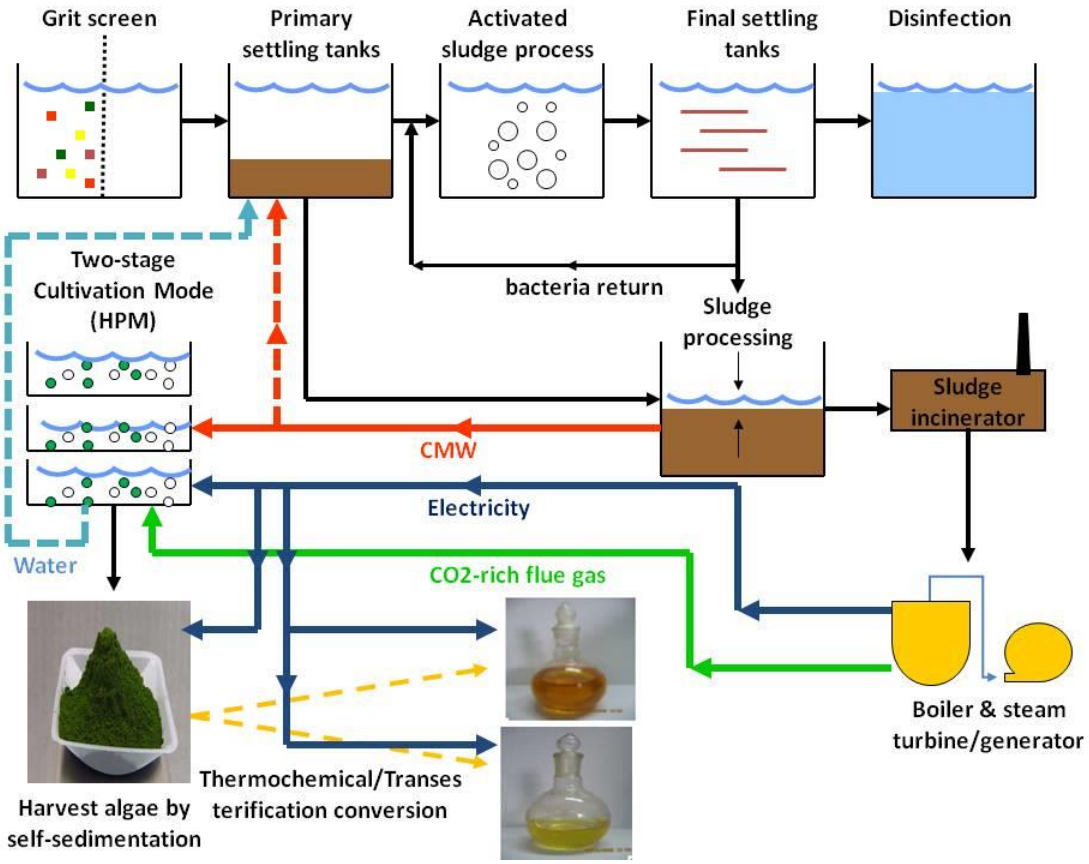


Figure 13. Integration of Pilot-scale HMP algae cultivation system into Metro Plant Municipal Wastewater Process Flow. CMW: Concentration municipal wastewater; HPM: hetero-photoautotrophic two-stage cultivation mode.

Based on the above fact that CMW was rich in organic carbon, N, P, and trace element, a hetero-autotrophic two-stage cultivation strategy was adopted in order to fully utilize above nutrients and at the same time to investigate the nutrient removal efficiency and algal biomass and lipid accumulation in such strategy. The present study was part of the ongoing major collaborative efforts on mass cultivation of algae on municipal wastewaters for fuels and products between the University of Minnesota and Metropolitan Council Environmental Services of Minnesota (MCES). One of MCES facilities, the St. Paul Metro plant utilizes a biological system to treat municipal wastewater and the sludge generated at the Metro plant are dewatered using centrifuges and then combusted in fluid bed incinerators equipped with heat recovery boilers. The process requires no additional inputs of fuel and creates heat and electricity for the buildings (Figure 13). The CO_2-rich flue gas during combustion could be sequestered by sparging into algae culture bioreactor and electricity produced could be used to run bioreactor and harvest algae as well as to convert algae to refined bio-oil directly by thermochemical processes or biodiesel by transesterification. Thus the integrated system and process could be incorporated into typical metro Plant municipal wastewater process flow to achieve significant cost reduction of algal based renewable biofuel production as well as mitigation of GHGs emission (Figure 13).

Investigated the potential of screened algae growing in a highly concentrated swine manure

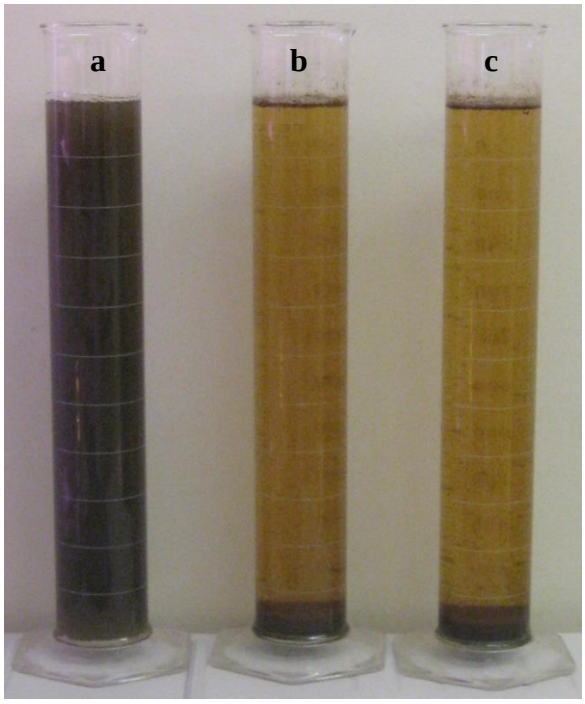
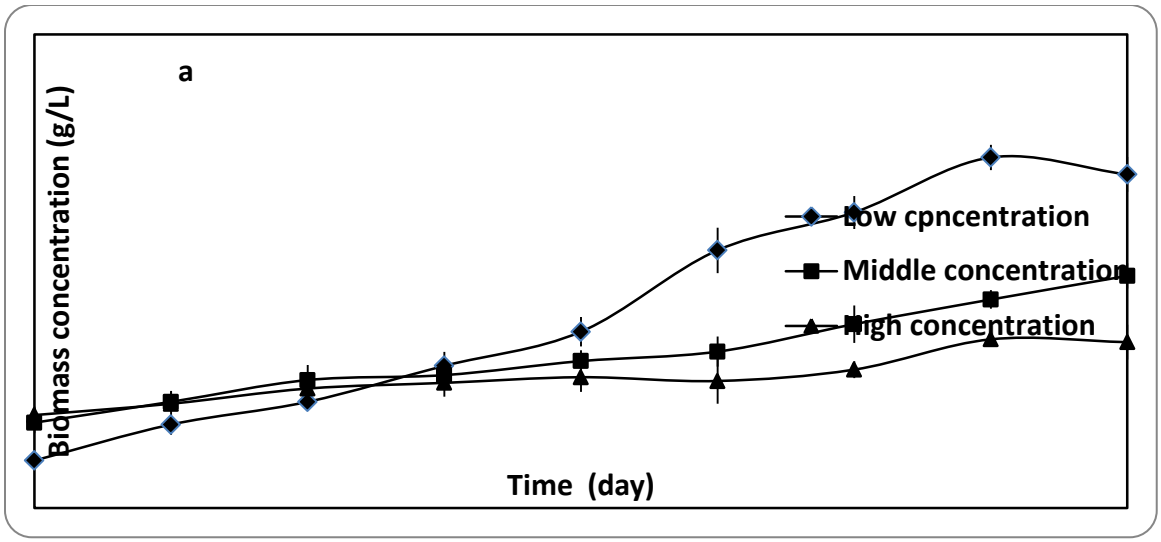
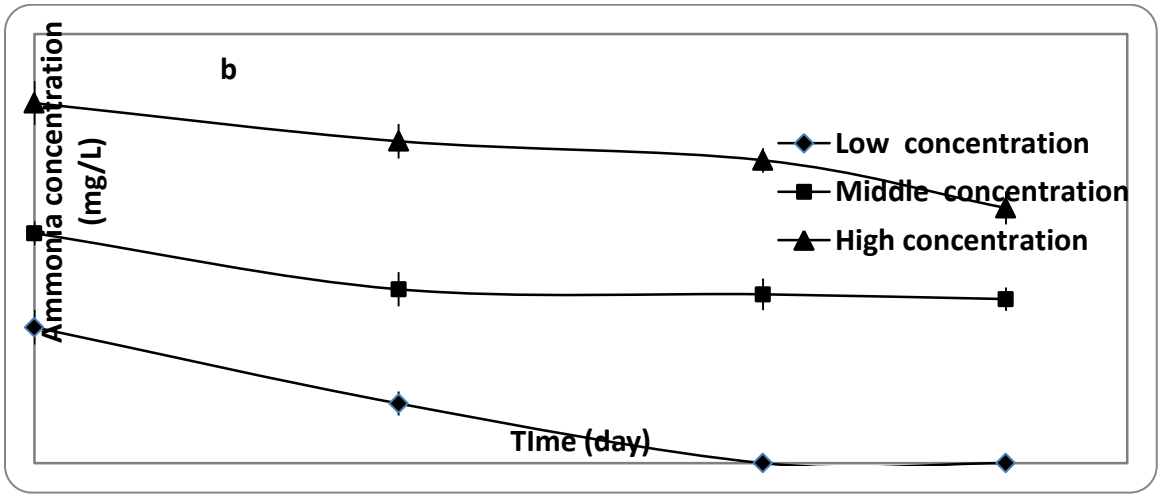


Figure 14. Investigation of precipitation of concentrated swine manure before and after adding cationic starch (a) 5× dilution of digested hog manure without cationic starch addition; (b) and (c) 5× dilution of digested hog manure with cationic starch addition

Table 5. Nuritent profile of DSM before and after starch addition

5X dilution of DSM	Ammonia (mg/L)	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	COD (mg/L)
Before cationic starch addition	475±8.96	7.11±0.13	564±6.72	1360±23.76
After cationic starch addition	460±7.81	6.48±0.07	532±9.90	1180±33.21


a
Biomass concentration (g/L)
Time (day)
Low cpncentration
Middle concentration
High concentration


b
Ammonia concentration (mg/L)
TIme (day)
Low concentration
Middle concentration
High concentration

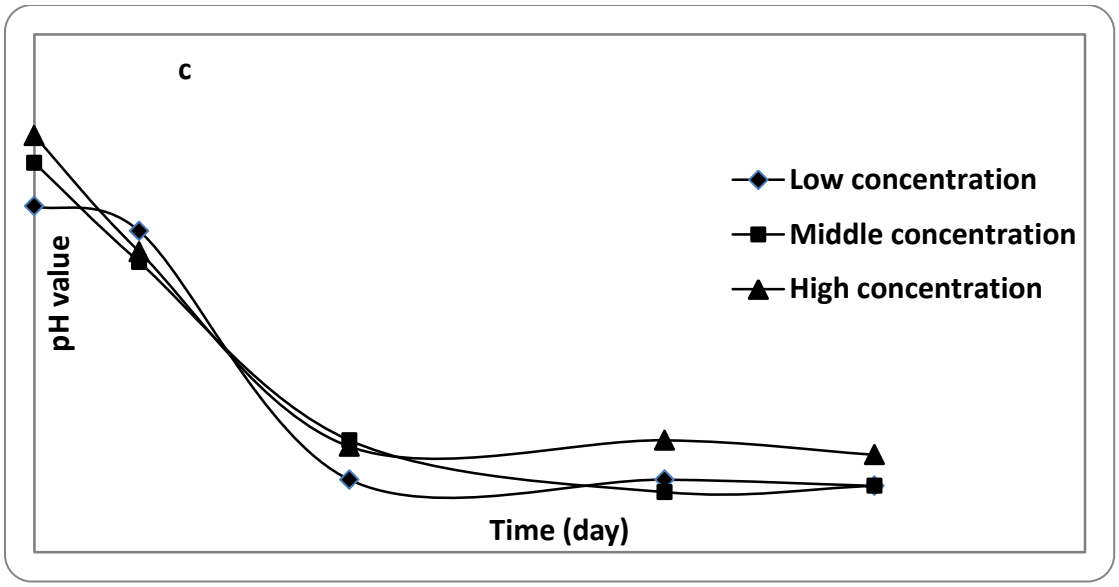


Figure 15. Growth ,nutrient removal profile and pH curves of microalgal strain *A. protothecoides* UMN280 during batch culture grown on 5× diluted hog manure (a) Growth profile; (b) Ammonia removal profile; (c) pH curves.

It is well known that effluent derived from anaerobic digestion of swine manure must be further treated due to high turbidity and opacity. The traditional treatment method is to dilute the digested swine manure with tap-water to final concentrations of 0.6-3.0%. However, dilution has to be reasonably small in order to reach the practical demand for treatment large amounts of swine manure daily. Therefore, investigation of impact factors affecting algae growth on highly concentrated swine manure wastewater of 5-fold dilution was studied in this section.

When grown algae on highly concentrated swine manure, the opacity of the algal growth medium will play an important role for final algae biomass concentration. In this study, biodegradable cationic starch was first used to treat 5-fold swine manure to reduce the turbidity. The results of before and after adding cationic starch for digested swine manure wastewater was shown in Figure 14. It was observed that the 5-fold swine manure showed more clear opacity after adding 2g/L of the cationic starch and treatment only 30 minutes while the nutrients profile of digested swine manure was similar before and after cationic starch addition except for COD concentration (Table 5). After treatment with cationic starch, the digested swine manure with relatively low turbidity was used as culture media to grow algal strain UM271 and examine the influence of turbidity on algae growth. However, the cultivation failed and algae could not grow whatever the initial algal inoculums used (0.1-0.6 g/L) and levels of CO2 injected. It was detected that concentration of ammonia in 5-fold dilution digested swine manure was over 400 gm/L. It was reported that algae grown on medium with high concentration of ammonia will inhibit algae grown. Thus, the probable reason for this case maybe the ammonia existed in 5-fold dilution DWM was still high. Therefore, in the subsequent experiment, different concentration of ammonia was examined for algae growth on 5-fold dilution.

As discussed above, it seems ammonia concentration should be the key factors affecting algae growth in 5-fold diluted DSM. Thus, in this study, different concentrations of ammonia were used to examine the influence of ammonia concentration on algae growth and nutrient removal. Air bubbling method was used to control the ammonia concentration in 5-fold diluted DSM. As

shown in Figure 15, when ammonia concentration was controlled between 50-150 mg/L, algae could survive in such highly concentrated DSM. And the optimal concentration was 55 mg/L, under which the maximal biomass reached 1.85 g/L(Figure 15a). Ammonia removal followed a pattern consistent with biomass production for three different concentrations of ammonia and was removed completely in 7-day cultivation for low concentration of 55 mg/L (Figure 15b). It is worth noting that, while the removal of the ammonia was significant at the end of first stage, the ammonia concentration remaining in the CMW was still very high for middle concentration and high concentrations of ammonia (96.4 mg/L and 151 mg/L), which may reflect the positive affect of ammonia concentration on algal biomass and nutrient removal. PH value also showed the similar pattern among three different concentrations when aerated with 5 % CO_2 in the culture medium.
Moreover, it was found that ammonia concentration played an important role on algae growth, and high lipid content of 34% of dry weight was obtained when grown on concentrated DSM (5 fold dilution) probably due to high C/N ratio.

Result Status as of *June 30, 2012*

Activity 1: An integrated swine wastewater-based algal platform sequential biofuel and omega-3 rich animal feed production

The use of algae cultivation in the treatment of digested swine manure (DSM) wastewaters, during which nutrients are converted into valuable algal cell components such as lipids for bio-fuel production, and protein/carbohydrate for animal feed use, could maximize the potential utilization of algae biomass. In addition, some algal species are excellent source of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), which are valuable PUFAs and contain important dietary compounds and have beneficial effects in human health as functional ingredients.
In order to cultivate algae in swine manure wastewater as feedstock for biofuel production, algae strains need to meet several criteria, including (1) the ability to survive in wastewater; (2) capability of growing to high cell density with high content of lipids, preferably TAGs; (3) Some algae strains could accumulate omega-3 unsaturated fatty acid under certain conditions, and (4) the capability of growing heterotrophically or mixotrophically since swine manure contains both organic carbon and inorganic carbon.
Thus the objectives of this study were to (1) establish microalgae pool from candidate strains derived from local area and algae banks adequate for digested swine manure wastewater treatment and energy crop production; and (2) further screen top-performing strains for simultaneous swine wastewater treatment and bio-fuel and omega-3 rich animal feed production with a two-stage cultivation strategy; (3) exam an integrated process and system for simultaneously swine wastewater treatment, high lipid accumulation for biofuel feedstock and omega-3 included animal feed production as well as mitigation of GHGs emission.

Experimental Design
In this study, the experiments were carried out in three consecutive phases. The first phase was aimed at testing the survival capability of all selected algae strains, including strains purchased from algae bank and strains isolated from local area, and determining the best facultative heterotrophic candidates to grow in digested swine manure cultivation system. The second phase was targeted at determination of top-performing strains based on high growth rate and omega-3 unsaturated fatty acid containing microalgae for animal feed. In this phase, top-performing strain UMN 271 was first evaluated for algae growth and nutrient removal profile under aeration with four different levels of CO_2, i.e., air, 1% CO_2, 5% CO_2, and 10% CO_2, respectively, in 1 L Roux culture bottles (Corning Inc., USA) containing 550 mL sterilized DSMW

media with a magnetic stir bar on the bottom mixing the solution at 100 rpm in 13-day batch culture under designed conditions. The third phase was to develop an integrated approach for simultaneous wastewater treatment and biofuel as well as animal feed production in a sequential mixo-photoautotrophic two-stage culture mode using the selected two most promising algal strains, UMN 271 and UMN 231. In this phase, UM271 was cultivated mixotrophically at first-stage, after removal of algal biomass by centrifuge, the residual supernatant was reused as media to culture omega-3 containing strain UM231 at the second-stage with 5% CO_2 aeration to determine the algal biomass and biochemical composition, as well as nutrient removal in 1L Roux culture bottles (Corning Inc., USA) containing 550 mL sterilized DSMW media. All experiments were carried out in triplicates, and the average values were reported. Results were analyzed using Excel and software SPSS.

Wastewater Resource

Digested swine manure wastewater was collected from Rosemount Research and Outreach Center (Rosemount, MN) and used for algae cultivation. A digester installed above the ground is a covered 1000 gallon plastic tank. The digester was designed with a maximum treating capacity of 800 gallon of fresh swine wastewater and a retention time of 21 days. Large solid particles in the wastewater streams were removed by centrifugation followed by filtration with filter cloth (Wypall X70, Kimberly-Clark Professional). After filtration, the wastewater were autoclaved at 121 ºC, allowed to cool to room temperature, and stored at 4 ºC for 5 days to allow settling of visible solid particles, and the supernatant was diluted 20-fold for algae cultivation.

Algal Strains and Growth Media

In this study, 97 different algae strains (Table 1) from the family of *Chlorella*, *Haematococcus, Scenedesmus*, *Botryococcus*, *Ankistrodesmus*, *Tetraadron*, *Tetracystis*, *Nannochloris*, *Crucigenia*, *Dictyochloris Chlamydomonas, Cosmarium, Characium*, and *Chlorococcum* purchased from the Culture Collection of Algae at the University of Texas and 50 algae strains isolated from local waters in Minnesota were tested (Table 2). Prior to being transferred to the digested swine manure wastewater, they were preserved in BG-11 medium containing (g/L): $K_2HPO_4 \cdot 3H_2O$, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; Citric acid, 0.006; Ferric ammonium citrate, 0.006; EDTA, 0.001; $NaNO_3$, 1.5; Na_2CO_3, 0.02; trace metal mix A5, 1.0ml. Trace metal mix A5 solution consisted of (g/L) H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $NaMoO_4 \cdot 2H_2O$, 0.39; $CuSO_4 \cdot 5H_2O$, 0.079; and $CoCl_2 \cdot 6H_2O$, 0.05

Table 1. List of UTEX strains for the bioprospection in this study

UTEX ID	Species	UTEX ID	Species	UTEX ID	Species
16	*Haematococcus lacustris*	302	*Cosmarium botrytis*	1779	*Chlorococcum paludosum*
20	*Chlorella ellipsoidea*	305	*Cosmarium subtumidum*	1782	*Chlorococcum oviforme*
25	*Chlorella protothecoides*	325	*Selenastrum gracile*	1786	*chlorococcum salsugineum*
26	*Chlorella vulgaris*	326	*Selenastrum minutum*	1787	*Chlorococcum sphacosum*
32	*Chlorella zofingiensis*	343	*Chlorella fusca var.fusca*	1788	*Chlorococcum texanum*
46	*Protosiphon botryoides f.pariet*	398	*Chlorella kessleri*	1789	*Chlorococcum typicum*
55	*Haematococcus droebakensis*	414	*Scenedesmus dispar*	1904	*Chlamydomonas zebra*
63	*Crucigenia tetrapedia*	415	*Selenastrum Acuminatus*	2096	*Characium bulgariense*
78	*Scenedesmus obliquus*	416	*Scenedesmus acutiformis*	2097	*Characium californicum*
79	*Scenedesmus basiliensis*	417	*Scenedesmus dimorphus*	2108	*Characium typicum*
101	*Ankistrodesmus falcatus var.*	572	*Botryococcus braunii*	2168	*Chlorella sp.*
117	*Chlorococcum minutum*	580	*Chlorella sp.*	2219	*chlorella minutissima*
120	*Tetraedron bitridens*	674	*Navicula pelliculosa*	2222	*Chlorococcum aquaticum*
127	*Dictyochloris pulchra*	748	*Ankistrodesmus falcatus var.*	2240	*chlorella minutissima*
151	*Monodus subterraneus*	750	*Ankistrodesmus braunii*	2248	*Chlorella sp.*
187	*Ankistrodesmus braunii*	773	*Tetracystis aplanosporum*	2252	*Dictyochloris schumacherensis*
189	*Ankistrodesmus angustus*	972	*Chlorococcum ellipsoideum*	2341	*chlorella minutissima*
190	*Ankistrodesmus densus*	1054	*Chlamydomonas moewusii var.*	2438	*Chlorococcum sp.*
208	*Chlamydomonas sphaeroides*	2911	*Chlorella saccharophila*	2442	*Coelastrum astroideum*
228	*Chlamydomonas dorsoventralis*	1233	*Chlorococcum scabellum*	2445	*Tetrastrum heteracantum*
230	*Chlamydomonas applanata*	1236	*Scenedesmus longus*	2459	*Scenedesmus minutum*
241	*Ankistrodesmus angustus*	1237	*Scenedesmus dimorphus*	2498	*Chlorococcum pamirum*
242	*Ankistrodesmus falcatus var.*	1338	*Chlamydomonas noctigama*	2502	*Nannochloris eucaryotum*
244	*Ankistrodesmus braunii*	1344	*Chlamydomonas debaryana var*	2505	*Haematococcus pluvialis*
245	*Ankistrodesmus braunii*	1450	*Scenedesmus obliquus*	2527	*Dictyochloris pulchra*
246	*chlorella sorokiniana*	1591	*Scenedesmus sp.*	2532	*Scenedesmus subspicatus*
251	*Chlorella fusca var.vacuolata*	1648	*Selenastrum capricornutum*	2551	*Scenedesmus armatus*
252	*Chlorella fusca var.vacuolata*	1767	*Chlorococcum arenosum*	2629	*Botryococcus sudeticus*

256	*Chlorella protothecoides*	1768	*Chlorococcum aureum*	2630	*Scenedesmus obliquus*
261	*chlorella sorokiniana*	1769	*Chlorococcum citriforme*	2714	*chlorella vulgaris*
280	*Coelastrum microporum*	1774	*Chlorococcum macrostigmatum*	2805	*chlorella sorokiniana*
287	*Oocystis marssonii*	1776	*Chlorococcum loculatum*		
299	*Cosmarium impressulum*	1777	*Chlorococcum microstigmatum*		

Table 2. Collection dates and sites of local microalgal strains established in the study

Local strain ID	Collection date	Collection site	Local strain ID	Collection date	Collection site
UMN220	Oct, 2006	a Twin Cities lake	UMN253	Jun, 2006	Theodore Wirth Lake 2 on the beach
UMN221	Apr, 2006	RoseLawn Pond	UMN254	Jun, 2006	Lake Calhoun
UMN223	Apr, 2006	Como Park Golf Course Pond #2	UMN255	Jun, 2006	Moore Lake
UMN224	Apr,2006	Como Lake	UMN258	Jun, 2006	Rice Creek after the bridge inside the park
UMN225	Apr, 2006	McCarrons Lake	UMN259	Jun, 2006	MayFlower drainage pond
UMN226	Apr, 2006	Rosville Park, Lexington south of County Rd C	UMN260	Jun, 2006	Drainage pond behind Bachmans
UMN227	Apr, 2006	Oxford and County Rd C	UMN261	Jun, 2006	Pond of Assisting Living banfill acrss apartments
UMN228	Apr, 2006	County Rd C and Victoria: north Pond	UMN263	Jun, 2006	Marlenes's drainage at the park
UMN229	Apr, 2006	County Rd C and Victoria: north Pond	UMN264	Jun, 2006	Metro Wastewater Treatment Plant dreft side
UMN230	Apr, 2006	Falls to the lake on County Rd C and Victoria	UMN265	Jun, 2006	3M Innovation Plant lake
UMN231	Apr, 2006	Lake Johanna, west side	UMN266	Jun, 2006	Kaller Lake
UMN232	May, 2006	swamp in west side of Lake Johanna across the road	UMN267	Jun, 2006	Pond on Keller Lake
UMN233	May, 2006	next swamp across Lake Johanna	UMN268	Jun, 2006	Maplewood: Lakewood and Maryland
UMN238	May, 2006	Lake Josephine east side	UMN269	Jun, 2006	Mcarron's lake
UMN240	May, 2006	Drainage to Lake Josephine #2	UMN270	Jun, 2006	Margolis pond on Lapenteur
UMN241	May,	Pond #1 across Rosville High School	UMN271	Jul, 2006	Loon Lake, Waseca

UMN242	2006 May, 2006	Pond #1 across Roseville High School	UMN272	Jul, 2006	Loon Lake, Waseca
UMN243	Jun, 2006	Como Park lake	UMN273	Jul, 2006	White Bear Lake
UMN244	Jun, 2006	Channel on Ripley road, Litchfield (at the golf course)	UMN274	Jul, 2006	Bold Lake, east site
UMN245	Jun, 2006	Lake Ripley picnic area, Litchfield	UMN275	Jul, 2006	Amelia Lake
UMN246	Jun, 2006	Pond between County Rd 1 and County Rd 23, Litchfield	UMN276	Jul, 2006	Coon Rapids Dam #1
UMN247	Jun, 2006	Lake Hope, Litchfield	UMN277	Jul, 2007	Pond at Marine City
UMN250	Jun, 2006	Theodore Wirth Parkway, Pond #3 on the right coming from 394	UMN278	Jul, 2007	Pond at Marine City
UMN251	Jun, 2006	Theodore Wirth Parkway, left, right Pond #2b around the bridge	UMN279	Jul, 2007	Spring brook 1, Fridley
UMN252	Jun, 2006	Theodore Wirth Lake 1, farther than the beach	UMN281	May, 2006	Itasca main lake

Results

Characteristics of Swine Manure Wastewater Before and After Digestion

The characteristics of the digested and undigested swine wastewater were summarized in Table 3. During the anaerobic digestion, 39% of the COD, 60% of TSS and 55% of TVSS were reduced. The total phosphorus did not change much since anaerobic digesters are known to reduce negligible amounts of phosphorus, while the concentration of total nitrogen and ammonium increased obviously, from 3272 mg/L and 2820 mg/L up to 4317 mg/L and 3630 mg/L, respectively, due to the anaerobic bioconversion of proteins contained in manure into amino acids and then to ammonia. Other micro nutrients such as Cu^{2+} and Zn^{2+}, also did not change much before and after digestion in this study (Table 3).The organic carbon profile in swine wastewater was also detected and listed in Table 4. The major substances in the swine wastewater were sugar, acetic acid, propionic acid, and butyric acid. Thus, the majority of the nutrients were retained in the swine wastewater after anaerobic digestion, which must be further reduced through conventional treatment or algal cultivation before discharge.

Establishment of a Microalgae Strains Pool Capable of Growing Robustly on DSMW

Previous reports demonstrated that BG-11 was ideal medium and commonly used for identifying autotrophy and heterotrophy of unicellular blue-green algae. Thus, in this study, 97 algae strains purchased from algae bank (Table 1) and 50 local isolated algae strains (Table 2) were tested for the ability of growing on both autotrophic BG-11 media ($NaHCO_3$ as carbon source) and heterotrophic BG-11 media (glucose as carbon source with $NaHCO_3$ eliminated from media). The results showed that all the tested strains were capable of using both inorganic and organic carbon source and assimilating them into biomass, suggesting that these strains may be able to grow in the inorganic and organic carbon containing DSMW wastewater stream (Table 3 and Table 4). A multi-step screening strategy described previously was adopted to identify candidate strains which showed facultative heterotrophic capability and were capable of surviving a 20-fold diluted DSMW-based culture system (Zhou et al., 2011). By this screening strategy, 99 among 147 tested strains could grow both photoautotrophically and heterotrophically under light and in dark, and 79 strains were found to be able to survive in DSMW. When continuously screening colonies on the 20-times diluted swine manure agar plates under continuous illumination for weeks, only 41 of them adapted well in cultures without further acclimation (Table 5). Thus an adequate microalgae pool in which all algae strains could tolerate DSMW was established successfully.

Table 3 Characteristics of swine manure before and after anaerobic digestion

Parameter	Digested manure	Raw undigested manure
PH	8.48±0.29	7.45±0.31
NH_3-N (mg/L)	3630.1±1250.0	2820.3±225.7
Total nitrogen (mg/L)	4317.0±1263.2	3272.1±323.6
Total phosphorous(mg/L)	38.90±1.57	48.20±3.43
COD (mg/L)	8933±666.7	14707±3668.9
TSS (g/L)	14.03±1.12	35.43±1.49
TVSS (g/L)	9.85±0.87	22.00±1.23
Al (mg/L)	1.9	2.32
B (mg/L)	2.5	2.5
Ca (mg/L)	99.46	64.02
Cu (mg/L)	1.4	1.06
Fe (mg/L)	11.66	11.14
K (mg/L)	3389.2	3494.8
Mg (mg/L)	133.66	81.92
Mn (mg/L)	0.38	0.2

Na (mg/L)	973.5	970.76
Ni (mg/L)	0.64	0.64
Zn (mg/L)	4.94	4.14

Table 4. Organic carbon profiles in swine manure wastewater before and after digestion

Wastewater streams	Reduced Sugar (mg /L)	Volatile fatty acids (mg/L) Acetic acid	Propionic acid	Butyric acid	Alcohols (mg/L) Ethanol	Propanol	Butanol
Before digestion	576	153.33	163.95	162.65	ND	ND	ND
After digestion	428	120.76	284.31	313.64	ND	ND	ND

Note: ND-not detectable

Table 5. The strain code and growth rate of selected top-performing microalgal strains adapted well to digested swine manure in batch culture

Strain ID	R_{TVSS} (g L^{-1} d^{-1})	Strain ID	R_{TVSS} (g L^{-1} d^{-1})
UTEX26	0.146±0.052	UMN242	0.005±0.014
UTEX78	0.167± 0.029	UMN243	0.200±0.052
UTEX230	0.189±0.043	UMN244	0.386±0.025
UTEX326	0.223±0.025	UMN245	0.245±0.038
UTEX343	0.160±0.029	UMN247	0.359±0.018
UTEX251	0.029±0.025	UMN251	0.158±0.029
UTEX252	0.114±0.029	UMN259	0.201±0.038
UTEX1236	0.129±0.016	UMN260	0.173±0.025
UTEX1591	0.208±0.014	UMN263	0.214±0.038
UTEX1787	0.209±0.020	UMN264	0.069±0.032
UTEX2240	0.194±0.038	UMN265	0.327±0.035
UTEX2498	0.040±0.018	UMN266	0.275±0.027
UTEX2551	0.085±0.016	UMN267	0.186±0.041
UTEX2714	0.282±0.058	UMN269	0.311±0.058
UMN220	0.264±0.035	UMN270	0.298±0.028
UMN224	0.209±0.043	UMN271	0.536±0.025
UMN228	0.158±0.058	UMN274	0.259±0.029
UMN231	0.433±0.038	UMN276	0.390±0.038
UMN232	0.250±0.029	UMN277	0.149±0.056
UMN238	0.185±0.035	UMN279	0.231±0.029
UM240	0.02±0.014		

Note: R_{TVSS} represents the growth rate of microalgae. And Each data indicates the mean ± SD (error) and were measured from three independent cultures.

High-throughput screening of robust strains from both purchased and local isolated algal strains and establishment of an adequate microalgae pool specifically for simultaneous energy crop

production and animal manure wastewater (e.g. DSMW) treatment, to the best of our knowledge, has not yet been reported before. The selected candidate strains in this pool have great potential to treat different kind of animal manure derived from dairy, swine, and poultry farms. Moreover, some of the above mentioned robust strains could be selected for mixed-cultivation to improve nutrient removal efficiency and culture system stability as well as tolerance to invasion of other species.

In order to obtain the most promising strains with high growth rates and higher biomass productivity for further studies, the candidate strains were further screened based on their growth rate (R_{TVSS}) in 7-day batch cultivation in autoclaved 20-time diluted DSMW on an orbital shaker at light intensity of 60 μmol $m^{-2} s^{-1}$. The growth data in terms of TVSS for the 41 strains are summarized in Table 5. Among 41 candidate strains, 21 strains showed higher growth rates (0.200-0.536 d^{-1}) than the rest (0.005-0.194 d^{-1}). It is worth noting that among the 21 top performing strains, 17 strains were isolated from the local sites and only 4 strains, namely UTEX 326, UTEX 1591, UTEX 1787 and UTEX 2714, were from the purchased strains. Our results showed that algal strains isolated from local strains tend to adapt well in local environments compare with purchased strains, which coincided with the findings from other researchers. In addition, among these top-performing strains isolated from local area, UMN 271 and UMN 231 showed much higher growth rates (0.536 d^{-1} and 0.433 d^{-1}) than other strains. And it was demonstrated that UMN 271 could accumulate lipid content as high as 27.51% of dry weight when grown on wastewater environment. Therefore, strain UMN 271 was chosen for further study based on the high lipid content and high growth rate in the next section. It was also found that UMN 231 was able to produce omega-3 unsaturated fatty acid (EPA), making it a good candidate feedstock for animal feed. Thus strain UMN 231 also was chosen for further study.

It was demonstrated in our previous studies that algae grew faster in 20-fold diluted digested animal manure compare to other different dilution (e.g. 10-fold, 15-fold, and 25-fold) (Wang et al, 2010), thus 20-fold dilution was used throughout the experiments in this phase. However, when algae were grown on 20-fold diluted digested animal manure in flask on shaker without CO_2 injection, the biomass productivities of different facultative heterotrophic strains selected above hardly exceeded 0.8 g L^{-1} (data not shown), while the maximum biomass density of some algae strains could be higher than 1.6 g L^{-1} in standard BG-11 media with glucose supplement [26]. By analyzing the nutrient profile, it was reasonable to conclude that the concentration of organic and inorganic carbon was not high enough in 20-fold diluted DSMW and could not maintain algal heterotrophic growth without a continuous supply of organic carbon, or grow autotrophically without the addition of inorganic carbon or CO_2 (Table 3 and 4). Thus, supply of exogenous CO_2 as carbon source may be beneficial for algal biomass accumulation, which will be examined and discussed in the next phase.

Investigation of Different CO_2 levels on Algae Growth and Nutrient Removal in the First Mixotrophy Dominated Stage for UMN 271

The profiles of biomass concentration and pH were shown in Figs. 1a and 1b. It was clear that algae cells cultivated on 5% CO_2 reached the highest biomass concentration of 2.03 g L^{-1} among the four levels of CO_2. However, the biomass declined slightly when the level of CO_2 was increased to 10%. Chiu et al. studied the effects of CO_2 concentration (at concentrations of 2%, 5%, 10% and 15%) on the growth of *Chlorella sp.* and found that algae growth could be inhibited heavily when grown on high CO_2 level such as 10% CO_2 and 15% CO_2. Chang and Yang [28] selected the mutant of microalgae tolerant to elevating CO_2 and found the maximal biomass produced by selected algal mutant was at 5% CO_2 aeration. Our results confirmed these previous studies and provided a useful alternative that can be applied to wastewater-based algae cultivation system for energy crop production. The pH values declined greatly with the increasing exogenous CO_2 concentration from 1% to 10% CO_2 probably due to more CO_2

dissolved in the water as $HCO3^-$ form. Direct CO_2 injection into the culture media is the best and most convenient method of pH control and at the same time supplying CO_2 for high algal biomass production.

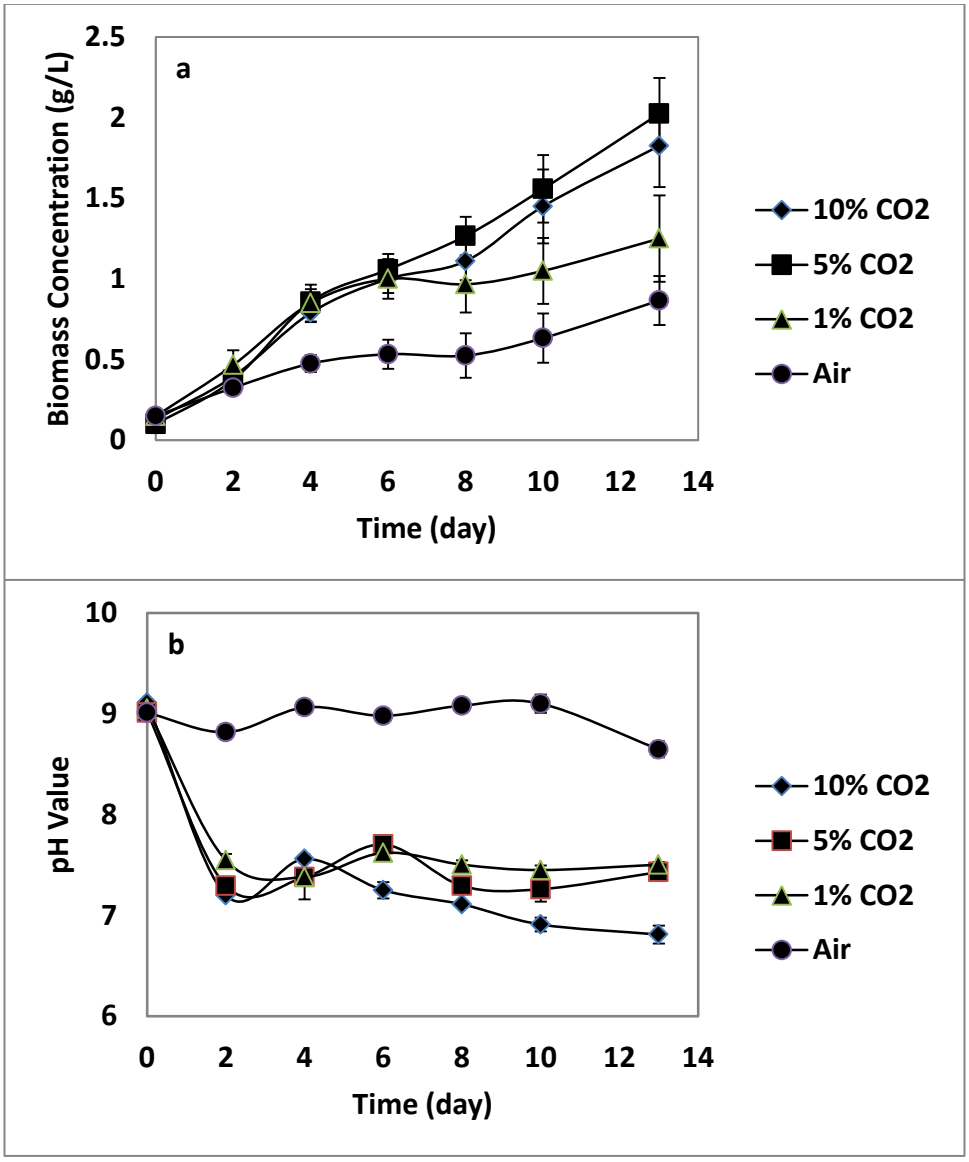


Figure 1. Growth profile and pH curves of microalgal strain UMN271 grown on digested swine manure at four levels of CO2 aeration (a) Growth profile; (b) pH curves. Each data indicates the mean ± SD (error) and were measured from three independent cultures.

The profile of total phosphorus and NH_4-N as a function of time in DSMW for 13 days in batch culture were shown in Figs 2a-b, respectively.

It shows that total phosphorus concentration reduced dramatically during the first 4 days and stayed at the similar removal rate until completely removed at the end of experiment (Fig. 2a). In our previous researches it was reported that algae might cause coagulation and adsorption of inorganic phosphates when the pH of culture media was increased to 8. However, in this study, the phosphorus removal was not due to pH rise since the pH was controlled below 8.0 by

constant CO_2 injection. It was reported that algae biomass typically contains 0.5-3.3% phosphorus [29]. Considering the low pH value in the system and the fact that some algae strains are capable of luxury uptake of phosphorus, it is reasonable to conclude that complete removal of phosphorus in this study was solely attributed to algae assimilation.

The NH_4-N concentration dropped from about 160-168 mg/L to 17.7 mg/L, 109.2 mg/L, 72.3 mg/L and 75 mg/L for air, 1% CO_2, 5% CO_2 and 10% CO_2 supplement, respectively (Fig. 2b). It was obvious that the algae culture aerated with air obtained highest removal efficiency of 89.5% followed by algae aerated with 5% CO_2. It was reported that NH_3 stripping occurred under alkaline pH value, and in this study, the range of pH aerated with air was between 8.6 and 9.1, thus the loss of NH_4-N in the air-aerated system probably was attributed to both NH_3 stripping and the assimilation by algae.

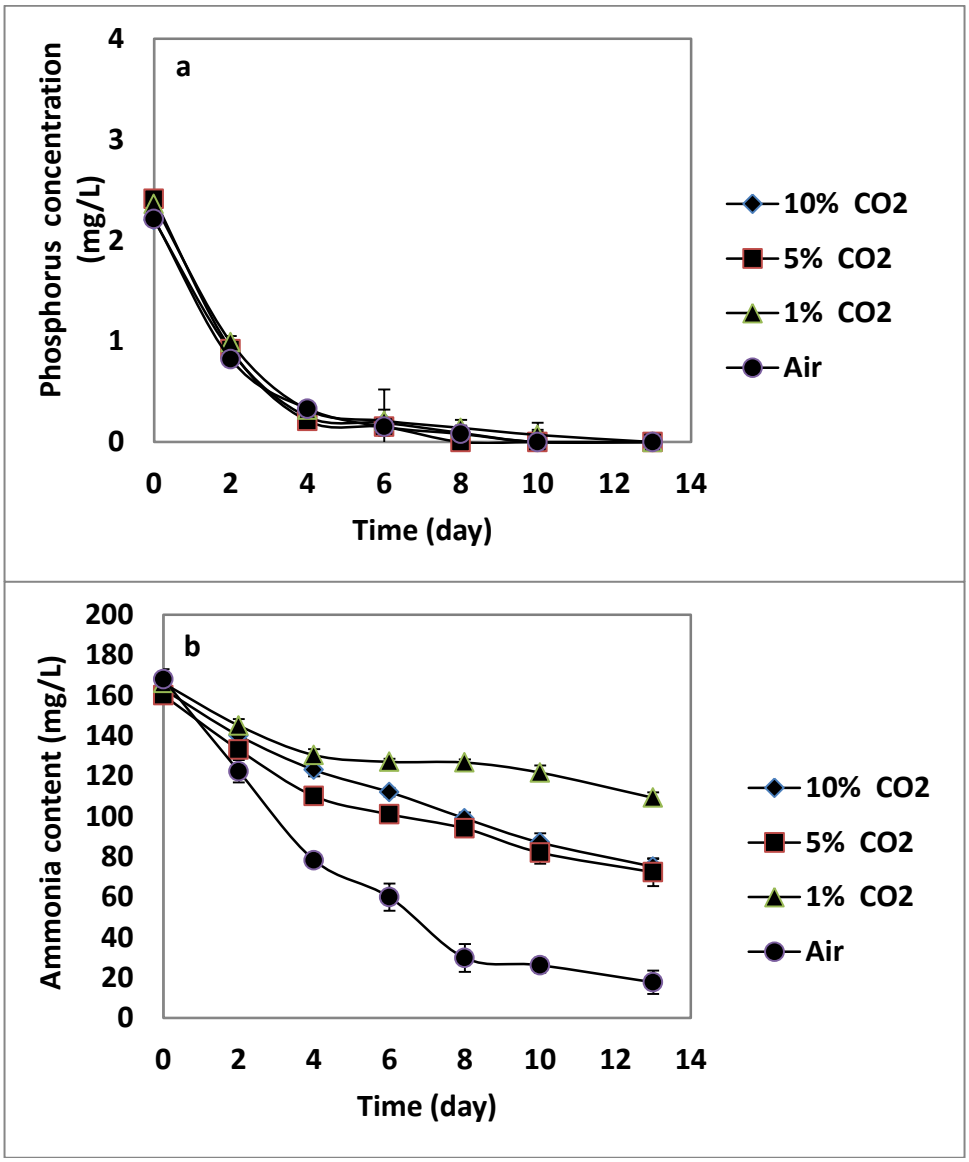


Figure 2. Nutrient removal profile of microalgal strain UMN271 grown on digested swine manure at four level of CO2 aeration (a) Ammonia removal profile; (b) Phosphorus removal

profile. Each data indicates the mean ± SD (error) and were measured from three independent cultures.

It is worth noting that, while the removal of the nutrient was significant at the end of experiment as mentioned above, the nutrient concentration remaining in the DSMW was still very high (Figs 2a and 2b). Thus further nutrient removal processes are needed for improved nutrient reduction before the discharge of wastewater.

Growth Profile and Nutrient Removal Efficiency for UMN 231 Specifically for Animal Feed Production in the Second Autotrophic Dominated Stage

The culture was centrifuged at the end of the first stage cultivation. The supernatant of wastewater from centrifugation was reused as the medium for the second stage cultivation to examine the recycling of nutrient for the growth of omega-3 containing algae UMN231 specifically for animal feed production under autotrophic dominated mode. It was demonstrated from the above data that algae grown on 5% CO_2 produced the highest biomass yield among four levels of CO_2. Thus in this stage, 5% CO_2 was used to supply inorganic carbon source using recycled DSMW and the profile of biomass concentration was shown in Fig. 3. The maximal concentration of algal biomass (0.83 g/L) was obtained during 8-day batch cultivation using recycled wastewater, which was lower than the 2.025 g/L yield obtained in the first stage (Fig. 2a). The possible reasons for the lower biomass yield could be due to: (1) the toxic materials produced during the first stage may present in the recycled wastewater and inhibit algae growth in the second stage; And (2) light penetration was low due to the high opacity of DSMW, which would influence the photosynthesis efficiency heavily and eventually limit algae fast growth. One way to solve above problem of low light penetration when growing algae on DSMW is the recently described new method of forming fungus-algae pellets as immobilized cells for efficient dark-color swine wastewater treatment. After pretreatment by unique spherical structure of fungi-algae complex, the swine manure wastewater became transparent and clear compared with the raw swine manure, thus could easily be penetrated by light. Moreover, the potential toxic materials produced also could be efficiently removed by fungi-algae pellets.
The NH_4-N profile in recycled DSMW for the 8 days batch culture was depicted in Fig. 3. The NH_4-N was reduced from 72.3 mg/L to 47 mg/L at the end of experiment with 5 % CO_2 aeration. It is reported that NH_3 stripping occurred only under alkaline condition. In this experiment, pH was controlled at 7.0-7.4, and thus the loss of NH_4-N could be largely attributed to the CO_2 promoted assimilation by algae rather than NH_3 stripping.

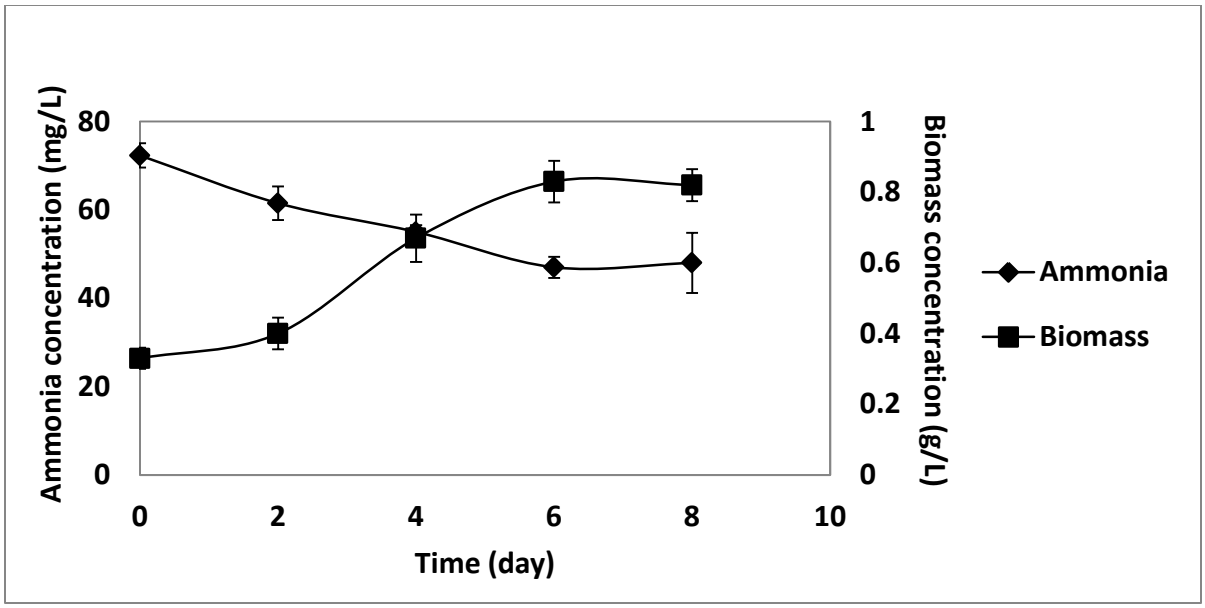


Figure 3. Growth and nutrient removal profile of microalgal strain UMN231 during batch culture grown on recycled 20-fold digested swine manure. Each data indicates the mean ± SD (error) and were measured from three independent cultures.

The Biochemical Composition Analysis of UMN 271 and UMN 231 Cultivated on 20-fold DSM

Growing algae for biofuel and bio-based products production using wastewater as cultivation media involves altering the culture conditions and processes toward maximum accumulation of biomass, including lipid for biofuel feedstock production, carbohydrate and protein for animal feed with minimal inputs and at lowest costs. Therefore, in this study, the lipid, carbohydrate and protein contents of algal biomass produced from 20-fold diluted DSMW were examined.

As shown in Table 6, the lipid and carbohydrate contents for strain UMN 271 were higher than those of strain UMN 231 while the protein content for strain UMN 271 was lower compared with that for strain UMN 231. The difference of chemical composition between these two strains probably was mainly attributed to strain-specific.

In our previous report it was demonstrated that UM231 could accumulate high value unsaturated fatty acid (EPA, C20:5), which accounted for 5.05 % of total fatty acid, when algae were grown on municipal wastewater. In this study, omega-3 fatty acid, carbohydrate, and protein contents were further examined and found to be 3.75%, 14.7%, and 45.7%, respectively, when grown on the recycled 20-fold dilution DSMW in the second stage and aerated with 5% CO_2 (Table 6). EPA is an important omega-3 polyunsaturated fatty acid which plays an important role in the prevention of various human diseases. Moreover, some algae species have amino acid profile similar to those of traditional foods such as egg, soybean etc. Thus, the algal biomass produced in the second stage could be an ideal feedstock for animal feed.

Table 6. The biochemical analysis of composition of top-perfroming strain UMN 231 and UMN271 grown on 20-fold DSMW.

Algae species	Protein (% DW)	Carbohydrate (% DW)	Lipid (% DW)	Omega-3 UFA (% DW)
UMN 231	45.7±4.13	14.7±3.75	19.4±1.66	3.75
UMN 271	39.5±7.71	17.5±3.99	23±4.32	ND

DW, dry weight; UFA, unsaturated fatty acid; ND: not determined.

With these benefits, the sequential two-stage cultivation strategy facilitates better nutrient recycling and protein-rich biomass accumulation for potential high value animal feed production (Fig. 4) and may offer an economically viable and environmentally friendly means for sustainable renewable algal based energy production with enormous savings of water and nutrient (e.g., nitrogen and phosphorus) required for algae growth and significant reductions in production costs with credits for wastewater treatment as well as mitigation of the greenhouse gas emissions.

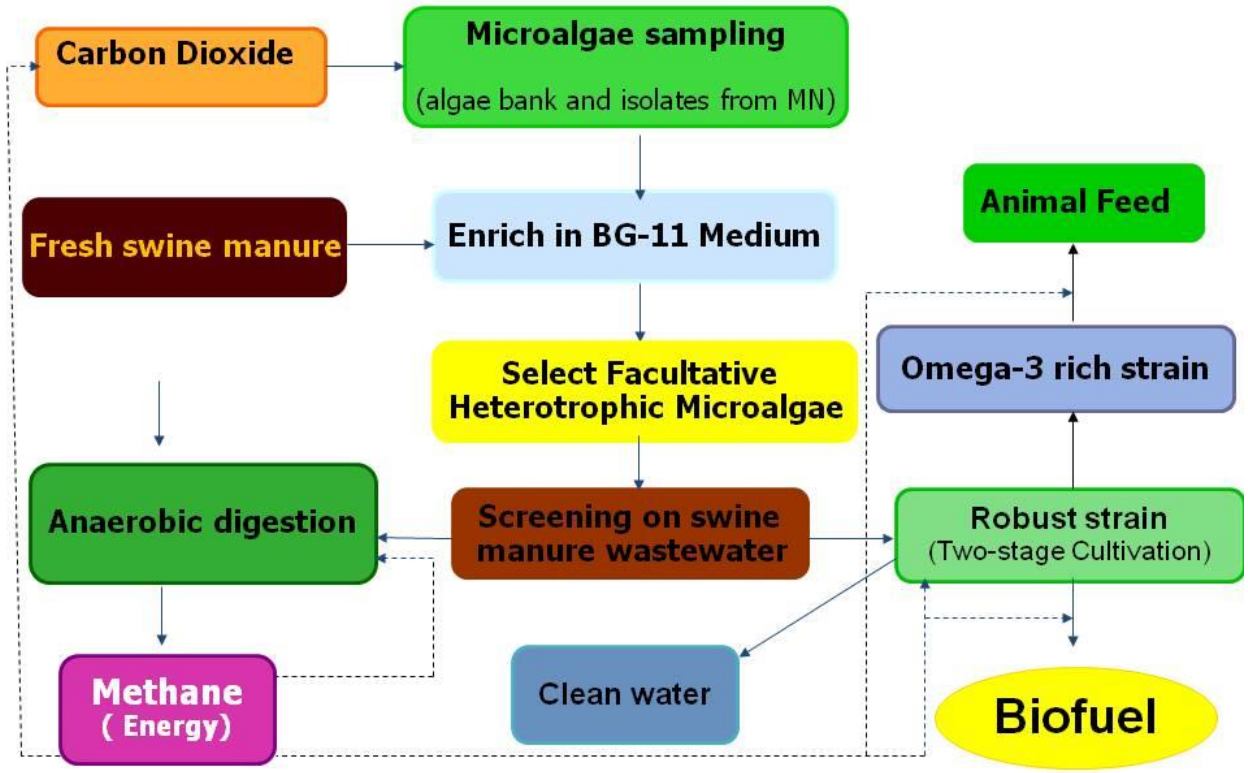


Figure 4. The schematic diagram of algae-based integrated system for digested swine manure wastewater treatment and biofuel & animal feed.

Summary

Previous research suggests a great potential of mass production of algal biomass using wastewaters (e.g., municipal and animal manure wastewaters) in literature. The integrated algal platform developed in this study showed multiple advantages of improving DSMW nutrient removal, enabling water recycling andCO_2 fixation, and accumulating algal lipid for low cost production of biofuel feedstock as well as producing omega-3 rich PUFA particularly for animal feed purposes. Such strategy could potentially be extended to treating other organic carbon-rich wastewaters and offer economically viable and environmentally friendly methods to produce algae-based bio-energy and high-value products.

Activity: Modification of anaerobic digestion on swine manure to enhance algae growth

1. Materials and Methods

Algae strain: a facultative heterotrophic alga strain Chlorella sp. (UMN271), isolated in our laboratory.

Manure sources: The fresh and anaerobically digested LSM effluents from a farm 5 miles northeast of the University of Minnesota Outreach, Research and Education (UMore) Park were collected. Because LSM had a high concentration of total suspended solids which led to poor transmission of light, and ammonia inhibition existed for microorganism growth, the manure samples were diluted with deionized water to obtain initial manure concentrations of 0.5% (78~180mg NH_3-N L^{-1}) before being used for algae cultivation.

Experiment I is used to determine whether the biomass productivity rates of microalga UMN271 were closely related to the three major soluble organic carbon substrates, namely acetic, propionic and butyric acids, in traditionally digested liquid swine manure (LSM).
A 7-day batch cultivation of UMN271 on diluted digested LSM with 0.1% (v/v) exogenous acetic, propionic and butyric acids under a continuous cool-white fluorescent light illumination at 100µmol $m^{-2}s^{-1}$ at room temperature was performed.

Experiment II is used to determine how VFA composition changes during our acidogenic fermentation of fresh LSM. In this experiment, fresh LSM with acidification pretreatment, which was used to inactive methanogens, was anaerobically digested for 72 h at pH5.3 to obtain VFAs accumulation in B2 treatment, by taking 15-day anaerobic digestion of untreated LSM as a control (B1 treatment).

Experiment III is used to determine how algae growth and oil yield could be affected by the LSM after the modified anaerobic digestion. In this lab-scale experiment, 5-day batch cultivation of algae UMN271 on 4 kind of media, including raw 20-fold diluted B1 manure effluent, sterilized 20-fold diluted B1 manure effluent, raw 20-fold diluted B2 manure effluent, and sterilized 20-fold diluted B2 manure effluent, labeled as C1, C2, C3 and C4 treatment, respectively, were operated at the same culture conditions of Exp I. All the treatments included four replicates. TVSS, pH, COD, NH_3-N, TN and PO_4-P were analyzed daily, VFAs contents at the beginning and ending of the culture period were determined, and fatty acid profile was performed on the harvested algal samples after the 5-day cultivation.

2. Results

Results of Exp I: As shown in Fig 1, the biomass accumulation of *Chlorella* sp. (UMN271) grown on VFA-amended manure was much greater than that on unamended manure, suggesting that the locally isolated microalgae strain *Chlorella* sp. was able to use not only acetic acid but also propionic and butyric acids for the growth. The final biomass concentrations in terms of TVSS for *Chlorella* sp. after 7-day batch growth were 0.53, 1.15, 1.58 and 1.25 g L^{-1} for A1, A2, A3 and A4 treatments, respectively.

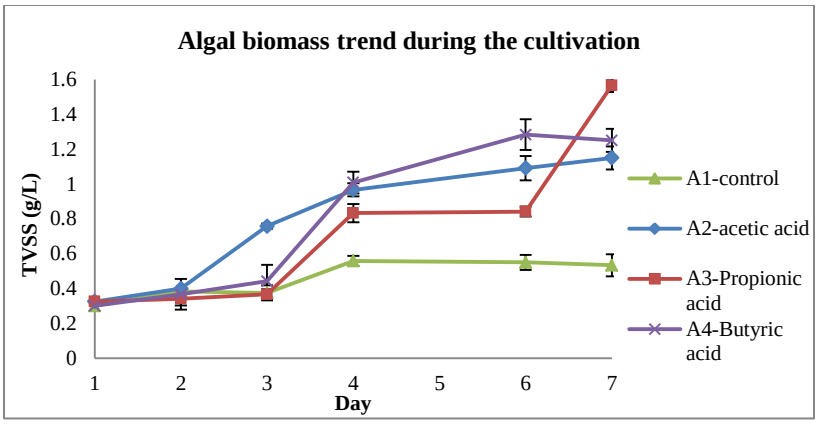


Figure 1. Biomass production of *Chlorella* sp. grown in batch on 20-fold diluted digested swine manure samples with no additive (A1), acetic acid (A2), propionic acid (A3) and butyric acid (A4).

Over the course of the experiment, COD, NH_3-N, total nitrogen, and PO_4-P in manure samples were measured daily during the growth period, and the data was shown in Table 1. Algae removed COD, NH_3-N, total nitrogen, and PO_4-P by -4.27 ~ 72.58%, 26.73 ~ 99.99%, 12.99 ~ 55.85%, and 79.08 ~ 88.56%, respectively, in the 4 treatments. It was observed that nutrient removal efficiencies of UMN271 on VFA-amended LSM media (A2, A3 and A4) were always higher than those of UMN271 on the control medium (A1). Therefore, it seems reasonable to conclude that faster growth of algae is beneficial for nutrient removal, and to confirm the feasibility of algae-based LSM treatment.

Table 1. Growth rates and nutrient removal efficiencies of *Chlorella* sp UMN271 in Experiment I

Treatment	Growth rate (d^{-1})	Average nutrient removal efficiency (%)			
		COD	NH_3-N	TN	PO_4-P
A1	0.398	-4.27	26.73	12.99	79.08
A2	0.640	62.45	88.99	51.78	87.07
A3	0.821	68.66	99.98	55.82	87.55
A4	0.825	72.58	99.99	55.85	88.56

As shown in Fig 2, in the experiment, the percentages of lipids were not significantly different among the algae samples cultivated on swine manure with and without VFAs ($p>0.5$), which were 29.97%, 26.74%, 26.25% and 27.43% for A1 ~ A4 treatments, respectively. the total lipids production (mg L^{-1}), which was the total weight of lipids obtained from 1 L algae culture, was significantly higher for algae on VFAs-amended LSM than that for algae on unamended LSM ($p<0.05$). Therefore, the wild-isolated *Chlorella* sp. grown on LSM had the potential as raw materials for biofuel production in terms of the quantity.

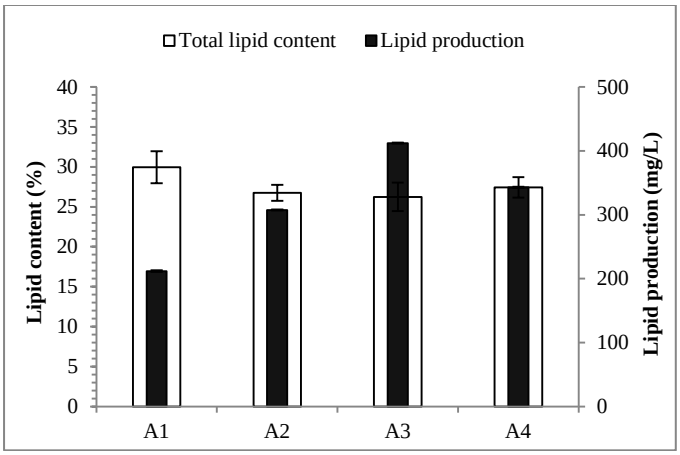


Figure 2 Lipid content of algae harvested from A1 (control), A2 (acetic acid), A3 (propionic acid) and A4 (butyric acid) cultures. White columns indicate the total lipid content (%) of dry weight; Black columns indicate the total lipid production (mg L^{-1}) of TVSS on day7.

Results of Exp II : 72-hour acidogenic fermentation of swine manure effectively increased the major VFA content from 6772.9 mg L^{-1} to 11748.32 mg L^{-1}, which was significantly higher than that manure effluent from a 15-day traditionally anaerobic digestion process (8586.2 mg L^{-1}). This indicates that the modified anaerobic digestion was effective for VFAs production in LSM. Considering the data, it is concluded that the best hydraulic retention time (HRT) in the particular study was at 48 h when the highest VFAs concentration (13811.91 mg L^{-1}) was achieved.

Table 2. VFAs concentrations (mg L^{-1}) in swine manure

	Fresh Manure	B1 treatment	B2 treatment			
			0 h	24 h	48 h	72 h
Acetic acid	2899.12	4535.2	3553.84	3797.15	6487.45	5744.43
Propionic acid	3540.26	3517.8	3923.73	4181.7	7066.33	5678.02
Butyric acid	333.52	533.2	264.96	293.41	258.13	325.87
Total	6772.9	8586.2	7742.53	8272.26	13811.91	11748.32

Results of Exp III : The initial contents of acetic, propionic and butyric acids of the diluted manure samples with a pH7.0 used in the experiment were shown in Table 3. As shown in Fig 3a, after the 5-day batch growth, the biomass accumulation of *Chlorella* sp. (UMN271) on the raw, diluted acigoenically fermented LSM (0.65 g TVSS L^{-1}) was the greatest among the 4 treatments, indicating that algae-available carbon content in the study significantly affected the growth of algae.

Table 3. The initial VFA concentrations in manure samples for experiment III

Treatment	medium	Acetic acid (mg L^{-1})	Propionic acid (mg L^{-1})	Butyric acid (mg L^{-1})	Total (mg L^{-1})
C1	20x raw, traditionally digested manure	226.76	175.89	26.66	429.31
C2	20x sterilized, traditionally digested manure	204.38	147.21	24.16	375.75
C3	20x raw, acidogenically fermented manure	304.04	237.26	26.97	568.27

C4	20x sterilized, acidogenically fermented manure	241.55	177.95	22.76	442.26

The nutrient removal efficiencies of UMN271 in the 4 cultures during the 5-day bacth cultivation were shown in Table 4. It was observed that the initial concentrations of COD, NH_3-N, TN and PO_4-P on the sterilized manure cultures were similar with those on the raw manure cultures, indicating that sterilization had no significant effect on the quantitative change of manure nutrients (data not shown). Although the nutrient removal efficiencies of UMN271 in Exp III were always lower than those in Exp I (Table 1), the amount of nutrients removed from the acidogenically fermented LSM by UMN271 were similar or higher than those removed from the exogenous VFA-amended LSM mentioned Exp I and those reported in literatures.

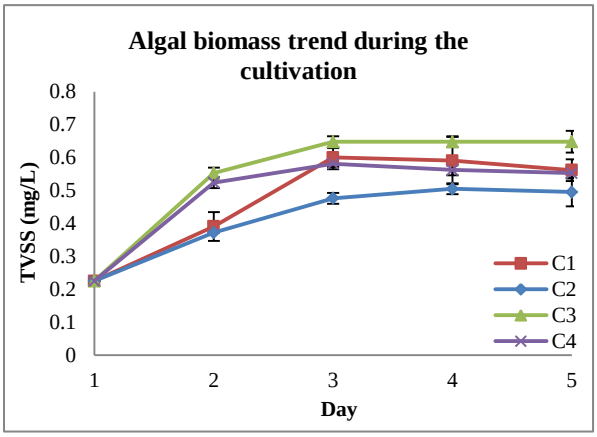


Figure 3. Biomass production of *Chlorella* sp. in C1, C2, C3 and C4 treatments during the 5-day batch experiment

Table 4. Nutrient removal efficiencies of *Chlorella* sp. UMN271 in Exp III

Treatment	COD	NH_3-N	TN	PO_4-P
C1	61.67	42.57	33.62	70.86
C2	58.82	31.58	28.67	68.48
C3	58.02	44.73	31.58	34.16
C4	58.16	36.89	22.32	23.37

The fatty acid profiles derived from triacylglycerol (TAG), phospholipid and free fatty acids, and the percentages of fatty acid components in the harvested algae samples (dry weight base) were shown in Table 5. Algae cultivated on raw and sterilized, acidogenically fermented manure samples showed higher fatty acid content (10.93% and 9.14%, respectively) than the rest two. GC-MS analysis showed that microalgal lipids for all the four algae samples were mainly composed of saturated and polyunsaturated fatty acids with C16 and C18 fatty acids as the major compounds. Among the 4 treatments, only the fatty acid profile of algae on raw acidogenically fermented manure (C3 treatment) present linolenic acid (C18:3) content (10.51%) within the EN14214 standard specification (2004), in which a limit of 12% linolenic acid was specified for a quality biodiesel. This meant that oil from *Chlorella* sp. grown on acidogenically digested LSM may be used for good quality biodiesel. Also, as shown in Fig 4, the fatty acid production from algae in C3 treatment was as high as 70.78 mg L^{-1}, which was 1.41times that of algae in C2 treatment (37.04 mg L^{-1}). Therefore, algae grown on acidogenic fermented LSM could be used as raw material for biodiesel and other biofuel production without restrictions.

Table 5. Fatty acid profile derived from triacylglycerol, phospholipid and free fatty acids in *Chlorella* sp. after 5-day batch cultivation on different manure samples

	C1 treatment	C2 treatment	C3 treatment	C4 treatment
Total fatty acid/dry weight (%)	8.33	7.48	10.93	9.14
Saturated fatty acids (% of total fatty acids)	48.37	43.16	57.1	50.68
C14:0	0.28	NA[a]	0.26	0.38
C16:0	17.37	22.02	16.84	20.72
C18:0	28.15	19.14	37.83	27.61
C20:0	1.06	0.88	1.01	1.02
C24:0	1.51	1.12	1.16	0.95
Monounsaturated fatty acids (% total fatty acids)	9.95	8.6	7.91	8.17
C16:1	2.57	2.71	1.21	1.84
C18:1	7.38	5.89	6.7	6.33
Polyunsaturated fatty acids (% total fatty acids)	41.68	48.24	35	41.15
C16:2	5.87	6.26	5.05	3.97
C16:3	5.46	5.98	4.25	5.62
C18:2	16.87	20.09	15.19	15.88
C18:3	13.48	15.91	10.51	15.68

[a] NA means 'not available'.

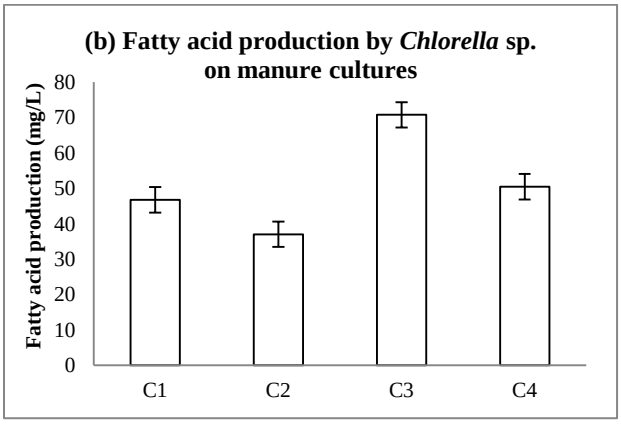


Figure 4. The algal fatty acid accumulation from 5-day batch cultures in C1 ~ C4 treatments

Result Status as of *December 31, 2012*
Activity 1: Algal biomass production at Rosemount site
1. Summary

In this period of time, we tested the growth characteristic of screened algal spice UMN271 at Rosemount site on a hog manure based wastewater, we tested the biomass productivity, water recycle ability, lipid content and profile, and waste removal rate. The results were listed below.
Bioreactor

The designed algae production system was a greenhouse-based vertical-distributed multilayer structure. The system included a green house, a temperature control system, a digester (not shown), and a photobioreactor (PBR) that consisted of a supporting frame, a stack of four trays, a mixing tank, a sub-pump, a CO_2 tank and a pH controller. In order to create a relative controllable environment, a standard high tunnel greenhouse (30' x 40' x 12') was set up at the UMore Park, Rosemount, MN in which two sets of PBRs were installed. The PBR had overall dimension of 4'(W) x 32'(L) x 8'(H) in which each tray can hold up to 6" water equivalent to volume of 1800 L. With the mixing tank and four trays, the total capacity of each PBR was 7500 L. Figure 1 illustrated the setup of the greenhouse and the PBR.

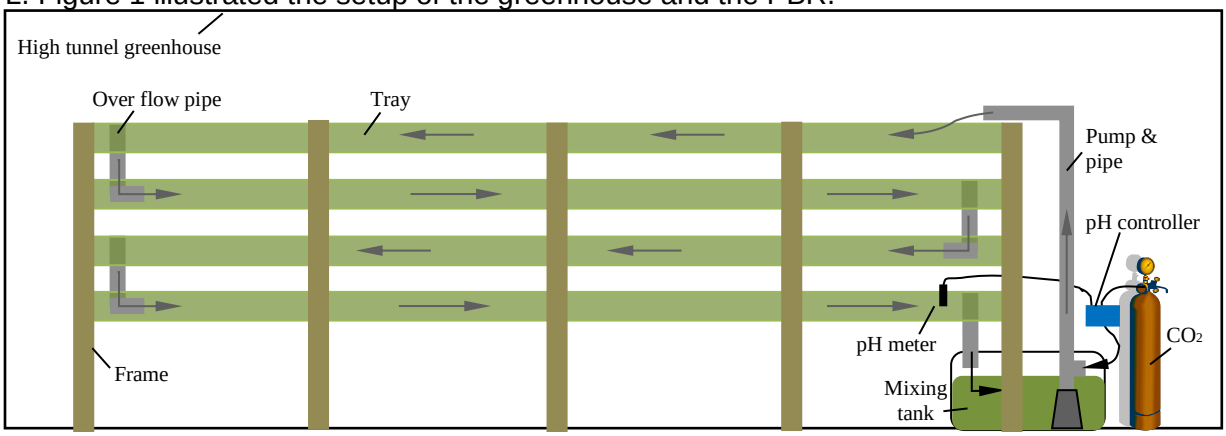


Figure 1. The greenhouse-based algal biomass production system.

In the PBR, the tray was made by folding a 3.5 mm thick twin-wall plastic panel which was made of high density polyethylene infused with UV inhibitors (Solexx, OR). In each tray, the water level was hold up by using an overflow pipe. The sub-pump lifted the mixed water up from the mixing tank to the top layer of the PBR, flew down to next layer according to its gravity, and finally came back to the mixing tank. A CO_2 injection that was used as carbon source and at the same time for adjusting pH was installed in line at the outlet of the sub-pump. A pH meter was installed to monitor the pH value. When pH value greater than 7.5, the pH controller would open a solenoid valve and CO_2 would be injected into the system.

Due to the unique design, the PBR had advantages of (1) High scalability. The system was easy to scale up by height and area due to the simple design and low cost materials; (2) Efficient land usage. The multilayered vertical distributed design reduced the demand for land use and increases water surface; (3) Reduced photo-inhibition by partially covered surface. Since the system had multilayer design, the layer above can provide shade for the layer below which prevented photo-inhibition caused by high intensity sun light; (4) Self clean design. The suction action at the top of the over flow pipe helped clean the scum/foam floating at the surface of the tray, therefore the light enters the water without blockage and there is no cleaning needed for this semi-open system; (5) Mixotrophic dominated cultivation. Since swine manure contained lot of organic carbon, the system adopted mixotrophic cultivation method which requires less light input than photoautotrophic growth for produce same amount of biomass.

Scale up procedures

The screened specie UMN271 was firstly prepared in a sterilized artificial medium BG11 (Hu et al., 2012) with 5g/L glucose in four 250-mL flasks, 100 mL medium in each flask. When reaching near maximum density, generally after 3~4 days cultivation, the seeds were transferred evenly to two 4-L flasks that 2000 mL medium was in each flask; when algal density reaching near maximum density, transferring the seeds to a 20-L bio-coil (Zhou et al., 2012). When reaching near maximum density, about 20-L seeds were transferred to a multilayer PBR at a greenhouse on campus and scaled up to 100 L by using diluted swine manure as culture medium. After the seeds adapted to the new environment and reaching algal density of 0.5-0.8 g/L, it was doubled every 2-3 days. When scaled up to 800 L, the seeds were transferred to the

Rosemount site, also after the seeds adapted to the new environment, double every 2-3 days till the PBR was fully scaled up.

During the seeds transferring process, such as transfer algae seeds from a BG-11 based environment to a manure-based environment and transfer seeds from campus greenhouse to Rosemount, the algae culture would generally experience environmental shock. This shock could be from the change of nutrient profile, water resources, temperature, or light exposure. When experiencing the environmental shock, the algae cells tended to precipitate at the bottom of the reactor; the cell and water were separated so that the water looked “clearer”. After two or three days, the algae cell would adapt to the new environment and start to float in the water, and the water would turn back to green color gradually.

Experimental design and operational procedure

The experiment was designed to compare algal biomass/algal lipid productivity and nutrient removal rate by using water recycle and without water recycle procedures. Since two PBRs were available at Rosemount inside the greenhouse, the PBR on the north side (namely BRN thereafter) used water recycle procedure which water was recycled after algae biomass being harvested. The PBR on the south side (namely BRS thereafter) didn’t recycle the water that the harvested algae and water were discharged to outside field and fresh water and manure were refilled after each harvesting. The algae culture was harvested using ¼ harvesting rate. Two month period (July-August, 2012) was used to run these experiment. In July 2012, BRS was run by using fed batch method in which ½ of the volume was harvested every other day and new water and manure was replenished. Due to environmental shock was observed, in August the BRS was changed to continuous harvesting method in which fresh water and manure was fed continuously and algae culture was also harvested continuously by using a controlled pump.

For BRN, cation polymer Clarifloc C-6244 (PolyDyne, GA) was used for algae biomass harvesting. About 5~7.5 ml polymer was used for harvesting 100 L algae solution. Polymer was diluted 200 times before added to algae solution to enhance harvest efficiency. After mixing with mixer for five minutes then allowing five minutes settling, the algae biomass was ready to separate with a filter cloth (50 μm pore size). The water after removing the algae biomass would be pumped back to the BRN.

2. Results and Discussion

The algal cultivation system was developed for dual purposes which were algal biofuel production and wastewater treatment. In order to minimize capital cost and energy input, minimum control was used in the system. Some of the operational parameters such as, dilution rate, harvesting rate, water input, CO_2 addition and related pH adjustment, and pump usage for circuiting water, were able to be controlled. Some parameters such as weather associated temperature and sunlight radiation and external organism were unable to be controlled. The general operational strategy is to control adjustable parameters to optimize the procedure for the best algal biomass production according to the cultivation condition.
Figure 2 and table 1 illustrated the GC-MS analysis result of the organic compound of the digested hog manure in which there was lot of organic carbons that were unable to be used by algae. It explained why COD could not be reduced further when reached at certain level.
Figure 3 illustrated the BRN photo-bioreactor running status. In BRN the harvested water was recycled back into the system. Considering that there were unused nutrient left in the recycled water, the amount of manure added should maintain the nutrient level at a relative constant

level to prevent toxic accumulation. The nutrients level and suspended solid concentrations before and after harvesting in BRN with water recycle procedure.
Figure 4 illustrated the nutrients level and suspended solid concentrations in BRS without water recycle procedure.
The table 2 listed the biomass productivity and nutrient removal rates in which the best overall biomass productivity was 23.16 g/m2.day. The nutrients removal rates were 68% for NH3-N, 28% for TN, 26% for COD, and 20% for TP. Due to high conductivity in the algae broth, the polymer used for flocculation took about 12-18% of the dry weight.
From the table 3, we can see that when reusing the recycled water, due to the salt and other waste could not be totally uptake by algae, the ion conductivity caused by salt accumulation increased from 1618 to 3360 µs. Other nutrient levels were also increased accordingly.
Although the greenhouse can help maintain the temperature at certain level, it was hard to keep the temperature at ideal level during winter time at northern climate without a heating source. Relocating the system with a power plant that could supply waste heat might be able to maintain optimum temperature for fast algal biomass growth.
The total FAME lipid is about 1±0.28% of the dry weight. The lipid profile was listed in table 4.
The elemental analysis was listed in table 5 in which N had relative high value compare to other studies. The ash content was high due to external particles were introduced when cultivating the algae.

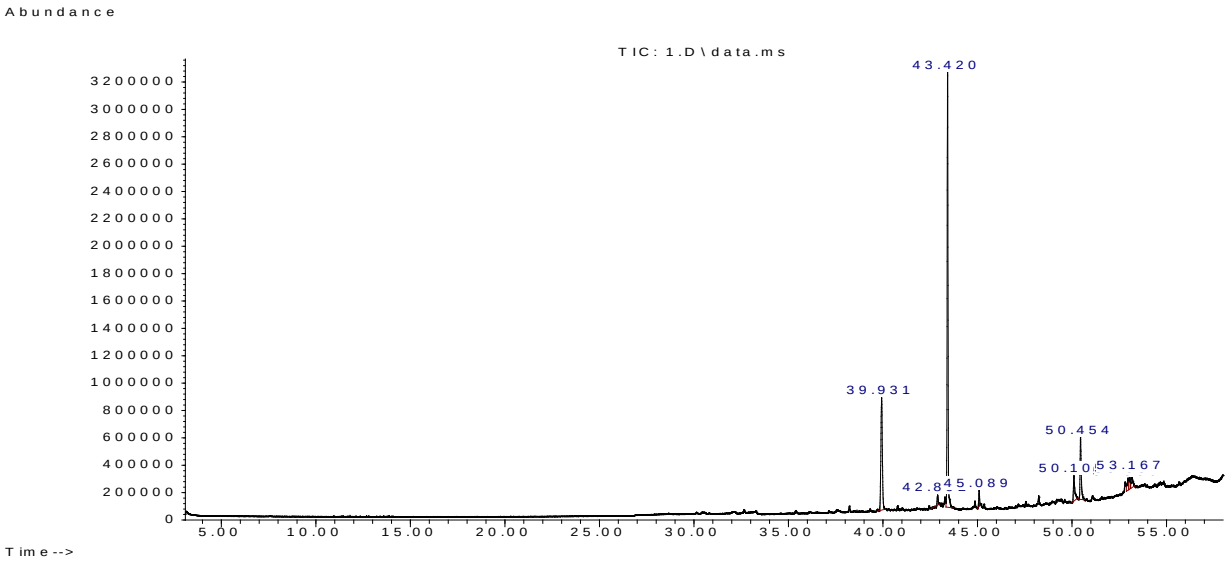


Figure 2. GC-MS analyze result of the organic compound of the digested hog manure.

Table 1. Organic compound of the digested hog manure.

	Component	%	Quality
1	Benzonitrile, m-phenethyl-	22.19	27
2	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	0.9	47
3	Benzonitrile, m-phenethyl-	53.77	27
4	1H-Indole, 1-methyl-2-phenyl-	1.76	42
5	1,1':3',1"-Terphenyl, 5'-phenyl-	4.62	99
6	1,1':3',1"-Terphenyl, 5'-phenyl-	9.53	99
7	Benzo[h]quinoline, 2,4-dimethyl-	2.24	18

8	2-Propenoic acid, 3-[(phenylmethyl)thio]-, methyl ester	2.08	16
9	8-Benzylamino-7-chloro-5,6-quinolinedione	2.91	10

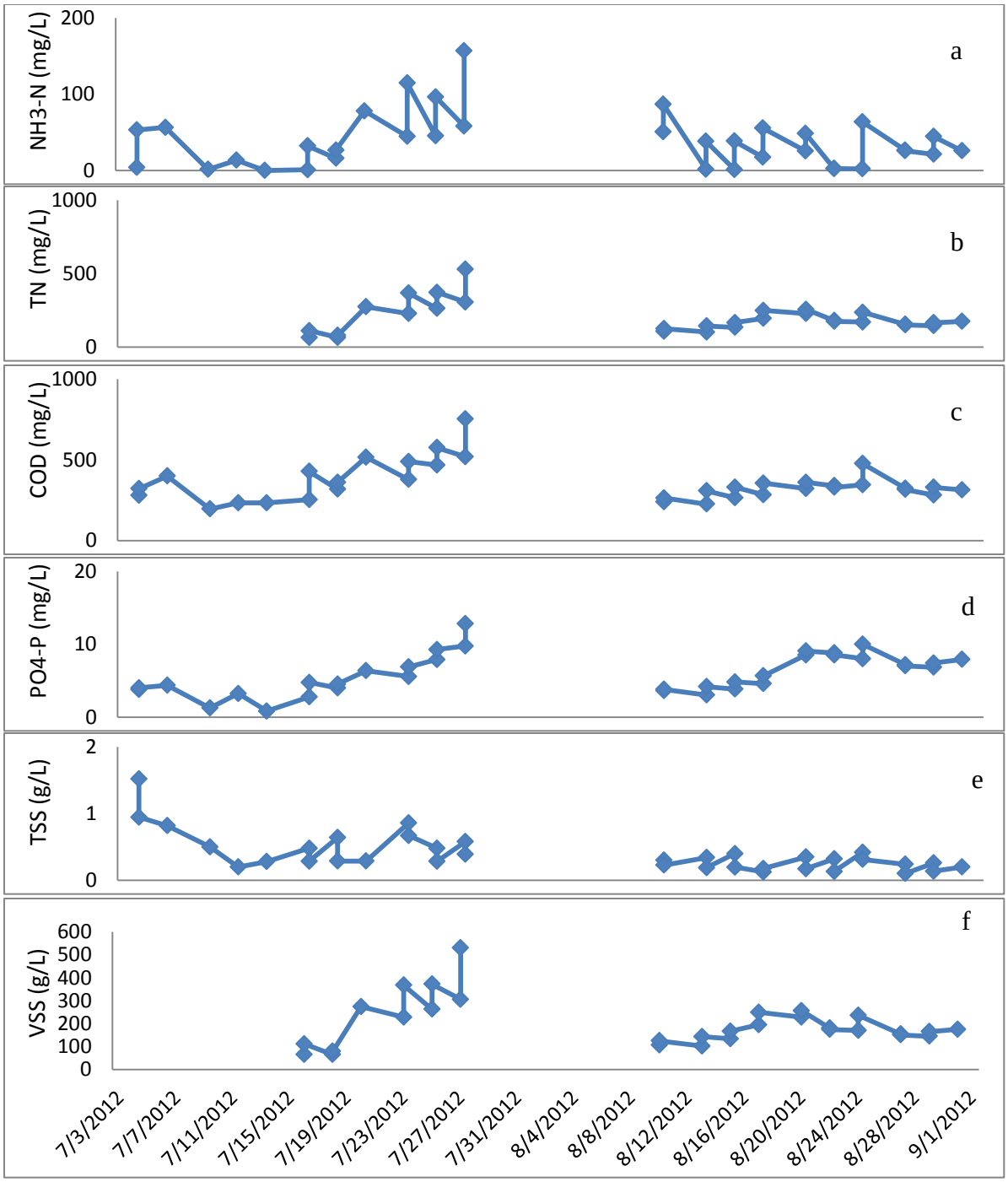


Figure 3. The nutrients level and suspended solid concentrations before and after harvesting in BRN with water recycle procedure. (a) Ammonia concentration (b) TN concentration (c) COD concentration (d) PO4-P concentration (e) TSS density (f) VSS density.

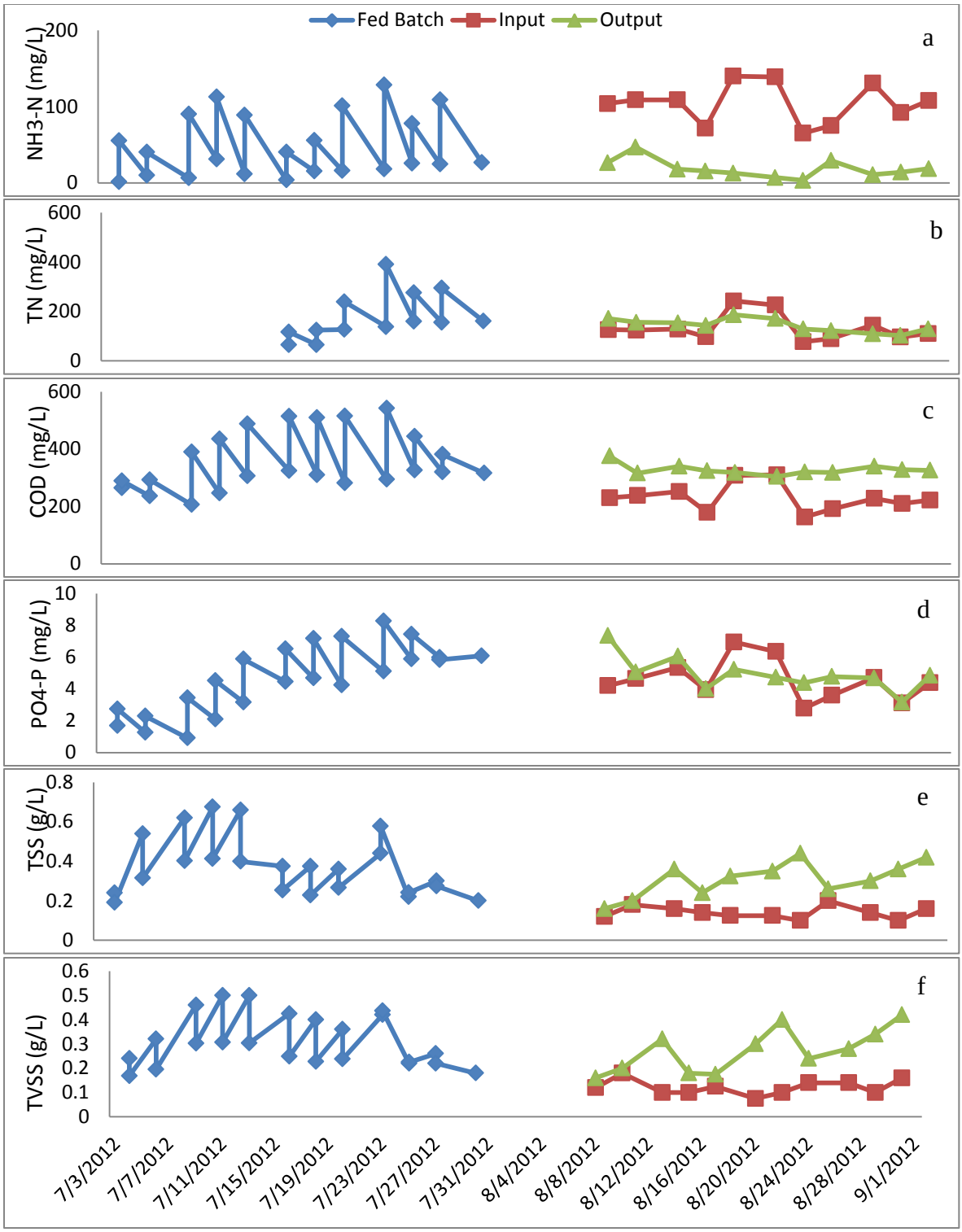


Figure 4. The nutrients level and suspended solid concentrations in BRS without water recycle procedure. In July the BRS was run in a fed batch mode, the line (-♦-) presented the concentrations before and after harvesting. In August the system was run in a continuous mode. The two lines presented the input and output concentrations (a) Ammonia concentration (b) TN concentration (c) COD concentration (d) PO4-P concentration (e) TSS density (f) VSS density.

Table 2. Results from summer 2012 experiments.

	July	July	August	August	
	BRN	BRS	BRN	BRS	Note
Productivity (g/m2.day)					
TSS	15.16	10.66	5.38	6.42	Subtract manure input
TSS	23.19	19.15	8.34	11.27	Total weight
AFDW (TVSS)	14.59	8.08	5.25	5.90	Subtract manure input
AFDW (TVSS)	19.03	16.61	7.49	10.61	Total weight
Nutrient Removal (%)					
NH3-N	68.07	78.47	59.54	83.93	
TN	17.78	28.20	12.32	-20.98	
COD	26.01	18.26	11.18	-45.12	
PO4-P	20.37	14.07	2.74	-16.88	
Nutrient Removal (g/m2.day)					
NH3-N	2.65	4.37	2.07	2.99	
TN	3.19	4.61	1.57	-0.63	
COD	7.21	4.51	3.18	-3.43	
PO4-P	0.067	0.044	0.026	-0.021	
Polymer (% dry weight)	12.11	-	18.86	-	
Ash content (%)	11.97	10.42	11.27		
Ion conductivity (µs)					
Initial	1618	1489	-	-	
Final	3360	1680	-	-	

Table 3. Nutrient level at the beginning and end of the tests.

Initial Nutrient Level (mg/L)	July	August
	BRN	BRN
NH3-N	12	1.69
TN	65.3	102
COD	226	226
PO4-P	3.85	3.04
Final Nutrient Level (mg/L)		
NH3-N	58.1	2.06
TN	306	170
COD	520	346
PO4-P	9.76	8.03

Ion conductivity (µs)		
Initial	1618	1304
Final	3360	2270

Table 4. Fatty acid profile derived from triacylglycerol, phospholipid and free fatty acids in Chlorella sp. from the Rosemount pilot study.

Name of the compounds	Content
Total fatty acid/dry weight (%)	0.97±0.28
Saturated fatty acids (% of total fatty acids)	32.28±3.95
C8:0	0.27±0.69
C15:0	10.13±12.67
C16:0	15.31±12.39
C17:0	1.72±2.81
C18:0	4.71±3.27
Monounsaturated fatty acids (% total fatty acids)	7.26±4.65
C12:1	0.11±0.52
C16:1	2.51±3.51
C18:1	4.64±2.59
Polyunsaturated fatty acids (% total fatty acids)	60.45±6.44
C8:2	1.19±1.21
C16:2	3.69±1.74
C16:3	2.37±5.09
C18:2	12.34±2.16
C18:3	40.40±5.18
C20:3	0.47±2.13

Table 5.CHN concentration.

	C	H	N	Residue
BRN	46.73±0.99	6.56±0.20	8.20±0.33	38.51 ±1.38
BRS	45.12±3.58	6.35±0.52	7.97±0.49	40.56±4.47

Activity 2: Enhanced mixotrophic growth of microalga Chlorella sp. on pretreated swine manure for simultaneous biofuel feedstock production and nutrient removal

1. Summary

In this period, we developed an effective semi-continuous process for the growth of the locally isolated green microalga *Chlorella* sp. (UMN271) on pretreated swine wastewater in bench scale for improved algal biomass production and waste nutrient assimilation using central composite design (CCD). The influences of two key parameters, namely wastewater dilution rate (DR) and hydraulic retention time (HRT), on algal biomass productivity and nutrient removal rates were investigated. The optimal parameters estimated from the significant second-order quadratic models (p<0.05) were 8-fold DR and 2.26-d HRT. The cultivating experiment in a bench-scale multi-layer photobioreactor with the optimized conditions achieved stable algal productivity and nutrient removal rates, which fitted the predictive models well. Moreover,

relatively high and stable protein and lipid contents (58.78% and 26.09% of the dry weight, respectively) were observed for the collected algae sample, indicating the suitability of the algal biomass as ideal feedstock for both biofuel and feed production.

2. Experimental design and operational procedures

For the seed culture preparation, the screened alga strain *Chlorella* sp. (UMN271) was cultivated in sterilized BG11 medium with 2 g L^{-1} glucose at 25 ± 2 °C under a continuous cool white fluorescent light illumination of 100 µmol m^{-2} s^{-1} for 1 – 2 weeks. Before being inoculated into manure cultures in experiments I and II (sections 2.2 and 2.3, respectively), the seeds were separated from the culture broth using centrifuge and then were washed with deionized water.

2.1 Acidogenic digester setup and operation

The fresh swine manure and the inoculum sludge were collected from the University of Minnesota Southern Research and Outreach Center, Waseca MN. The fresh swine manure was used as the substrate during the acidogenic digestion in the study, and its characteristics are shown in Table 6. The sludge was anaerobically cultivated for 5 days with 5 g L^{-1} glucose at temperature 38 ±1 °C to get the activated and concentrated inoculum, and then was heat-treated at 80 °C with a water bath (Thermo Fisher Scientific Inc., Waltham, MA) for 30 minutes to kill methanogenic bacteria from the community according to Wang et al (2009)

Table 6. Characteristics of fresh swine manure

Parameter	Value
pH	7.58±0.31
TVSS (mg L^{-1})	2580.01±300.01
Total nitrogen (mg TN L^{-1})	2031.43±66.19
Ammonia-nitrogen (mg NH_3-N L^{-1})	1602.86±84.72
Phosphate-phosphorus (mg PO_4-P L^{-1})	407.43±99.58
COD (mg L^{-1})	17240±816.66
Total VFAs (mg L^{-1})	7676.26±576.37
Acetic acid (mg L^{-1})	4957.08±357.48
Propionic acid (mg L^{-1})	1612.03±116.13
Butyric acid (mg L^{-1})	1107.16±105.48

An Erlenmeyer flask with the working volume of 4 L was used as the anaerobic digester. The reactor was operated in semi-continuous mode. At the beginning, the mixture of concentrated inoculum and fresh manure substrate was adjusted to approx pH 5.3 with sulfuric acid (4 mol H_2SO_4 L^{-1}) solution, and was maintained at pH 5.3 ~ 5.6 and 38 ± 1 °C for the acidogenic fermentation for 48 h as described by Hu et al. (2012). The 2-day batch fermentation was followed by a 10-day semi-continuous operation with the HRT of 3 days determined in the previous study (Hu et al., 2012). The pH and temperature were kept at 5.3 ~ 5.6 and 38 ± 1 °C, respectively, during the 10-day operation. Samples were taken from daily effluent and feeding substrate for the concentration test of volatile fatty acids (VFAs). In the study, the initial drained effluent was used in experiment I (section 2.2), and the effluents in the rest 9 days were used in experiment II (section 2.3).

2.2 Optimization of conditions for the semi-continuous mode (Experiment I)

2.2.1 Factorial design

The experiments were performed to develop a mathematical model for the response variables, including biomass productivities and nutrient removal rates, and to predict the optimum DR and HRT for biomass production and wastewater treatment. The experiments were designed according to a 2^2 circumscribed central composite response surface methodology (RSM) to build a second order model for the response variables without employing a full factorial experiment design. Each factor was designed with 5 coded levels (-α, -1, 0, +1, +α) which could constitute 25 combinations of DR and HRT at different levels. The typical value of α is a function of the number of variables $\alpha = (2^k)^{1/4}$, where k is the number of independent variables. According to the two-variable CCD approach, only 9 variable level setting combinations with the α value of 1.41 were needed for experiment runs in the study, which were presented in the forms of 1 center point, 4 factorial points and 4 star points in Fig. 5. The DR and HRT in both coded levels and actual values, and the designed matrix using SAS 8.1 software ((SAS Institute Inc, Cary, NC) are shown in Table 7 and Table 8, respectively.

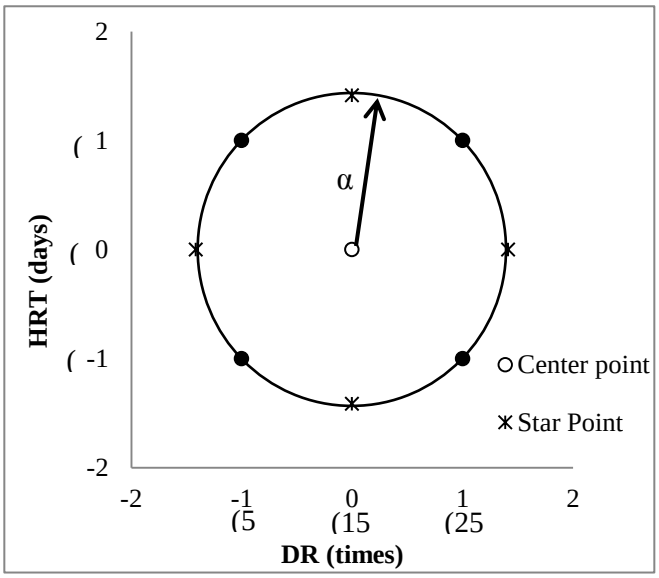


Figure 5. Two-variable central composite design

Table 7. Coded levels and true values of potential significant variables

Independent variable	Variable	(-α [a]) level	(-1) level	0 level	(+1) level	(+α [a]) level
Dilution rate (DR, time)	X_1	1	5	15	25	30.
Hydraulic retention time (HRT, day)	X_2	1.6	2	3	4	4.4

α [a] is the distance from the center of the design space to a star point.

Table 8. Full factorial central composite design matrix

Experimental runs	**Coded value**		**Real value**	
	X_1	X_2	DR (time)	HRT (day)
1	-1	-1	5	2
2	-1	+1	5	4

3	+1	-1	25	2
4	+1	+1	25	4
5	-α	0	1	3
6	+α	0	30	3
7	0	-α	15	1.6
8	0	+α	15	4.4
9	0	0	15	3
10	0	0	15	3
11	0	0	15	3
12	0	0	15	3
13	0	0	15	3

2.2.2 Algae growth experiments

The designed 13 runs (Table 9) were carried out in 250 mL Erlenmeyer flasks containing 150 mL of unsterilized acidogenically digested swine manure with different DRs and an initial *Chlorella* sp. biomass concentration of 0.3 g L^{-1}. All the cultures were adjusted to around pH7.0 at the beginning of the incubation, and were maintained at 25 ± 2 °C under 100 μmol $m^{-2}s^{-1}$ white fluorescent light on a 16:8 h light/dark cycle for one week to obtain the stationary-phase algae. The one-week batch cultivation was followed by 10-day semi-continuous cultivation with various HRTs (Table 9) at the same temperature and light conditions as described above. Samples were taken from the daily harvested cultures and feed solutions for the TVSS and nutrient concentration tests. The data collected after the cultivation system reached quasi steady states were used for the statistical analysis in section 2.2.3. To avoid bias, 13 runs were performed in a totally random order.

2.2.3 Quadratic model analysis

Data of algal biomass and nutrient contents collected from samples on the 9th and 10th days of the semi-continuous cultivation were used to calculate the algae biomass productivity (μ_1, mg L^{-1} d^{-1}) and removal rates of COD, PO_4-P, TN, and NH_3-N (μ_2 ~ μ_5, respectively, mg L^{-1} d^{-1}) according to Eq. 1.

$$\mu_i = \left| i_{10} - \left(i_9 \frac{X_2 - 1}{X_2} + i_{M9} \frac{1}{X_1 X_2}\right) \right| \quad \text{Eq. 1.}$$

where μ_i is the daily change of i's concentration; i_9 and i_{10} are concentrations of i in the harvested cultures on day 9 and 10, respectively; i_{M9} is i's concentration in the feeding swine manure on day 9; X_1 and X_2 are the values of DR and HRT for the experiment run, respectively. The results of μ_1 ~ μ_5 were analyzed statistically through analysis of variance (ANOVA) at 95% confidence interval and RSM with SAS8.1 ADX Interface software. Second-order quadratic models were established to evaluate the effects of DR and HRT on the responses, including algal biomass productivity (Y_1, mg L^{-1} d^{-1}), COD removal rate (Y_2, mg L^{-1} d^{-1}), PO_4-P removal rate (Y_3, mg L^{-1} d^{-1}), TN removal rate (Y_4, mg L^{-1} d^{-1}), and NH_3-N removal rate (Y_5, mg L^{-1} d^{-1}), as in Eq. 2 by using the method of least squares:

$$Y_i = a_{i0} + a_{i1}X_1 + a_{i2}X_2 + a_{i12}X_1X_2 + a_{i11}X_1^2 + a_{i22}X_2^2 \quad \text{Eq. 2.}$$

where Y_i is the predicted response; X_1 and X_2 are the real values of DR and HRT, respectively; a_{i0}, a_{i1}, a_{i2}, a_{i12}, a_{i11} and a_{i22} are the coefficients inEq.2 to be determined by the statistical analysis.

2.3 Application of the predictive optimum in a bench-scale multi-layer photobioreactor (Experiment II)

The objective of this experiment was to confirm the feasibility of the algae–acidogenically digested swine manure system using the bench-scale photobioreactor with the predicted optimal DR and HRT according to the regression models. The novel bench-scale photobioreactor system consisted of a proprietary 2-layer reactor and a recycling peristaltic pump (Cole-Parmer Co, Vernon Hills, IL). The detailed structure and description of the system were mentioned in Fig. 1. In the experiment, *Chlorella* sp. seeds were inoculated at approx 0.2 g L^{-1} in the 2-layer photobioreactor containing 4 cm-high unsterilized manure effluents from the acidogenic digester with the optimal DR. Seven-day batch cultivation followed by 10-day semi-continuous cultivation with the predicted optimal HRT was operated at 25 ± 2 °C under 100 μmol $m^{-2}s^{-1}$ white fluorescent light on a 16:8 h light/dark cycle. During the incubation period, the culture unit was daily refilled with deionized water to compensate for evaporation. TVSS, pH, COD, PO_4-P, TN and NH_3-N were assayed daily, while lipid content, protein content, and the fatty acid profile were analyzed on the harvested algae samples on the last three days of the 10-day semi-continuous cultivation.

2.4 Analytical methods

TVSS, pH, COD, PO_4-P, TN and NH_3-N were analyzed in accordance with the standard methods (APHA, 1998) and instructions in the Hach DR5000 spectrophotometer manual (Hach, 2008).

The concentrations of acetic, propionic and butyric acids were measured with an Agilent 7820A gas chromatography with flame ionization detector (GC-FID) according to the method of Zhou et al. (2012a).

Total lipid contents in the harvested algae cells were analyzed using the one-step extraction method adapted from Folch method (Folch et al., 1956). The fatty acid composition of the harvested cells were extracted with a one-step extraction-transesterification method as described by Li et al. (2011), and was determined through the GC-MS analysis using an Agilent 7890-5975C gas chromatography–mass spectrometry with a HP-5 MS capillary column.

The protein content of the freeze-dried algal biomass was determined from the nitrogen content data evaluated with a CE-440 elemental analyzer (Exeter Analytical Inc., Chelmsford, MA), using the nitrogen-to-protein conversion factor (NTP) of 6.35 (Safi et al., 2012).

3. Results and discussion

3.1 Semi-continuous acidogenic fermentation of fresh swine manure

As shown in Fig. 6, the VFAs production reached the steady state on the 10th day of the semi-continuous aciodgenic fermentation in the digester, with the VFAs productivity of 2002.25 mg L^{-1} d^{-1} on average. The total VFAs concentration in the acidogenically digested manure effluents from the semi-continuous process was in the range of 12500 ~ 14000 mg L^{-1}, which was close to the highest VFAs concentration in the acidogenic, batch fermentation of liquid swine manure (LSM) in our previous report (Hu et al., 2012), indicating that the semi-continuous, acidogenic fermentation was effective in promoting VFAs production in swine manure. It is observed from Fig. 6 that the semi-continuous process had a noticeably higher VFAs yield than the batch mode (12000 mg L^{-1} on day 2 before manure exchange). Therefore, the moderate-to-high yields obtained with the batch-to-semi-continuous process used in this work demonstrates the advantage of acidogenic, semi-continuous fermentation in the production of VFA-enriched swine manure. The daily drained effluent from the semi-continuous digestion system was used for the cultivation of *Chlorella* sp. in Exp I and Exp II.

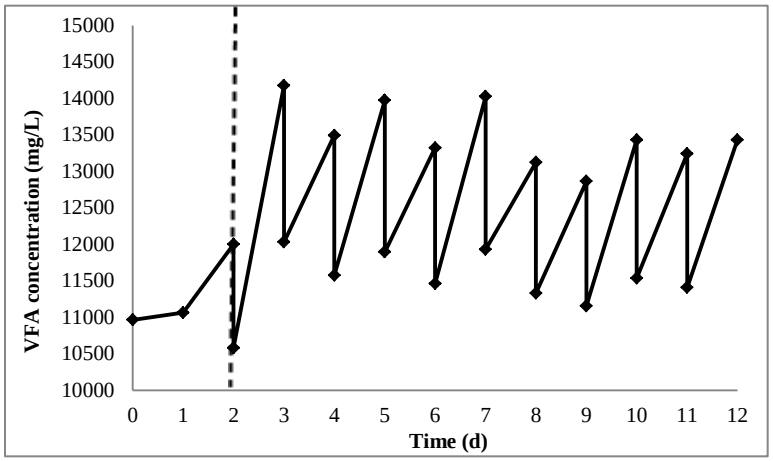


Figure 6 Change in total VFAs during acidogenic fermentation of fresh swine manure at 38 ±1 °C during the 2-day batch fermentation (before the vertical dotted line) followed by a 10-day semi-continuous mode with an HRT of 3 days (after the vertical dotted line)

3.2 Optimization of DR and HRT in CCD for the microalgae–acidogenically digested swine manure system (Exp. I)

The 2^2 CCD experimental results, including algal biomass productivity (μ_1, mg L^{-1} d^{-1}), COD removal rate (μ_2, mg L^{-1} d^{-1}), PO_4-P (μ_3, mg L^{-1} d^{-1}), TN (μ_4, mg L^{-1} d^{-1}), and NH_3-N (μ_5, mg L^{-1} d^{-1}), for each CCD run are listed in Table 9.

Table 9**.** Central composite design matrix and the results of the response variables including biomass productivity (μ_1, mg L^{-1} d^{-1}), COD removal rate (μ_2, mg L^{-1} d^{-1}), PO_4-P (μ_3, mg L^{-1} d^{-1}), TN (μ_4, mg L^{-1} d^{-1}), and NH_3-N (μ_5, mg L^{-1} d^{-1}) in the CCD runs

Run	DR (times)	HRT (days)	μ_1 (mg L^{-1} d^{-1})	μ_2 (mg L^{-1} d^{-1})	μ_3 (mg L^{-1} d^{-1})	μ_4 (mg L^{-1} d^{-1})	μ_5 (mg L^{-1} d^{-1})
1	5(-1)	2(-1)	332.67	1027.33	6.77	36.20	29.33
2	5(-1)	4(+1)	121.89	349.18	0.42	14.06	10.41
3	25(+1)	2(-1)	98.82	299.33	0.19	9.57	8.95
4	25(+1)	4(+1)	95.35	285.58	0.08	5.69	5.12
5	1(-α)	3(0)	89.26	275.33	0.39	6.56	6.48
6	30(+α)	3(0)	76.10	254.88	0.14	6.39	6.28
7	15(0)	1.586(-α)	164.27	445.18	0.72	15.70	14.83
8	15(0)	4.414(+α)	102.20	243.10	0.12	6.12	5.89
9	15(0)	3(0)	220.50	678.90	2.75	22.75	16.35
10	15(0)	3(0)	221.50	688.90	2.81	24.35	15.80
11	15(0)	3(0)	225.50	692.90	2.65	24.55	14.98
12	15(0)	3(0)	219.50	704.10	2.92	21.05	18.21
13	15(0)	3(0)	215.50	691.00	2.55	20.98	17.56

The SAS ADX interface program was used to analyze the CCD data sets in Table 9 to build up the quadratic mathematical models of DR and HRT responding to the algal biomass productivity and removal rates of COD, PO_4-P, TN and NH_3-N. According to the statistical analysis of the variable estimates (data not shown), the independent variables of DR (X_1, X_1^2) and HRT (X_2, X_2^2) had significant effects on the biomass production and the removal of COD, PO_4-P, TN and NH_3-N, while the interactions between DR and HRT (X_1X_2) had low significances on *Chlorella* sp to remove PO_4-P, TN and NH_3-N (p>0.05). The quadric models predicted for the response variables of Y_1 ~ Y_5 using significant coefficients are given as Eq. 3 ~ 7 in Table 10.

Table 10 Regression equations for the response variables, including the algal biomass productivity and removal rates of COD, PO_4-P, TN and NH_3-N (Y_1 ~ Y_5, respectively)

Model	Equation label
$Y_1 = 2.143\times10^2 + 1.397X_1 + 25.234X_2 - 0.186X_1^2 + 1.082X_1X_2 - 8.505X_2^2$	Eq. 3
$Y_2 = 4.132\times10^2 - 9.489X_1 + 3.658\times10^2X_2 - 1.559X_1^2 + 15.554X_1X_2 - 1.189\times10^2X_2^2$	Eq. 4
$Y_3 = -1.104\times10^2 + 4.062X_1 + 84.115X_2 - 0.182X_1^2 - 16.145X_2^2$	Eq. 5
$Y_4 = 8.169 + 1.315X_1 + 15.375X_2 - 0.054X_1^2 - 3.183X_2^2$	Eq. 6
$Y_5 = 31.660 + 1.863X_1 + 16.752X_2 - 0.078X_1^2 - 4.003X_2^2$	Eq. 7

In the study, the quadratic models predicted for the response variables of Y_1 ~ Y_5 are all statistically valid. The statistical significance of each quadratic model was evaluated by using ANOVA technique as shown in Table 11. It was observed that the *F*-values of the 5 models were more or less higher than the critical *F*-value (critical $F = 3.972$), and the *p*-values of the five models were all relatively low ($p < 0.05$), indicating that the regression models were significant at high confidence levels.

Table 11. ANOVA for the quadratic models predicted for the response variables of the algal biomass productivity and removal rates of COD, PO_4-P, TN and NH_3-N (Y_1 ~ Y_5, respectively)

	Source	[a] SS	[b] DF	[c] MS	*F*-value ($p < 0.05$)
Y_1	Model error	2.292	5	0.458	8.205 ($p = 0.008$)
	Residual error	0.391	7	0.056	
	Total	2.683	12		
	[d] R^2	85.42%			
	[e] Adjusted R^2	75.01%			
	[f] RMSE	0.236			
	[g] CV	4.706			
Y_2	Model error	5.853×10^5	5	1.171×10^5	5.048 ($p = 0.028$)
	Residual error	1.623×10^5	7	2.319×10^4	
	Total	7.476×10^5	12		
	[d] R^2	78.29%			
	[e] Adjusted R^2	62.78%			
	[f] RMSE	152.279			
	[g] CV	29.836			
Y_3	Model error	24.612	5	4.922	13.482 ($p = 0.002$)
	Residual error	2.556	7	0.365	
	Total	27.168	12		
	[d] R^2	90.59%			
	[e] Adjusted R^2	83.87%			
	[f] RMSE	0.604			
	[g] CV	-224.715			
Y_4	Model error	3.839	5	0.768	4.914 ($p = 0.030$)
	Residual error	1.094	7	0.156	
	Total	4.932	12		

	[d] R^2	77.83%			
	[e] Adjusted R^2	61.99%			
	[f] RMSE	0.395			
	[g] CV	15.052			
	Model error	2.799	5	0.560	5.601 (p = 0.022)
	Residual error	0.700	7	0.100	
	Total	3.498	12		
Y_5	[d] R^2	80%			
	[e] Adjusted R^2	65.72%			
	[f] RMSE	0.316			
	[g] CV	12.943			

[a] Sum of Square; [b] Degree of Freedom; [c] Mean Squares; [d] Coefficient of Correlation; [e] Coefficient of determination; [f] Root-mean-square Error; [g] Coefficient of Variation.

The three-dimensional (3D) response surface plots for Y_1 ~ Y_5 against the two experimental factors of DR and HRT are depicted in Fig.7. The plots for Y_1, Y_2, Y_4 and Y_5 showed that higher biomass productivities and nutrient removal rates were generally obtained with short HRT, but there was a strong effect of DR (Fig.7a, 7b, 7d and 7e). Considerably lower biomass production and nutrient removal rates were attained at high DR values and short HRTs. Generally, it was found that the highest biomass productivity, COD, TN and NH_3-N removal rates were obtained at the DR of 8.00 times and the HRT of 2.26 d. From the Y_3 surface plot (Fig.7c) and the counter plot (not shown), it can be seen that PO_4-P removal rate was sensitive to higher values of both DR and HRT, and the maximum PO_4-P removal rate was observed for DR of 11.4 time and HRT of 2.66 d. Nevertheless, since the drop in PO_4-P removal rate from the apex of the Y_3 surface plot (21.80 mg PO_4-P L^{-1} d^{-1}) to the point with DR of 8.00 times and HRT of 2.26 d (18.11 mg PO_4-P L^{-1} d^{-1}) was not very much, the optimal values of DR and HRT in the microalgae – acidogenically digested swine manure system is still determined to be 8.00 times and 2.26 d, respectively. The research presented herein was the first to use CCD for the optimization of culture conditions for both algal mass production and wastewater treatment.

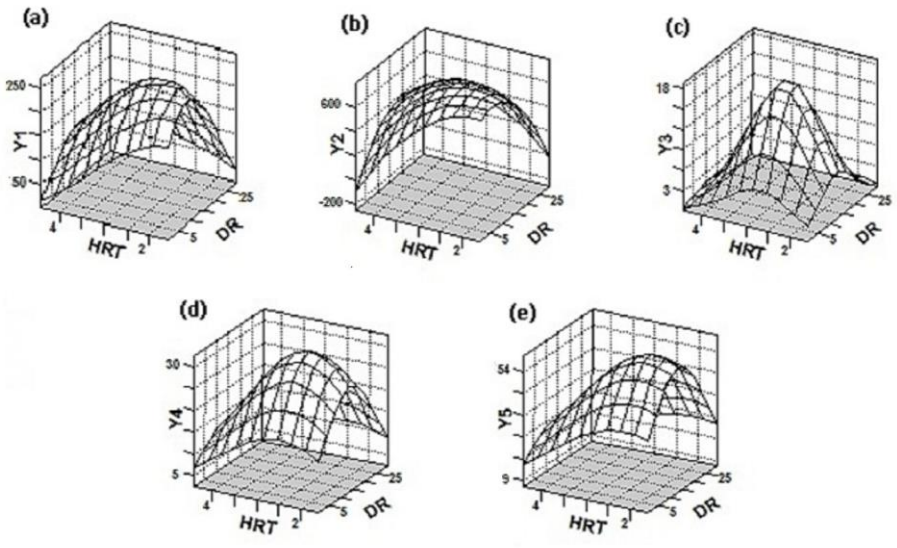


Figure 7. Response surface plots for biomass productivity, Y_1 (a), COD removal rate, Y_2 (b), PO_4-P removal rate, Y_3 (c), TN removal rate, Y_4 (d), and NH_3-N removal rate, Y_5 (e) as functions of DR and HRT

3.3 Applicability of the optimal conditions for fast algae growth and nutrient removal using a bench-scale 2-layer photobioreactor (Exp. II).

The predictive optima (DR_{opt} = 8.00 times, HRT_{opt} = 2.26 d) were utilized for *Chlorella* sp. cultivation on swine manure effluent obtained from the acidogenic digester as mentioned in section 3.1in a bench-scale 2-layer photobioreactor containing 17 L (4-cm water depth for each layer).

The process parameters used in this experiment demonstrated a good performance in growing algae and removing nutrients from the acidogenically digested swine manure. The algal cell density as TVSS, nutrient concentrations including COD, PO_4-P, TN and NH_3-N in the manure culture, and the culture pH were measured daily during the 17-day cultivation, and the data are shown in Fig.8 and 9. At the end of the period I (7-day batch mode), *Chlorella* sp. reached stationary phase with the cell density of 780 mg L^{-1} (Fig. 9a), and the nutrient levels of 238 mg COD L^{-1} d^{-1}, 28.6 mg PO_4-P L^{-1} d^{-1}, 48.3 mg TN L^{-1} d^{-1} and 28.2 mg NH_3-N L^{-1} d^{-1}(Fig.9b ~ 9d). As shown in Table 12, the cultivation performance achieved stable algal productivity of 276.18 mg L^{-1} d^{-1} and nutrient removal rates of 751.33 mg COD L^{-1} d^{-1}, 20.21 mg PO_4-P L^{-1} d^{-1}, 38.35 mg TN L^{-1} d^{-1}, and 60.39 mg NH_3-N L^{-1} d^{-1} during period II (10-day semi-continuous mode). It is noticed that TN removal rate was lower than NH_3-N removal rate, which was probably due to the fluxes of dissolved organic nitrogen (DON) from the suspended manure particles by the algae strain. Tyler et al. (2001) found that the opportunistic green macroalgae *Ulva lactuca* leaked DON from sediment into water column during its active growth in shallow lagoon in Hog Island Bay, Northampton County, VA. As shown in Table 12, the experimental values were close to the predicted response variables, which was very good for the goodness of fit. The result that the experimental values were slightly higher than the theoretically predicted values can be associated to the ammonia volatilization from the 2-layer photobioreactor and, therefore for the reduced ammonia content in the culture medium during algae growth. We believe that the removal of NH_3-N in the system was mainly attributed to the nitrogen uptake by *Chlorella* sp. other than ammonia volatilization, since the pH of the culture during the course of the growth

experiment (Fig.8) was always below pH 9.3 which was reported as pK_a of NH_4^+/NH_3 at room temperature (Ferrara and Avci, 1982). Moreover, the time course of pH values in Fig.9 demonstrated that the fed VFA-enriched manure medium could not only be used as a nutrient source, but also had a neutralizing effect against the culture alkalization which was mainly caused by the consumption of dissolved CO_2 in the culture during algal photosynthesis (Shiraiwa et al., 1993).

Table 12. Theoretically predicted and experimental values for algal biomass productivity, removal rates of COD, PO_4-P, TN and NH_3-N during the semi-continuous process in Exp II

	Biomass productivity (mg L^{-1} d^{-1})	Nutrient removal rate (mg L^{-1} d^{-1})			
		COD	PO_4-P	TN	NH_3-N
Predictive	246.76	738.04	18.11	33.73	58.96
Experimental	276.18	751.33	20.21	38.35	60.39

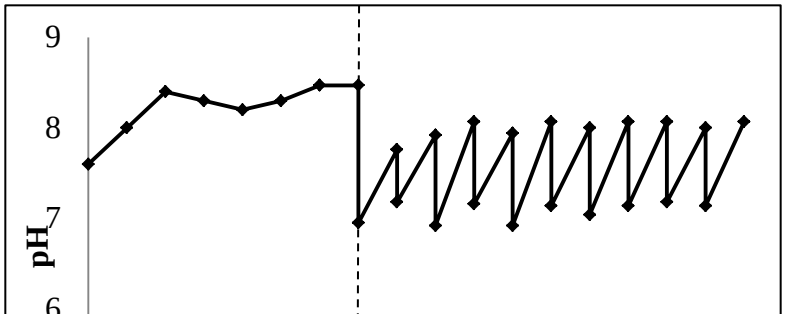


Figure 8. pH changes during the 17-day cultivation of *Chlorella* sp. in Exp II. The vertical dotted line represents the interface between the batch and semi-continuous periods.

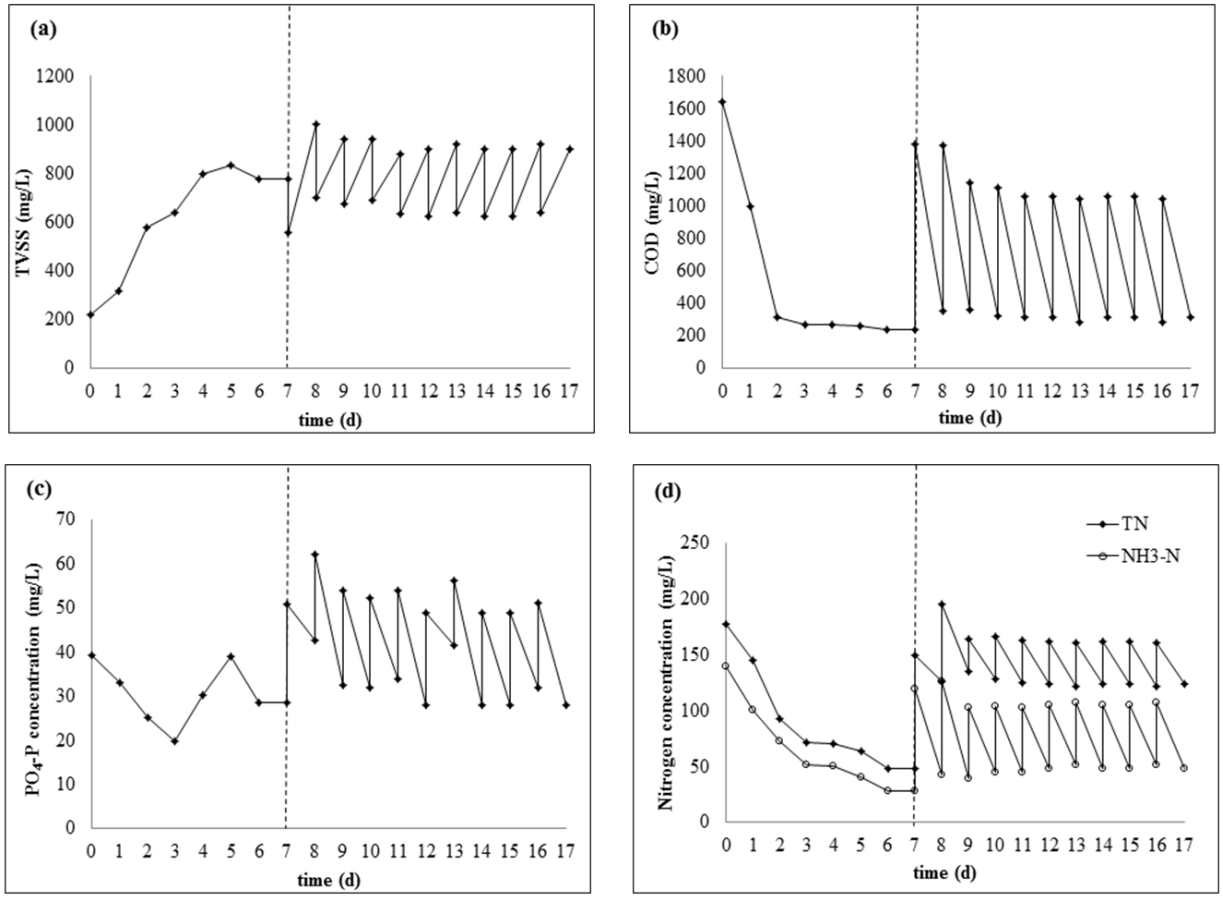


Figure 9. Time courses of algal cell density as TVSS (a), COD concentration (b), PO_4-P concentration (c), TN and NH_3-N concentrations (d) during the 17-day cultivation of *Chlorella* sp. on 8-fold diluted, acidogenically digested swine manure. The vertical dotted line represents the interface between the batch and semi-continuous processes

The chemical compositions of algal biomass collected from the culture effluents on the last three days of the semi-continuous process were measured using Folch extraction, elemental analysis, and GC-MS analysis to elucidate the potential of *Chlorella* sp. as algal feedstock for biofuel and feed production.

As shown in Table 13, the lipid content (on a dry matter basis) of *Chlorella* sp grown in the semi-continuous system was around 26.09% of dry biomass. According to our previous reports, the alga strain *Chlorella* sp. had similar lipid contents to the one in the study when it was grown on concentrated municipal wastewater (CMW) and 20-fold diluted, conventionally digested swine manure (DSM), which were 27.50% and 23±4.32%, respectively (Zhou et al., 2011; Zhou et al., 2012b). Therefore, it is considered that the lipid contents of the locally isolated *Chlorella* sp. grown on wastewaters are relatively stable and high, in the range of 25 ~ 30%. According to the data in the study, the lipid productivity of the microalgae-acidogenically digested swine manure system (3.63 g m^{-2} d^{-1}) corresponds to about 1.00×10^4 L ha^{-1} y^{-1}. Though our algal lipid productivity is much lower than the 5.87×10^4 ~ 1.37×10^5 L ha^{-1} y^{-1} reported for algal oil yield from the artificial media, it is 16.74 times greater than that for soybean oil production (598.6 L ha^{-1} y^{-1}; Pradhan et al., 2011), and thus, presents an exciting possibility as a low-cost feedstock for biofuel production.

Table 13. Chemical composition of *Chlorella* sp. on a dry matter basis
(%)

Composition	Content
Protein	58.78±1.05
Lipids	26.09±1.13
Carbohydrates and nucleic acid	15.13±0.69

Around 37.03% (on a dry matter basis) of total lipids in the frozen-dried algal biomass were free fatty acids and fatty acids derived from triacylglycerol and phospholipids (Table 14). GC-MS analysis showed that the fatty acids in the algal cells were mainly composed of saturated C14 ~ C18 fatty acids and monounsaturated C16 ~ C18 fatty acids (62.83±8.96% and 31.80±8.32% of total fatty acid weight, respectively) with C18:0 and C18:1 as the major compounds (22.46±2.81% and 19.00±1.62%, respectively). It is considered that transesterification of the fatty acid composition in our study could produce high-quality biodiesel. Xu et al. (2006) found that the biodiesel from algal oil of *Chlorella protothecoides* was abundantly composed of 18 carbon acid methyl esters, and had comparable physical and fuel properties to diesel fuel. Moreover, linolenic acid (C18:3), which should be lower than 12% for a quality biodiesel according to EN14214 standard (2004), was not detected in the fatty acid composition in our study.

Table 14. Fatty acid profile derived from triacylglycerol, phospholipid and free fatty acids in *Chlorella* sp. collected from the semi-continuous microalgae-acidogenically digested swine manure system

Name of the compounds	Content
Total fatty acid/dry weight (%)	9.66±1.83
Saturated fatty acids (% of total fatty acids)	62.83±8.96
C14:0	12.34±1.69
C15:0	5.51±0.45
C16:0	17.41±1.57
C18:0	19.00±1.62
C20:0	8.57±1.53
Monounsaturated fatty acids (% total fatty acids)	31.80±8.32
C16:1	9.34±1.86
C18:1	22.46±2.81
Polyunsaturated fatty acids (% total fatty acids)	1.53±0.08
C20:2	1.53±0.08

The *Chlorella* sp. cells or the remaining biomass fraction after oil extraction from algae can be used as a high protein feed for livestock to further help offset costs of algal mass production. As reported in Table 13, the crude protein content of the algal strain was 58.78±1.05%, which was comparably high among various microalgal species (6~71% of dry biomass; Becker, 2007). The use of microalgae as animal feed, such as in poultry farms and aquaculture, has been investigated for many years, and the research on waste-grown algae as food is more recent (Marimura and Tamiya, 1954). It is predicted that the acidogenically digested swine manure-grown algal biomass could be a valuable feed substitute for conventional protein sources.

4. Conclusion

It is concluded that the combined CCD approach and response surface analysis were effective to determine the optimum conditions of DR and HRT for algal biomass production and nutrient removal from the acidogenically digested swine wastewater. By semi-continuously cultivating *Chlorella* sp. (UMN271) on acidogenically digested swine wastewater under the optimal conditions (DR_{opt} = 8.00 times, HRT_{opt} = 2.26 d) using a bench-scale multi-layer photobioreactor,

stable mass productivity and nutrient removal rates slightly higher than the predicted values were obtained, and the algal biomass produced in the system can be a good feedstock for biofuel and feed production.

Activity 3: Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process

Summary:

This study investigated the feasibility of using recovered nutrients from hydrothermal carbonization (HTC) for cultivation of microalga Chlorella vulgaris. Different dilution multiples of 50, 100 and 200 were applied to the recycled process water from HTC and algal growth was compared among these media and a standard growth medium BG-11. Algae achieved a biomass concentration of 0.79 g/L on 50x process water after 4 days. Algae removed total nitrogen, total phosphorus and chemical oxygen demand by 45.5–59.9%, 85.8–94.6% and 50.0–60.9%, respectively, on differently diluted process water. The fatty acid methyl ester yields for algae grown on the process water were 11.2% (50x), 11.2% (100x) and 9.7% (200x), which were significantly higher than 4.5% for BG-11. In addition, algae cultivated on process water had 18.9% higher carbon and 7.8% lower nitrogen contents than those on BG-11, indicating that they are very suitable as biofuel feedstocks.

2. Methods

2.1. Characteristics of process water from HTC The process of HTC was described in details in our previous work (Du et al., 2012). Process water obtained under 200 ºC/40 min was recycled for use in this study. The main characteristics of process water are listed in Table 1. Chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), ammonia (NH4-N), nitrate (NO3-N), nitrite (NO2-N) and total phosphorous (TP) of the process water were determined using specific test kits on a Hach DR 5000 Spectrophotometer (Loveland, CO). Metal ion concentrations were analyzed on an inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000, Waltham, MA) by Soil Testing Laboratory at University of Minnesota, St. Paul.

2.2. Algal strain and culture conditions

Algal strain was a wild-type C. vulgaris isolated from local freshwater and its selection procedure was described in details in our previous study (Li et al., 2011). The algal strain was enriched in 100 ml BG-11 medium (Stanier et al., 1971) with 2 g/L glucose to obtain enough starting cultures for this study. Since the nutrient levels were too high for microalgae to survive, the process water was diluted to 50x, 100x and 200x times' volume with distilled water. BG-11 medium was used as the control to evaluate the growth efficiency of C. vulgaris on diluted process water. Algae were inoculated with the starting dry weight of 0.15 g/L in 250 mL Erlenmeyer flasks containing 150 mL autoclaved medium.

The flasks were placed on a shaker with 100 rpm rotation speed. All cultures were kept at 25 ± 2 ºC under continuous cool-white fluorescent light illumination at 100 lmol /m^2.s . Each growth condition was carried out in triplicates and a fourth culture was used to supplement the medium after sampling.

2.3. Algae growth and chemical analysis

Algal growth was determined daily by measuring total volatile suspended solids (TVSS) using 4 mL algae suspension collected from each flask. The biomass productivity was calculated from the equation:

$P(\mathrm{gL^{-1}day^{-1}}) = (\mathrm{TVSS} - \mathrm{TVSS_0})/t$ where t (day) was the time between the two measurements, TVSS and TVSS0 were the concentration of biomass at day t (growth curve leveling off) and day 0, respectively. A one-way analysis of variance (ANOVA, at 0.05 significance level) and the least significant difference method (LSD) was carried out for the statistical analysis of algal growth on the four different media. A volume of 8 mL algae suspension was collected daily from each flask for nutrient analysis. The samples were centrifuged at 7000 rpm for 10 min and supernatants were collected and diluted to suitable concentrations for analysis. TN, TP and COD were measured using test kits on a Hach DR 5000 Spectrophotometer.

Algae harvested at the end of the 5-day batch culture were analyzed for their C, H and N contents with an Exeter Analytical CE-440 Elemental Analyzer (Chelmsford, MA). Fatty acid content and composition were analyzed using acid-catalyzed in-situ transesterification method (Indarti et al., 2005). Dried algae (ca. 0.05 g) were weighed in 25-ml screw-top glass tubes, and 10 ml of a mixture of methanol, concentrated sulfuric acid and chloroform (volume ratio 4.25:0.75:5) was added. The glass tubes were sealed and placed into a 90 ———————————C water bath for 90 min. Upon cooling, the tubes were shaken and centrifuged at 7000 rpm for 5 min after adding 3 ml of distilled water into the reaction mixture. The chloroform layer containing fatty acid methyl esters (FAME) was carefully collected and subjected to gas chromatography–mass spectrometry (GC–MS). An Agilent 7890–5975C GC–MS with a HP-5MS (30 m x0.25 m x 0.25 lm) capillary column was used for FAME analysis. The carrier gas was helium at a flow rate of 1.2 mL/min. The oven temperature was initially 80 ºC for 1 min, then increased to 290 ºC at a rate of 4 ºC/min, and held at 290 ºC for 5 min. The injector and detector were maintained at constant temperature of 250 and 230 ºC, respectively. Compounds were identified with the National Institute of Standards and Technology (NIST) mass spectral data library and quantified with external standard calibrations of C14-C22 FAME standards (Sigma–Aldrich).

3. Results and discussion

3.1. Algal growth

Fig. 1 shows the growth curves for the four different media. The C. vulgaris could survive in all media as evidenced by the increase of biomass concentration. The biomass productivities were 0.013, 0.160, 0.092 and 0.054 g/ L.d for BG-11, 50x, 100x and 200x process water, respectively. Algae had significant higher productivities and biomass concentrations on the three dilutions of process water than BG-11 medium. Different from HTL carried out at high temperatures with many growth inhibitors produced, polysaccharides and proteins were mainly hydrolyzed to mono-sugars and amino acids in HTC. These mono-sugars and amino acids provided adequate carbon and nitrogen nutrients which can be readily used by algae. However, algae need to sequester CO2 as the sole carbon source when grown on inorganic BG-11 medium photoautotrophically. Many reports showed that mixotrophic growth can result in higher biomass production than phototrophic growth (Bhatnagar et al., 2011; Zhou et al., 2012). Among the three dilutions of the process water, both biomass productivity and final biomass concentration were in the following order: 50x > 100x > 200x. This indicates that algae can endure the higher concentration of potential growth inhibitors in the more concentrated process water. It is noticed that algae grew rapidly in the first 4 days and then decreased significantly on the fifth day on the 50x process water.

However, algae grew at a lower rate on the 100x and 200x process water and then leveled off after the third day. The stationary phase occurred so early mainly due to the limitation of phosphorus which will be discussed later.

3.2. Nutrient removal

Variations of TN, COD and TP in different media for the 5-day batch culture are depicted in Fig. 2. TN was significantly reduced by 45.5–59.9% on different dilutions of process water. As shown

in Table 1, most of the nitrogen in the process water was in the form of soluble organic nitrogen. This again indicates that algal proteins were hydrolyzed into simple amino acids during HTC, since algae can only use ammonia, nitrate and simple organic nitrogen, such as urea and amino acids as the nitrogen source
(Barsanti and Gualtieri, 2006). However, only 5.4% of TN was used in BG-11 medium, which coincides with the slight increase of algal biomass concentration under autotrophic growth. CODs also decreased drastically by 50.0–60.9% in the process water. This indicates that TOCs hydrolyzed from polysaccharides and proteins can be efficiently used by algae. A significant reduction (85.8–
94.6%) of TP was achieved for all of three diluted process water media. Since the concentration of TP was relatively low compared with other nutrients, it was quickly consumed in the first 2 days of growth. For green algae, the suitable N/P ratio should be in the range of 5:1 to 12:1 so that both N and P can be efficiently used (Li et al., 2010). However, the N/P ratio was 28:1, and P concentration reached to the range of 1.96–6.23 mg/L, leading to a P starvation condition. The depletion of the TP was at the end of the cultivation period could be the main reason that biomass growth leveled off.

3.3. Lipid and elemental compositional analysis

Fatty acids in algae can be converted to biodiesel via transesterification reaction (Li et al., 2011) or pyrolyzed into simple hydrocarbons (Du et al., 2012). Thus, it is very important to determine the fatty acid content of algae grown on recycled process water. Table 2 shows the fatty acid profiles of algae grown on different media.
The FAME yields for algae grown on the process water were 11.2% (50x), 11.2% (100x) and 9.7% (200x), which were significantly higher than that of 4.5% for BG-11. This is probably because microalgae were in the exponential phase on BG-11 medium when harvested at the fifth day, but lipid accumulation mainly happens at the stationary phase for algae. The fatty acid composition of algae is very similar among all these media. Hexadecanoic acid (C16:0), octadecadienoic acid (C18:2) and octadecatrienoic acid (C18:3) were the most abundant fatty acids under all conditions. Long chain fatty acids (C22:0 and C24:0) were detected in 100x process water in small quantities. Algae cultivated on BG-11 contained higher amount of saturated fatty acids and lower monoenoic fatty acids than those on the process water.
The elemental analysis results of the algae are shown in Table 3.
The elemental composition of the algae had no significant difference among the three dilution multiples of the process water, which is because the nutrient ratios (C:N:P) were the same, although their concentrations were different. Algae grown on the process water were higher in carbon and hydrogen and lower in nitrogen contents. This is in accordance with the lipid analysis results, since carbon denser products such as lipids will lead to higher carbon content. Also, lower nitrogen content indicates that algae grown on the process water had lower protein content. These properties are desirable for fuel application since high lipid and low protein will lead to better fuel quality and simpler downstream processing needs.

4. Conclusions

Algae grew much faster and achieved significantly higher biomass concentrations on the process water from HTC than those on BG-11 medium. 45.5–59.9% of TN, 85.8–94.6% of TP and 50.0–60.9% of COD were utilized by algae, on differently diluted process water. The results proved the feasibility and great potential of using the process water from HTC. The recycling of the process water for algae production could significantly increase the overall nutrient efficiency and reduce the production cost for the algae based biorefineries.

Table 1
Characteristics of process water from HTC.

Parameters	Concentration (mg/L)	Metals	Concentration (mg/L)
COD	134800 ± 2287	K	775.45
TOC	45700 ± 1513	Mg	4.025
TN	9650 ± 1582	Mn	0.01
Ammonia	1343 ± 75	Fe	3.085
Nitrate	211 ± 20	Na	8966
Nitrite	3.63 ± 0.73	B	1.855
TP	343 ± 43	Ni	0.005
		Cr	0.115

*Each data point indicates the mean ± standard deviation for three independent measurements; data for metal analysis were the average value of duplicate measurements.

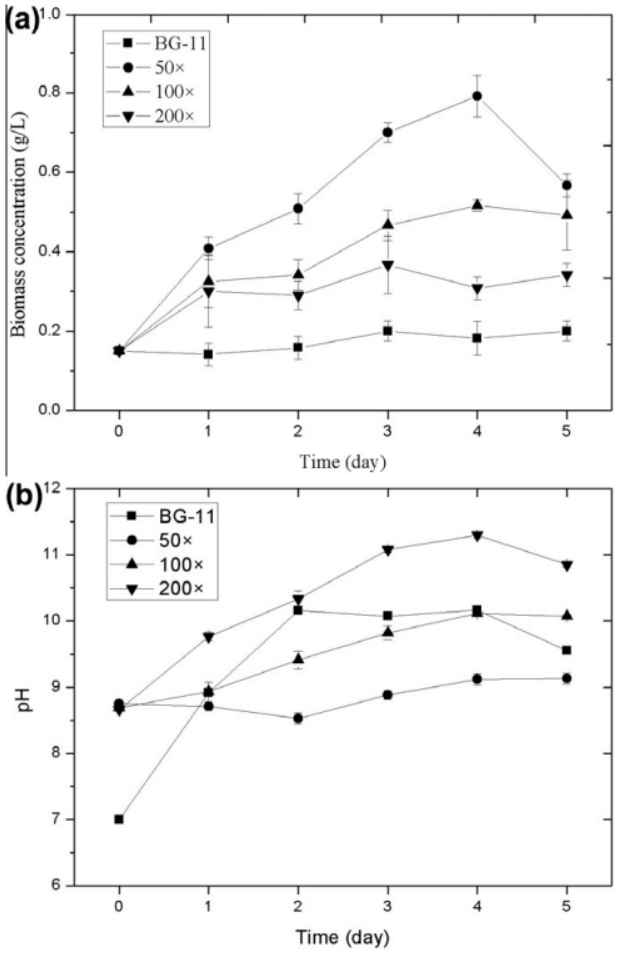


Fig. 1. (a) Growth curves for algae grown on the four different media; (b) pH values Error bars indicate the standard deviations of three replicates.

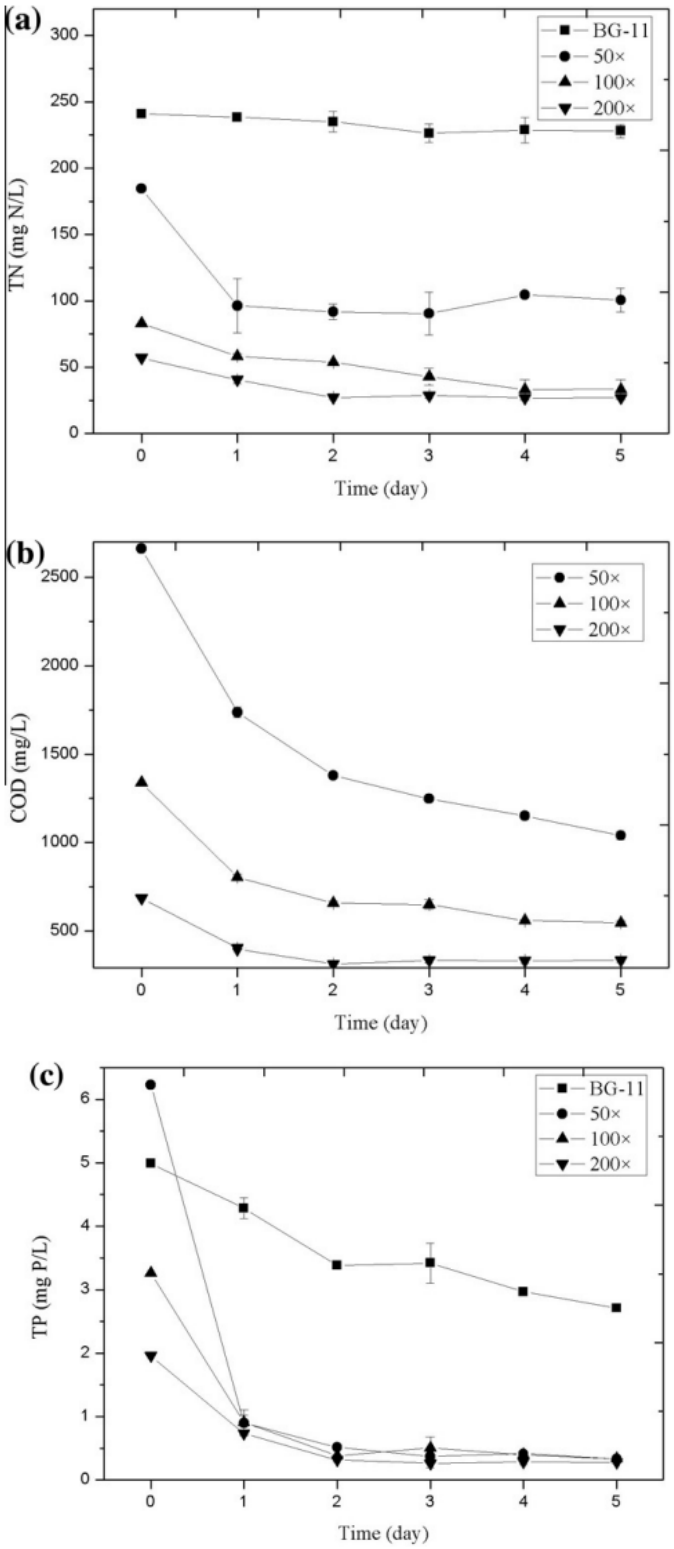


Fig. 2. Nutrient removal profiles. (a) TN removal; (b) COD removal; (c) TP removal. Error bars indicate standard deviations of three replicates.

Table 2
FAME profile for algae cultivated in different media.

FAME composition	BG-11	50×	100×	200×
Saturated fatty acids subtotal (% of total FAME)	34.7	26.6	29.5	22.9
16:0	34.5	24.5	25.2	22.5
18:0	0.2	1.4	1.0	0.4
20:0		0.7	1.0	
22:0			1.1	
24:0			1.2	
Monoenoic fatty acids subtotal (% of total FAME)	5.4	11.9	9.0	10.0
16:1	1.3	5.5	4.4	4.9
18:1	4.1	6.4	4.6	5.0
Polyenoic fatty acids subtotal (% of total FAME)	59.9	61.5	61.5	67.1
16:2	1.9	8.5	6.8	7.0
16:3	11.1	5.1	7.1	10.5
18:2	17.6	28.7	23.5	23.9
18:3	29.3	17.5	22.2	24.0
20:5		1.7	1.8	1.6
Total (% of TVSS)	4.5	11.2	11.2	9.7

Table 3
Elemental composition of algae cultivated in different media. Each data point indicates the mean ± standard deviation for three independent measurements.

Elemental composition (%)	BG-11	50×	100×	200×
C	42.9 ± 0.1	51.0 ± 2.6	49.9 ± 0.4	50.8 ± 1.3
H	6.1 ± 0.0	7.4 ± 0.2	7.2 ± 0.1	7.1 ± 0.2
N	9.7 ± 0.8	9.0 ± 0.6	8.9 ± 0.3	8.8 ± 0.1

Aquaculture has become the fastest growing food production sector, requiring matching increase in the production of aquafeeds, especially those for carnivorous species, which have heavily depended on fish meal to meet their critical protein requirements and fish oil for omega-3 fatty acid requirements. The overall goal of the proposed project is to develop a novel approach to the production of high quality alternative fish feed (aquafeed) from high protein, high oil, and low ash microalgae strains. So the objective of this study is to develop processes to produce microalgae with high protein and low ash content and microalgae with high content of long chain omega-3 fatty acids through selecting algae strains, developing special culture media and cultural conditions, and developing bio-based harvest techniques. The proposed project addresses key problems of national, regional, and multi-state importance in sustaining aquaculture and its profitability and human nutrition by producing alternative aquafeeds from waste streams and satisfying human food needs and at the same time cleaning the water and reducing pollutants emission.

Results

Microalgae strains adapted well to wastewater culture environments

Our large microalgae inventory includes 97 strains acquired from UTEX and 60 from local waters in Minnesota (Zhou et al. 2011). These strains belong to the families of *Chlorella*, *Haematococcus*, *Scenedesmus*, *Botryococcus*, *Ankistrodesmus*, *Tetraadron*, *Tetracystis*, *Diatom*, *Nannochloris*, *Crucigenia*, *Dictyochloris Chlamydomonas*, *Cosmarium*, *Characium*, *Chlorococcum, Scenedesmus*, *Heynigia, Micractinium and Hindakia*. Most of these strains have been subjected to a rigorous high-throughput screening procedure to determine their metabolic pathways, their growth characteristics, and their ability to adapt to different wastewaters

including municipal wastewater, dairy manure, swine manure, and industrial wastewater (Zhou et al. 2011). Among those candidate strains, more than 40 of them adapted well in diluted digested swine manure wastewater (Table 1), and more than 20 strains showed high growth rates in the range of 0.200-0.536 d^{-1}. Our results also showed that algal strains isolated from local strains tend to adapt well in local environments compared with those purchased from commercial algae bank. Among these top-performing strains isolated from local area, UMN 271 showed highest growth rate at 0.536 d^{-1}.

Table 1. The strain code and growth rate of selected top-performing microalgal strains adapted well to digested swine manure in batch culture (Zhou et al. 2012).

Strain ID	R_{TVSS} (g L^{-1} d^{-1})	Strain ID	R_{TVSS} (g L^{-1} d^{-1})
UTEX26	0.146±0.052	UMN242	0.005±0.014
UTEX78	0.167± 0.029	UMN243	0.200±0.052
UTEX230	0.189±0.043	UMN244	0.386±0.025
UTEX326	0.223±0.025	UMN245	0.245±0.038
UTEX343	0.160±0.029	UMN247	0.359±0.018
UTEX251	0.029±0.025	UMN251	0.158±0.029
UTEX252	0.114±0.029	UMN259	0.201±0.038
UTEX1236	0.129±0.016	UMN260	0.173±0.025
UTEX1591	0.208±0.014	UMN263	0.214±0.038
UTEX1787	0.209±0.020	UMN264	0.069±0.032
UTEX2240	0.194±0.038	UMN265	0.327±0.035
UTEX2498	0.040±0.018	UMN266	0.275±0.027
UTEX2551	0.085±0.016	UMN267	0.186±0.041
UTEX2714	0.282±0.058	UMN269	0.311±0.058
UMN220	0.264±0.035	UMN270	0.298±0.028
UMN224	0.209±0.043	UMN271	0.536±0.025
UMN228	0.158±0.058	UMN274	0.259±0.029
UMN231	0.433±0.038	UMN276	0.390±0.038
UMN232	0.250±0.029	UMN277	0.149±0.056
UMN238	0.185±0.035	UMN279	0.231±0.029
UM240	0.02±0.014		

Note: R_{TVSS} represents the growth rate of microalgae. And Each data indicates the mean ± SD (error) and were measured from three independent cultures.

High protein and low ash algal biomass

UMN 271 was further tested in large-scale experiment using our multi-layered bioreactors installed in the UMN Rosemount Research and Outreach Center (Rosemount, MN). The algal biomass was harvested using a starch based floccurant we developed. The composition of harvested algal biomass was analyzed and compared with other samples derived from different culture media (Table 2). The results show that our algae samples have high protein content and low ash content, which is desirable as a feed replacement.

Table 2. UMN algae sample compositions as compared with two other sources (The data were provided by USDA ARS lab in Aberdeen)*.

Algae sample	**Moisture**	**Protein**	**Oil**	**Ash**	**Total CHO**
UMN 271	9.23a	52.04a	1.12c	12.63c	32.21a

Algae source A	7.31b	32.05c	2.63a	31.45a	33.86a
Algae source B	6.16c	46.60c	4.04a	26.89b	22.46b

*Columns labeled with the same letters are not significantly different at p = 0.05.

Microalgae strains with high PUFAs

We analyzed the fatty acid profiles of a number of strains in our algae inventory. It was found that UMN231, UMN 258, UMN268 contained EPA ranging from 0.13-34.3% of total FFA but no DHA (Table 3). No EPA or DHA was detected in the high protein strain UMN 271, suggesting that UMN 271 is a good fish protein replacer but not fish oil replacer. We also include data for several UTEX strains with high PUFAs (Table 3), which can be potential sources of fish oil replacements. To further improve the FFA and PUFA contents in these algae strains, we may employ different cultivation strategies such as changing growth mode (heterotrophs) (Ganuza et al. 2008), temperature shift, adding PUFA precursor, etc.

Table 3. Main fatty acid compositions (% of total fatty acid) of UMN microalgal strains (Zhou et al. 2011) and UTEX strains (Vazhappilly and Chen 1998).

FFA (%)	**UM231**	**UM258**	**UM268**	**UM271**	**UTEXLB1002**	**UTEX151**	**UTEXL1649**	**UTEX2341**
C12:0	N/D	N/D	0.31	N/D	N/D	N/D	N/D	N/D
C14:0	0.89	4.12	5.13	1.03	5.4	0.1	4.4	0.7
C16:0	22.15	26.92	35.66	27.31	30.9	18.7	20.6	11.9
C16:1	0.2	5.77	0.12	6.89	7.1	10.1	22.6	15.0
C16:2	2.86	2.75	3.78	9.61	N/D	N/D	N/D	N/D
C16:3	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
C18:0	3.74	0.87	2.24	2.80	10.5	0.9	9.0	7.8
C18:1	10.40	31.73	N/D	5.24	0.3	5.4	0.3	12.0
C18:2	22.81	15.86	23.91	31.57	5.6	2.4	2.3	6.3
C18:3	30.16	0.56	26.30	13.03	3.1	0.4	1.1	4.6
C20:0	N/D	0.14	N/D	0.44	N/D	N/D	N/D	N/D
C20:1	0.23	0.13	N/D	N/D	N/D	N/D	N/D	N/D
C20:5 (EPA)	**5.05**	**7.58**	**3.96**	**N/D**	**15.1**	**34.2**	**N/D**	**31.3**
C22:0	N/D	N/D	0.33	0.39	N/D	N/D	N/D	N/D
C22:6 (DHA)	**N/D**	**N/D**	**N/D**	**N/D**	**17.0**	**N/D**	**19.9**	**N/D**
FFA in biomass (% w/w)	7.08	9.77	8.77	9.43	6.85	9.93	11.1	6.53
FFA in oil (% w/w)	30.41	38.02	32.66	29.78	N/A	N/A	N/A	N/A
Oil in biomass (% w/w)	23.28	25.70	26.85	1.12	N/A	N/A	N/A	N/A
Protein in biomass (% w/w)	45.7	N/A	N/A	52.04	N/A	N/A	N/A	N/A
Ash in biomass (% w/w)	N/A	N/A	N/A	12.63	N/A	N/A	N/A	N/A
Carbohydrates in biomass (% w/w)	14.7	N/A	N/A	32.21	N/A	N/A	N/A	N/A

N/D = not detectable, N/A = not available

To address concerns over the metals in animal wastewater, we will focus on anaerobically digested wastewater for culture. It is well known that animal wastewater contains much lower metals than industrial and municipal wastewaters, and anaerobic digestion will further precipitate metals and kill potential pathogens. Thus the potential of toxicity for fish and human consumption will be minimal if not eliminated. However, potential effects will be confirmed by feeding tests.

Final Report Summary:

Extensive work was conducted to screen algae strains, optimize cultivation processes, and develop the pilot scale facility. High performance algae strains have been developed for specific applications such as oil accumulation, nutrient removal, growth under low temperature and low light conditions, and accumulation of high value lipids. The growth characteristics of these algae strains were systematically studied and used to guide cultivation process optimization for specific applications. Process was developed to improve the suitability of wastewaters for algae growth and enhance nutrient removal and water cleaning. Innovative harvest techniques, such as fungi assisted palletization, starch based flocculants, were developed. Multi-layer photobioreactors enclosed in a simple and low cost greenhouse were developed and demonstrated. These new developments are significant because algae can be effectively used to simultaneously remove nitrogen, phosphorus, chemical oxygen demand (COD), and other nutrients in municipal and animal wastewaters, sequester carbons in organic matters and flue gas, and at the same time accumulate biomass for production of high vale biofuels and bioproducts.

Result 2: *Lab Scale Algae to Fuel Conversion Technology*

Description:
Processing is another important factor in the algae-to-fuel equation. The processing costs may be reduced by improving the processing efficiency and generating multiple value-added by-products. The objective of this project result is to develop cost effective conversion processes and a product portfolio for best economic returns. Figure 1 shows the possible product streams from algae.

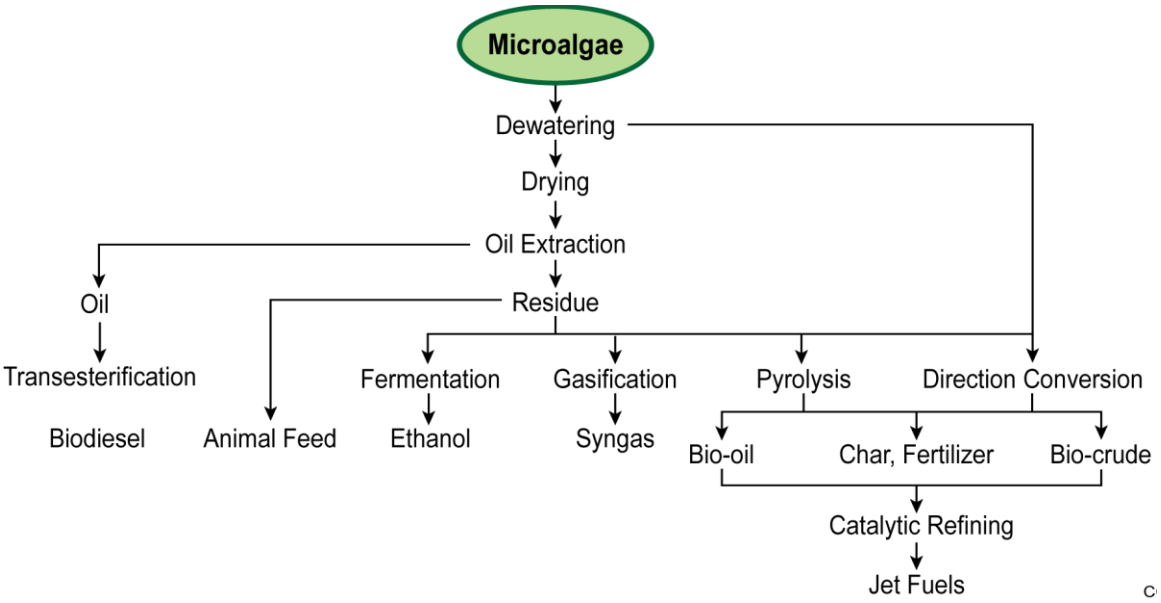


Figure 1. Product possibilities from algae.

The planned activities for this project result include development and demonstration of technologies for converting algal biomass to a range of products. We will advance research on direct conversion processes which either extract lipids from wet algae or convert wet algae directly to fuels without the expensive drying process. In this project, we will (1) optimize direct conversion and catalytic upgrading processes, (2) design and construct large lab scale systems, (3) develop P and N recovery process through algae processing, and (4) test and demonstrate the systems.

Summary Budget Information for Result 2:

ENRTF Budget:	**$ 414,010.00**
Amount Spent:	**$ 414,010.00**
Balance:	**$0.00**

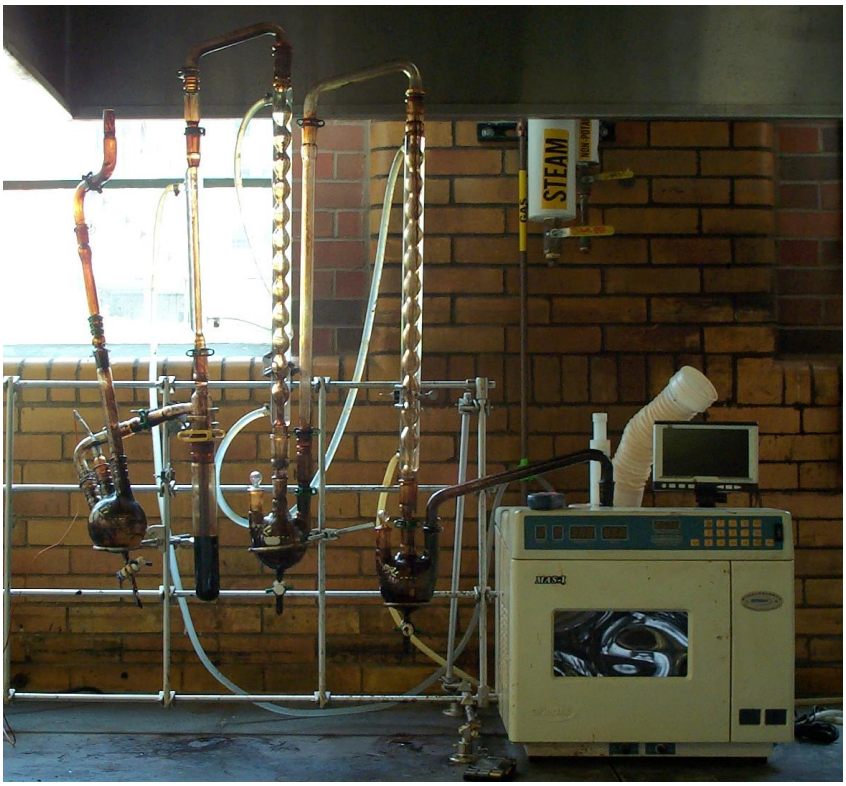
Deliverable	**Completion Date**	**Budget**
1. Optimize direct conversion and catalytic upgrading processes	*06/30/2011*	$56,304
2. Design and construction of the conversion systems	*06/30/2012*	$250,906
3. Develop P and N recover process	*09/30/2013*	$44,980
4. Evaluation data and demonstration	*09/30/2013*	$56,003

Result Completion Date: *09/30/2013*

Result Status as of December 31, 2010: Wet algae paste (about 15% solids) was processed using hydrothermal liquefaction (HTL) method with or without catalysts to produce bio-crude and biochars. Our preliminary data show that the bio-crude and biochar yields are affected by temperature, pressure and residence time. More studies are under way to understand and improve the process. HTL could be an ideal process for algae processing because it does not require expensive drying. We are also exploring the possibility of using HTL process as a pretreatment of microalgal cells for improved oil extraction operation. We also converted dried

algae using microwave assisted pyrolysis (MAP) method. The algal bio-oil obtained from MAP process is much better than cellulosic feedstock derived bio-oil in terms of heat value, viscosity, acid numbers, and other fuel properties, and can be mixed directly with gasoline for engine use. We are developing catalytic processes to remove oxygen and nitrogen from algae derived bio-oils.

Result Status as of *June 30, 2011*
We continued to test microwave assisted pyrolysis (MAP) of algal biomass. The physical, chemical, and energetic properties of the liquid fuels obtained from pyrolysis of algae are favorably compared with the commercial fuels of similar category. New process is being developed to treat algal biomass using hydrothermal process to convert wet algae to higher energy density materials which may be further converted to fuels and chemicals. Different direct transesterification methods were examined. The fatty acids profiles of selected strains were determined and conversion of fatty acids under different conditions was studied. We are able convert more than 90% of fatty acids to biodiesel using our methods.



Bench scale MAP reactor used for converting algal biomass to bio-oil.

Result Status as of *December 31, 2011*
We continued to develop in situ transesterification of algal biomass. New pretreatments including solvent soaking, ultrasonic treatment and microwave treatment were tested. Microwave treatment was found quite effective in speeding up the reaction rate. Hydrothermal treatment of wet algal biomass produced intermediates with higher energy content and ease of oil extraction. Transesterification of the hydrothermally treated samples is being studied.

We will continue to study pyrolysis of algal biomass to directly produce high quality bio-oil as liquid fuels. The photo shown below is the mobile MAP system developed through efforts

funded in part by LCCMR. This system can be used for distributed conversion of algal biomass on algal biomass production sites.

Mobile MAP system on trailer.

Result Status as of *June 30, 2012*

Activity 1: Hydrothermal Pretreatment of Wet Algal Biomass

1. Summary

Previous studies indicate that microalgae can be converted to an energy-dense bio-oil via pyrolysis. However, the relatively high nitrogen content of this bio-oil presents a challenge for its direct use as fuels. Therefore, hydrothermal technique was employed to pretreat microalgae *Nannochloropsis oculata*, in order to reduce the N content in the algal feedstock by removing proteins without requiring significant energy inputs. The effects of reaction conditions on the yield and composition of pretreated algae were investigated by varying the temperature (150−225 °C) and reaction time (10−60 min). Compared with untreated algae, pretreated samples had higher carbon content and enhanced heating values under all reaction conditions. Additionally, decreased N content was observed under relatively severe conditions. Follow-up studies confirmed that pyrolytic bio-oil from pretreated algae biomass contained less N-

containing compounds and was dominated by long-chain fatty acids which could be more readily converted into hydrocarbon fuels in the presence of simple catalysts.

2. Materials and Methods

2.1. Materials

For consistent comparison, *Nannocloropsis oculata* slurry (80% moisture content) was purchased from Reed Mariculture, Inc. (Campbell, CA) and was used within three months before spoilage occurred. The main characteristics of dry *Nannochloropsis oculata* are listed in Table 1.

Table 1. Characteristics of dry algal biomass (dry basis)

Biochemical composition (wt.%)		Elemental analysis (wt.%)	
Proteins	39	C	39.9
Carbohydrates	20	H	5.5
Lipids	17	N	6.2
Ash	24	O[a]	24.0

[a] Calculated by difference, $O(\%) = 100 - (C + H + N + Ash)$.

2.1. Apparatus and experimental procedure

Six inches long T316 stainless steel tubes (3/8" outside diameter) with one end plugged and one end capped were used as the batch reactors. Each reactor was loaded with 5.0 g of algal slurry (1.0 g on a dry basis) and flushed with nitrogen to ensure an inert atmosphere before sealing. The reactors were then inserted in a preheated Techne IFB-52 sand bath (Burlington, NJ) which can provide fast, uniform and stable heat transfer. After a pre-determined period of time, the reactors were lifted and quenched with a cold water bath to terminate the reactions.The total residence time in the tubular reactors counted from inserting reactors in the sand bath until quenching. Upon cooling, the reactors were opened and weighed after the gas was released. The weight of gaseous products was recorded as the total weight difference of reactor and biomass before and after reaction. After that, the inner chambers, caps and plugs were flushed with 50 mL deionized water to ensure complete collection of the products. The reaction mixtures were separated by filtration and the pretreated algae solid was freeze-dried to remove the remaining water. The pretreated algae samples were weighed before and after drying to determine the moisture content. The weight of aqueous phase products was calculated according to mass balance, i.e.

$$Weight\ of\ aqueous\ phase\ products = Weight\ of\ algae\ before\ treatment - Weight\ of\ gaseous\ products - Weight\ of\ pretreated\ algae$$

2.2. Analysis of products

Untreated and pretreated algae were analyzed for their C, H and N contents by using an Exeter AnalyticalCE-440 Elemental Analyzer (Chelmsford, MA). Ash content was determined by weighing the residues after complete combustion of the biomass in a furnace at 550 °C. All measurements were performed in duplicates with the mean values reported. Their HHVs were calculated according to following equation (Friedl et al., 2005)

$$HHV\ (MJ/kg) = (3.55 \times C^2 - 232 \times C - 2230 \times H + 51.2 \times C \times H + 131 \times N + 20600) \times 10^{-3}$$

Total organic carbon and total nitrogen in the aqueous phase were measured using Hach analysis kits on a Hach 5000 Spectrophotometer.

Lipids in untreated and pretreated algae were analyzed to determine the fatty acid retention after HP. Fatty acid composition and content was analyzed using acid-catalyzed *in-situ* transesterification method (Indarti et al., 2005). Dried untreated and pretreated algae (ca. 0.1 g) were weighed in 25 ml screw-top glass tubes, in which 10 ml mixture of methanol, concentrated sulfuric acid and chloroform (volume ratio 4.25:0.75:5) were added afterwards. Then, the glass tubes were tightened up and placed into a 90 °C water bath for 90 min. Upon cooling, the tubes

were shaken and centrifuged after adding 3 ml distilled water into the reaction mixture. After that, the chloroform layer containing fatty acid methyl esters (FAME) was carefully collected and subjected to gas chromatography-mass spectrometry (GC-MS) analysis. An Agilent 7890-5975C GC-MS with a HP-5MS (30 m × 0.25 m × 0.25 μm) capillary column was used for FAME analysis. Helium was employed as the carrier gas at a flow rate of 1.2 mL/min. The initial oven temperature was 80 °C held for 1 min and then increased to 290 °C at a rate of 4 °C /min, and held at 290 °C for 5 min, while the injector and detector were maintained at constant temperature of 250 °C and 230 °C, respectively. The compounds were identified with National Institute of Standards and Technology (NIST) mass spectral data library and quantified with external standard calibrations of C14−C22 standards (Sigma-Aldrich). Each reaction condition was analyzed in duplicate with the average values reported.

Equilibrium moisture contents (EMC) of untreated and pretreated algae were measured using the static desiccator method (Bellur et al., 2009). Solid samples were exposed to an environment with constant relative humidity and temperature over a period of time, until the moisture content in the samples reached equilibrium. The humidity in the chamber were maintained at a constant value by keeping the air in equilibrium with a saturated salt solution, including potassium acetate, magnesium nitrate and sodium chloride, which corresponds to the humidity level of 22.5%, 52.9% and 75.3% at 30 °C.

The micro-structures of untreated and pretreated algae were analyzed with a Hitachi scanning electron microscopy (SEM, model S3500N) at the Imaging Center of University of Minnesota, Twin Cities.

Pyrolysis experiments were performed using an analytical pyrolyzer coupled with a GC-MS (Py-GC-MS). About 0.5 mg of untreated or pretreated algae was filled into a quartz tube, which was then inserted in a platinum coil and pyrolyzed in a CDS2000 pyrolyzer at 500 °C. Upon pyrolysis, the pyrolysis vapors were directly swept into the Varian 3400 CX-Saturn 3 GC-MS with a DB-5MS (30 m × 0.25 m × 0.25 μm) column. An injector temperature of 250 °C and a split ration of 1:100 were used. The initial oven temperature was 40 °C held for 3 min and then increased to 250 °C at a rate of 5 °C /min, and held at 250 °C for 10 min.

Statistical analysis was carried out using software SPSS 18.0.

3. Results and Discussion

3.1. Pretreated algae yields and elemental composition

The yields of the pretreated algae under different temperatures and reaction times are shown in Fig. 1. Two-way ANOVA revealed that the pretreated algae yield was significantly influenced by temperature ($p<0.05$) and time ($p<0.05$) with no interaction between the two ($p>0.05$). As it can be observed, the mass of recovered pretreated algae dropped significantly with increasing temperature, suggesting a greater decomposition of biomass at higher temperatures. Varying reaction time also displayed a similar trend, although pretreated algae yield started to level off after 30 min of reaction. These results are consistent with some other studies on different biomass materials (Heilmann et al., 2010; Hoekman et al., 2011).

On the other hand, higher carbon content and energy densification were achieved with increasing temperature and time as a consequence of carbonization process, in which oxygen was removed by dehydration and decarboxylation (Funke and Ziegler, 2010). In all cases, the heating values of pretreated algae increased between 22−95% compared with those of the untreated algae. These increases are comparable to other reports (Heilmann et al., 2010; Yan et al., 2009). The N retention in the pretreated algae decreased with increasing process severity, which was mainly because of the hydrolysis of algal proteins. N remaining in the pretreated algae could be from some residual hydrophobic peptides, amino acids or Maillard reaction products, as the pretreated algae had a rich brown color. Pretreated algae with a lower N are expected to lead to the pyolytic bio-oil with lower N compared with the untreated samples, which will be discussed in details in another section. Also, our preliminary results revealed that the organic C and N in the water phase could be recycled for algal cultivation and will be discussed

in future publications. Fig. 2 shows the C and N distribution in the products of HP under 200 °C, 40 min condition. In addition, ash content in the pretreated algae greatly reduced, which could be due to the “wash out” effects of water on the minerals. This is important because the high ash content in microalgae can cause slagging and fouling problems in thermochemical conversions (Ross et al., 2008).

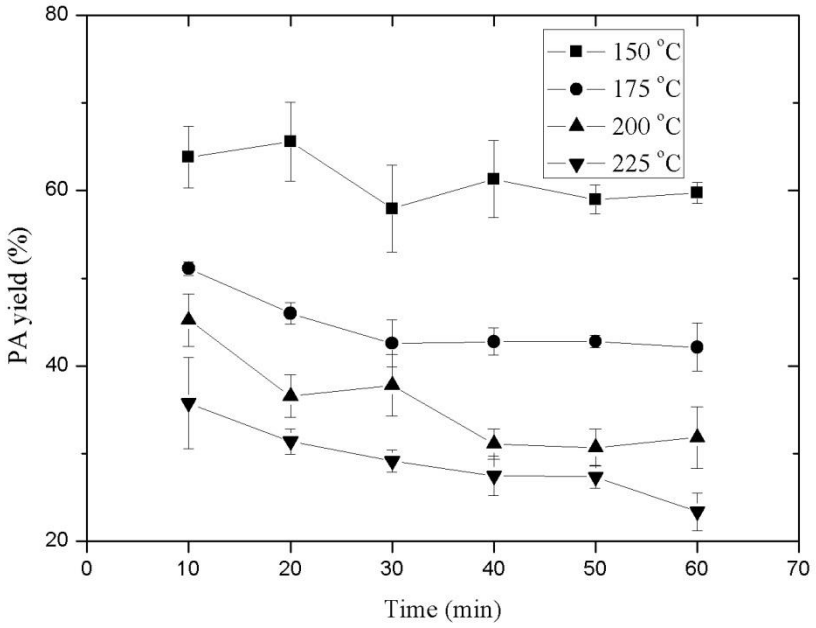


Fig. 1. Effects of temperature and reaction time on the yield of pretreated algae

Table 2. Elemental composition of the pretreated algae

			Elemental composition (%)						HHV(MJ/kg)
			C	H	N	Ash	C retention	N retention	
untreated algae			39.9	5.5	6.2	24.4			16.8
pretreated algae	Temperature (°C)	Time (min)							
	150	10	51.0	7.0	8.3	14.4	81.6	84.9	21.7
		20	51.0	7.0	8.0	14.3	83.9	84.5	21.7
		30	51.1	6.9	7.8	12.4	74.3	73.0	21.7
		40	50.5	6.9	7.7	14.7	77.7	76.2	21.4
		50	51.3	7.0	7.7	14.6	75.9	73.1	21.8
		60	52.0	7.0	7.6	12.8	77.8	73.2	22.1

175	10	49.9	6.9	7.0	18.8	64.0	57.8	21.0
	20	52.7	7.2	6.7	17.2	60.7	49.5	22.4
	30	53.1	7.2	6.5	14.3	56.8	44.9	22.7
	40	54.8	7.4	6.1	14.0	58.8	42.0	23.6
	50	54.6	7.3	6.0	14.8	58.6	41.1	23.4
	60	53.7	7.1	6.2	14.0	56.7	42.4	22.9
200	10	49.1	6.6	6.6	22.1	55.7	48.3	20.5
	20	54.0	7.1	5.7	16.7	49.5	33.5	23.0
	30	51.1	6.8	5.8	19.9	48.4	35.1	21.4
	40	57.5	7.6	5.1	13.6	44.8	25.7	25.1
	50	56.9	7.6	4.8	13.5	43.8	23.9	24.7
	60	54.4	7.2	5.1	12.8	43.4	26.0	23.1
225	10	51.1	6.8	6.1	18.8	45.8	34.9	21.4
	20	58.2	7.7	5.4	13.9	45.9	27.1	25.6
	30	58.7	7.8	4.8	13.6	42.9	22.5	25.9
	40	63.6	8.3	4.8	12.8	43.8	21.3	29.4
	50	63.1	8.1	4.4	12.2	43.3	19.2	28.8
	60	67.5	9.1	3.6	13.5	39.6	13.4	32.7

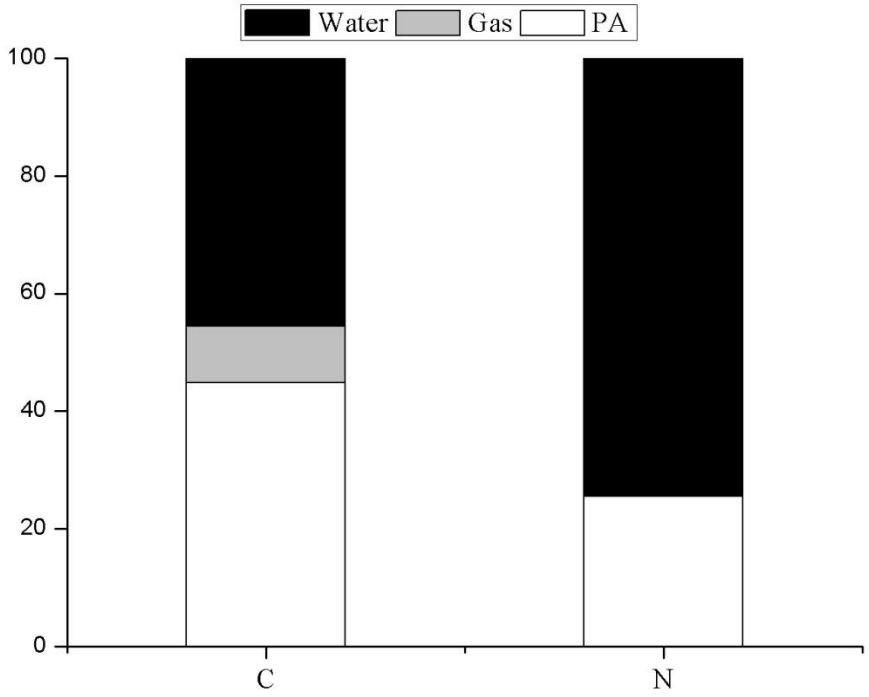


Fig. 2. C and N distribution among the products of HP under 200 °C, 40 min condition.

3.2. Fatty acid retention after HP

Lipids are desirable compounds in microalgae, because they can be converted to diesel-range hydrocarbons in pyrolysis (Idem et al., 1996; Schwab et al., 1988). Hydrocarbons in the algal pyrolytic bio-oil have been generally considered as the derivatives of lipids (Miao and Wu, 2004; Vardon et al., 2012). Therefore, it is important to determine the lipid retention (i.e. percentage of lipids in the untreated algae that is still retained in the pretreated algae). Since hydrophobic compounds may be produced from proteins and polysaccharides in HP (Torri et al., 2012), lipids quantification by gravimetric analysis of organic solvent-extracts could be inaccurate. For this reason, the method in section 2.2 was used for the determination of fatty acids. Detected fatty acids included C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:4 and C20:5. As can be seen in Fig. 3, the fatty acid retention ranged from 73 to 99%. Generally, there was a trend of decreasing for the fatty acid retention with increasing temperature. This could be because of greater emulsification between intracellular fatty acids and water when algal cells were more severely destroyed at higher temperatures. Also, polyunsaturated fatty acids could be decomposed to some extent in HP (Holliday et al., 1997). Similar high lipid retention has been reported on heterotrophically cultivated *Chlorella vulgaris* with 45.6% lipid content (Levine et al., 2010). Although other studies show that thermal treatment could hydrolyze triglycerides in microalgae into free fatty acids (FFA) (Levine et al., 2010; Torri et al., 2012), which can generate undesirable soaps in transesterification, pyrolysis of microalgae after HP in this study does not have this issue.

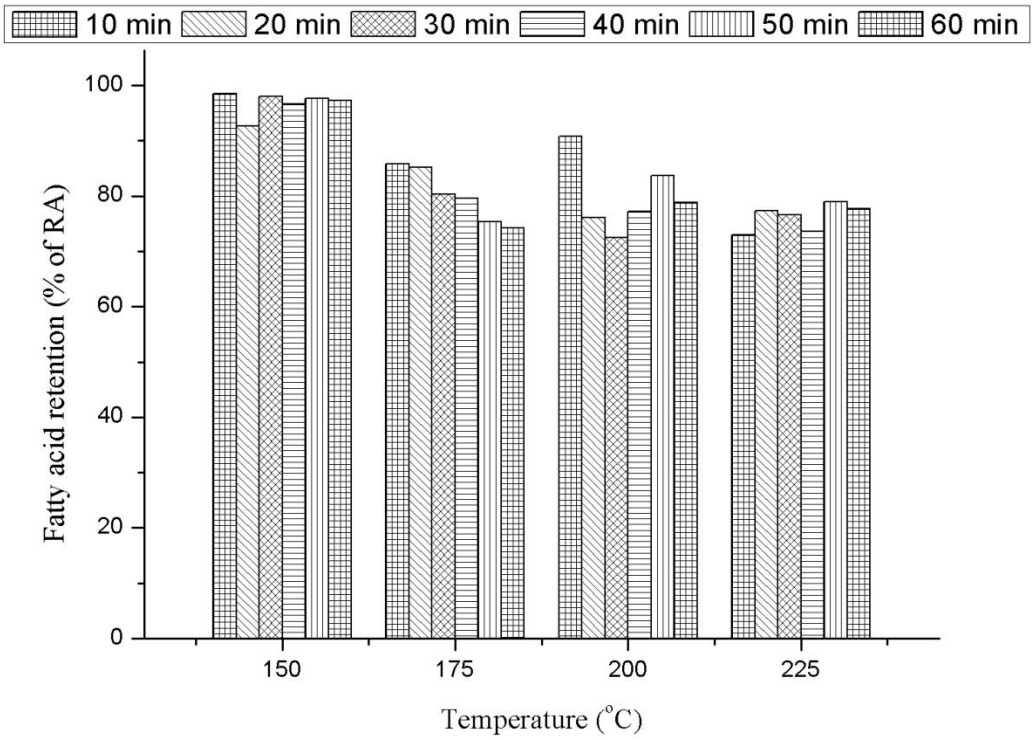


Fig. 3. Fatty acid retention in the pretreated algae.

3.3. Microstructure of algal cell before and after HP

Based on the pretreated algae yield and lipid retention data under different reaction conditions, we suspected that their variations could be related to structural changes of algal cells, considering that intracellular compounds may become more accessible when cell wall is destroyed. Fig. 4 shows SEM images of the algal cells obtained before and after HP. It can be seen that the cells under 150 °C/50 min appeared unbroken and their morphology was very similar to the untreated algae. This may explain the high pretreated algae yield and lipid retention under this condition. The temperature was not high enough to break the cell wall even though 50 min processing time was applied. At 200 °C, cells started to cluster and compact, although some cells were still recognizable. Cells deformed more seriously and the compacting effects became stronger with longer reaction time, which could lead to a greater release of intracellular compounds. At 225 °C, there was an abrupt cellular morphological change with no individual cells could be differentiated for a short reaction time of 10 min. These results could explain the decreasing yield of pretreated algae and lipid retention with increasing process severity, as greater cell wall breakage occurred with more intracellular materials decomposed and released. More accessible intracellular proteins and polysaccharides could be hydrolyzed and dissolved in the aqueous phase. Lipids, although insoluble in water, can be hydrolyzed to

FFAs, which are easier to be emulsified, leading to a lower lipid retention in pretreated algae.

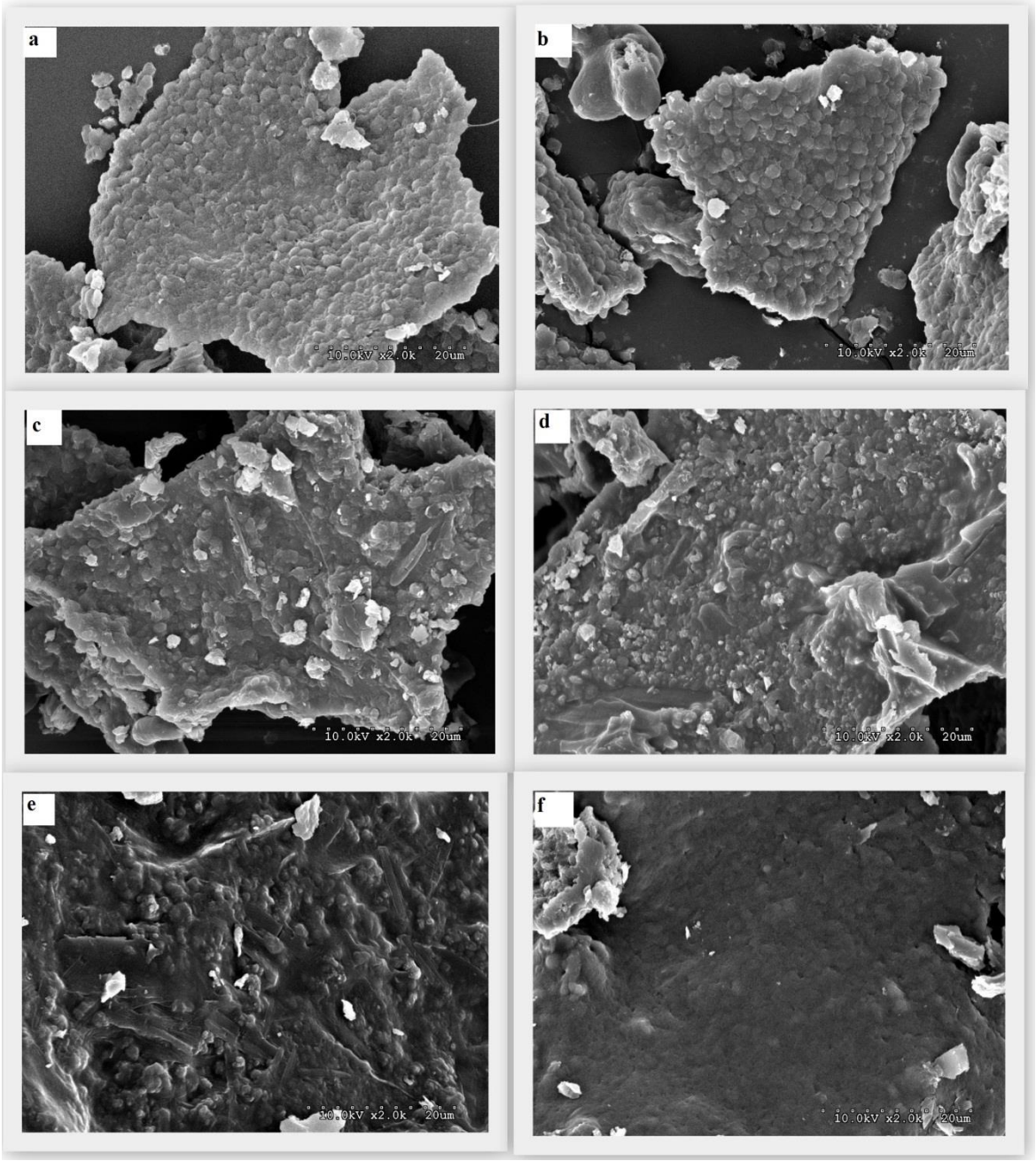


Fig. 4. SEM images: (a) untreated algae and (b-f) pretreated algae at: (b) 150 °C/50 min, (c) 200 °C/10 min, (d) 200 °C/30 min, (e) 200 °C/50 min and (f) 225 °C/10 min.

3.4. EMC analysis

Previous literature has suggested that hydrothermally pretreated biomass are relatively hydrophobic (Acharjee et al., 2011). The hydrophobicity of solids could be characterized with EMC, which is defined as the moisture content in the biomass in equilibrium with that in the surroundings at a certain temperature and relative humidity. The EMC of freeze-dried untreated and pretreated algae of 200 °C/40 min and 225 °C/40 min were analyzed in this study. Fig. 5 shows that pretreated algae was more hydrophobic compared with untreated algae and the difference was more evident at higher relative humidity. The EMC of pretreated algae decreased with increasing pretreatment temperature, which could be because of greater cell

wall degradation, since it was suggested that moisture was absorbed by forming hydrogen bond with the hydrophilic hydroxyl groups of the cell wall components (Andersson and Tillman, 1989). The relatively hydrophobic pretreated algae can provide the following advantages: firstly, pretreated algae can be easily dried to remove the residual moisture after filtration; secondly, dry pretreated algae can be stored stably with less biological deterioration to solve the seasonal availability problem, especially in northern regions where climate dependent year-around algal production facilities are impractical; thirdly, transportation of biomass, if needed, is less expensive when less water is transported.

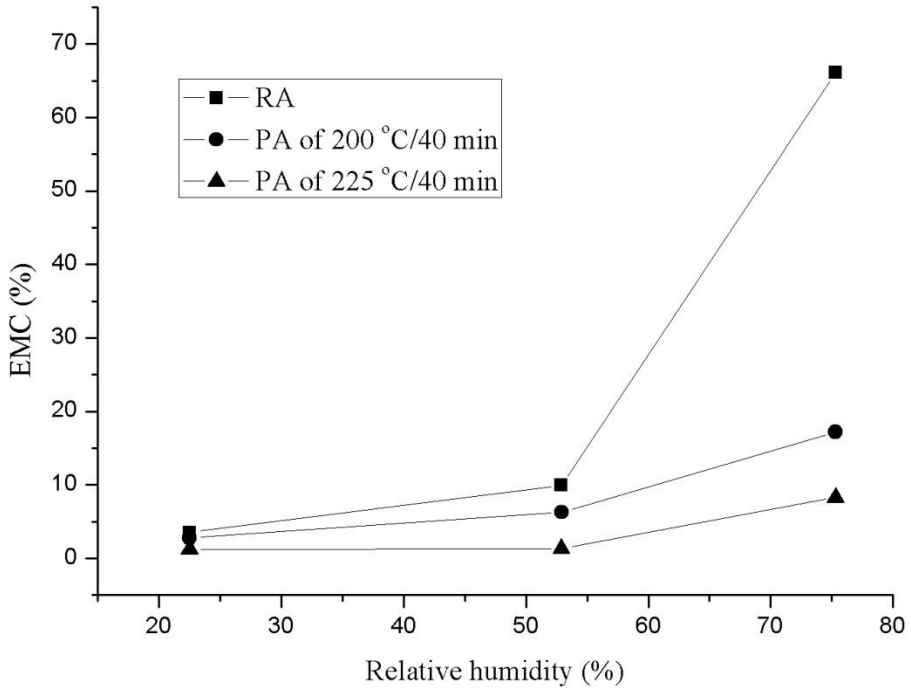


Fig. 5. EMC of untreated and pretreated algae

3.5. Energy balance

The energy consumption ratio (ECR), which was defined as the ratio of energy required in a process to the available energy of the products, was calculated to determine the process energy balance. An ECR value above 1 means a negative energy balance.

For HP,

$$ECR_{HP} = \frac{\left[W_1 C_{pw} T + (1 - W_1) C_{pa} T + W_2 C_{pw} \times 75℃ + W_2 L\right][1 - HR]}{Y Q_1 (1 - W_1) R}$$

For thermal drying,

$$ECR_{Combustion} = \frac{\left[W_1 C_{pw} \times 75℃ + W_1 L\right][1 - HR]}{Q_2 (1 - W_1) R}$$

where $W_1$is the initial moisture content of the algae slurry (80%), T is the temperature increase (assume an initial temperature of 25 °C), C_{pw} and C_{pa} are the specific heats of water and algae (4.18 kJ/kg/K and 1.00 kJ/kg/K assumed for algae according to Grierson et al. (2009)), HR and R are the efficiencies of the heat recovery after the reaction and combustion energy (assumed to be 0.5 and 0.7 respectively (Minowa et al., 1998)), W_2 is the residual moisture content in pretreated algae after filtration, L is the latent heat for water vaporization, Y is the yield of pretreated algae, Q_1 is the HHV of pretreated algae and Q_2 of untreated algae.

For HP at 200 °C with 40 min reaction time, W_2 is around 37% and the ECR_{HP} value is 0.31, compared with 0.44 for direct drying of algae. The ECR values less than 1 indicate that both processes had a net energy gain. In addition, HP shows more favorable results than direct drying of algae, because of the large amount of heat required for water removal.

3.6. Py-GC-MS

Py-GC-MS was used to study the pyrolytic bio-oil differences between untreated and pretreated algae. The total ion chromatograms of untreated and pretreated algae obtained at 200 °C with 40 min reaction time are shown in Fig. S1 in supplementary data and major compounds with >1% peak area are listed in Table 3. Although peak area does not represent the actual concentration of each compound, it could illustrate well the influence of HP on the changes of pyrolytic bio-oil composition.

Table 3. Major chemical compounds present in pyrolytic bio-oil of untreated algae and pretreated algae

Untreated algae			Pretreated algae		
RT[a] (min)	Area /%	Compound	RT (min)	Area /%	Compound
34.1	5.1	Pentadecanenitrile	35.7	29.6	Oleic acid
22.9	4.8	1-Decanol, 2-methyl-	35.2	13.1	9-Hexadecenoic acid
32.9	4.4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	39.4	2.9	Hexadecanamide
1.6	4.1	L-Alanine, N-methyl-	39	2.5	9-Octadecenamide
20.2	4	Indole	31.5	2.2	Tetradecanoic acid
39.3	3.9	Octadecenamide	32.9	2.1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
33.7	3.7	9, 17-Octadecadienal	33.8	1.4	13-Heptadecyn-1-ol
38.9	3.4	cis-11-Eicosenamide			
29.8	2.9	2-Cyclohexen-3-ol-1-one, 2-[11-tetradecenoyl]-			
20.3	2.7	Indolizine			
4.5	2.6	Toluene			
25.4	2.3	Pentadecane			
38.2	2.3	Oleanitrile			
33	1.9	1,9-Tetradecadiene			
27.6	1.9	1-Dodecanol, 3,7,11-trimethyl-			
25.2	1.7	7-Tetradecene			
48.7	1.6	9,17-Octadecadienal, (Z)-			
30.1	1.6	1,4-Benzenediol,2,5-dimethyl-			
18.7	1.6	Benzenepropanenitrile			
27.7	1.6	1-Dodecanol, 3,7,11-trimethyl-			

22.7	1.5	Z-10-Pentadecen-1-ol
27.3	1.3	Pyrrolizidine-3-one, 5-acetylmethyl-
17.3	1.3	1-Teidecene
44.9	1.3	5,8,11,14,17-Eicosapentaenoic acid
17.1	1.2	2-Piperidinone
37.9	1.1	Oleic acid
17.5	1.0	Hydroxylamine, O-decyl-

[a] Retention time

Consistent with other reports on pyrolysis of microalgae (Campanella et al., 2012; Du et al., 2011; Vardon et al., 2012), pyrolytic bio-oil of untreated algae contained very complex compounds, including hydrocarbons, alcohols, fatty acids, N-containing compounds, etc. Compounds, such as indole, 2-Piperidinone and indolizine, are typical derivatives of protein pyrolysis (Moldoveanu, 1998). However, they were not found in the pyrolytic bio-oil of pretreated algae, which indicates that the related proteins were removed during HP. On the other hand, it is very clear that the bio-oil composition of pretreated algae was much simpler and dominated by long-chain fatty acids. They did not decompose to smaller molecules because of fast heating in the pyroprobe. However, fatty acids can be easily converted to hydrocarbons in the presence of catalysts during pyrolysis (Milne et al., 1990; Twaiq et al., 1999). Also, amides of fatty acids were detected, most likely formed due to the reaction of fatty acids and ammonia released from the residual protein or amino acids in pretreated algae. Overall, pretreated algae produced higher quality pyrolytic bio-oil with reduced percentages of heteroatom (N) compounds compared with untreated algae. The influence of HP on the overall yield of bio-oil was not investigated in this study because of the small amount of pretreated algae from batch reactions; future studies utilizing scaled-up pyrolysis facilities will be employed to investigate this question.

Activity 2: Development of Continuous Hydrothermal Liquefaction System

The large-scale algal PBR's located at UMORE Park in Rosemount, MN have provided pivotal operational and experimental observations for algae-based bioenergy crop production. This test bed site has provided not only the opportunity to test the efficiency of novel harvesting methods, but also assess their viability at scale. Nearly all of the harvesting technologies explored effectively captured the suspended algal culture, however, few could address the prominent self-precipitated algal flocs that naturally developed within the PBRs. Simply scraping the accumulated algal paste (~15% solids w/w) could be an effective low-cost harvesting strategy that improves the energy balance of the cultivation system by alleviating mixing penalties. Traditionally, harvested algal pastes would be dried and either pressed to release their lipids to undergo transesterification to make biodiesel, or pyrolyized to produce bio-crude, syn-gas and char. Both methods require very low feedstock water contents which increase the pretreatment costs of each respective conversion pathway. Ideally, downstream conversion technologies that can accommodate wet algal biomass, thus eliminating the energy intensive dewatering and drying steps, are preferred. Recently, a thermochemical process known as direct hydrothermal liquefaction has been researched to fulfill this role.

Hydrothermal liquefaction (HTL) technologies employ high temperature (200-500°C) and high pressures (5-30MPa) to rapidly depolymerize, degrade and reform biomass constituents into bio-crude, aqueous, gas and char phases. At its core, HTL technologies aim to enhance the

geologic processes that converted ancient biomass into the petroleum crude we use today. Recent literature reports that 11-18%, 6-16% and 55-80% of the protein, carbohydrate and lipid portions respectively of algal biomass are successfully converted to bio-oil. The total energy recovered in the bio-crude fraction of HTL processed algae can be 19.3-66.1% of the initial feedstock heating content. Indeed, recent studies have shown that the energy recovered by HTL processing is greater than the energy releases via direct combustion alone after the intensive drying costs are factored in.

HTL technologies, although ideally suited for algal feedstock's, are currently limited in their application. A variety of challenges hinder mature HTL processing reactors so of which include:

- Poor thermal transfer.
- Excessively long heat-up and cool-down times.
- Limited heat recovery potential.
- Poor process control.
- Limited feedstock loading and conversion product output.
- Product separation and extraction difficult.

Given HTL's enormous potential we have begun work on a scalable, continuous hydrothermal liquefaction system capable of processing a host of aqueous feedstock solutions. Figure 1 outlines the process flow diagram of the CHTL we are developing.

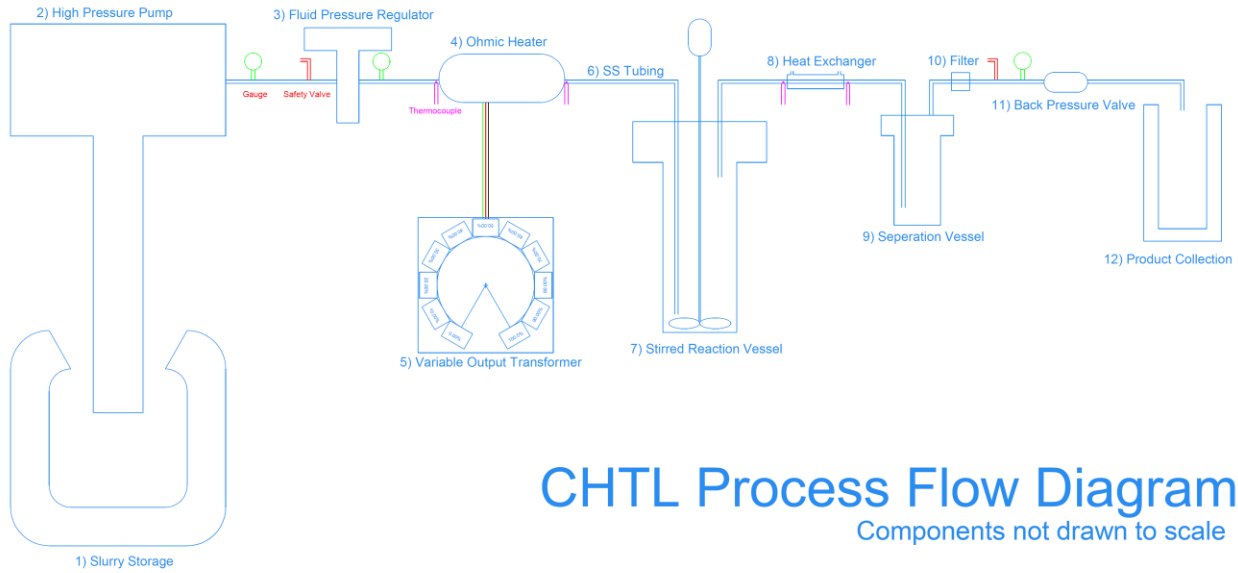


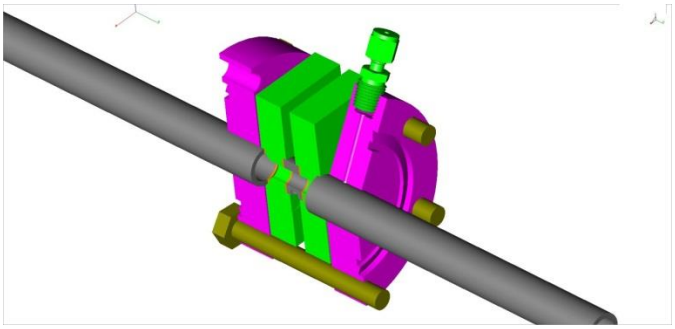
Figure 1. UMN CHTL Process Flow Diagram.

Rapid heating of the biomass slurry has been identified as one of the most promising avenues to improve CHTL functionality and product yields. We are assessing and developing a variety of traditional and novel heating methods to improve the overall performance of the system. Preliminary results suggest that coupling our recently developed rapid heating method provides marked improvements to the energy efficiency, system reliability, and product quality of our CHTL system. Additionally, our CHTL system as pictured in figure 2 can be tuned to pre-treat algal slurries for dry conversion technologies. By treating the algal cells under mild HTL conditions their water holding capacity can be greatly reduced, this reducing the amount of energy needed to dry the feedstock for oil extraction or pyrolytic conversion. Further design refinements are being implemented to assess the overall performance of converting wet algal pastes produced at our remote test bed site into high value products.

Figure 2. UMN CHTL system prototype system under development.

Result Status as of *December 31, 2012*

Continued work has been carried out to produce a reliable and scalable Continuous Hydrothermal Liquefaction (CHTL) System. Initial trials utilizing a propriety direct ohmic heating technology (Figure 1) yielded promising results. After incorporation into our existing CHTL framework heating rates in excess of 16,000 °C min^{-1} where observed. This initial device was able to raise our trial slurry from 50 °C to 240 °C in ~0.01 seconds.



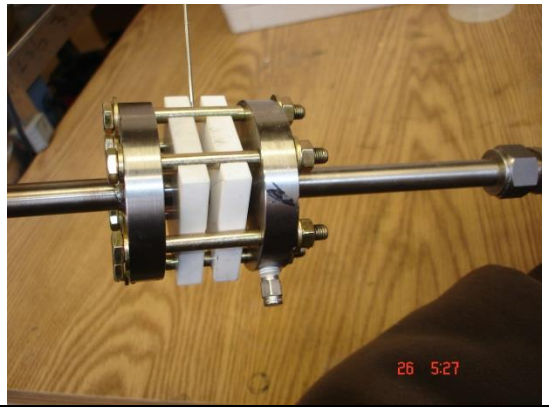
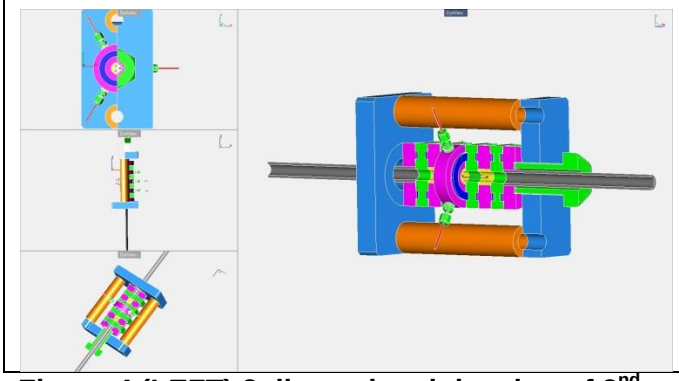


Figure 3 (LEFT) 3 dimensional drawing of 1st-generation ohmic heating prototype. Image provided by Dave Hultman Design. (RIGHT) Fully assembled heater as tested with CHTL system. Image provided by Michael Mohr.

Additional trials conducted using the prototype ohmic heater revealed areas where operational improvements could be made. Firstly, it was found that the primary sealing mechanism of this design could be improved to reduce the electrochemical attack from the electrolyte in an intense electric field. Secondly, the dielectric (electrical insulating) ceramic materials used in this design could be substituted with a more robust material. Static stresses from the mechanical compression of bolts coupled with the dynamic stresses induced by the non-symmetrical thermal expansion coefficients of different materials lead to a breakdown of our insulating material. Intense degradation of the initial electrode material (T316SS) was also recognized as a key area of improvement.

Given the initial promise of the first generation prototype consultations where held with industry specialists to help resolve the aforementioned issues. A 2nd prototype (Figure 2) was developed to meet these challenges head on. The main sealing mechanism was redesigned to incorporate a compressible gasket located away from the high intensity treatment region. A high temperature ceramic was also incorporated to provide the dielectric isolation necessary for this process. A singular screw-type clamp coupled with a helical washer was developed to ensure a uniform compression load on the ohmic heating components. Finally, 1/16" tube adapters where incorporated to allow for swapping of the electrode materials. This design also allowed us to test various 1/16" OD electrode materials to identify which best suited our application.



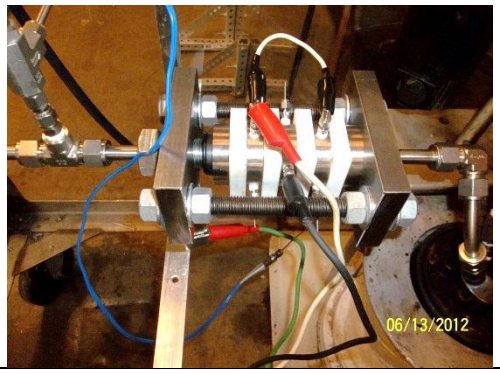
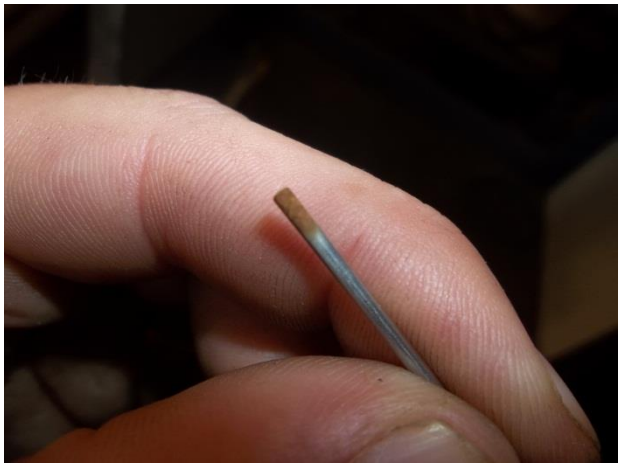


Figure 4 (LEFT) 3 dimensional drawing of 2nd-generation ohmic heating prototype. (RIGHT) Fully assembled heater plumbed into the CHTL system. Images provided by Michael Mohr.

Performance data gathered from this prototyped produced a few interesting observations. It was found that Stainless Steel, Copper, Brass, and Tungsten electrodes exhibited rapid tip passivation where the electrolyte was in direct contact with the electrode. Graphite was identified as a superior electrode material; minimal degradation was observed when graphite was employed. With this design the ceramic insulators where still prone to failure. Minimizing the static stress caused by angular misalignment did little to produce a safer and more robust heating device.

Upon further consultation with multiple vendors, a thin Mica-based gasket was suggested for our application. Minor machining



Electrochemical attack of the T316SS electrode tip. Orange discoloration is highly dielectric. Image provided by Michael Mohr.

work was carried out to retrofit the 2nd generation prototype to incorporate a double concentric sealing ring on the electrode housing faces. This sealing mechanism, coupled with the Mica gaskets proved to be a superior sealing solution. Extensive testing proved that Mica-based gaskets worked very well as the primary insulating material. These gaskets also held a 4,000 psi seal during our trial runs which is more than adequately needed to achieve supercritical conditions.

The simplicity of ohmic heating technologies, along with the ability to gather real-time data with our experiments allowed us to produce and test a heating model for our heating process. The model produced is outlined below:

According to *Joules 1st Law,* heating of a resistive media is proportional to the square of the current multiplied by the material's resistance which can be expanded to include voltage. All ohmic heating applications loosely abide by the fundamentals laid out by this law given by equation 5.

$$P \sim I^2 * R = \frac{E^2}{R} = E * I \quad \textbf{(Eq 5)}$$

Only two additional equations are necessary to describe the continuous ohmic heating process. The *Resistance* equations (eq. 6), which can be expressed in terms of slurry resistivity or conductivity, coupled with the *Heat Capacity* equation (eq. 7) are all that is necessary to build the slurry heating models.

$$R = \frac{\rho L}{a} = \frac{L}{\theta a} \quad \textbf{(Eq 6)}$$
$$Q = C_{ps}\dot{m}\Delta\mathrm{T} \quad \textbf{(Eq 7)}$$

By condensing the *Ohm's Law* and *Resistance* equations into one model and setting it equal to the slurry *Heat Capacity* equation the resultant heating models in terms of slurry resistivity (Eq 8) or conductivity (Eq 9) can be readily derived.

$$\Delta\mathrm{T} = \frac{I^2\rho L}{aC_{ps}\dot{m}} = \frac{E^2 a}{\rho L C_{ps}\dot{m}} = \frac{EI}{C_{ps}\dot{m}} \quad \textbf{(Eq 8)}$$
$$\Delta\mathrm{T} = \frac{I^2 L}{\theta a C_{ps}\dot{m}} = \frac{E^2 a\theta}{L C_{ps}\dot{m}} = \frac{EI}{C_{ps}\dot{m}} \quad \textbf{(Eq 9)}$$

Where:

$\Delta\mathrm{T}$ is temperature rise of the slurry measured in Kelvin ($\boldsymbol{K}$)

E is potential difference across gap measured in Voltage ($V \sim \frac{\boldsymbol{kg*m^2}}{\boldsymbol{A*s^3}}$)

I is current flow measured in Amperes (A)

θ is conductivity of slurry measured in Siemens per meter $\left(\frac{S}{m} \sim \frac{\boldsymbol{s^3*A^2}}{\boldsymbol{kg*m^3}}\right)$

ρ is resistivity of slurry measured in ohm meters $\left(\Omega m \sim \frac{\boldsymbol{m^3*kg}}{\boldsymbol{s^{3*}A^2}}\right)$

a is the cross sectional area of gap measured in square meters ($\boldsymbol{m^2}$)

L is length of gap measured in meters (m)

C_{ps} is specific heat capacity of slurry ($\frac{J}{kg*K} \sim \frac{\boldsymbol{m^2}}{\boldsymbol{s^{2*}K}}$)

$\dot{m}$ is mass flow rate of slurry ($\frac{\boldsymbol{kg}}{\boldsymbol{s}}$)

*Bold indicates SI base or derived units.

This heating model was referenced while conducting the lab trials with the 2nd prototype heater. Preliminary results produced with graphite electrodes are provided below. As can be noted from figures 3 and 4 the observed slurry temperature rise is proportional to the square of the supplied voltage and/or amperage. This raw data supports the heating models derived in Eqs 8 & 9. Furthermore, when the raw temperature rise of the slurry is checked against the developed models (figure 5) a strong correlation can be seen suggesting our model can be used predict what power requirements will be necessary to operate a heater with fixed physical characteristics.

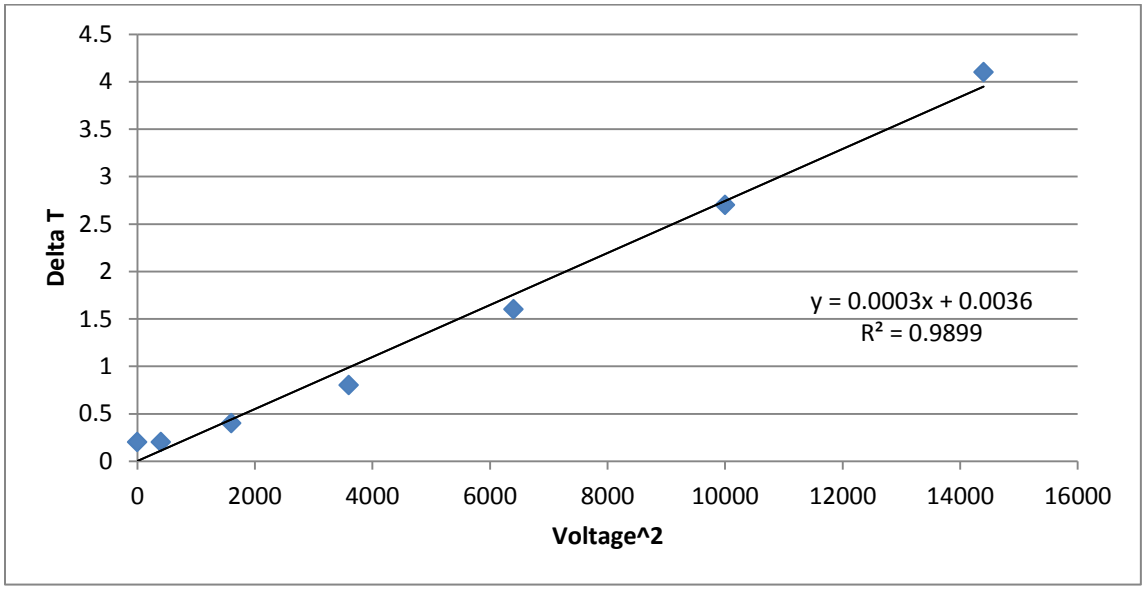


Figure 5 Slurry temperature rise over the voltage squared.

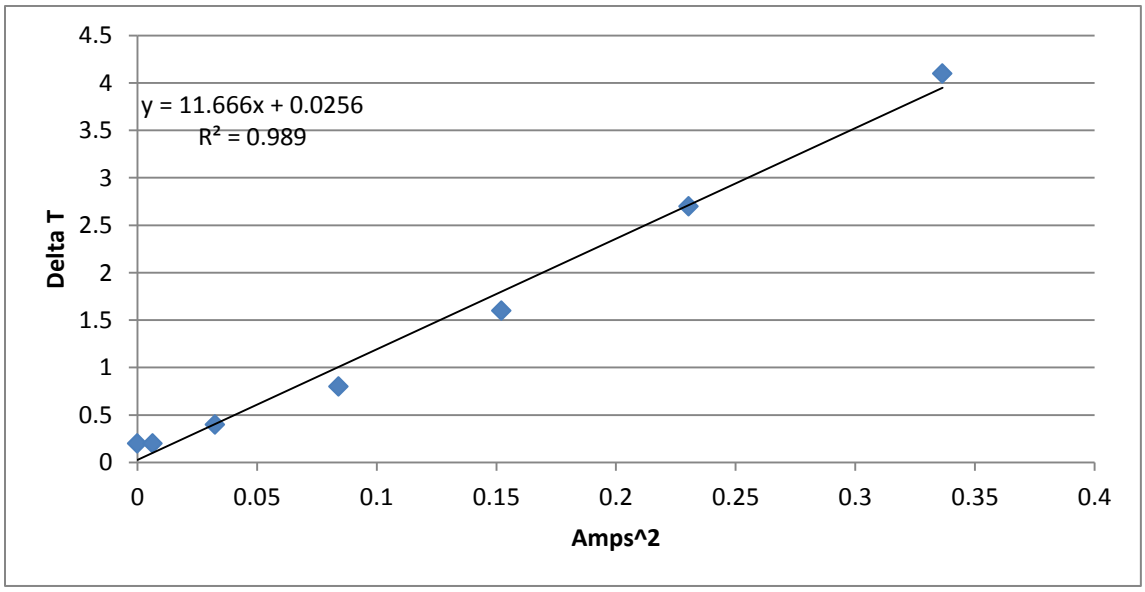


Figure 6 Slurry temperature rise over the amperage squared.

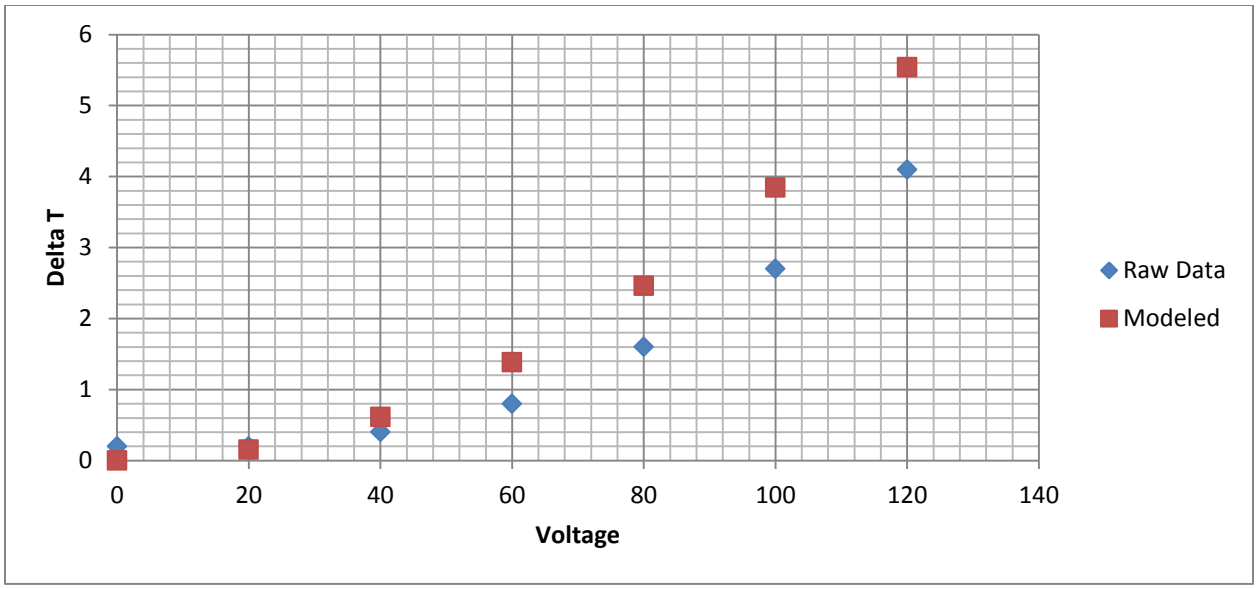


Figure 7 Observed temperature rise the slurry (blue) vs the temperature rise predicted by the model (red) in Eq 9 with experimental variables supplied.

The development of a third and final prototype ohmic heater is currently underway. Refinements from the second prototype aim to resolves arching issues caused by adjacent electrode faces. Delivery of the final prototype is expected in early 2013 with lab trials following soon after. A sketch of the 3rd prototype is provided below. Major improvements include:

1. Tapered Electrode housing flange (blue) to minimize arching between adjacent flanges.
2. Graphite electrode (grey) washer energized by a flexible spring (yellow). This will provide and higher surface area for the electrode and a more robust sealing mechanism for graphite.
3. Double set of concentric sealing rings to produce a better seal against the Mica (green) gasket.
4. Spacer bushings (pink) to further reduce potential arching.

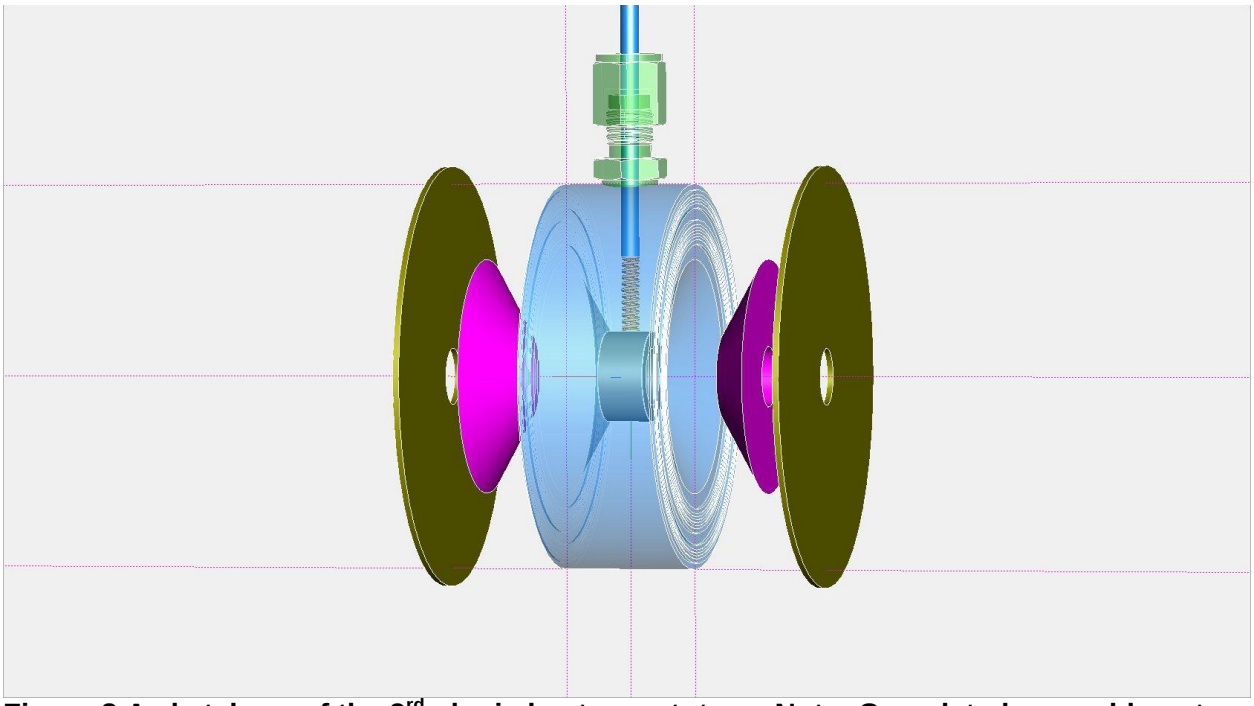


Figure 8 A sketch-up of the 3rd ohmic heater prototype. Note: Completed assembly note shown. Image provided by Michael Mohr.

Final Report Summary:

Extensive work was conducted to develop methods to convert harvested algal biomass to valuable products. The microwave assisted pyrolysis (MAP) process was improved for algae processing. Our findings show that MAP process produced bio-oil with excellent fuel quality. The tests results from a commercial lab indicated that the bio-oil from pyrolysis of algal biomass can be directly mixed with commercial fuels. Our second focus was the hydrothermal process (HTP). We used existing HTP equipment to convert wet algae to bio-oil and syngas and to pretreat wet algae so that algae can be easily dewatered. The results show that HTP is an excellent technique to facilitate energy-efficient dewatering. We also invented an ohmic heating mechanism for rapid internal heating of algal paste without relying on conventional conducting heating. Three generations of the heating device were developed and tested. While the concept is sound, there remain engineering challenges in fabricating a durable device for this purpose. Our research findings and new development significantly suggest that MAP is an excellent conversion alternative to conventional oil extraction based biodiesel process, and that HTP is a cost effective pretreatment process to improve dewatering of algal biomass.

Result 3: *Evaluation, Demonstration, and Outreach*

Description:
We will evaluate and demonstrate the systems and present our results to the general public, in scientific and trade journals, and to funding agencies. Specifically, we will (1) evaluate the systems against designed technical specifications; (2) evaluate and quantify the green impacts and benefits with respect to pollutant removal, water usage and quality, carbon sequestration, energy balance, and fuel quantity and quality, and conduct economic and environmental life-cycle analysis; (3) demonstrate the systems and processes to stakeholders; and (4) present the

project data to funding agencies, academic community, and the general public through reports, seminars, meetings, and journal publications.

Summary Budget Information for Result 3:

ENRTF Budget:	**$155,925**
Amount Spent:	**$155,925.34**
Balance:	**-$0.34**

Deliverable	**Completion Date**	**Budget**
1. Technical evaluation data	*12/31/2012*	$50,821
2. Environmental, ecological, and techno-economic evaluation, and life-cycle analysis data	*09/30/2013*	$16,864
3. Demonstrations	*09/30/13*	$60,944
4. Project report and presentations	*02/28/2014*	$8,432

Result Completion Date: 02/28/2014

Result Status as of *December 31, 2010:* No progress for this result to be reported at this time.
Result Status as of *June 30, 2011:* No progress for this result to be reported at this time.
Result Status as of *December 31, 2011: No progress for this result to be reported at this time*

Result Status as of *June 30, 2012:*
Activity: Life cycle environmental impacts of novel technologies in production of algae-derived transportation fuels with wastewater

A well-to-wheel life cycle assessment is undertaken to evaluate various algal biofuels derived from high strength wastewater (centrate and swine manure) with novel cultivation processes (photobioreactor (PBR) and open pond) and conversion technologies (microwave pyrolysis, combustion and lipid extraction). The purpose of this study is to evaluate environmental performance of new nutrient sources (centrate and swine manure) and new cultivation (PBR and open pond) and conversion technologies (microwave pyrolysis, combustion and lipid extraction) in production of wastewater based algal biofuel. Several algal biofuel production pathways are modeled and compared in terms of fossil fuel consumption, GHGs emissions, eutrophication, land and water use. Key factors that determine the environmental performance are identified and their effects on LCA results are explained as well. In contrast to most LCA studies that utilize lab-scale data or literature review data to model algal biofuel production, this study uses actual data of PBR and microwave pyrolysis processes obtained from pilot-scale reactors feed with wastewater. The research is to present a more accurate picture of the true environmental impacts and trade-offs in algal biofuel production. Through studies in this paper, we can improve the viability of algal biofuel production, scale up the algal production to industrial level, and expand utilization of algal energy use in the United State.

This analysis focuses on comparing three nutrients sources, two algae cultivation reactors and three algae conversion options in the life cycle of algal biofuels production. The boundary is shown in Figure 1. The life cycle is divided to three stages: 1) algae cultivation stage, including wastewater pretreatment, algae growing and dewatering processes; 2) algae conversion stage, including conversion processes (i.e. algae conversion to raw bio-oil, and bio-oil upgrading),

biofuels transportation (i.e. transportation of raw bio-oil from cultivation field to a centralized oil refinery, and transportation and distribution of product fuels), and wastes (ash/digestate) transportation; 3) transportation stage, where the biofuel burned in different engines.
The three algae conversion technologies produce both liquid fuels, i.e. diesel and gasoline, and non-liquid fuel, i.e. electricity. In liquid fuels production, GHGs emit in all three life cycle stages. But electricity production only has GHG emissions in cultivation and conversion stages. In order to reflect GHG emissions correctly, a well-to-wheel LCA were conducted. The functional unit is set as per mile vehicle traveled so as to compare different fuels and vehicles on a uniform basis.

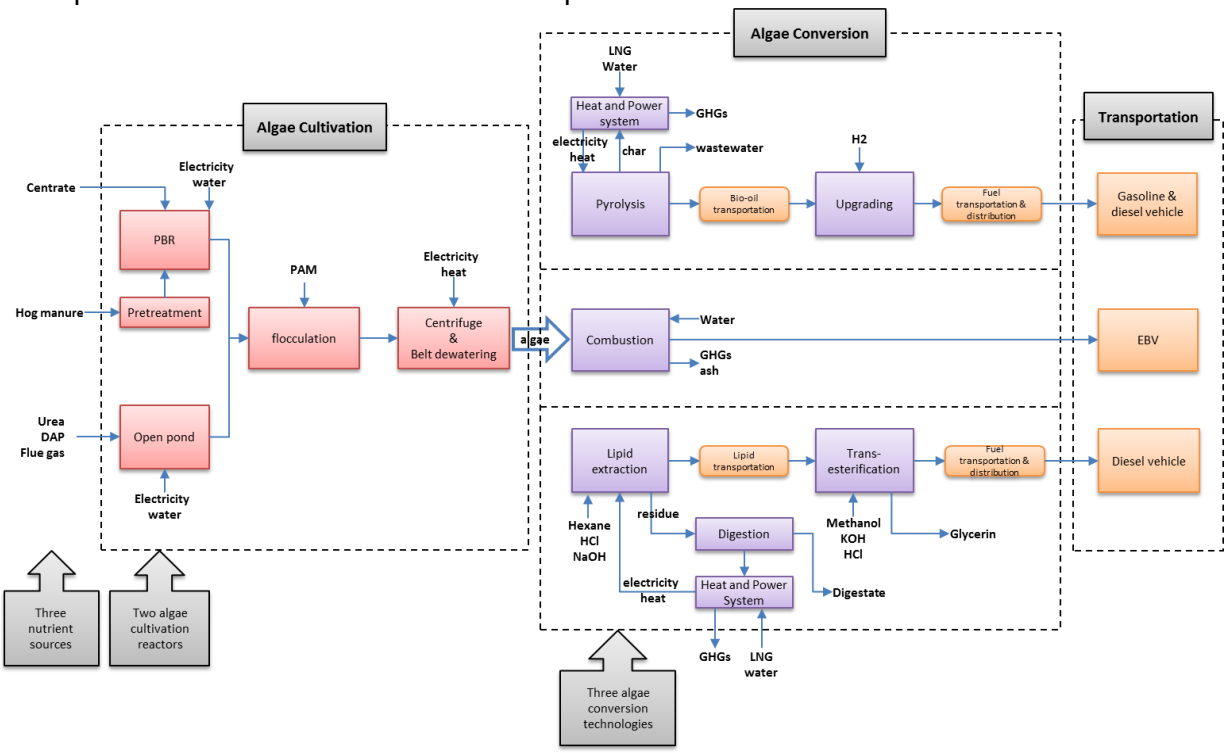
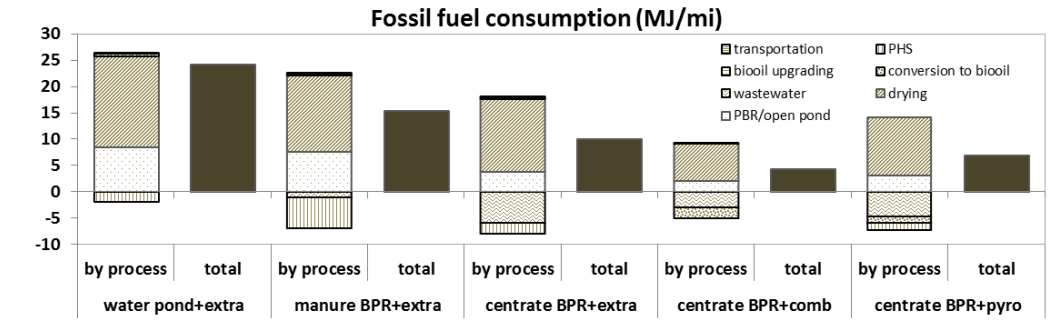
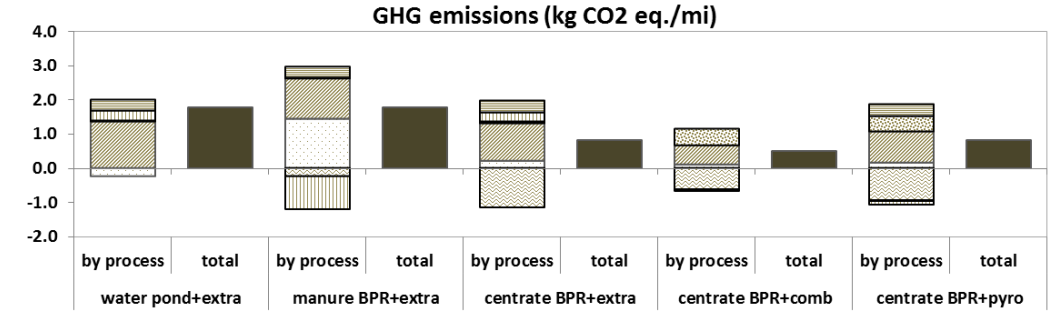
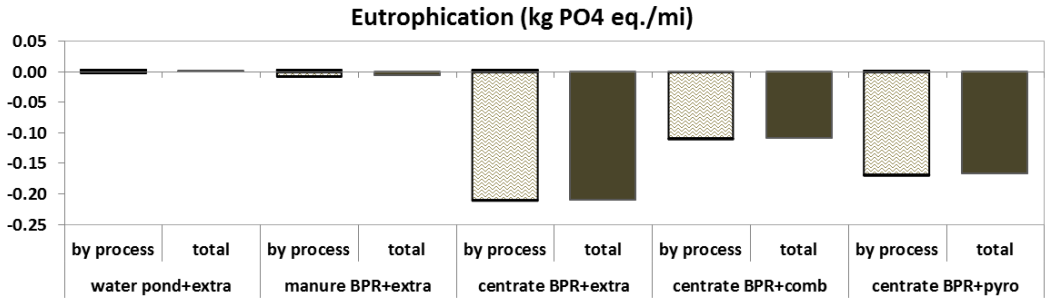


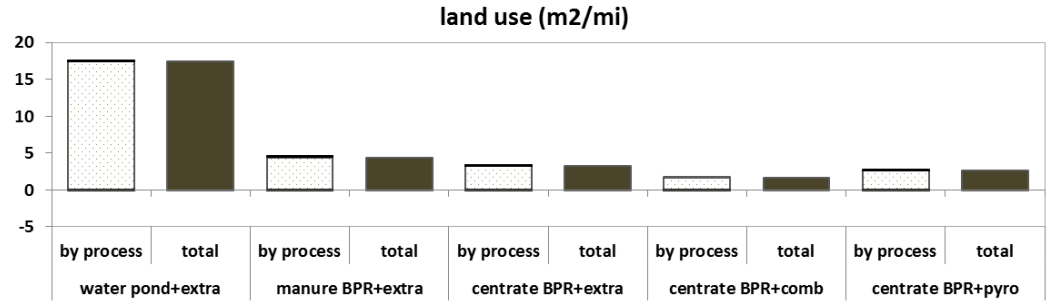
Figure 1. System boundary.

The well-to-wheel results are shown in Figure 2. The results show that application of wastewater as nutrient sources improves environmental performance of algae biofuels in all impact categories when the wastewater treatment credits are added. But the degree of improvement depends on various wastewater sources and technologies selected. For all pathways discussed, the centrate PBR with algae combustion scenario has the best performance in all impact categories except eutrophication reduction. The algae drying processes are the most energy intensive processes in algae biofuel production, and consume more than 70% of total life cycle energy. Algae cultivation (PBR/open pond) is the second largest energy consumers, and contributes 21% energy use. The algae drying processes are also the major contributors to GHG emissions because of the intensive electricity and heat use in these processes. Generally, the algae cultivation stage contributes major environmental burdens in algal biofuel production.


Fossil fuel consumption (MJ/mi)
transportation
biooil upgrading
wastewater
PBR/open pond
PHS
conversion to biooil
drying
30
25
20
15
10
5
0
-5
-10
by process
total
water pond+extra
manure BPR+extra
centrate BPR+extra
centrate BPR+comb
centrate BPR+pyro


GHG emissions (kg CO2 eq./mi)
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
by process
total
water pond+extra
manure BPR+extra
centrate BPR+extra
centrate BPR+comb
centrate BPR+pyro


Eutrophication (kg PO4 eq./mi)
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
by process
total
water pond+extra
manure BPR+extra
centrate BPR+extra
centrate BPR+comb
centrate BPR+pyro


land use (m2/mi)
20
15
10
5
0
-5
by process
total
water pond+extra
manure BPR+extra
centrate BPR+extra
centrate BPR+comb
centrate BPR+pyro

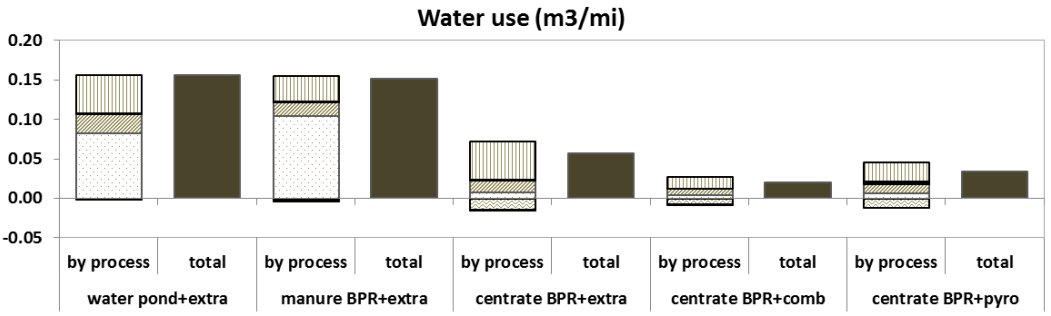

Water use (m3/mi)
0.20
0.15
0.10
0.05
0.00
-0.05
by process
total
water pond+extra
manure BPR+extra
centrate BPR+extra
centrate BPR+comb
centrate BPR+pyro

Figure 2. LCA results

Comparing life cycle impacts of three algae cultivation systems

The comparison of first three scenarios shows that the centrate PBR system has the best performance in all impact categories of all three cultivation systems. The life cycle fossil fuel use of water based algal biodiesel is 9.2MJ/MJ diesel produced. Whereas, the life cycle energy use reduced to 3.8 MJ/MJ diesel with centrate feed and 5.8MJ/MJ diesel with manure feed. The reduction of fossil fuel use is 60% for centrate and 37% for manure. When comparing fossil fuel use by unit processes, results show that the open pond system uses much more energy in the algae growing and the dewatering processes than the wastewater PBR system, and this is caused by three reasons: 1) more upstream impacts of fertilizers and indirect impacts of facility construction in the water open pond system. 2) Less biomass density in the open pond reactor. The solid content of algae slurry from the open pond is half of that from the centrate PBR, as a result, the electricity consumption in the centrifuge process is twice of the centrate PBR system. 3) The open pond system has no wastewater treatment credits. However, the manure PBR system uses more fossil fuel than the centrate PBR system, which is mainly caused by two reasons. One is the additional electricity use for supernatant recycling and CO_2 injection in the manure PBR. Another is the higher electricity use in centrifuge process because of higher water content in algae slurry from the manure PBR.

The life cycle GHG emissions for water grown algal diesel is 0.68 kg CO2 eq /MJ diesel produced. Whereas, GHG emissions reduce to 0.31 (-54%) and 0.67 (-1%) kg CO2 eq /MJ diesel produced for centrate and manure. LCA results show that the wastewater treatment credits contribute significantly to GHG emissions reduction for wastewater based algal biofuels. However, the GHG emission reduction of the manure system is not as significant as the centrate system. This is cause by two reasons. 1) The waste (COD, N and P) removal per kg algae in manure PBR is less than that in centrate PBR. Consequently, less wastewater treatment emission credits are added. 2) Some CO2 emits from the pretreatment process of the manure PBR.

The centrate PBR has the highest eutrophication reduction, which is caused by highest COD, N and P removal in the wastewater. In contrast, the manure PBR scenario has limited eutrophication reduction. The open pond scenario has positive eutrophication impact but not significant. The wastewater provides large amount of organic carbon, therefore, supports mixotrophic algal growth that characterized by faster growth rate and lower requirement of light intensity than that of autotrophic growth.[28] This makes multiple layer structure of PBR work well for wastewater grown algae. The vertical multi-layer structure design of PBR reduces land use in algae cultivation. The centrate totally replaces the freshwater in the algae cultivation reactor. The life cycle water use of the centrate PBR is ¼ of the water open pond system. However, in the manure PBR system, manure dilution and frequent clean increase freshwater use. The life cycle water consumption of the manure PBR is three times of the centrate PBR, and almost equals to the open pond.

Comparing life cycle impacts of three algae conversion pathways

Results of comparison of last three scenarios show that direct combustion of algae has the best performance in fossil fuel consumption, GHG emissions, land use, and water use of all three conversion pathways. This agrees with previous study by Clarens et al.[13] However, battery electricity vehicles are still under development. As a result, the large scale application is not applicable in the near future. Also, the algal electricity is not a compatible to our current transportation systems. In addition, the impacts of the electricity vehicle are not included in the analysis. There are still uncertainties on life cycle impacts of algal electricity production.

When comparing the pyrolysis and the lipid extraction pathways, the pyrolysis pathway has better performance than the lipid extraction in all environmental categories discussed except the eutrophication reduction. As the pyrolysis process produces some wastewater (21% by algae weigh) containing COD, N and P, it has less eutrophication deduction than the lipid extraction.

Pyrolysis can convert some non-lipid content in algae into bio-oil,[20] which increases biofuel productivity when lipid is low in algae, whereas in the lipid extraction process, major part of energy remains in non-lipid residue. As a result, the pyrolysis pathway can produce more transportation fuels than the lipid extraction pathway and also more miles driven by vehicles. Therefore, the pyrolysis pathway has less environmental burdens than the lipid extraction pathway in per mile driven basis. In addition, the upstream impacts in the lipid extraction pathway are higher than the pyrolysis pathway, which leads to higher environmental burdens.

Unlike fossil fuel use and water use, the GHG emissions of the pyrolysis pathway are almost equal to the lipid extraction pathway. There are three reasons for relative higher GHG emissions in the pyrolysis pathway. First, in the pyrolysis process, some GHGs emits to atmosphere with flue gases (0.57 kg CO2 eq./mi). In contrast, no direct GHG emissions in the lipid extraction and transesterification process. Second, two pathways use different energy recovery processes. In the pyrolysis pathway, char is combusted to generate electricity, in which C in char converts to CO2. In contrast, in the lipid extraction pathway, the digestion process is used for energy recovery. Around 10% of C stays in digestate. Third, the GHG emissions are very sensitive to electricity use, as the upstream GHG emissions of electricity generation are as high as 0.75 CO2 eq per kWh. The microwave pyrolysis uses more electricity (0.24 kwh/kg algae) than the lipid extraction, which lead to higher upstream GHG emissions (0.18 kg CO2 eq/kg algae).

Discussion

Based on the comparison of different nutrient sources and pathways for algal biofuel production, it is evident that integration wastewater in algal cultivation can improve environmental performance of algal biofuels when adding wastewater treatment credits into the LCA model. By replacing freshwater with nutrient containing wastewater, environmental burdens associated with fertilizer and freshwater consumption can be largely reduced. In addition, wastewater supports mixotrophic algal growth that characterized by higher algae yield and higher density that reduces the energy inputs for light and GHG emissions related to algae cultivation. However, there are also some limitations of growing algae in wastewater. The characteristics of nutrient amount, nutrient ratio, and algae growth inhibitors in wastewater highly affect environmental performance of algal biofuels. Swine manure, for example, although has high N concentration, the algae yield is lower than that from centrate because of the nutrient limitation on soluble P and organic C. The high ammonia concentrations in manure could inhibit algae growth as well. In addition, wastewater always has high turbidity which could block light penetration and reduce photosynthesis efficiency and consequent algae yields. If the characteristics of wastewater are not desirable for algae growth, action need to be taken to either improve the quality of wastewater before feeding, or develop proper management methods during cultivation, such as diluting the wastewater, adding fertilizers, or recycling supernatant. All those efforts need additional energy, water or chemicals; therefore lead to additional direct and indirect environmental burdens which could compromise the environmental benefits carried by wastewater treatment. Generally, the environmental performance depends significantly on the characteristics of wastewater, cultivation conditions, water management, algae characteristics, and technology options for cultivation, conversion, and residue treatment.

The algae energy yield ($MJ/m^2/day$) is important factors in algae cultivation that could influence environmental performance. The algae energy yields are the total energy with algae collected, which depend on algae growth ($kg/m^2/day$) and energy content (MJ/kg dry algae).

Ideally, the higher energy yield leads to higher travel distance, consequently, the lower environmental burdens per miles traveled. However, the energy yields are highly related to the nutrients in the media and cultural condition (e.g. temperature, light intensity and mixing). The environmental performance is always in a tradeoff between algal biomass/lipid productivity and input energy for light, heat and nutrients. The biomass density is another important factor in algae cultivation that is positively related to environmental performance. The higher biomass density leads to lower volume of algae slurry effluent, and less energy consumption in algae drying processes.

The water management includes strategies such as wastewater dilution, supernatant recycling and tank clean. Dilution is used to reduce turbidity or concentration of inhibiting substances in wastewater. Wastewater dilution could increase freshwater use in cultivation, and reduce nutrient concentration and decrease algae yields. Supernatant recycling is a way to save freshwater use and recycles nutrients. But the supernatant recycling causes accumulation of inhibiting substances in cultivation tank which reduces algae yields and leads to frequent clean of reactors and subsequently with increase of freshwater and electricity use. Therefore, the supernatant recycling always has some environmental burdens that compromise water saving credits. In the centrate PBR, there is no supernatant recycling where many problems are avoided. However 10% of algal biomass loss could happen due to 90% of harvesting efficiency could be reached when using the flocculation process.

Of all factors discussed above, the characteristics of the wastewater are the most important one to the life cycle performance, since they could influence algae yields, biomass density, water management methods, and algae characteristics. The LCA shows that centrate and hog manure both have high C, N and P concentration, but their environmental burdens are hugely different in algae cultivation stage. Centrate contains optimum nutrient profile supporting fast algal growth with selected local algal species. In contrast, hog manure has extremely high N concentration and has to be diluted with freshwater prior to feeding. This leads to lower algae yield and lower algae density in the manure PBR. In addition, as organic C in manure is too low to algae grow, additional flue gas has to be injected, which increases energy use in algae growing. In order to save water use, the supernatant is recycled in manure PBR system which increases energy inputs as well. Combined with dilution and supernatant cycling, the manure PBR has 1.7 times energy use and 14000 times water use per kg diesel produced comparing to the centrate PBR. In manure based cultivation systems, as the non-balanced nutrients sources and inhibitors, cultivation conditions and water management method have to be adjusted, which leads to significant increase of energy use and water use, and the improvement of environmental performance by manure treatment is limited. In order to improve performance of manure-based algal biofuel, it is necessary to improve its nutrient profile before it feeding into cultivation tanks. A nitrogen removal process is suggested to be installed to lower down the N concentration in manure.

The selection of technologies is important to the life cycle performance as well. As the wastewater grown algae allows multiple layer structure in cultivation, the novel PBR developed by UMN is a good reactor for wastewater grown algae and characterized with higher yield, higher density and less land use in cultivation. However, the benefit of PBR could be compromised by certain wastewater. For example, the manure-based algae consume some inorganic carbon (CO_2 in flue gas), sun-light is crucial to its algae growth. The multiple layers structure will influence the contact of algae to sun light and reduce the yields.

The selection of algae conversion technologies are mainly based on the characteristics of algae. For example, the biofuel yields in the pyrolysis pathway depend mainly on total energy contents in algae. In contrast, the fuel yields of the lipid extraction pathway are more related to lipid content. A sensitivity analysis is conducted for different lipid content scenarios to demonstrate selection between lipid extraction and pyrolysis pathways. The results show that as the increase of lipid content, the life cycle impacts of the lipid extraction pathway decrease

gradually. When the lipid content is lower than 20%, the pyrolysis has better performance in all categories than lipid extraction. In contrast, when the lipid content is higher than 40%, the lipid extraction has better performance in all categories than pyrolysis. However, this sensitivity analysis is based on a simply assumption that energy and nutrients inputs are not changed when the lipid content increases. Based on the analysis of algae energy yields, the energy and nutrients increase with the increases of lipid content as well as environmental burdens in this pathway. Therefore, the lipid content limits to selected lipid extraction would be higher than current analysis (i.e. 20%~40%). Finally, as developing of electricity vehicle and infrastructure, algae combustion technology could be the choice in the far future.

Table 3. Sensitivity analysis on lipid content

	lipid content	**fossil fuel**	**GHG**	**eutrophication**	**land use**	**water use**
	%	MJ/mi	$kgCO_2$ eq./mi	$kgPO_4$ eq./mi	m_2/mi	m_3/mi
centrate PBR + pyrolysis	20	6.79	0.83	-0.17	2.64	0.03
centrate PBR + lipid extraction	10	18.90	1.41	-0.42	6.57	0.11
	15	12.96	1.02	-0.28	4.39	0.07
	20	10.00	***0.83***	-0.21	3.30	0.06
	25	8.22	***0.71***	***-0.17***	***2.65***	0.05
	30	***7.03***	0.64	***-0.14***	***2.21***	***0.04***
	40	***5.55***	0.54	-0.10	1.67	***0.03***

The LCA also identified energy intensive processes, i.e. algae dewatering processes in algal biofuel production. Technology selection should focus on technologies that are capable of treating high water content algae, or could separate algae from water without energy intensive processes. A new technique, wet lipid extraction has been proposed to treat algae with high water content (solid content in 20%). The direct process energy consumption is only ¼ of the conventional dry lipid extraction in algae drying and lipid extraction stages.[10] But this technology has been only demonstrated at the lab-level and is at the very early stage of development.[18] The wet extraction is modeled for comparison with dry lipid extraction and petroleum diesel production, shown in Table 4. When wet traction is applied with the centrate system, the environmental performance is improved significantly. The environmental performance of algal biodiesel with the wet extraction pathway is better than petroleum diesel as well.

Table 4. Life cycle impacts of three different diesel production

	fossil fuel	**GHG**	**eutrophication**	**land use**	**water use**
	MJ/mi	**kg CO2 eq./mi**	**kg PO4 eq./mi**	**m2/mi**	**m3/mi**
centrate PBR + dry lipid extraction	9.996	0.829	-0.209	3.303	0.056
centrate PBR + wet lipid extraction	-0.113	0.009	-0.246	3.879	-0.001
petroleum diesel	1.37	0.102	1.3E-5	0.0003	0.020

Even with huge energy potential in wastewater, there are still many difficulties in extracting energy form wastewater in an environmentally friendly way. Especially for animal wastewater,

the composition is complicated and containing many algae growing inhibitors. In addition, the lack of efficient conversion of algae biomass into usable components is a challenge for algal biofuel production. The LCA study shows that advances in technologies are necessary to make wastewater based algal biofuels a commercial and sustainable reality.

Result Status as of *December 31, 2012*

Despite algal biofuels have attracted increasing interests in recent years, it is still questionable that algal biofuels can be produced environmentally sustainably in a commercial scale.[34] Many studies suggests applying wastewater into cultivation to improve environmental and economic performance of algal biofuels, given huge nutrients and energy potentials containing in wastewater and strong ability of microalgae grown in harsh aqueous conditions.[11-13] The previous studies show that the theoretical internal chemical energy of wastewater is range around 10-15 mega joules (MJ) / kg Chemical Oxygen Demand (COD).[2,3,4] Based on those studies, the municipal and industrial wastewater generated in the U.S. today, approximately 1.2×10^8 m^3/day,[5] contains energy potentials as high as 1.2×10^9 MJ. At the same time, there is about 7.2×10^6 m^3 dairy and swine manure generated every day,[6] which contains 7.5×10^8 MJ of more energy potentials. The total energy potential in those wastewaters equivalents to 5.7 billion gallon of gasoline consumed in the transportation sector per year, which counts for 4% of gasoline consumption in the US.[7] As a result, wastewater, rather than being perceived as a waste, has come to be viewed as a valuable resource for water, energy, and plant nutrients.[1]

The current literature describes merits of growing algal biomass with wastewater in several aspects. (1) Studies found out that over 50% of energy use and GHG emissions are associated with fertilizer use and water extraction in algae cultivation.[14,15] Wastewater provides nutrients, N and P, for algae growth, which reduces the energy and GHG emissions related to water extraction and fertilizer production as well as the cost of algal biofuel production. (2) Wastewater treatment consumes approximately 3% electricity generated in the US.[2] Stringent discharge standards especially for P will put additional burden on wastewater treatment facilities. The removal of organics, N and P, by algae can reduce the electricity use in treatment facilities and in turn decrease the environmental burdens related to electricity generation. (3) There is an increasing pressure on livestock producers to treat their wastewaters prior to discharge,[16] which is becoming a significant financial burden on the livestock producers. Using manure to grow algae can treat manure, produce feedstocks for bioenergy, and create a new source of revenue for livestock producers. Therefore, wastewater based algal biofuels have received increasing interest, and algae production has been examined in various wastewaters, such as municipal wastewater,[18, 19] industrial wastewater,[20,21] and animal manures.[22, 23]

However, algae cultivation with wastewater is still at an early stage of research with many uncertainties and challenges.[24] In particular, the composition of wastewater is complicated and varies by source, colleting infrastructure, weather conditions, and methods applied in pre-treatment. Thus, the nutrient profile of wastewater may not always be suitable for algae cultivation. For example, low nutrient levels or inhibitors present in wastewater can result in poor nutrient assimilation and significantly reduce algal biomass productivity.[18, 25] The benefits of integrating algae production with the wastewater treatment may not be significant in those situations. In addition, existing algae cultivation and conversion technologies vary greatly in productivity, efficiency and operating conditions,[26, 27] which leads to different biofuel yields, utilities use, and environmental impacts. In order to evaluate wastewater based algal biofuels, it is important to understand the environmental implications of different wastewater sources and production technologies and identify key factors that determine the environmental burdens and benefits in the whole life cycle.

Several studies have used life cycle assessment (LCA) to describe the environmental profiles of different technologies in wastewater based algal biofuels. For example, Clarens and his colleagues[14] compared municipal wastewater grown algal biomass with terrestrial crops, i.e. corn, switchgrass and canola. Their study shows a significant reduction in the environmental burdens of algae cultivation because conventional fertilizers are replaced by wastewater. The same authors[28] compared several algal energy pathways for algal biodiesel and bioelectricity production via combustion, digestion and lipid extraction. The results show producing bioelectricity by combustion outperformed the other two pathways in energy use and GHG emissions, because a large portion of the feedstock energy remains unused as process residue, and a greater upstream usage of electricity, heat, and chemicals in the digestion and lipid extraction pathways. Sander and Murthy,[29] created an LCA model for open pond cultivation and wet lipid extraction technologies for the production of algal biodiesel from the effluent of the activated sludge process and the flue gases. The well-to-pump results show that for 1000 MJ of algal biodiesel produced, the total energy input was -3778 MJ, which is lower than that of petroleum diesel (1020 MJ/1000 MJ diesel produced), but CO_2 emissions were 135.7 kg, which is higher than petroleum diesel (81.3 kg/1000 MJ diesel produced).

Although efforts have been made in this area, the LCAs of wastewater based algal biofuels are still limited. Moreover, the results of existing LCA studies differ significantly in their assumptions and scopes in wastewater feed, production technologies, algae growth parameters, co-product allocation method, and the functional unit applied.[30] There is still a paucity of studies that describe the key factors that influence the environmental profile of wastewater based algal biofuels. In addition, as interest in algal biofuels has increased, new technologies and new algal biofuel pathways have been proposed. For example, microwave pyrolysis followed by hydro-upgrading has been considered for the conversion algae to high quality bio-oil. This process is claimed to have higher biofuel yields than the conventional lipid extraction process with low lipid algae.[31] Also, the search for new nutrient sources has stimulated new algae-to-biofuel systems. Researchers have shown particular interest in high nutrient loads wastewater because it has enough nutrients to support high algae productivity.[32,33] To date, LCAs of these new nutrient sources and novel technologies have not been conducted.

The purpose of this study is to evaluate the environmental performance of new nutrient sources (centrate and swine manure), cultivation systems (photobioreactor (PBR)), pretreatment technologies (hydrothermal treating), and conversion technologies (microwave pyrolysis) for the production of wastewater based algal biofuel. Several algal biofuel production pathways are modeled and compared to the petroleum diesel in terms of fossil fuel consumption, GHG emissions, eutrophication, and water use. Key factors that determine the environmental performance are identified and their effects on LCA results are explained. In contrast to most LCA studies that utilize lab-scale data or literature review data to model algal biofuel production, this study uses actual data of PBRs, microwave pyrolysis, and hydrothermal treating reactors obtained from pilot-scale experiment. The goal of this study is to present a more accurate picture of the true environmental impacts and trade-offs of wastewater based algal biofuel production. Knowledge gained will facilitate to "achieve an economic viable and environmental sustainable biofuels in a large scale".[34]

Methodology

System boundary for analysis.

This analysis focuses on comparing three nutrients sources, two algae cultivation reactors, three algae pre-treatment options, and three algae conversion pathways in the life cycle of algal biofuels production. The system boundary is shown in Figure 1. The life cycle is divided to four stages: 1) algae cultivation stage, including algae growing, algae-water separation processes, and a dewatering process; 2) algae pre-treatment stage, including processes to meet requirement of algal biomass conversion; 3) algae conversion stage,

including bio-oil conversion processes (i.e., algae conversion to raw bio-oil, and bio-oil upgrading), raw bio-oil transportation (i.e., transportation of raw bio-oil from cultivation field to a centralized oil refinery), and residue reuse and waste disposal (ash/digestate); 4) end-use stage, where the biofuel is transported and distributed and burned in different engines. As each conversion pathway has different products, i.e. diesel, gasoline and electricity, a well-to-wheel LCA was conducted in order to reflect GHG emissions correctly. The functional unit was set as one vehicle-mile traveled so as to compare different fuels and vehicles on a uniform basis.

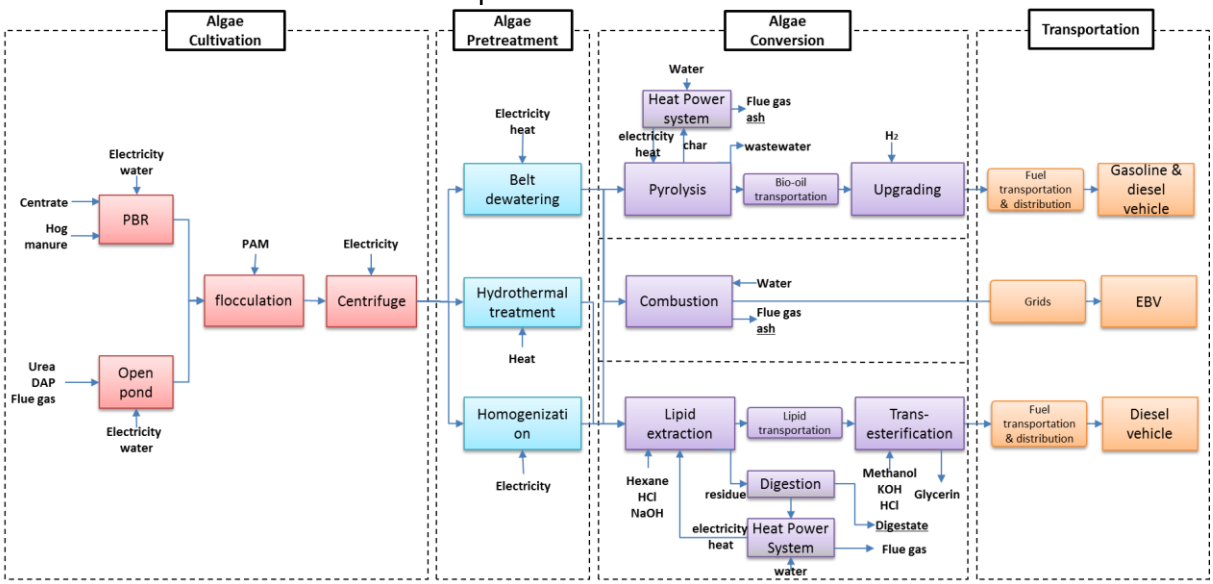


Figure 1. System boundary for analysis. The study compare three nutrients sources (centrate, swine manure, and freshwater with fertilizers), two algae cultivation reactors (photobioreactor (PBR) and open pond), three algae pretreatment options (belt drying, hydrothermal treating and homogenization), and three algae conversion pathways (microwave pyrolysis, direct combustion and lipid extraction) in the life cycle of algal biofuels production. Only major inputs and outputs of unit processes are shown in this figure. Ash and digestate are final wastes and assumed to dispose at landfills.

Nutrient sources.

Two types of high nutrient load wastewaters, centrate, and hog manure, are included in analysis according to ongoing projects conducted at the University of Minnesota. Both wastewaters have high average COD, SS (suspended solid), N, and P concentrations, shown in Table 1. Centrate is the stream in wastewater treatment facilities generated during the sludge dewatering process. The molecule ratio of C: N: P (1000:120:120) in centrate is close to the nutrient requirement for algae growth (103:10:1)[35] making centrate a good source of nutrients for algae production. The source of centrate referenced in this study is from the Metropolitan Wastewater Treatment Plant in Saint Paul, Minnesota, which has a capacity of 185 million gallons per day (MGD) and generates 1 MGD of centrate with a P concentration, 250 mg/L.[33] Normally, the centrate generated in the sludge dewatering process is recycled back to the activated sludge process and combined with untreated wastewater. As a result, it adds over 30% of the soluble P loading of influent and significantly increases energy consumption for treatment facilities. Therefore, using centrate to feed algae can remove P before it is recycled back to the activated sludge process and reduce P loading in wastewater treatment facilities.

Swine production is an important agricultural activity in the Upper Midwest region of the U.S., and could provide abundant manure for algae growing. A farm with 1,000 hogs can produce 1,360 gallon of manure per day.[36] Currently, the manure is directly discharged to agricultural land and serves as a fertilizer. But such waste management practices have come

under increased scrutiny as their environmental impacts have been assessed, such as contamination of local water resources with excessive nutrients, microbial pathogens, and pharmaceuticals present in the waste.[17] As a result, there is increasing pressure on livestock producers to treat their wastewaters prior to discharge. The use of manure as nutrient sources for algae production has been proposed as a method to significantly reduce both treatment loading and economic burdens for livestock producers.[32] However, there is considerable uncertainty as to whether these benefits can be realized. For instance, manure often contains high ammonia concentrations that can inhibit algae growth,[25] which needs addition fertilizers and energy for increasing algae yields. Therefore, the environmental performance of using manure has yet to be assessed and compared to tradition fertilizing methods.

Table 1. Characteristics of wastewater.

	COD	**TSS**	**VSS**	**NH_3-N**	**TN**	**soluble TP**
	mg/L	g/L	g/L	mg/L	mg/L	mg/L
Centrate[1]	3027	0.46	0.35	113	150	250
Manure before primary digestion[2]	9300	35.43	22	2960	3150	24.05
Manure after primary digestion	6480	14.03	9.85	3200	3440	20.75

1. Centrate is generated during sludge dewatering process and is directly applied in algae cultivation reactors;
2. Manure undergoes pretreatment—digestion before being applied to algae cultivation.

Algae cultivation systems.

Algae cultivation systems include all processes of water extraction, wastewater pretreatment, algae growing, and algae-water separation. In this study, one of the modeled cultivations systems is the novel wastewater photobioreactor (PBR) developed by the algae research group at the University of Minnesota. This patent pending reactor consists of multiple layers of open plastic tanks for algae growing, and combines advantages of both open pond reactors and closed PBRs including low cost, easy scale up, high algal biomass productivity, and easy maintenance.[33] A detailed description of the PBR can be found in the supporting information (SI). Data for PBR system's modeling are collected from pilot scale systems that were composite with unit modules for centrate (1,200 L) at Saint Paul Metro Plant, and for hog manure (15,000 L) at the Rosemount Research and Outreach Center, Rosemount, MN.

Due to the different nutrient compositions of the two wastewater sources, the operational strategies of the two systems are different. Centrate, with the optimum nutrient balances, is directly fed into the centrate PBR system. The organic C in the centrate becomes the major C source for algae growth, so no additional CO_2 is injected into the PBR. Based on the pilot scale study, the average biomass productivity rate is 34.6 g/m^2/d. The average nutrient removal rates for COD, N, and P are 52.2, 2.0 and 4.6 g/m^2/d, respectively. The average biomass density before harvesting is approximately 1.5~2 g/L, which is higher than the open pond reactor (0.2-0.5 g/L).[37] The harvesting rate is one third of the PBR water volume daily, and the flocculation and sedimentation processes are used for algae-water separation. The flocculant (PAM: 5 g/m^3) is added to the algae slurry before it is sent to a separation tank. The algae slurry after sedimentation process contains 1~2% solid, and requires further dewatering.

No supernatant recycling is applied in this system, and the supernatant is sent back to the activated sludge process in wastewater treatment plant.

The pilot studies show that the swine manure contains a high concentration of organics and inorganics but the C/N ratio is much lower than that of centrate. In the pilot scale manure-based PBR system, digested manure is used in the PBR, shown in Table 1. Due to the high ammonia concentration and high turbidity, dilution of the hog manure is generally required in order to avoid inhibiting the algae growing. The dilution rate is typically 60 times by volume. The dilution of the manure reduces the organic C concentration in the PBR at the same time. In order to increase algae productivity, flue gases from the in-plant heat and power system (HPS) are recycled to provide CO_2 (1.42 g/g dry algae) for algae growth. The average biomass productivity of the manure PBR system is 24.6 g/m^2/d based on pilot scale study, which is lower than the centrate PBR system. The average nutrient removal rates for COD, N and P are 45.7, 16.8 and 0.425 mg/L/d, respectively. The average biomass concentration in the manure PBR is 0.8 g/L. The harvesting rate is half of the tank volume in every 2 days. The slurry collected from the manure PBR proceeds to the same flocculation process as the centrate PBR system, and the solid content in algae slurry after sedimentation is around 0.8%. The collected supernatant after separation is recycled back to the PBR to reduce freshwater use and recycle nutrients of C and P. Water recycling results in the accumulation of nitrogen, which can be detrimental to algal growth. To solve the problem, half of the volume of water in the PBR is replaced with freshwater every 45 days to reduce nitrogen concentration.

The open pond system cultivates algae in freshwater with Urea (N) and Diammonium phosphate, DAP (P) as nutrient sources. It is involved in this study as a baseline to compare to two wastewater-based PBR systems. The data for open pond system modeling are based on reviewing of current literature. The system structure and operation of an open pond system is primarily based on the design by Beneman and Oswald.[38] It is composite of 10 unit ponds each with 1000 m^3 surface area and 300 m^3 in volume. The harvesting rate is one sixth of the pond volume per day. The average gross biomass productivity is assumed as 13.7 g/m^2/d in Minnesota area (13.6-24.7 g/m^2/d across the US,) based on the calculation by Murphy and Allen.[15] Selection of the local growth rate but not US average is for a fair comparison to PBR reactors that is operated in Minnesota area. The biomass density before harvesting is assumed as 0.5 g/L. The nutrient requirements are 0.055 g N/g dry for Urea algae and 0.0125 g P/g dry algae for DAP, which is based on the stoichiometric of algae cells, and CO_2 is added into the system at a rate of 1.9 g CO_2/g algae.[35] The urea and DAP are purchased from fertilizer production industries, and flue gases collected from the in-plant HPS provide CO_2 for algae growing. Algae slurry is concentrated by flocculation and sedimentation processes in a separation tank. The water collected in the separation tank is recycled back to the open pond to reduce water use and recycle nutrients.

It is better to model a commercial scale cultivation facility as we focusing on large scale production and commercialization of algal biofuels. However, data are scarce and often proprietary because there is no real commercialized algal biofuel production facility under operation. In this study, the algae cultivation is modeled with pilot scale facilities under development in the US, which could lead to variability and uncertainty in LCA results. However, because the facilities are designed with unit ponds and unit PBR modules, we assume that design parameters are linearly related in expansion of facilities. Detailed designs of the two PBR systems and the open pond system are provided in SI.

In pilot scale PBR systems, *Chlorella sp*. is the algae species cultivated. The *Chlorella sp*. has high productivity and ability to tolerate high ammonia environment,[39] and therefore are suitable for growing in various wastewaters. The algal biomass collected in PBRs has an average lower heating value (LHV) of 21.2 MJ/kg. The neutral lipid content is low for wastewater based algae, 4-6% for centrate and 1-2% for manure. The low neural lipid is caused by high contaminants and high N concentration in wastewater. Other characteristics of

chlorella sp. can be found in SI. In the open pond system modeling, no specific species is applied. The average neutral lipid content is assumed as 14% and LHV is 20.8 MJ/kg dry algae based on literature review of 8 species by Clarens, 2011.[28]

After the flocculation process, the algae slurry goes through the centrifuge process to remove water in algae slurry. After centrifuge, the solid content reaches 20% in all three cultivation systems. Based on study of Lardon,[38] the energy use in the centrifuge process is 8 kWh for increasing the solid content to 20% for per m^3 slurry.

Algal biomass pretreatment processes.

Following the centrifuge, three different pretreatment options are included in the model for comparison. The belt dewatering process is a thermal dying process and can increase the solid content to 90%. It consumes 0.4 kWh electricity and 13.8 MJ heat to treat one kg of dry algae, which is an energy intensive process comparing to other two pretreatment processes. Use thermal drying is to meet the requirement of following biomass conversion processes. For example, the popular dry lipid extraction process requires the input biomass has a higher solid content than 85%.

In order to lower the energy use in pretreatment processes, presently, there are several new technologies proposed for treating wet algal biomass. In this study, two novel pretreatment processes are modeled. The first one is a hydrothermal technology which is developed at University of Minnesota. Based on the lab data, the process can treat wet algae (80% water content) and to remove proteins without requiring significant energy inputs.[42] Compared with belt drying process, the treated algae yield is only 30 wt.% of algae input, and 88% of neutral lipid can be reserved in the treated algae. In this process, around 37% of energy in algae is used to keep the system operating. The treated algae have higher C content (57.5wt.%) and enhanced heating value 25 MJ/kg dry treated algae, and can be easily treated in lipid extraction processes. Therefore, it can get higher biofuel yields in the following biomass conversion processes.

The second novel technology for comparison is the homogenization technology that is commonly used in wastewater treatment. It can break cell wall of algae and facilitate the lipid extraction. The average efficiency of algae disruption of homogenization is around 93%,[28] but the energy use is 67 MJ/m^3 algae slurry that is lower than dry lipid extraction. The homogenization following by lipid extraction is normally called as wet lipid extraction that is modeled in many recent literatures of algal biofuel production. In this study, parameters of homogenization are based on literature review.

Algal biomass conversion pathways

In this study, three algal biomass conversion pathways are modeled. First microwave pyrolysis followed by hydro-upgrading is modeled based on data collected in the pilot scale system developed at the University of Minnesota. Microwave pyrolysis' advantages over traditional pyrolysis are: uniform internal heating of large biomass particles, ease of control, and no need for agitation or fluidization.[31] The belt-drying is the pretreatment process for this pathway as low moisture content is required. In the microwave pyrolysis process, temperature is controlled around 500-600 C and the electricity requirement is 1.94 kWh/kg bio-oil produced. The average bio-oil yield from the pilot scale system is 0.286 g/g dry algae. Biochar, syngas, and water are co-produced with bio-oil in the pyrolysis process. Biochar contains 18% of the energy in the dry algae and is burned in HPS to generate electricity and heat that is used in the pyrolysis and then in belt drying process. The heat from the syngas combustion is used in the belt drying process to reduce imported energy for the algal biofuel production. The bio-oil produced is stored in the pyrolysis plant, and then sent to a centralized biofuel refinery to be upgraded by hydrotreating and hydrocracking. The modeling of bio-oil upgrading processes is mainly based on the paper by Wright.[40] The hydrotreating takes place in a hydrogen rich

environment at 300-400 C and 10-14 MPa pressure with a cobalt-molybdenum catalyst. It is necessary to remove impurities that can affect downstream equipment. Hydrogen is imported and added at a rate of 5 wt.% of bio-oil. The hydrocracking process is undertaken at 10-14 MPa and 400-450 C using a nickel-molybdenum catalyst. It breaks down heavy hydrocarbon molecules into shorter chain fuels, such as diesel and gasoline. In the paper by Wright, the yields of transportation fuels from woody bio-oil are 21 wt.% diesel and 21 wt.% gasoline of bio-oil input. Algal biomass has less lignocellulose content than woody biomass and thus produces a higher quality bio-oil. In this study, diesel and gasoline yields from algal biomass are assumed to be 35% and 35% by weight of algal bio-oil.

In the second pathway, algal biomass is directly combusted to produce electricity to power electric vehicles. Similar to the pyrolysis process, combustion is a thermo-chemical conversion process. Thus, the combustion was chosen for this study as a baseline to compare to the novel microwave pyrolysis process. The modeling of algae combustion is based on the GREET model. The efficiency of boilers is 32.1% for electricity generation and 32.9% for heat co-generated. All electricity generated is assumed to use for in-plant need, and then be exported to power electricity battery vehicles (EBV), and heat generated in combustion is reused in the belt drying process. The EBV is modeled as more such vehicles are available in the market and with high energy use efficiency.

The third pathway is the technology of lipid extraction followed by transesterification, which is a technology applied in commercial scale for soybean biodiesel production. If the belt drying is applied as pretreatment, the pathway is called dry lipid extraction that has a maximum 99 wt.% lipid extraction efficiency.[41] In contrast, if the homogenization is applied, the pathway is called wet extraction in which the extraction efficiency reduces to 90 wt.% by lipid as the influence of high moisture content.[28] The wet lipid extraction technology is still in the very early stages of development and has only been demonstrated at the lab-level.[26] In the model of the lipid extraction pathway, algal lipids are first extracted with hexane and stored on site. Then the lipids are sent to a centralized biofuel refinery and converted to biodiesel through the transesterification process. Glycerin is co-produced with biodiesel. The design parameters for the model are based on existing literature. The remaining lipid-free algal biomass goes through an anaerobic digestion process. The biogas from digestion generates electricity and heat for in-plant process use with gas turbines. The modeling of digestion of biomass and biogas turbines is based on paper by Clarens[28] and GREET model.

Life cycle inventory (LCI).

The life cycle inventory is calculated for thirteen pathways for algal biofuel production shown in Table 3. These five scenarios combine different nutrient sources, cultivation systems, and conversion technology options for comparison. The pathway is modeled only if there is an experiment or literature data available for analysis. For example, there is no data for hydrothermal pretreatment followed by the pyrolysis and combustion process. These two pathways are not modeled.

Table 3. Scenarios for comparison

Name of Pathways	Nutrients	Algae cultivation	Pre-treatment	Algae conversion technology	Products	Residue recovery
Centrate + dry extraction	Centrate	PBR	Centrifuge + belt drying	lipid extraction+ transesterification	Biodiesel	Digestion
Centrate + wet extraction	Centrate	PBR	Centrifuge+ Homogenization	lipid extraction+ transesterification	Biodiesel	Digestion
Centrate + combustion	Centrate	PBR	Centrifuge + belt drying	combustion	Electricity	-
Centrate +	Centrate	PBR	Centrifuge +	Pyrolysis +	Diesel+	Char

pyrolysis			belt drying	hydro-upgrading	gasoline	combustion
Manure + dry extraction	Digested hog manure, flue gas	PBR	Centrifuge + belt drying	lipid extraction+ transesterification	Biodiesel	Digestion
Manure +wet extraction	Digested hog manure, flue gas	PBR	Centrifuge+ Homogenization	lipid extraction+ transesterification	Biodiesel	Digestion
Manure + combustion	Digested hog manure, flue gas	PBR	Centrifuge + belt drying	combustion	Electricity	-
Manure + pyrolysis	Digested hog manure, flue gas	PBR	Centrifuge + belt drying	Pyrolysis + hydro-upgrading	Diesel gasoline	Char combustion
Openpond + dry extraction	Water, Flue gas, Urea, DAP	Open pond	Centrifuge + belt drying	lipid extraction+ transesterification	Biodiesel	Digestion
Openpond + wet extraction	Water, Flue gas, Urea, DAP	Open pond	Centrifuge + Homogenization	lipid extraction+ transesterification	Biodiesel	Digestion
Openpond + combustion	Water, Flue gas, Urea, DAP	Open pond	Centrifuge + belt drying	combustion	Electricity	-
Openpond + pyrolysis	Water, Flue gas, Urea, DAP	Open pond	Centrifuge + belt drying	Pyrolysis + hydro-upgrading	Diesel gasoline	Char combustion
Openpond + hydrothermal	Water, Flue gas, Urea, DAP	Open pond	Centrifuge + hydrothermal treatment	lipid extraction+ transesterification	Biodiesel	Digestion

- A total of thirteen pathways for algal biofuel production are modeled for comparison. The dry lipid extraction treats algae with belt drying followed by lipid extraction, whereas the wet lipid extraction applies homogenization followed by lipid extraction.

The life cycle inventory (LCI) is established by following assumptions and databases

- LCI includes direct and upstream (supply chain) impacts of the process as well as impacts of construction and building of cultivation systems. The data for construction are based on pilot scale studies of PBRs and literature review of the open pond system. The direct impacts are based on the analysis of all unit processes within the system boundary. All upstream impacts of the system's inputs, such as fertilizers, electricity, heat, and chemicals are based on Ecoinvent 2.2 database. The EU data is applied when there is no US data available in Ecoinvent. Other indirect impacts, such as labor, capital and machinery are not included.
- Impacts are allocated between co-products using the displacement method. Bio-electricity displaces a US mix of electricity generation, bio-glycerin displaces petroleum glycerin from an epichlorohydrin plant, and heat collected from flue gases displaces heat generation by natural gas. The wastewater treatment is considered as a co-product for algal biofuel production and displaces treatment of sewage from residence with class 2. Based on Ecoinvent 2.2, the COD removal is assumed as 0.15 kg/m^3 sewage from residence. The replacement of wastewater treatment (m^3/d) by algae cultivation equals to the total COD removal (kg/d) in the PBR divided by 0.15 (kg/m^3).
- Supernatant from the centrate PBR system is discharged every day without recycling. It is assumed that there is no environmental burden for this waste stream. This is because the

pollutant concentration of supernatant is lower than the centrate, and there is no new pollution added to environment. The environmental burdens of blowdown (wastewater discharged during cleaning of cultivation reactors) from all cultivation systems is not account for in the LCI because the total amount of blowdown is small.

- The model counts the GHG emissions of algal biofuels in transportation sector. The fossil fuel use and GHG emissions from car manufacturing are included based on GREET, but the no data are available for eutrophication and water use of car manufacturing. Three different vehicles are modeled. They are gasoline engine vehicles, diesel engine vehicles and electricity battery vehicles (EBV). The respective efficiencies are 28.1 MPG for diesel, 23.4 MPG for gasoline based on GREET, and 0.81 mi/MJ for electricity[28]. The GHG emissions of algal gasoline and diesel in vehicle operation are assumed to be the same as the petroleum gasoline and diesel. All electricity exported is assumed to be used in EBV in order to simplify the model and focus the study on impacts of wastewater to algal biofuel production.
- Conventional petroleum fuels are assumed to be used in the transportation of bio-oil, wastes and the final products. The emission factors of petroleum fuels are based on the GREET model. Impacts of bio-oil storage are not considered.
- A petroleum diesel is list in LCI to make as a baseline for comparison. The data is obtain from GREET model. In this study, algal biofuels are only compared to petroleum diesel but not gasoline and electricity. This is because the focus of this study to examine influence of wastewater to algae cultivation and algal biofuels, comparing to petroleum fuels is not our major goal.
- The impact calculation is based on TRACI 2. All biogenic and fossil GHG emissions are included in the LCI. All water use for algae cultivation and for cooling makeup is assumed as freshwater from surface water.
- The calculation of impacts is based on the likeliest value of variables of unit processes. The best and the worst case of all pathways are calculated as well. The best/likeliest/worst values of variables are based on experiment and literature review of unit processes, and list in SI.

Results and Discussion

General comparison of thirteen pathways.

The well-to-wheel results in Figure 2 show that the using wastewater as a nutrient source improves the environmental performance of freshwater based (i.e., open pond system) algal biofuels if appropriate production pathways applied. For two wastewater sources considered, centrate is a good nutrient source for algae growing, as the results, the centrate based algae biofuels have better performance than freshwater and manure based algal biofuels. Due to the low neutral lipid content (2%) in manure based algae, the impacts are extremely high for manure based algal biofuels with lipid extraction pathways. But with pyrolysis and combustion pathways, the environmental performance of manure based algal biofuels is better than freshwater based. The pathway with novel hydrothermal treating process has better performance than the wet lipid extraction pathway, but it cannot compete with combustion pathways. The results show a significant difference between the best and the worst scenarios in all examined pathways, which is caused by the huge variances of design variables. The best/worst case analysis also implies that the fossil fuel use and GHG emissions are more sensitive to the change of variables than other two impacts, as their variances are higher than that of eutrophication and water use. Of all thirteen pathways examined, only centrate based algal biofuel can have better performance than petroleum diesel in all impact categories examined. The best pathway is the centrate BPR system followed by wet lipid extraction that has -38.9 MJ/km fossil fuel use, -3.0 kg CO2 eq./km GHG emissions, - 0.11 m^3/km water use and -2.0 kg N eq./km eutrophication potential based on the likeliest case. If the worst case is

applied, there is no algal biofuel has better performance than petroleum diesel. If the best case is applied, most open pond and manure based algal biofuels still have higher impacts than petroleum diesel in fossil fuel use, GHG emissions and water use.

The results of the likeliest case show higher impacts than previous studies in water based algal biofuels, such as studies by GREET model and by Batan. This is due to the lower algae yield (13.7 g/m2/d), lower lipid content (14%) and higher power use (0.11 kWh/m3) applied in this study. Most previous studies applied a yield over 20 g/m2/d and lipid content at 20-30%. However, it is questionable to obtain such a high yield and lipid content in the open pond as the yield and lipid content are always lower in large scale than in the lab scale, let alone the neutral lipid (TGA) that can really convert to diesel. The power use in this study is more reliable as it is based on calculation of pump performance curves. Compared to other wastewater based algal biofuel studies, such as the studies by Sander and Murthy that are based on effluent of the activated sludge process, the manure based algal biofuels have higher impacts than previous studies, whereas the centrate based biofuel have lower impacts. This is because the centrate PBR has higher algae yields and nutrients removal rates than cultivation facilities feed with conventional effluent of activated sludge process, which leads to lower environmental impacts. In contrast, manure PBR has lower yields and removal rates and consequently higher impacts.

When examining impacts by production stages, the algae cultivation is the most energy intensive stage in all pathways for comparison, and consumes over 50% of total life cycle fossil fuels. The second energy intensive stage is the pretreatment stage, and consumes 10-50% fossil fuels. These two stages contribute over 90% fossil fuel burdens together. Application of hydrothermal and homogenization can reduce life cycle fossil fuel use of algae pretreatment by 40%. The algae cultivation, pretreatment, and PHS are major contributors to GHG emissions and accounts for 50-90% emissions in algal biofuel production. Most of GHG emissions are indirect emissions by electricity and heat generation. GHG emissions of HPS are mainly direct emissions from biogas turbines. In pathways with the thermal conversion (i.e., combustion and pyrolysis), the biomass conversion accounts for 20-40% GHG emissions. In terms of water use, almost all pathways have higher water use than petroleum diesel except the centrate + wet lipid extraction pathway. This is due to the direct water use in algae cultivation to compensate evaporation, and also indirect water use related to electricity and heat generation in pretreatment. For eutrophication, the manure and centrate based pathways have negative eutrophication potentials because of the removals of nutrients in wastewater in algae cultivation stage. The reduction of eutrophication is not applicable in water-based algal biofuels. Finally, analysis of processes shows that the wastewater treatment credits contribute significantly to the reduction of life cycle impacts of algal biofuels. For example, in centrate based pathways (expect pyrolysis), wastewater credits are higher than environmental burdens (expect water use).

The LCA results imply that environmental performance of algal biofuels is highly related to nutrients sources and technologies applied in production. Fossil fuels use mainly in cultivation stage, whereas GHGs emit in all stages in the life cycle. As the many stages involved in production pathways, there are so many uncertainties in process design and operation. The following sections will discuss the influence of nutrients sources and compare influence of using different technologies.

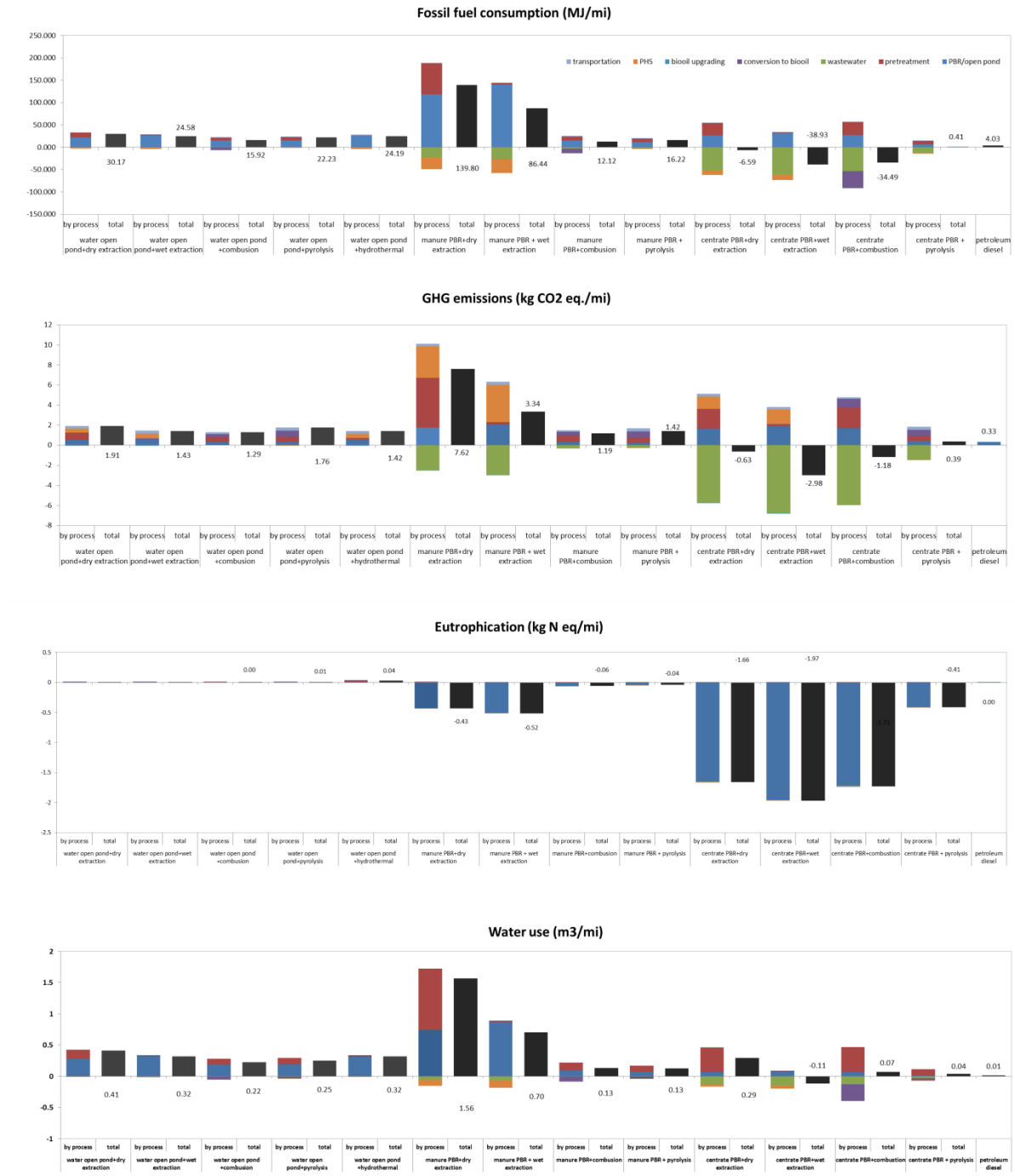


Figure 2. LCA results of pathways for comparison. A total of thirteen pathways are compared and algal biofuels are compared to petroleum diesel as well. For environmental impacts are examined including fossil fuel use, GHG emissions, eutrophication, and water use. The error bars show the best and worst case scenarios of pathways.

Comparison of algae cultivation systems.

The LCA results show that cultivation stage is a major contributor to environmental impacts of algal biofuel production. Comparing three different cultivation systems can help to understand the influence of nutrients sources and cultivation technologies to environmental

performance. Figure 3 shows life cycle impacts of three different cultivation systems by different inputs. The comparison of three cultivation systems show that the wastewater based systems have better performance than water based systems. This is because by reducing environmental burdens associated with the fertilizer and freshwater consumption, reducing electricity use for light and dewatering, and reducing impacts of reactor construction. Finally, the wastewater treatment credits further improve environmental performance.

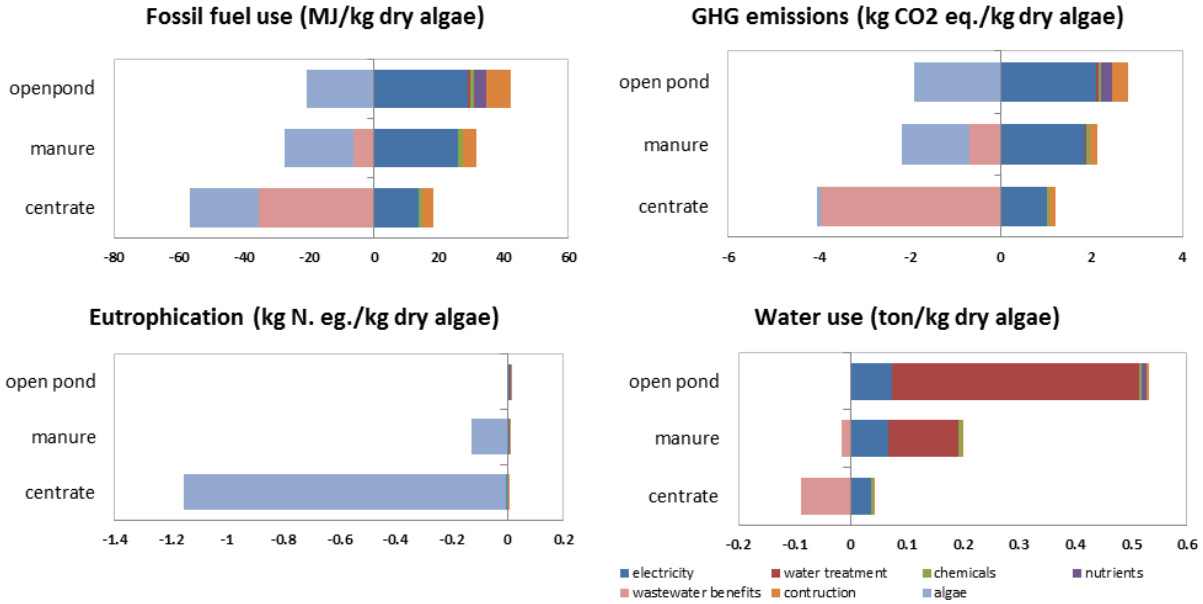


Figure 3. Comparison of impacts three algal cultivation systems with different inputs. The functional unit is 1 kg dry algae produced. The output algae slurry is with 20% solid content. The right side of each chart is the energy input, and the left side is the energy output. The 'chemical' refers to PAM in the flocculation process. 'Nutrients' include Urea and DAP for water open pond. The 'algae' refer to impacts by algae growing. For example, in the manure cultivation system, algae provide 21.2 MJ energy output, remove 1.42 kg CO2 from flue gases, and 0.13 kg N from manure per kg dry algae produced.

The characteristics of the wastewater, such as nutrient amount, nutrient ratios, and the presence of algal growing inhibitors highly influence the algae growth in cultivation reactors. The results show that centrate and hog manure both have high C, N and P concentrations, but their environmental impacts are significant different in the cultivation stage. Centrate contains the optimum nutrient profile for supporting fast algal growth and higher biomass density with selected algal species, which reduces energy use for light, water and dewatering. In contrast, hog manure has an extremely high N concentration (3000 mg/L) that can inhibit algae growing, and thus the manure has to be diluted with freshwater (60 times) prior to application. But manure dilution lowers the P and C concentration at the same time, and could lead to a lower algae yield than centrate PBR. To increase algae yield, additional flue gas is injected to provide C in the water, which increases the energy use for flue gas collection and injection. Also, to increase nutrient concentration and reduce water use, the supernatant from the algae-water separation is recycled. This further increases energy use for pumping in the manure PBR system. Combined with manure dilution and supernatant recycling, the manure PBR has 2.2 times the electricity use and 750 times the water use per kg dry algae produced to the centrate PBR. In addition, as manure PBR has less nutrients removal rate than centrate PBR, less wastewater treatment credits are assigned to manure PBR (2 m^3 wastewater removal/kg dry algae produced) than centrate PBR (11.2 m^3/kg algae). Therefore, the environmental performance of the manure system is much worse than the centrate system and only

demonstrates limited improvements in compared with freshwater open pond (e.g., LCA results show the fossil fuel use of centrate PBR is around -100% of the open pond, but fossil fuel use of manure PBR is 55% of the open pond). In order to improve performance of manure-based algal biofuel, it is necessary to improve manure's nutrient profile before it feeding into cultivation tanks. The raw manure digestion process applied before the PBR reactor is good at removing inorganics and organics, but not good at lowering ammonia concentration, shown in Table 1. New technologies should be considered for algae cultivation to reduce impact of N on algae growing.

In algae cultivation systems, the algae yield ($kg/m^2/d$), lipid content (%) and lower heating value (LHV, MJ/kg) are key parameters determining system's performance. The algae yield depends on nutrient inputs in the growth media and culturing conditions (e.g. temperature, light intensity and mixing), and determines biomass density at harvesting and energy/lipid productivity (MJ/day or kg/day), i.e. the total energy/lipid collected in algae per day and calculated by total algae yield (kg/d) * LHV(MJ/kg)/lipid content (%). Ideally, the increased biomass density can reduce electricity use in centrifuge and reduce environmental burdens. Also the higher productivity leads to higher biofuel yields and higher possible travel distance per nutrient inputs, and consequently, lower environmental burdens per mile traveled. However, as the density and productivity increases, the nutrient inputs and energy for light or mixing will increase significantly. This could make the environmental improvement by increase of productivity faded quickly. In addition, for algae species, the lipid content and yields are inversely related, i.e., algae with higher lipid content always have lower yields. To improve both lipid and yield, more energy for light and mixing or nutrients such as sugar have to put into the system and significantly increase energy use. Many previous studies ignore this and discuss how performance can improved by constantly increasing yields/lipid and fix the other one, which could lead to wrong conclusions. Finally, based on experiment of wastewater based algae, the lipid content of algae in outdoor reactors is always lower than lab cultivation, as outdoor reactors cannot operate in the optimum conditions. The neutral lipid content is low as well and caused by excess N and contaminants in wastewater. In the manure PBR even the total lipid content can reach 20% and the neutral lipid is only 2%. In water based algae cultivation systems, many studies uses lipid content range from 20-30%, which is too high to obtain in large scale cultivation. Currently, the relationship of yields, lipid content and energy use has not been well studied and should be an area of active research.

Comparison of algal biomass conversion pathways.

The LCA results show pretreatment is another major contributor to environmental impacts of algal biofuel production, and biomass conversion is a major contributor to GHG emissions. As the pretreatment is closely connected with biomass conversion pathways, two stages are discussed together on their roles in performance of algal biofuels. Based on comparison of 13 pathways, even with the same algae cultivation system, environmental performance is of significant difference in different conversion pathways. Figure 4 shows life cycle impacts of three different conversion pathways to treat one kilogram centrate based algae with 20% solid content. Three conversion pathways are compared: belt drying + pyrolysis, belt drying + combustion, and homogenization + lipid extraction (wet lipid extraction). The impacts in of stage of final use of algal biofuels are also included. The Figure 4 only shows algae from centrate PBR, algae from other cultivation systems can be found in SI. Analysis shows the environmental burdens are mainly from direct emissions and discharges of processes and also indirect impacts of heat and electricity use. Comparing three conversion pathways, combustion can produce more electricity that displaces electricity generation, thus, has lowest fossil fuel use of all three pathways. Wet lipid extraction has lest GHG emissions of all pathways, which is caused by less heat and electricity use in pretreatment and less emissions in PHS. The pyrolysis pathway consumes electricity, 2.0 kWh/kg biooil, for microwave generation and discharges wastewater in pyrolysis process, which leads to the highest impacts of three

pathways in all categories. Water use is mainly from heat generation in belt drying, therefore, pyrolysis and combustion pathways have much more water use than wet lipid extraction. Algae from different cultivation systems do influence the impacts of three technologies, and their influences are around 10-30%. For example, the fossil fuel use of the wet extraction pathway is 13.5 MJ/kg dry algae for centrate algae and 11.2 MJ/kg dry algae for open pond algae. The change is around 20 %.

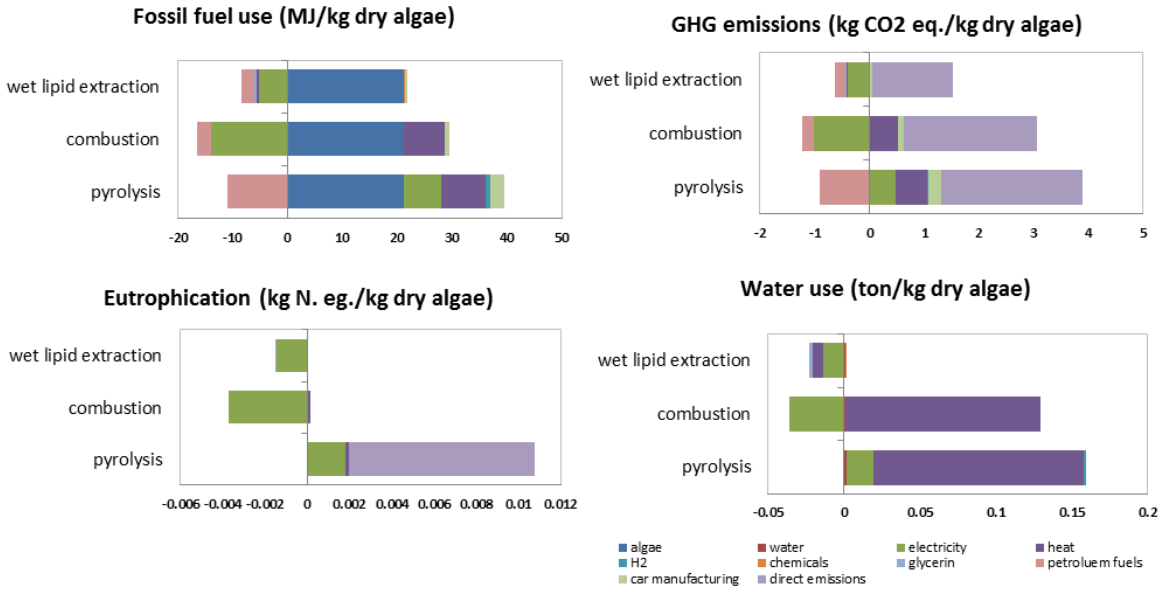


Figure 4. Comparison of impacts of three algal biomass conversion pathways with different inputs. The algae are from centrate PBR with 20% solid content. The right side of each chart is the energy input, and the left side is the energy output. The 'chemical' refers to all chemicals used in lipid extraction and transesterification.

However, the total life cycle impacts of algal biofuels are not the sum of impacts shown in Figure 3 and Figure 4. For example, based on results in Figure 3 and 4, the open pond + wet lipid extraction should have better performance than open pond + pyrolysis pathways. But based on life cycle analysis in Figure 2, the wet extraction and pyrolysis pathways have a mixed performance. The pathway with wet extraction uses more fossil fuel and water than that of pyrolysis, but has less GHG emissions and less eutrophication potentials. This can be explained by different function units applied in LCA calculation. The life cycle results in Figure 2 are based on mileage driven by vehicles, but not on mass of algae. The driven mileage of wet extraction per kg algae (1.63 km/kg) is lower than pyrolysis (2.77 km/kg), which lead to higher fossil fuel use of wet extraction (13.9 MJ/km) in algae cultivation than pyrolysis (8.15 MJ/km). Even fossil fuel use of wet extraction (6.83 MJ/km) are lower than pyrolysis (11.1 MJ/km), the overall impacts of wet extraction (20.7 MJ/km) is higher than pyrolysis (19.3 MJ/km). In manure based pathways, on the contrary to results of conversion pathway analysis, the wet lipid extraction pathways have higher impacts than the pyrolysis pathway. This is caused by lower biofuels yields in manure + lipid extraction pathways, which leads to higher impacts in cultivation stage and higher overall impacts. In summary, the influence of algae conversion technologies to the entire life cycle impacts of algal biofuels is complicated and depends heavily on functional units as well.

Based on LCA analysis, the PHS is a major contributor of GHG emissions in the biomass conversion stage, particular in lipid extraction stage PHS accounts for 90% direct GHG emissions. In wet lipid extraction, the non-lipid portion of algae, residue is digested and energy is recovered by biogas turbines. A primary comparison is conducted with the technology to treat

residue as land amendment where GHG emissions could be reduced. In this process, electricity is used for residue dewatering to reduce volume to facilitate residue transportation. The data of land amendment, such as nutrient recover efficiency are based on GREET. The results show that the environmental performance is not improved by the new technology. The life cycle GHG emissions are even higher than the pathway with digestion. This is because the credits assigned to fertilizer displacement in the land amendment scenario are lower than credits are assigned to electricity and heat generation in the digestion scenario. Although the land amendment scenario has less direct emissions, but the overall emissions is still higher. The study points out that the land amendment technology should focus more on increasing efficiency of nutrients use in lands and improve fertilizer displacement.

Sensitivity of critical design parameters.

In light of huge differences of environmental performance of cultivation and conversion technologies in algal biofuel production. A sensitivity study has been conducted to identify key parameters of different technologies that contribute most directly to life cycle impacts. These also represent areas of future work of technology improvement. As many parameters are included in process design, key parameters are identified in each technology, and then their impacts to the life cycle performance are discussed. The results of the sensitivity study are shown in Figure 5. In the best and worst case analysis, fossil fuel use and GHG emissions are identified as more sensitive to process inputs and design parameters, therefore, the sensitivity analysis only includes these two impacts. The results (bars) show the % change to the likeliest value associated to 10% change of a key design parameter from its likeliest value. Other parameters are kept constant. Figure 5 only shows three centrate based pathways, two manure based pathways, and one open pond pathway. Other pathways can be found in SI.

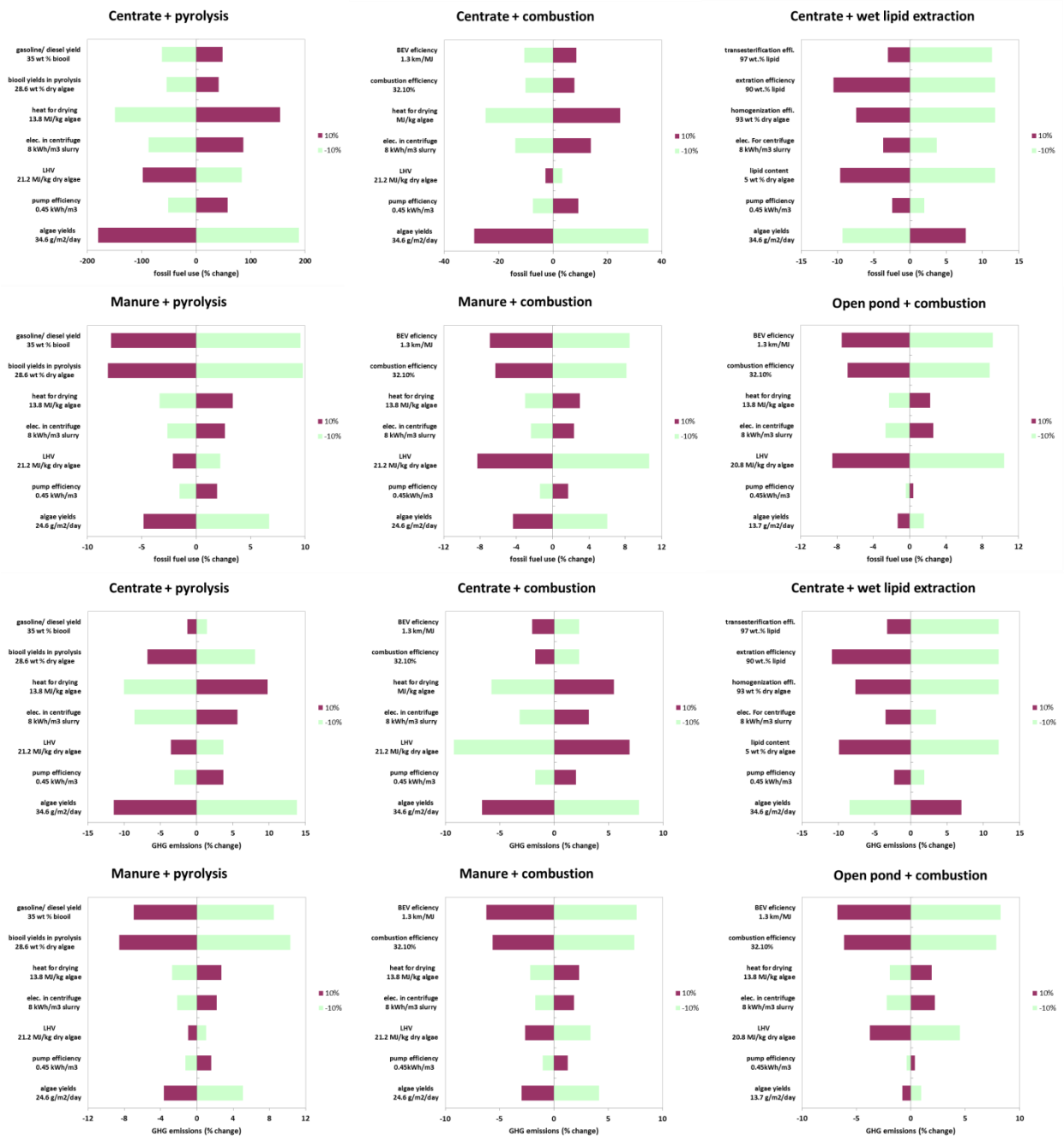


Figure 5. Sensitivity analysis of fossil fuel use and greenhouse gas emissions for pathways of algal biofuel production to a 10% change in design parameters. Only key parameters are selected and their likeliest value presented in the Figure. The centerline represents the likeliest case. The bars indicate % change of the life cycle fossil fuel use and GHG emissions. For example, increasing algae yield by 10% reduces fossil fuel use by 180% in the centrate + pyrolysis pathway.

As seen in Figure 5, there are huge differences in sensitivity of key parameters to algal biofuel production. Generally, the fossil fuel use and GHG emissions are sensitive to algae energy/lipid productivity, heat efficiency in belt drying process, and efficiency of biomass conversion processes, which is in accordance with the current research areas of improving algae cultivation, replacing energy intensive dewatering processes, and developing conversion

technologies to treat algae with high moisture. In centrate based systems, the fossil fuel use and GHG emissions are sensitive to algae productivity; whereas water open pond systems are not as sensitive to algae yields as centrate based systems. Therefore, improving environmental performance of centrate based algal biofuels should focus on the algae cultivation stage, whereas water open pond should focus more on algae pretreatment and conversion stages. The manure based systems are sensitive to both algae productivity and conversion efficiencies, thus, manure based systems need much more work on both cultivation and conversion technologies.

Implications and recommendation of wastewater based biofuel production.

Based on the comparison of different nutrient sources and pathways for algal biofuel production, it is evident that integrating wastewater into algal cultivation can improve the environmental performance of algal biofuels. Normally, the municipal wastewater has a desirable nutrient profile and less growing inhibitors for algae cultivation than industrial and animal wastewater. But the widely discussed wastewater of effluent of secondary wastewater treatment facilities cannot obtain high algae yield and high biomass density because the activated sludge system has removed most of nutrients and the effluent often has low nutrient concentration.[42,43] Utilization of high strength municipal wastewater e.g., centrate could obtain high algae yield, but the amount of centrate could not support large scale production.[33] For example, in the large scale Metropolitan Wastewater Treatment Plant in Saint Paul, Minnesota, 1 MGD of centrate is generated every day, but it can only produce around 100 gallon diesel in the wet lipid conversion pathway or around 480 gallon of gasoline + diesel in the pyrolysis pathway. Manure has high nutrients concentration and huge amount that is available for algae growth, but it has an unbalanced nutrient profile and growth inhibitors. Actions need to be taken to either develop proper management methods during cultivation, such as diluting the wastewater, adding additional fertilizers, and recycling supernatant, or improving the quality of manure before application. All those efforts need additional energy, water or chemicals, and therefore lead to additional direct and indirect environmental burdens that compromise the benefits carried by manure cultivation. Based on our analysis, even with increasing interest, wastewater based algae are unlikely to be a viable feedstock for transportation energy in a large scale with current technologies.

However, wastewater based algal biofuels provides an important perspective in wastewater treatment and beneficial reuse. To remove 1 kg COD, 21.3 MJ fossil fuels are required in the conventional wastewater treatment facilities where most energy is consumed in the active sludge process for aeration. In contrast, as no aeration is required, removal of 1 kg COD by producing algal biofuels requires only 12.4 MJ fossil fuels with the wet lipid extraction technology. This represents a considerable potential to improve the environmental impacts of wastewater facilities and beneficial reuse of wastewater. One problem is that algae cultivation needs more time for COD removal (normally 1-3 day), whereas the activated sludge process only needs 4-6 hours. As a result, algae cultivation requires more land than the activated sludge process, which could be not available for municipal wastewater treatment facilities as they are often located in the urban area. In order to solve the problem, it is better to use a portion of wastewater to co-produce algal biofuels to address both rapid removal COD and beneficial reuse. In addition, given the current technologies, the study suggests applying other technologies to recycle energy in manure, such as direct digestion of manure to generate electricity. Based on the preliminary study conducted with this research, direct digestion of swine manure to generate electricity has better performance than producing algal biofuels, and also has better performance than petroleum diesel based on mileage driven.

Whether you are growing algae to produce transportation fuels or to treat wastewater and recycle energy, technologies of algal biofuel production must be adapted to wastewater based algae growing and biomass conversion. For the mixtrophic growing in wastewater, the algae

could grow in areas with less sunlight and in cold weather. This extends geographic distribution of algae cultivation and reduces environmental burdens of electricity and heat use. The reactors applied in this study have multiple layers, which can significantly save the land use and occupation. On the other hand, most wastewater could contain unbalanced nutrients and growing inhibitors that need to be removed before feeding to algae. The pretreatment process of wastewater is always required. In addition, to treat low lipid content wastewater based algae, the thermal conversion, such as pyrolysis pathways can be applied to maximize biofuel productivity, as the pyrolysis can convert some non-lipid content in algae into bio-oil[29] (the bio-oil yield is 28 wt.% of algae input). In summary, to increase application of wastewater based algal biofuels, improvement of technologies to fit in wastewater conditions in all life cycle stages are required.

Final Report Summary:

The algae cultivation facility and conversion equipment developed through this project were tested and demonstrated to the public, and the demonstrations were well received. The data obtained with these facilities were used in our life cycle assessment (LCA) to evaluate the environmental impacts of the technologies. The analysis shows that our technologies, which integrate wastewater into algal cultivation, can improve the environmental performance of algal biofuels. Our finding indicates that utilization of high strength municipal wastewater e.g., centrate could obtain high algae yield. However, wastewater based algae are unlikely to be a viable feedstock for transportation energy in a large scale with current technologies. We must explore other benefits and possibilities of algae based technologies in addition to wastewater treatment. For example, a more comprehensive plan for utilization of multiple major waste streams may be developed so that such an integrated approach could maximize the economic and environmental benefits of algae based technologies.

What this project was able to demonstrate which will contribute to both economic and environmental benefits was the following:

- The ability to combine biofuel production with production of animal feed and the optimization of both outputs. A marketable bi-product from biofuel production is important in improving the economic performance of the system.
- Minimizing the use of water in processing. One of the constraints to algae biofuel production is the water required to produce and process the algae. The technologies explored in this project address this issue.
- Providing a new technology for wet processing of biomass. A major cost and constraint of economic biofuel production from algae is the need to remove water from the biomass prior to conversion to a biofuel. The project has developed a preliminary innovative solution to address this constraint.

Although many of these advances are at a preliminary stage, they hold promise for enabling biofuel production from algae that will be more economically and environmentally feasible and beneficial.

V. TOTAL ENRTF PROJECT BUDGET: $900,000

Personnel: $707,005.14

Roger Ruan, PI, 2.5 months in the 3rd yr, including 32.3% benefits, project leader, system development and installation.	$8,551.36

Paul Chen, co-PI, 30%, 3yrs, including 32.3% benefits, project coordination, conducting R&D, project evaluation, progress report	$112,863.02
Dean Current, co-PI, 15%, 3yrs, including 32.3% benefits, conducting R&D, economic and environmental life-cycle analysis	$32,805
2 Postdocs, 100%, 3yrs, including 19.75% benefits, conducting R&D, operations, demonstration, data analysis	$239,366.89
2 Graduate Research Assistants, 50%, 3yrs, including 16.84% benefits and tuitions, conducting R&D, operating, demonstration	$302,055.11
Undergraduate assistants	$11,363.76
Civil Service staff	0.00

Equipment/Tools/Supplies~~:~~ *$182,966.19*

Non-capital Equipment / Tools: pumps, hoses, valves, control components, lights, tanks, mixers, for maintenance, repair and replacement	$14,000.00
Algal biomass conversion equipment including hydrothermal reactor system, oil extraction system, and catalytic upgrading reactors.	$86,999.66
Lab and operation supplies: materials for making lab scale reactors for testing, nutrients, cultures, chemicals for analysis, lab supplies, consumables for analytical instruments, external analysis services	$81,966.53

Travel: $10,028.67

In-state travel: daily usage of University and personal vehicles for researchers to travel between campus and research and demonstration sites.	$10,028.67

Explanation of Capital Expenditures Greater Than $3,500:
We requested $87,000 for custom-manufacturing of an algal biomass conversion system. We will develop a basic design through our research for the system. The requested budget is to cover the costs associated with engineering design and manufacturing of the system by a local company. The system will be used for this ENRTF project and for similar work throughout its useful life time for process development, demonstration, education, and pilot scale trials. It includes and automatic distillation analyzer and a shaker.

VI. PROJECT STRATEGY:

A. Project Partners:

The project will be carried out by a team of researchers and engineers from UMN and MCES.

Dr. Roger Ruan, Professor, Director, Center for Biorefining, Department of Bioproducts and Biosystems Engineering (BBE), UMN, will be the PI & project director. He will be responsible for overall project planning and budget control, development, design and evaluation of enclosed photobioreactors, development and evaluation of processes and lab scale systems for conversion of algae to fuels. He will also lead demonstrations and present project results. Dr. Ruan's salary will not be covered by this project.

Dr. Robert Polta, Director of Research, Metropolitan Council Environmental Services (MCES), a registered professional engineer in Minnesota, will be a co-PI of the project. He will be responsible for demonstration site preparation and coordinate construction, installation and operation of the production system. He will also lead efforts in evaluating the environmental and ecological benefits of the project. Dr. Polta's salary will not be covered by this project.

Dr. Paul Chen, Program Director, Center for Biorefining, Dept of BBE, UMN, will be a co-PI. He will be responsible for experiment design and coordination, monitoring and documentation of project progress and results, and publicizing the project. He will also be involved in development of conversion processes.

Dr. Dean Current, Program Director, Center for Integrated Natural Resources and Agricultural Management, UMN, will be a co-PI and will be responsible for conducting the economic and environmental life-cycle analysis.

B. Project Impact and Long-term Strategy:

The proposed project, built on our existing R & D efforts, does not need additional investment other than the requested financial support to be completed. However, further R & D leading to eventual technology transfer and commercialization will be our long-term goal and will require additional funding. Next level scale-up pilot facilities must be demonstrated with federal, state, and private funding before the technology can be commercialized.

C. Other Funds Proposed to be Spent during the Project Period:

The expected LCCMR funding level for this project was lower than initially requested. We have two options for the demonstration site and scale depending on level of additional funding received. Option 1, with the current LCCMR fund and no additional other funding, we are planning to build and expand a scaled-down demo system in our current small pilot reactor site in the St. Paul campus greenhouse facilities. Option 2 (most likely), the University of Minnesota IREE and Rosemount Research and Outreach Center and MCES come up with some funding to support building and operating a pilot demo system in the MCES St. Paul wastewater treatment plant and/or Rosemount Research and Outreach facility. MCES has been providing monetary and in-kind supports (staff salaries, facility construction/modification, operation and maintenance) for the past few years. They are very interested in and happy with the fact that algae are cost effective in removing COD, phosphors, and nitrogen. In fact, they have just built a small green house at the MCES Metro Wastewater Treatment Plant in St. Paul for us to test the photobioreactors for continuous wastewater treatment using algae and simultaneous algae production as an energy crop from wastewater.

D. Spending History:

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period: private gift funds will be used for algal biomass conversion research	$100,000	*secured*
Other State $ Being Applied to Project During Project Period:	$	*N/A*
In-kind Services During Project Period: spaces and utility, staff time (20% of Bob Polta and Adam Sealock) donated by MCES.	$100,000 + space and utility	secured
In-kind Services During Project Period: spaces and utility, staff time (20% of Roger Ruan time for two years) donated by UMN.	$60,000 + space and utility	secured
Remaining $ from Current Trust Fund		*N/A*

Appropriation (if applicable):		
Funding History:	$	
Xcel Energy (2007-2008): Development of mass algae culture systems	$150,000	secured
MCES (2006-2009): Mass Culture of Algae as an Energy Crop for Biofuel Production by Utilizing Wastewater and Flue Gas from Wastewater Plant	$540,000	secured
MCES (7/1/2009-6/30/2010): Mass Culture of Algae as an Energy Crop for Biofuel Production by Utilizing Wastewater and Flue Gas from Wastewater Plant	$55,000	secured
IREE (2006-6/30/2010): Mass Culture of Algae as an Energy Crop for Biofuel Production by Utilizing Wastewater and Flue Gas from Wastewater Plant	$540,000	secured
IREE (4/1/10 – 8/30/10) Algae Production System Using Centrate Wastewater from Met Council Treatment Plant	$156,840	Secured
Private industry gifts, renewable resources utilization	$220,000	Secured

VII. DISSEMINATION:

1) The research results were presented more than 10 times at national and international conferences.
2) The technologies developed as a result of the project were demonstrated to stakeholders 5 times.
3) Information about the project and results obtained were provided to participants of our demonstration events and on our website (http://biorefining.cfans.umn.edu).
4) More than 10 papers were published in peer-reviewed journals (See the list below).

- Du, Z.; Ma, X.; Li, Y.; Chen, P.; Liu, Y.; Lin, X.; Lei, H.; Ruan, R., Production of aromatic hydrocarbons by catalytic pyrolysis of microalgae with zeolites: catalyst screening in a pyroprobe. Bioresource Technology 2013.
- Hu, B.; Zhou, W.; Min, M.; Du, Z.; Chen, P.; Ma, X.; Liu, Y.; Lei, H.; Shi, J.; Ruan, R., Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production. Applied Energy 2013, 107, 255-263.
- Min, M.; Hu, B.; Mohr, M. J.; Shi, A.; Ding, J.; Sun, Y.; Jiang, Y.; Fu, Z.; Griffith, R.; Hussain, F.; Mu, D.; Nie, Y.; Chen, P.; Zhou, W.; Ruan, R., Swine Manure-Based Pilot-Scale Algal Biomass Production System for Fuel Production and Wastewater Treatment—a Case Study. Applied biochemistry and biotechnology 2013, 1-17.
- Wang, Z., Ma, X., Zhou, W., Min, M., Cheng, Y., Chen, P., Shi, J., Wang, Q., Liu, Y., Ruan, R. 2013. Oil Crop Biomass Residue-Based Media for Enhanced Algal Lipid Production. Applied Biochemistry and Biotechnology. 171(3): 689-703
- Zhou, W., Min, M., Hu, B., Ma, X. Liu, Y., Wan, Q., Shi, J., Chen, P and Ruan, R. 2013. Filamentous fungi assisted bio-flocculation: an efficient and low-cost technique for harvesting heterotrophic and autotrophic microalgal cells. Sep. Purif. Technol. DOI:10.1016/j.seppur.2013.01.030
- Du, Z., Hu, B., Ma, X., Cheng, Y., Liu, Y., Lin, X., Chen, P., and Ruan, R. 2012. Catalytic pyrolysis of microalgae and their three major components: carbohydrates, proteins, and lipids. Bioresource Technology. DOI: http://dx.doi.org/10.1016/j.biortech.2012.12.115

- Du, Z., Mohr, M., Ma, X., Lin, X., Liu, Y., Zhou, W., Chen, P., and Ruan, R.. 2012. Hydrothermal pretreatment of microalgae for pyrolytic bio-oil production. Bioresource Technology, 120:13-8. doi: 10.1016/j.biortech.2012.06.007
- Hu, B., Min, M., Zhou, W., Li, Y., Mohr, M., Cheng, Y., Lei, H., Liu, Y., Lin, X., Chen, P., Ruan, R. 2012. Influence of exogenous CO_2 on biomass and lipid accumulation of microalgae *Auxenochlorella protothecoides* cultivated in concentrated municipal wastewater. Applied Biochemistry and Biotechnology. 166(7):1661-73
- Zhou, W., Hu, B., Li, Y., Min, M., Chen, P and Ruan, R. 2012. Mass cultivation of microalgae on animal wastewater: a sequential two-stage cultivation process for biofuel feedstock and omega-3 rich animal feed production. Appl. Biochem. Biotechnol.168: 348-363.
- Zhou, W., Li Y, Min M, Hu B, Zhang H, Ma X, Cheng Y, Chen P, Ruan R. (2012) Growing Wastewater-born Microalga Auxenochlorella protothecoides UMN280 on Concentrated Municipal Wastewater for Simultaneous Nutrient Removal and Energy Feedstock Production. Appl Energ. 98: 433-440.
- Zhou, W., Min, M., Li, Y., Hu, B., Ma, X., Cheng, Y., Liu, Y., Chen, P, and Ruan, R. 2012. A Hetero-photoautotrophic Two-stage Cultivation process to Improve Wastewater Nutrient Removal and Enhance Algal Lipid Accumulation. Bioresour Technol. 110, 448-455.

VIII. REPORTING REQUIREMENTS: Periodic Work Program progress reports will be submitted not later than 6/30 and 12/31 of each year. A final Work Program report and associated products will be submitted between December 31 and January 31, 2014 as requested by the LCCMR.

Attachment A: Budget Detail for 2010 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: *Algae for Fuels Pilot Project*

Project Manager Name: *Roger Ruan*

Trust Fund Appropriation: $ *900,000*

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2010 Trust Fund Budget	**Result 1 Budget:**	**Revised Result 1 Budget 04/11/2013**	**Amount Spent** *(01/31/14)*	**Balance 01/31/14**	**Result 2 Budget:**	**Revised Result 2 Budget 04/11/2013**	**Revised Result 2 Budget 01/31/14**	**Amount Spent** *(01/31/14)*	**Balance (01/31/2014)**	**Result 3 Budget:**	**Revised Result 3 Budget 2/28/14**	**Amount Spent** *(01/31/14)*	**Balance** *(01/31/2014)*	**TOTAL BUDGET**	**TOTAL BALANCE**				
	Pilot Scale System for Production of Algae on Wastewater				*Lab Scale Algae to Fuel Conversion Technology*					*Evaluation, Demonstration, and Outreach*									
BUDGET ITEM																			
PERSONNEL: wages and benefits	244,265	244,265	244,265.00	0.00	~~229,810~~	~~284,810~~	320,917	320,916.53	0.47	~~139,780~~	141,823	141,823.61	-0.61	707,005	-0.14				
Roger Ruan, PI, 2.5 months in the 3rd yr.								4,860.31				3,691.05							
Paul Chen, co-PI, 20%, 3yrs, including 33.3% benefits, project coordination, conducting R&D, project evaluation, progress report			7,841.92					55,437.33				49,583.77							
Dean Current, co-PI, 10%, 3yrs, including 33.3% benefits, conducting R&D, economic and environmental life-cycle analysis								32,805.00											
Postdoc 1, 100%, 3yrs, including 20.22% benefits, conducting R&D, operations, demonstration, data analysis			39,604.85					37,552.11				36,394.00							
Postdoc 2, 100%, 3yrs, including 20.22% benefits, conducting R&D, operations, demonstration, data analysis			23,152.51					74,688.76				27,974.66							
Graduate Research Assistant 1, 50%, 3yrs, including 16.84% benefits and tuitions, conducting R&D, operationg, demonstration			72,761.06					56,623.14				10,584.47							
Graduate Research Assistant 2, 50%, 3yrs, including 16.84% benefits and tuitions, conducting R&D, operationg, demonstration			89,540.90					58,949.88				13,595.66							
Undergraduate assistants			11,363.76																
CS/BU staff																			
Non-capital Equipment / Tools *(pumps, hoses, valves, control components, lights, tanks, mixers, for maintenance, repair and replacement)*	9,800	9,800	9,800.00	0.00	4,200	4,200	4,200	4,200.00	-					14,000	0.00				
Capital equipment over $3,500 *(Algal biomass conversion equipment including hydrothermal reactor system, oil extraction system, and catalytic upgrading reactors.)*	~~50,000~~	27,007	27,006.66	0.34	~~150000~~	59,993	59,993	59,993.00	-					87,000	0.34				
Supplies *(materials for making lab scale reactors for testing, nutrients, cultures, chemicals for analysis, lab supplies, consumables for analytical instruments, external analysis services)*	22,000	44,993	44,993.00	0.00	~~30,000~~	~~65,007~~	28,900	28,900.47	(0.47)	10,000	8,073	8,073.06	-0.06	81,966	-0.53				-
Travel expenses in Minnesota	4,000	4,000	4,000.00	0.00						6,145	6,029	6,028.67	0.33	10,029.00	0.33				
Other *(Describe the activity and cost) be specific*																			
COLUMN TOTAL	**$330,065**	**$330,065**	**330,064.66**	**0.34**	**$414,010**	**$414,010**	**$414,010**	**414,010.00**	**$0**	**$ 155,925.00**	**$ 155,925.00**	**155,925.34**	**-0.34**	**$ 900,000.00**	**$0.00**				