M.L. 2015 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: A novel biofilm technology for water nutrient removal
PROJECT MANAGER: Bo Hu
AFFILIATION: University of Minnesota
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2015, Chp. 76, Sec. 2, Subd. 04b

APPROPRIATION AMOUNT: $281,000
AMOUNT SPENT: $281,000
AMOUNT REMAINING: $0

Overall Project Outcome and Results

Nutrient pollutants such as nitrogen and phosphorus from urban and agricultural fields is the leading cause of water quality issues in Minnesota. We proposed a novel biofilm technology to remove nutrients such as nitrogen and phosphorus from water, based on a concept of a "simulated lichen biofilm", mimicking the natural symbiotic lichen ecosystem, for efficiently removing and recovering nutrients and pollutants, by introducing a supporting matrix, binding filamentous fungal strains and microalgae. Different strain combinations, types of wastewater, reactor designs, and operational parameters were investigated. After laboratory scale experiments, the pilot demonstration was tested at the Sarita Wetland close to Saint Paul Campus of UMN and the pond next to the Frank and Sims Yard Waste Collection Site in East Saint Paul. Based on the results from the porotype model testing using a rotating paddle wheel design in Sarita wetland, we can conclude that the biofilm can be operated between 96-120 h with P removal efficiency of 80 %, N removal efficiency of about 66.2% and COD removal efficiency of about 74%, and needs replacement of biofilm for the next batch of operation. More future work is needed to address some technical challenges as it is applied in the field, including the competition from local microalgae in the wastewater, very effective in heavily polluted water while not effective with much diluted water nutrient pollution, and the biofilm as a food attraction to many insects, leading to the disintegration of biofilm. The technology developed from this project will contribute to a solution for both rural and urban communities to handle water sites polluted by nutrients. When communities can effectively manage their nutrient pollution in water systems, public health and the environment are adequately protected while the community has the management structure in place over the long-term.

Project Results Use and Dissemination

Even though a final applicable solution is still in needs of more research and development, we have presented our research in many national and local conferences, several publications either in press or in submission.

We published three journal articles and made a list of presentations to disseminate our research results and the Environment and Natural Resources Trust Fund was acknowledged at each of the presentation and paper publications. We also reached large amount of undergraduate students and high school teachers via the teaching module developed form this project. The project generates some excitement

from both the scientific community and industry. The technology developed from this project, together with the information obtained from the techno-economic analysis, can be beneficial to local communities to eventually find a solution for nutrient pollution issues. Besides the academic dissemination, a video of showcasing the pilot-scale testing system at Sarita Wetland will be posted on the group website for general public access. Below are the list of papers and publications and we are preparing for another two manuscripts for peer-reviewed publication.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2015 Work Plan

Date of Report: July 1, 2018

Date of Next Status Update Report: July 1, 2018

Date of Work Plan Approval: June 11, 2015

Project Completion Date: June 30, 2018

Does this submission include an amendment request? No

PROJECT TITLE: A novel biofilm technology for water nutrient removal

Project Manager: Bo Hu

Organization: University of Minnesota

Mailing Address: 1390 Eckles Ave

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Telephone Number: (612) 625-4215

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Location: The experiment will be primarily done at Biological Agricultural Engineering Building (BAE) 320, 1390 Eckles Ave, St Paul, MN, 55108. The impact of the project will be statewide

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Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$ 281,000**
	Amount Spent:	**$281,000**
	Balance:	**$ 0**

Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 04b

Appropriation Language:
$281,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to develop a simulated lichen biofilm system that can be used to remove pollutants and recycle nutrients from storm water runoff and polluted lakes, ponds, and lagoons. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2018, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: A novel biofilm technology for water nutrient removal

II. PROJECT STATEMENT:

Non-point source pollution is the leading cause of water quality impairment in Minnesota. Excessive loading of nutrient from stormwater runoff at urban and agricultural landscapes results in eutrophication and encourages the growth of invasive species. We propose a novel biofilm technology to remove and possibly recover nutrients such as nitrogen and phosphorus from water and the process will also remove pollutants such as heavy metal ions (lead, zinc, copper, arsenic etc.) and pesticides. This new technology will be developed based on the concept of a "simulated lichen biofilm", mimicking the natural symbiotic lichen ecosystem, for efficiently removing and recovering nutrients and pollutants, by introducing a supporting matrix, binding filamentous fungal strains and microalgae. The project will develop this technology through the lab experiments and evaluate its effectiveness on the Sarita wetland close to UMN St Paul campus.

Conventional practices use sedimentation methods to remove suspended pollutants; whereas dissolved nutrients (phosphorous and nitrogen), organics, pesticides and heavy metals (lead, zinc, copper, arsenic) require more complicated approaches to remove them from the runoff. Lichen, a natural ecosystem with phototrophic algae and heterotrophic fungi symbiotically growing on the solid surface of rock or roof, is not readily applied in engineering field due to their low growth rate. A concept of "simulated lichen system" is recently developed by our UMN research group that we can select different desired microalgae and fungal combinations that will be growing on the surface of some specific polymers to form the biofilm. We are proposing to study this concept and apply to remove and even possibly recover nutrients from water. Microalgae are naturally growing on the surface of the nutrient-rich water; however, biological treatment of polluted waters using microalgae is limited by problems associated with the settling and separation of algae downstream of the treatment site. The proposed methodology using bioaugment filamentous fungi in lichen biofilms overcomes this limitation, by efficiently retaining algae and recovering the nutrients and heavy metals with possible recycling of useful nutrients. The simulate lichen biofilm will also grow much faster than aquatic plants for removing nutrients.

Our preliminary research has shown that filamentous fungi and fresh water microalgae can naturally be grown attached on some specific bio-based polymers to form the "simulated lichen biofilm". Both types of cells can accumulate phosphorous, nitrogen and toxic heavy metals, and pollutants will be removed by removing this biomass from water. The development of a stable lichen biofilm will have multiple benefits over the current available technologies. This composite will have the capacity to remove the pollutants in a wide concentration range, and to possibly recycle valuable and non-renewable nitrogen and phosphorous. The technology can be incorporated into current practices for protecting our water bodies. A successful on-site demonstration of the prototype would transform the storm water runoff treatment, in polluted lakes, pond, and lagoon.

The project will develop this technology through the lab experiments with the samples from urban runoff sites at Twin Cities Metro, the swine manure wastewater lagoon at Waseca and a heavily polluted lake at Albert Lee and evaluate its effectiveness on the Sarita wetland close to UMN St Paul campus. We hypothesis that the lichen biofilm technology will significant improve the nutrient removal and recuperation with improved bioremediation potential. The beneficial interaction between the algae and fungi in the system will possibly enhance productivity and efficiently utilize the resources. Also understanding of the fundamental aspects of algae-to-fungi interactions underlying the observed phenomena is critical to expand the process to industrial scale and for other algae-related processes.

III. OVERALL PROJECT STATUS UPDATES:

Retro-Amendment Request (01/12/2018):
We are requesting to move $20,000 from personnel to equipment/tools/supplies for the third year. With the amendment, the budget for lab supplies for the third year will increase from $6,365 to $26,365. The reason for

this increase was due to the increase of scales when we move to the field tests. We are including two urban sites for testing our system and more lab supplies are needed to have more chemicals and analysis. We are currently building a demonstration device to use a paddle-wheel attached with our mycoalgae biofilm to treat wastewater. The system will be dedicated to this project and we will continue using this system for our future work remediation project. Originally, we proposed the Sarita Wetland as the demonstration site to test our system. We took water samples from the website for over a month and our lab culture experiments showed that the nutrients level in the Sarita Wetland were not high enough to support our mycoalgae biofilm growth and treatment. We continued monitoring the water quality and eventually chose the end of August to install our system at the Sarita Wetland because that was the week for the State Fair and the wetland water carried very high nutrients from state fair ground. The system was running for one week and we reported our data in this report. However, we felt that the Sarita Wetland might not be a suitable site for relative long-term demonstration because the water was only heavily polluted during the State Fair period. Therefore, we started to monitor another wetland (as shown in the photo) next to the Frank and Sims Yard Waste Collection Site on the Phalen Blvd in east St Paul. We put our system on that site to run for about a week before the winter came and will continue our work at this site in the Spring. We are also taking water from this site to work in our lab to modify our design for the treatment. Due to the increase of water sampling and analysis, and additional operations at two sites, we overspent around $14,000 on our materials and supplies. On the other hand, we originally planned to hire an additional Post-Doc researcher to assist our large-scale demonstration. Dr. Bruce Wilson's graduate student Lori Krider has the exactly right skills we need for the project and we have her to work on the project instead of the Post-Doc. Since Lori has already finished all of her PhD courses, we paid her PhD stipend with advanced status without need to pay her tuition fees; therefore, we have saved some funds in the personal expenses. We are requesting to transfer $20,000 from personal to supplies to cover the deficit we have already overspent and preserve some extra for the continuous experiments we are currently working with.

Amendment Request (01/01/2016):

We are requesting to move $7,121 from capital expenditures to equipment/tools/supplies for the first year. With the amendment, the budget for equipment/tools/supplies will increase from $6,000 to $13,121. The reason for this increase was due to the large amount of screening we had for the microalgae and fungal strains and we need to have more chemicals and analysis.

We are requesting to move $260 from the capital expenditures for HPLC parts addition to the capital expenditures for the automatic cell counter purchase. This cell counter was originally budgeted at $9,730 in 2013 and the final purchase price was $9,990 due to the inflation.

We spent $22,619 to purchase the addition parts for the HPLC. These items were originally budgeted at $30,000 and the final purchase price was $22,619. This gives us saving of $7,381 for the capital expenditures. We are proposing to transfer the funds from this saving to cover $260 shortfall for the automatic cell counter purchase and we are also requesting to transfer the rest $7,121 to the equipment/tools/supplies.

Amendment Approved (01/20/2016)

Project Status as of January 1, 2016:

Fungal strains were isolated from the Sarita wetland (located in the southeast corner of the University of Minnesota St. Paul campus) using the serial dilution method, and the fungal isolates were identified by genetic sequencing of fungal internal transcribed spacer (ITS) regions. The nutrient (Phosphorous and Nitrogen) removal efficiency of the strains as attached growth was determined using a synthetic media. It was found that the isolate Sa7 (*Mucor hiemalis)* has better nutrient removal efficiency compared to the other isolated strains. The selection of strain combination for the lichen type biofilm with the isolated strains and the strains from the culture collection

was done. The compatibility of the model algae strain *Chlorella vulgaris* is tested with different fungal species (isolated and type strains from culture collections) and the best strain combination in terms of biofilm formation with high attachment of algae was selected. Among all the fungi tested, *Mucor* sp. was found to have high Phosphorous recovery efficiency with high algae attachment efficiency (99%). The fungi, *Mucor circinelloides UMN-B34, Mucor hiemalis (B7 and SA7),* and *Mucor Indicus-Amylomyces rouxii (ATCC)* and algae *Chlorella vulgaris, Scenedesmus obliquus* and *Selenastrum capricornutum)* was selected for biofilm formation and nutrient recovery.

Project Status as of July 1 2016:

After cultivation tests with different microbial combinations, *Chlorella vulguris* and *Mucor circinelloides UMN B34* were chosen for their complete attachment and better Nutrient removal efficiency in the synthetic medium; and the polypropylene spun and tape yarns woven into a dimensionally stable matrix was chosen as the attachment matrix for the lichen biofilm formation. We found that adding 2 g/L of sugar in the beginning of the cultivation showed the best biomass production and nutrient removal. Different cultivation conditions were tested and several significant factors were determined for the nitrogen and phosphorus uptake by the microalgae and fungi. The batch cultivation experiments showed the process feasibility and provided solid foundation for us to move to the pilot scale experiments.

Project Status as of January 1, 2017:

The water samples form the Sarita wetland, lakes and lagoon waste water was collected and analyzed for the nutrient levels and the biofilm formation. The other point source nutrient pollution with different levels of phosphorous and nitrogen sources like corn ethanol wastewater and municipal waste water was also considered for the lichen type biofilm formation and nutrient removal. The biofilm growth is highly affected by the nutrient levels especially the available carbon in the medium in which it grows. The phosphorus removal was predominantly by the poly-phosphate accumulating fungi and nitrogen removal by the algae. The fungal growth requires the carbon source for the initiation of biofilm formation on the matrix to which the algae attaches. Different design configuration for the prototype as proposed were attempted for the nutrient recovery from the different sources. Submerged paddle wheel design and flow through design were the two designs developed for the nutrient recovery from different sources of nutrients.

Project Status as of January 1, 2018:

We built a large reactor to test the microalgae/fungal biofilm at UMN dairy center to treat dairy manure wastewater, and the research results showed great reduction of nutrient pollutions. We also set up our biofilm study and testing the process on the water from Frank and Sims Yard Waste Collection Site and water from dairy manure storage lagoon. For the pond at the Frank and Sims Yard Waste Collection Site, we designed a paddle-wheel system with the attachment matrix in order to form fungi and algae biofilm. The water pollution parameters seemed improved significantly with our treatment, and the biofilm formation was predominated by filamentous fungi. Further economic analysis will be needed to explore the process techno-economic feasibility.

Overall Project Outcomes and Results:

Nutrient pollutants such as nitrogen and phosphorus from urban and agricultural fields is the leading cause of water quality issues in Minnesota. We proposed a novel biofilm technology to remove nutrients such as nitrogen and phosphorus from water, based on a concept of a “simulated lichen biofilm”, mimicking the natural symbiotic lichen ecosystem, for efficiently removing and recovering nutrients and pollutants, by introducing a supporting matrix, binding filamentous fungal strains and microalgae. Different strain combinations, types of wastewater, reactor designs, operational parameters were investigated. After laboratory scale experiments, the pilot demonstration was tested at the Sarita Wetland close to Saint Paul Campus of UMN and the pond next to the Frank and Sims Yard Waste Collection Site in East Saint Paul. Based on the results from the porotype model testing using a rotating paddle wheel design in Sarita wetland, we can conclude that the biofilm can be operated between 96-120 h with P removal efficiency of 80 %, N removal efficiency of about 66.2% and COD removal efficiency of about 74%, and needs replacement of biofilm for the next batch of operation. More future work is needed to

address some technical challenges as it is applied in the field, including the competition from local microalgae in the wastewater, very effective in heavily polluted water while not effective with much diluted water nutrient pollution, and the biofilm as a food attraction to many insects, leading to the disintegration of biofilm. The technology developed from this project will contribute to a solution for both rural and urban communities to handle water sites polluted by nutrients. When communities can effectively manage their nutrient pollution in water systems, public health and the environment are adequately protected while the community has the management structure in place over the long-term.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Develop the biofilm technology through lab experiments

Description: The purpose of this task is to develop a lichen type biofilm with the algae and fungal type strains, and the native species found in the local wetlands. Effect of culture conditions on biofilm formation, cell distribution and the nutrient recuperation and pollutant removal capacity of the developed technology will be studied.

Water samples will be collected from Sarita wetland which will be used to isolate the native microalgae / fungi for the biofilm formation. Three different combinations of algae-fungal strains will be tried for the lichen-type biofilm formation. Meanwhile, we will identify one to three microalgae/fungal strain combinations suitable for both biofilm formation and growth on the local water. The fungi and algae in different ratio of cells will be co-cultured in a minimal medium with different matrix materials for cell attachment. The effect of different matrix type (ecofriendly polymer-cotton composite matrix; recycled polymer materials; lignocellulose matrix), agitation intensity, temperature and nutrient concentration on the algae-fungi cell concentration in biofilm formation will be estimated. The attachment material will be evaluated based on the degree of cell attachment, material durability, and material cost and the recycling capacity.

The biomass distribution and the nutrient recovery efficiency of the biofilm with the medium containing the nutrient and pollutant concentration mimicking the various runoffs will be tested in shake flasks. The synthetic runoff will be designed based on the chemical and physicochemical characteristics of typical runoffs. The lab-scale evaluation of the operating parameters will be carried out using a robust statistical methodology and mathematical models will be developed for the system under study. Central composite design will be implemented to estimate a second-degree polynomial model, expressing the relationship between the test variables and the response variables (cell population distribution, removal efficiency) to predict the behavior of this synthetic ecosystem.

Summary Budget Information for Activity 1:

ENRTF Budget: $106,822
Amount Spent: $ 106,822
Balance: $0

Outcome	Completion Date
1: Screening native microalgae and fungal species of the local wetlands to have better growth and nutrient removal performance in the regional climate.	Oct 2015
2: Identify algae/fungal combinations suitable for biofilm formation.	Jan 2016
3: Laboratory study of microalgae/fungal systems to remove nutrients.	Jun 2016

Activity Status as of January 1, 2016:

1.1 Screening native species of the local wetlands

Water and soil samples were collected from Sarita wetland to isolate the native fungi for the biofilm formation. Fig. 1 shows the stages in the isolation of fungi from water and soil sample collected from Sarita wetland.

a. Isolation of fungal species from Sarita wetland

Fungi were isolated from water and soil samples from Sarita wetland, using a tenfold serial dilution-plating technique on potato dextrose agar (PDA) plates into which 30 μg of penicillin-streptomycin (Penstrip) was added and was incubated at room temperature (27 ± 2°C). The soil samples (10 g) were suspended in 100 ml of sterilized saline, and subsequently 1 ml of this suspension was added to 9 ml saline to obtain desired dilutions. The culture was observed daily and fungal growth was sub-cultured onto fresh plates of PDA until pure isolates were obtained (Fig. 2). The pure cultures were then transferred to PDA slants and maintained by sub-culturing every four weeks. The isolates were screened for the Phosphorous and Nitrogen removal capacity for them to be used in the future experimental plan. The morphological characteristics of the pure cultures isolates were observed under microscope and identified using gene sequencing.

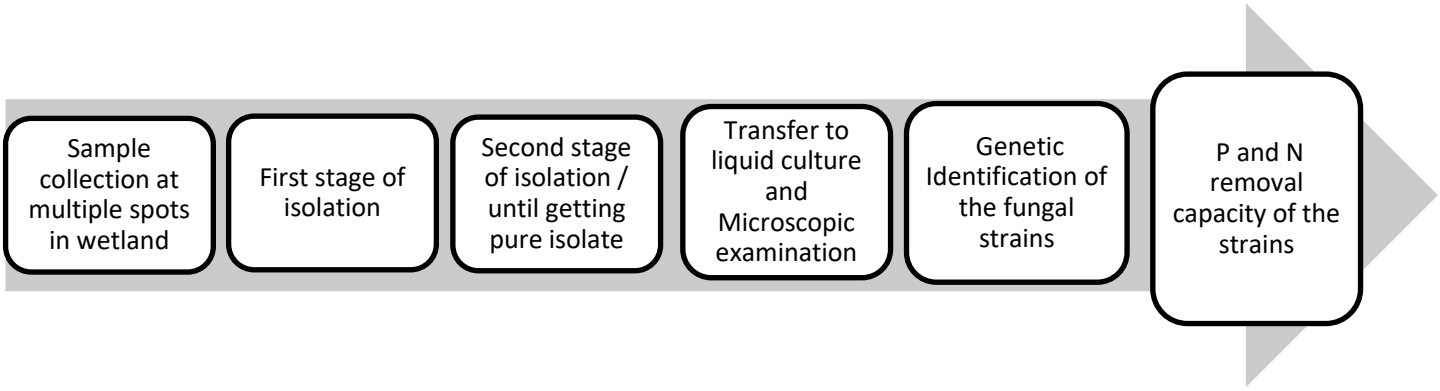


Fig. 1 Stages in the isolation of fungal cultures for nutrient removal studies

b. Sequencing for strain identification

Fungal isolates were identified by genetic identification of fungal internal transcribed spacer (ITS) regions. The genomic DNA of the isolated strain was extracted with the E.Z.N.A.®HP Fungal DNA kit. Amplification of ITS region (ITS1, 5.8s rRNA and ITS2) was performed using the universal primer ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATT GATATGC). PCR reactions were performed with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 1 min, and 72 °C for 1 min, with final extension at 72 °C for 10 min. Amplified products were purified by E.Z.N.A.® Gel Extraction Kit and then sequencing results were analyzed by NCBI blast.

The fungal isolates were found to be:

- *Antrodia hingganensis (isolate X1283) –SA1*
- *California Fungi—Amanita vernicoccora – SA2 and SA11*
- *Trametes versicolor –SA3 and SA12*
- *Irpex lacteus – SA5*
- *Gloeophyllum trabeum – SA6*
- *Mucor hiemalis –SA7*
- *Plectosphaerella cucumerina – SA8*
- *Fusarium solani – SA9*
- *Amyloporia Sinuosa –SA 10;* SA13 – unidentified strain

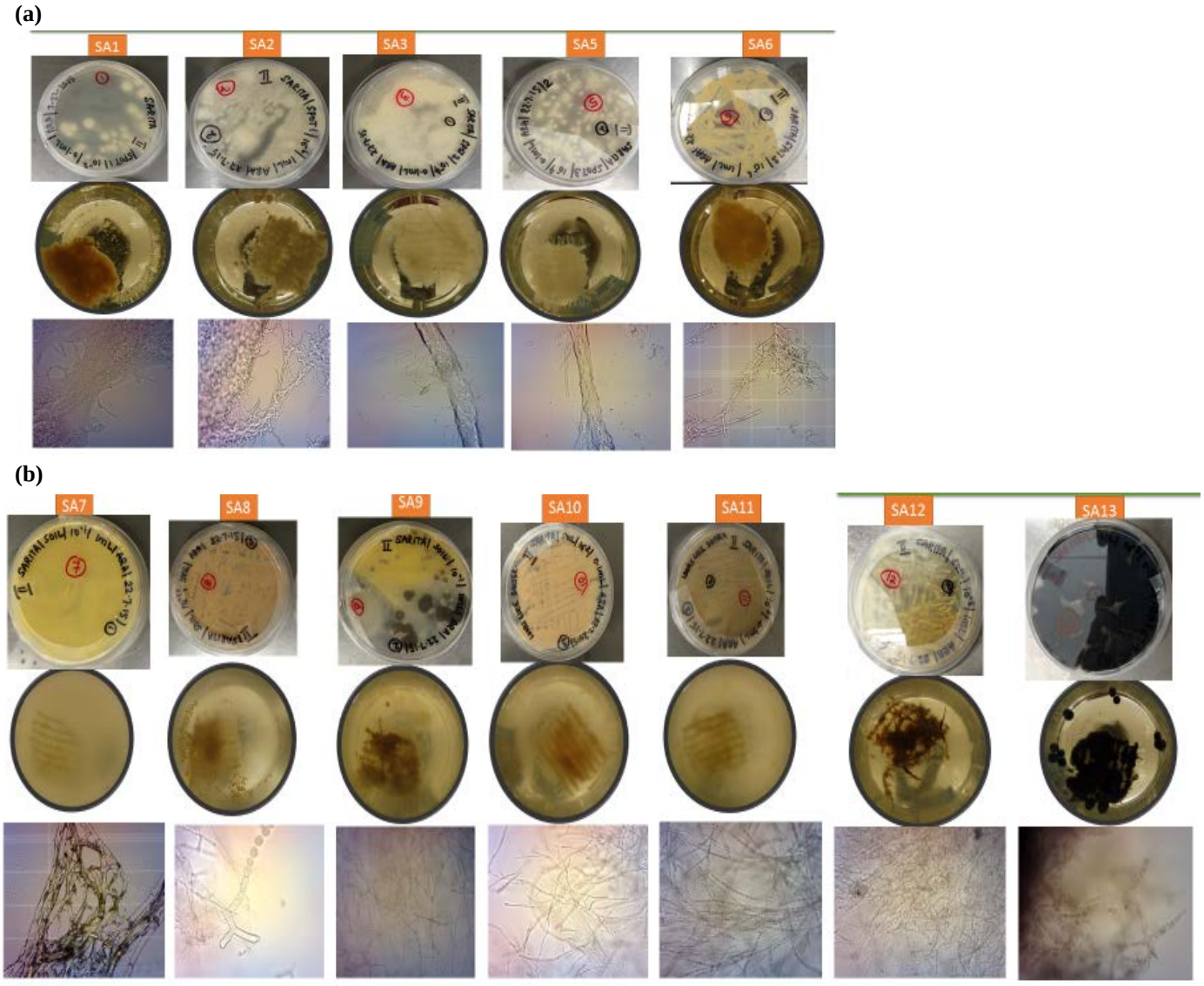


Fig. 2 Morphology of the isolated strains in Potato dextrose agar medium and the respective attached growth and microscopic pictures of the fungal strains. (a) Fungi isolates from the water samples (b) Fungi isolates from the soil samples.

c. Nutrient removal efficiency of the isolates

Since the biofilm will be used for nutrient recovery from an array of samples (two urban runoff sites at Twin Cities Metro, the swine manure wastewater lagoon at Waseca, a heavily polluted lake at Albert Lee and a possible site at Northern MN), a synthetic medium for the cell growth with the initial P (40 mg/L), N (200 mg/L) and other nutrients were used as the medium for the biofilm development. Attached fungal morphology of the isolated strains and the microscopic images of the fungi at 40x magnification was shown in Fig 3. Maximum Phosphorous and Nitrogen removal was observed in strain Sa7 (*Mucor hiemalis),* which will be considered for the biofilm formation with algae.

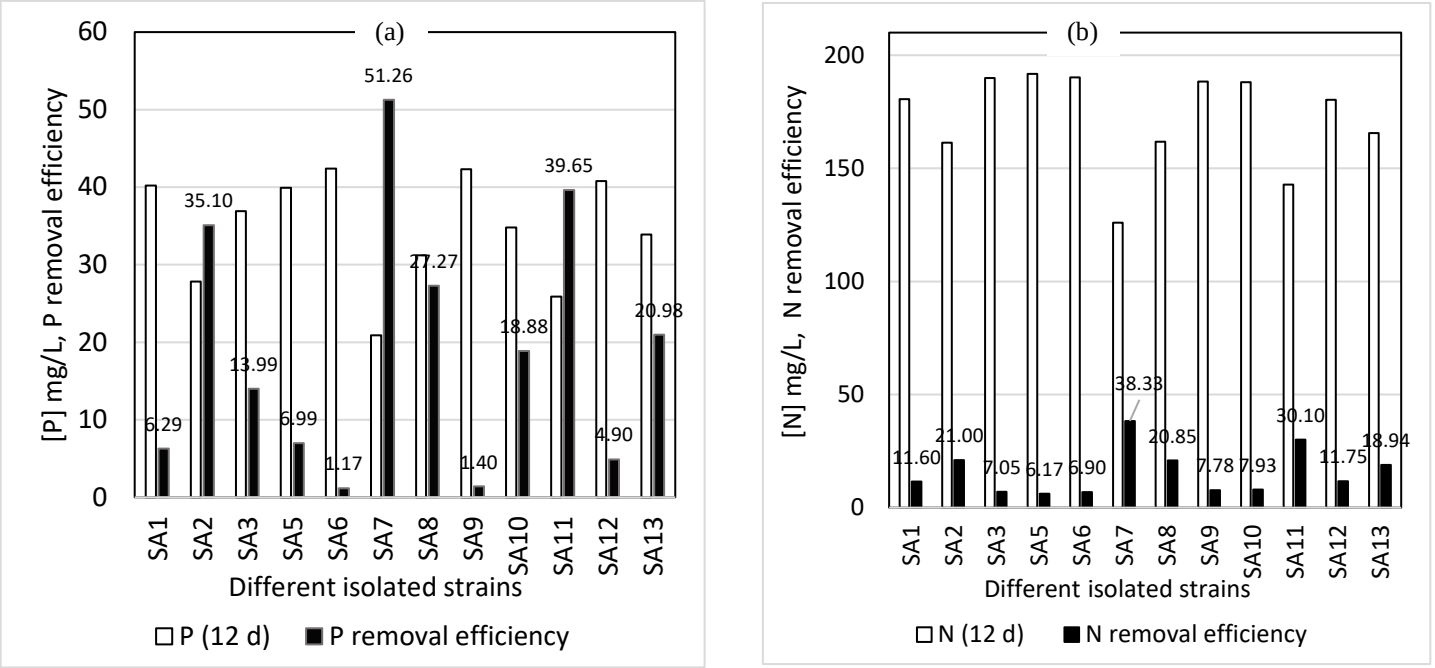


Fig. 3 Nutrient removal efficiency [a. Phosporous recovery b.Nitrogen recovery] of the different isolated fungi from sarita wetland

d. Algae selected for the biofilm formation

The common algae species (*Chlorella vulgaris, Scenedesmus obliquus, Selenastrum capricornutum)* given in Fig.4 was chosen for the biofilm formation with the fungal species isolate and the fungi species obtained from the culture collection (UTEX).

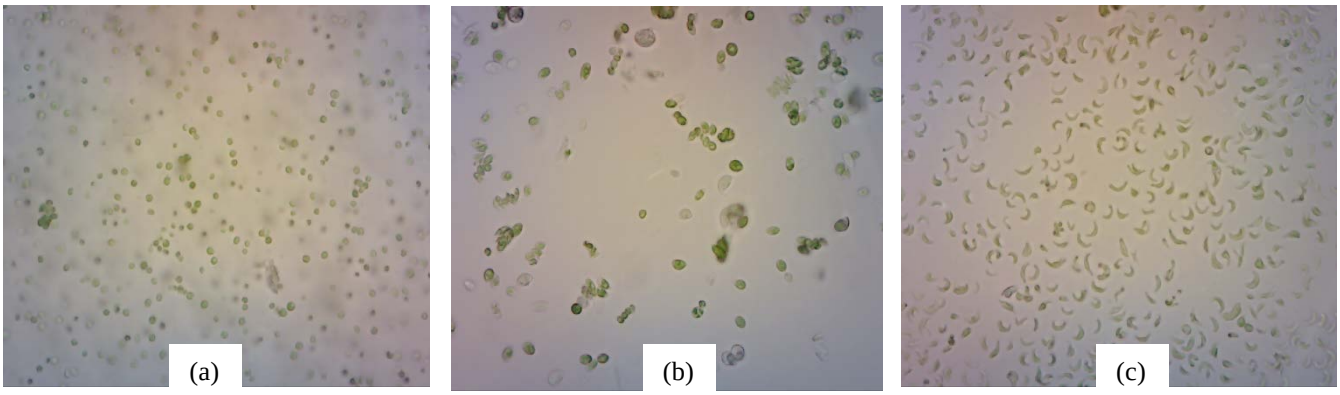


Fig 4. Different common algae species selected for the biofilm formation *(a) Chlorella vulgaris (b) Scenedesmus obliquus (c) Selenastrum capricornutum*

1.2: Identify algae/fungal combinations suitable for biofilm formation

The attachment of algae *Chlorella vulgaris, Scenedesmus obliquus* and *Selenastrum capricornutum* with various fungal cultures was tested to identify the best strain combination for biofilm formation and high algae attachment efficiency. To screen the best fungal strain, the algae *Chlorella vulgaris* was cultured with different fungal strains. The fungal strains used in our study were *Mucor circinelloides* UMN-B34; *Fusarium equiseti (A11); Fusarium lacertarum (A13); Nigrospora oryzae (A16); Altermaria alternate (A20); Fusarium equiseti (B5); Mucor hiemalis (B7); Mucor hiemalis –SA7, Mortierella isabellina (MI)* and *Aspergillus niger* Ted S-OSU (ATCC). Among the different fungal species tested with the algae *Chlorella vulgaris,* the algae attachment efficiency vary from 51% to 99.9 %

depending upon the type of fungal strain tested. The symbiotic biofilm was complete and all the algae cells are attached to the *Mucor sp*. with high harvest efficiency (99%), especially with the *M. circinelloides* in a synthetic medium (glucose concentration of 2 g/L) and agitation intensity of 150 rpm at 27 °C.

As the *Mucor sp.* shows better results in terms of biofilm formation with algae, three different *Mucor* species (*Mucor circinelloides UMN-B34, Mucor hiemalis (B7 and SA7), and Mucor Indicus Amylomyces rouxii (ATCC*)) was selected for the biofilm formation with *Chlorella vulgaris, Scenedesmus obliquus* and *Selenastrum capricornutum.* These strain combination of *Mucor* species with algae selected (Table 1), will be used for the further nutrient recovery studies in a simple media.

Table 1: Algae-fungi combination for the biofilm formation and Nutrient removal studies

S. No	Algae strain	Fungal strain	Biomass distribution in the lichen biofilm	Removal efficiency and rate		
				Nutrient	Pesticide	Toxic metals
1	*Chlorella vulguris*	*Mucor circinelloides UMN B34 / Mucor Indicus*	48.5% algae and 51.46% fungi	P:90-95% N:84% This strain combination is selected for further study		
2	*Scenedesmus obliquus*	*Mucor hiemalis (B7 and SA7)*	The removal efficiency of SO is comparable to CV. Fungi B7 and SA7 grows as yeast cells and the attachment of B7 /SA7 is not complete.	Not selected for further study		
3	*Selenastrum capricornutum*	*Mucor Indicus*	Biofilm formation is not complete and the algae is highly sensitive to the culture conditions.	Not selected for further study		

Activity Status as of July 1 2016:

1.3 Laboratory study of microalgae/fungal systems to remove nutrients

The compatibility of the *Chlorella vulguris, Scenedesmus obliquus, Selenastrum capricornutum* is tested with different *Mucor* species and the best strain combination in terms of biofilm formation with high attachment of algae and biomass production was selected for this study. Among the different combinations chosen from the previous study, *Chlorella vulguris-Mucor circinelloides UMN B34* shows complete attachment and better nutrient removal efficiency in the synthetic medium tested (Medium A). Medium (A) contained (g L^{-1}) Glucose 2-variable; KNO_3 1; KH_2PO_4 0.075; K_2HPO_4 0.1; $MgSO_4.2H_2O$ 0.5; $Ca(NO_3)_2.4H_2O$ 0.0625; $FeSO_4.7H_2O$ 0.01; Yeast extract 0.5; Trace metal solution 1 ml L^{-1}. The trace metal solution contained (mg L^{-1}): H_3BO_3 2.86; $Na_2MoO_4.2H_2O$ 0.39; $ZnSO_4.7H_2O$ 0.22; $MnCl_2.4H_2O$ 1.81; $CuSO_4.5H_2O$ 0.079; $Cu(NO_3)_2.6H_2O$ 0.049.

Among the different matrix materials tested including polymers, polymer-cotton composite, cotton mesh, metal coil (helically wound extension springs) and stainless steel mesh matrix were evaluated for the lichen biofilm development. The fungal strains exhibit different levels of attachment from no attachment in smooth surfaces either in polymer or metal surface to complete attachment in tape yarn mesh matrix, whereas pure cultures *C. vulgaris* cells shows poor or no attachment in any of the matrix tested. Complete attachment and better cell growth was witnessed in the polypropylene spun and tape yarns woven into a dimensionally stable matrix with the culture solution becoming clear after the attachment, which was chosen for rest of the study based on better cell growth, cost, and reusability.

The carbon source concentration and the process duration have a significant effect on the algae attachment to the fungal mycelium and lichen biofilm (*C. vulgaris and M. circinelloides*) formation. The effect of different initial glucose concentration was studied at concentrations ranging from 2 g/L to 10 g/L keeping all the other conditions constant as given in the methods. The algae attachment efficiency is high (>99%) at the low levels of initial carbon (2 g/L) and when nutrient availability is low especially phosphorous. The algae attachment efficiency drops from 99 % to 20 % as the glucose concentration increases from 2 g/L to 10 g/L. With the absence of carbon source (0 g/L), the fungal growth was considerably affected which hampers the initiation of fungal biofilm formation and subsequently affects the algae attachment. Also, the algae harvest efficiency varies during the process duration depending upon the individual cell growth rate and environmental conditions. At glucose concentrations higher than 4 g/L the attachment efficiency decreased considerably, which could be due to the fact that the algae concentration in the suspension increases with the increase in glucose concentration and outcompetes the fungal growth. It is also possible that at high concentrations of carbon, the dependency on each other reduces and does not favor the algae and fungi to grow together as attached lichen type biofilm, which implies that the lichen formation is favored at nutrient limiting conditions with a possible mutualistic relationship. With 2 g/L of initial glucose in the medium, the total soluble phosphorous dropped with the fungal growth but increased slightly at the end of the process period, when algae attachment reaches the maximum. Under these conditions, the algae experienced significantly increased growth, implying that the algae have the capacity to utilize the polyphosphate accumulated by the fungal cultures, as the P was insufficient in the solution to support the further microalgae growth. The release of phosphorous may also be due to the fungal cell lysis as there is not enough carbon to support the cell maintenance at the condition tested.

Lab scale Flask cultures

The experiments were conducted in 250 mL Erlenmeyer flasks with 100 mL of the synthetic waste water medium (containing different concentrations of P and N) and a submerged supporting matrix for biofilm formation. The medium was adjusted to an initial pH of 6.8 using 2 mol/L HCl or 1 mol/L NaOH (pH meter Oakton, SN 153400, Malaysia), and heat sterilized along with the matrix. The culture medium was inoculated with the co-cultures of fungal spores and algae cells at a ratio of 1:300 (Initial algae count: 2.50×10^9 cells) unless otherwise specified and incubated in an orbital sharer at 150 rpm and 26 ºC in the presence of continuous light for the entire cultivation period of about 8 days. Aliquots of samples from the cultivation broth were withdrawn at regular time interval for glucose analysis and cell count of suspended algae without much change in the culture volume to maintain constant oxygen transfer. The suspended cells were separated from the medium by centrifugation for 15 min at 4 ºC and 5030 *g* and filtered through 0.45 µ for residual glucose analysis and phosphorous analysis. The total phosphorous content in the culture liquid was measured using Hach analysis kits (Hach Company, Loveland, CO), following the standard protocol described in the kit manual. Control experiments with axenic cultures were also performed at the same conditions tested for the co-culture experiments. After the completion of biofilm formation or observing the complete attachment of algae in approximately 8 days of culture, the lichen-type biofilm is removed from the flask and analyzed for biomass distribution. Weight ratios of the wet to dry samples were measured to calculate the amount of dry biomass taken for chlorophyll analysis. Pictures were taken at different stages of the cell culture and biofilm formation with a digital camera (DSC-T20, Sony).

Biomass distribution and harvest efficiency

The microalgae cell numbers in the supernatant was measured after diluting the supernatant multiple times until the cell numbers can be counted under microscope (Cellometer Auto X4, Automated Cell Counter, Nexcelom Bioscience). Algal biomass in the biofilm samples was determined indirectly by measuring chlorophyll-a (Chl-a) concentration and determining the algal biomass using a standard chart of Chl-a concentration and dry biomass. Chl-a concentration is determined spectrophotometrically (Shimadzu UV spectrophotometer, UV-1800, Torrance, CA, USA) by homogenizing and extracting with methanol solution (90% v/v) at 650 nm and 665 nm. The chlorophyll a and algal biomass was correlated using the standard equation: Dry algae mass (mg) = 33.612 (A650nm). The fungal biomass in the biofilm was determined by the difference from the total dry weight of the biomass and the algae biomass in the matrix. The percentage of microalgae harvesting efficiency is the amount of microalgae biomass attached to the fungal biomass over the total algae biomass produced. The total biomass in the flask

cultures was determined by gravimetric method (oven-dried overnight at 105°C), with the weight of the matrix excluded. The lichen biofilm was viewed using a digital microscope (National DC5-163) connected to a computer using Motic Images plus 2.0 software. Glucose concentration was estimated using HPLC.

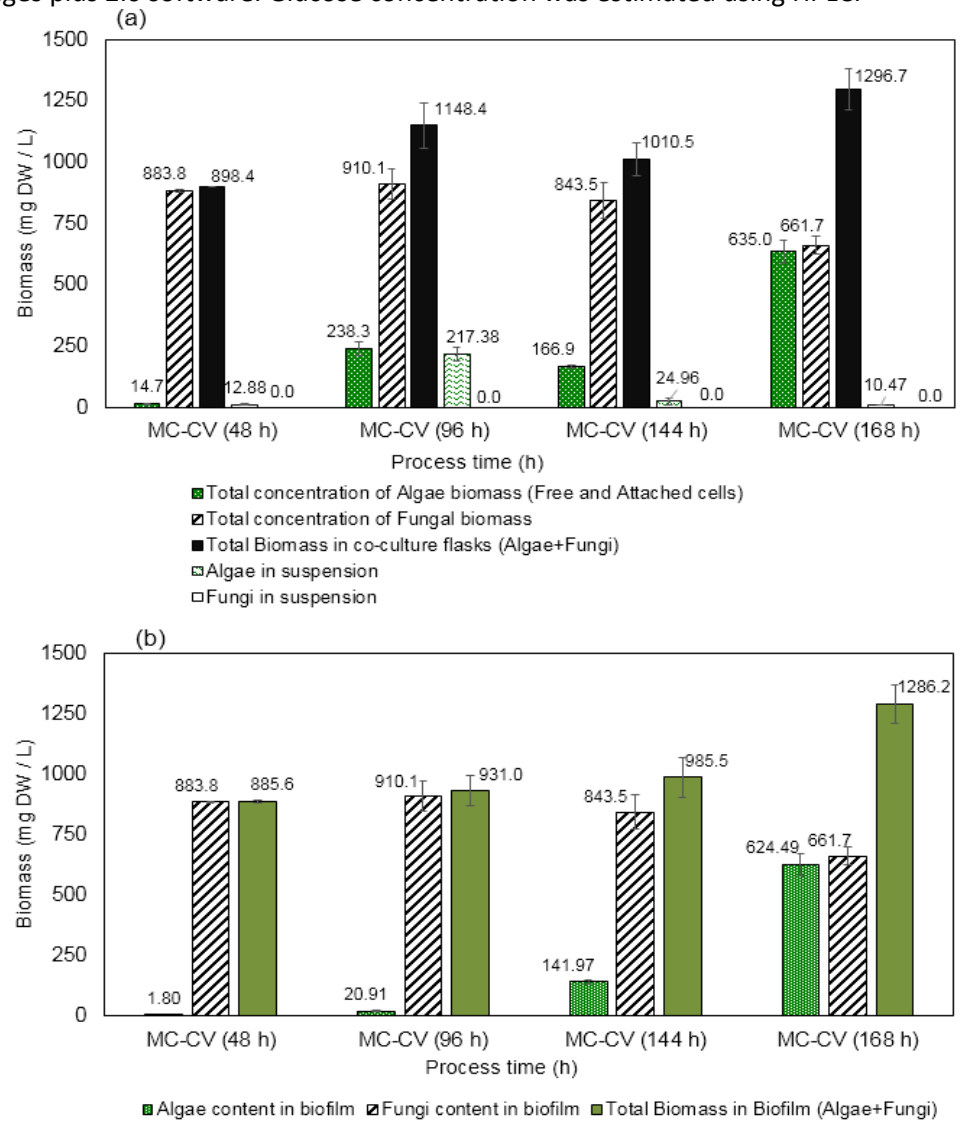


Fig. 5. Kinetic profile of the cell cultures of *Mucor circinelloides* UMN-B34 with *Chlorella vulgaris* attached on a polymer matrix (a) Total Biomass Distribution in the co-culture flasks (b) Biomass composition of the lichen biofilm. MC: *Mucor circinelloides;* CV: *Chlorella vulgaris*

The algae harvest efficiency varies during the process duration depending upon the individual cell growth rate and environmental conditions. To understand the growth profile of the individual cells in the biofilm and their nutrient requirements, measurements were made on the total biomass distribution in the co-culture flask, biofilm composition (Fig. 5), glucose and other nutrient concentrations. In co-culture flasks (*C. vulgaris* and *M. circinelloides*), the growth of fungi was predominant during the initial phase and the glucose was completely depleted within the initial 48 h. No suspended cell growth was evident in the liquid, so the total biomass was almost the same as the attached biomass until 48 h. The inoculated alga was completely attached to the fungal biomass at 48 h of the process. After 48 h, algae start to grow in liquid suspension overcoming the initial inhibition by the fungal cells and metabolites, possibly high CO_2 release as the growth rate of fungi was more during this period. Since the added carbon (2 g/L) was completely utilized within 48 h, mostly utilized by the fungal cells, it can be presumed that the algae growth after 48 h of the process was predominantly by photoautotrophic mode and partially by the chemicals or exudates released by the fungal biofilm in the liquid. The algae cells in the suspension increases after 48 h and biomass in the matrix also increases gradually. At this stage the attachment

efficiency was low due to the high concentration of algae in suspension. At 144 h, all the algae produced become attached to the fungal matrix and the algae attachment efficiency was about 99% and above. At the end of the process, the total biomass concentration is 1296.7 mg/L (Fig 5a) and 99.2% of the biomass is attached as biofilm in the matrix, with the biofilm composition being 48.5% algae and 51.46% fungi (Fig. 5b). The culture medium looks clear, which can be used for the next batch of cultures. Preliminary experiments with the recycled water show that the water can be recycled up to three cycles with nutrient addition, without affecting the total biomass production.

The pH of the culture medium gradually increases from 6.4 to 8.3 which may be due to the growth of algae and photosynthesis. The dissolved oxygen concentration of the medium and the pH is usually regulated by the algal photosynthesis and fungal growth; and the net addition or removal of carbon dioxide. Rapidly growing algae remove CO_2 from the water during photosynthesis, which increases the pH. The constant increase of pH with time after the complete attachment also shows that the algae cells are growing on the fungal surface. Since the initial glucose concentration used was 2 g/L, the organic acid production by fungi was not perceptible, as observed with the gradual rise in the pH. There was a sharp decline in the phosphorous concentration from 35 mg/L to 3.36 mg/L within 48 h, which shows the P accumulating capacity of *M. circinelloides*. The cellular phosphorous content of the total biomass was approximately 3.6% at 48 h, in which the *M. circinelloides* contribution will be major as the algae cell concentration was low at this stage. At the later stages of the process the total phosphorous in the culture medium was found to increase marginally which could be the result of fungal cell lysis or may be due to the release of fungal surface phosphorous due to the algal attachment.

The nutrient removal efficiency at different N/P ratios at fixed levels of phosphorous and glucose shows that the Nitrogen availability influences phosphorus removal in the process (Fig. 6). The P removal efficiency of *Mucor* species was higher than the algae C. *vulgaris* but the N removal efficiency was higher with the algae *C. vulgaris* compared to the fungi. In the co-culture lichen biofilm flasks, the removal efficiency was comparable to the pure cultures at the conditions tested. P that has to be removed from different sources will have different concertation ranges, for instance the urban runoffs will have relatively low P, carbon and N compared to the industrial waste water especially ethanol coproducts or the manure samples which will have high concentrations of P and N. The results show that nutrient recovery of P depends on the external factors and the availability of carbon in the medium.

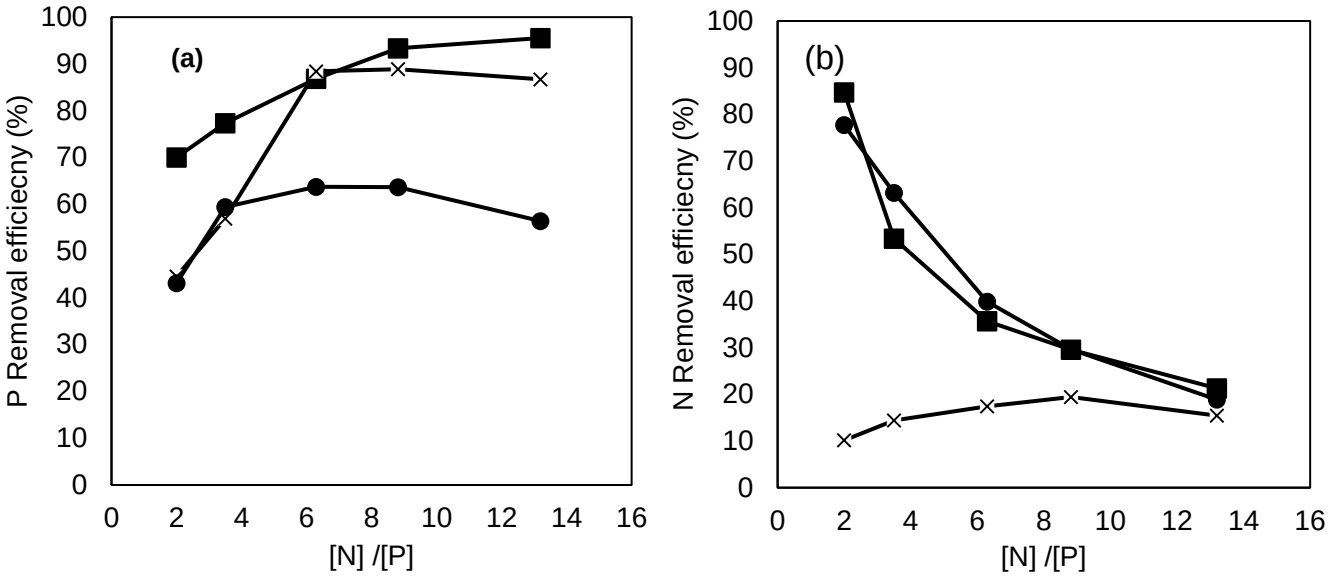


Fig. 6. Nutrient removal efficiency of *Mucor circinelloides* UMN-B34 with *Chlorella vulgaris* attached on a polymer matrix at different N/P concentration (a) P removal efficiency 72 h of the process and (b) N removal efficiency 72 h of the process

Central composite design for the process optimization

The experimental plan to determine the effect of carbon availability (0.52-4.38 g/L) on the N (98 – 500 mg/L) and P (25-100 mg/L) removal was studied using a full factorial Central composite design. Multiple regression analysis of the experimental data obtained using CCD for P and N removal gave the following second order polynomial equation,

(Y_1) P removal efficiency (%) =67.52+34.4x_1-0.032 x_2-1.25x_3-5.90E-003 x_1x_2-0.12 x_1x_3+1.78E-003 x_2x_3-3.52 x_1^2 -1.067E-004 x_2^2 +3.45E-003 x_3^2

(Y_2) N Removal efficiency (%) =+65.79+8.14 x_1-0.315x_2-0.123 x_3-0.0387 x_1x_2-0.0157 x_1x_3-1.4E-004x_2x_3+1.99 x_1^2 +5.49E-004 x_2^2 +2.83E-003 x_3^2

Where Y_1 and Y_2 are the P and N removal efficiency; x_1 - Glucose, x_2- Nitrogen and x_3- Phosphorous

The response surface plots (Fig. 7) are used to describe the individual and cumulative effects of the variables as well as the mutual interactions between the variables on the dependent variable (P and N removal). The second-degree polynomial equation was maximized by a constraint search procedure to obtain the optimal levels of the independent variables and the predicted maximum Nutrient recovery.

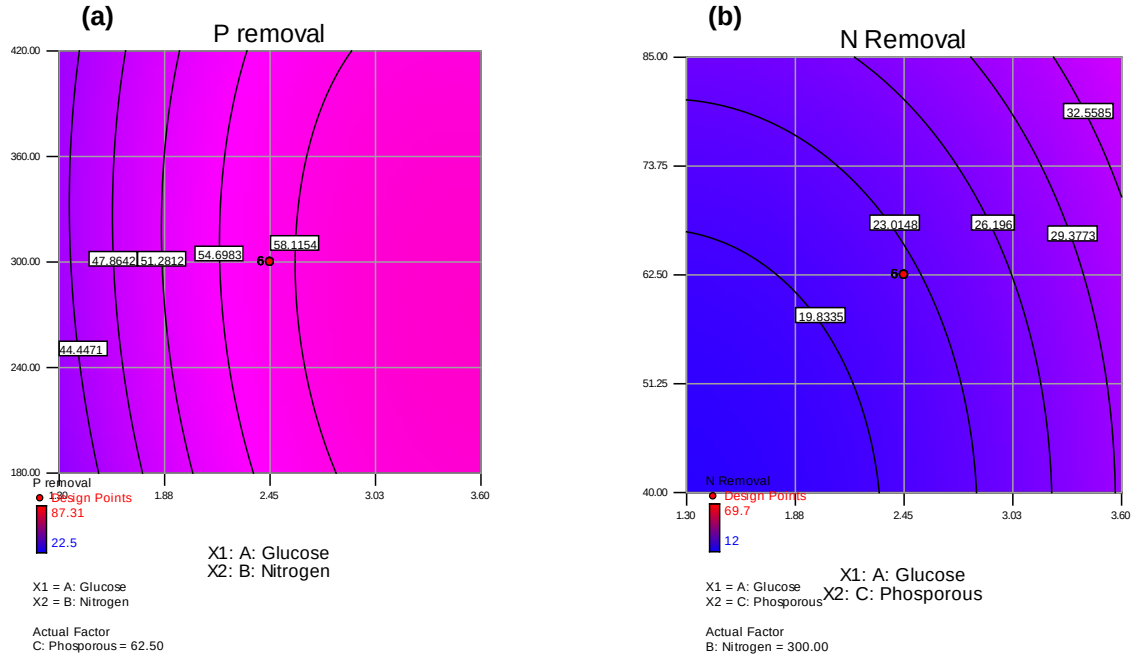


Fig. 7 Response surface contour plots of (a) P removal and (b) N removal at different levels of carbon and Nutrients with the remaining process factors held constant

For the P removal model the F-value of 14.47 implies the model is significant. For P removal with the biofilm the components glucose, phosphorus and interactive effects of Nitrogen-Phosphorous were found to be significant model terms. With R^2 value of 0.9287 and Adeq Precision value of 14.098, this model can be used to navigate the design space. The model for N removal is significant as the F-value is 5.99 and the model has the R^2 value of 0.8434. The significant parameters for N removal is the initial carbon levels and the Nitrogen concentration.

Activity Status as of January 1, 2017:

This activity is completed

Activity Status as of January 1, 2018:

Final Report Summary:
After cultivation tests with different microbial combinations, *Chlorella vulguris* and *Mucor circinelloides UMN B34* were chosen for their complete attachment and better nutrient removal efficiency in the synthetic medium; and the polypropylene spun and tape yarns woven into a dimensionally stable matrix was chosen as the attachment matrix for the lichen biofilm formation. We found that adding 2 g/L of sugar in the beginning of the cultivation showed the best biomass production and nutrient removal. Different cultivation conditions were tested and several significant factors were determined for the nitrogen and phosphorus uptake by the microalgae and fungi. The batch cultivation experiments showed the process feasibility and provided solid foundation for us to move to the pilot scale experiments.

ACTIVITY 2: PILOT SCALE ACTIVITY – Prototype design and testing with the polluted water.

Description: The samples from two urban runoff sites at Twin Cities Metro, the swine manure wastewater lagoon at Waseca, a heavily polluted lake at Albert Lea and a possible site at Northern MN will be collected and the physicochemical characteristics of the samples will be quantified. The microbial composition analysis of the samples and the possible effect on the biofilm will also be evaluated.

Prototype development for this technology through the model wetland setup with the biofilm will be developed in our laboratory with three different configurations: (1) S*ubmerged paddle wheel design:* The paddle wheel will serve dual purpose, both for mixing and as a matrix for the attachment of fungal cultures for lichen biofilm formation that will be partially immersed inside the medium. The removable paddle wheel enables harvesting the biofilm in the attached fungal cultures. These reactors will be more suitable for cultivations if mixing plays an important role in the biofilm development. (2) *Floating cascade design*: Cascades of the attached biofilm will be used as a float on the polluted waters and the movement of the cascade of biofilm will be mechanized in the model wetland-reactor system. (3) *Flow through design:* is an enclosed system with biofilm where the water movement will be facilitated to flow through an attached lichen biofilm continuously. This design will have the biofilm entrapped in the enclosure and will arrest the movement of cells.

The pilot reactor will be operated with the samples from different source under conditions as optimized previously in the flask level experiments. The effect of different polluted samples on the biofilm composition and nutrient recovery will be tested. The time of harvest should also be evaluated, as the thickness of the biofilm will have a significant effect on the process. The biomass grown on the surface needs to reach optimum thickness; then it will be harvested by scraping off, and the polymer matrix can be returned for the next cycle of growth. Microscopic examination and thickness evaluation will also be performed. The ideal biofilm should be thick enough to facilitate the cell harvest, but thin enough to allow for transfer of nutrients, byproducts, and, sometimes, light. This thickness can be affected by many factors and may be controlled by the cultivation time.

Summary Budget Information for Activity 2:

ENRTF Budget: $74,105
Amount Spent: $ 74,105
Balance: $0

Outcome	Completion Date
1: Water sample collection from multiple sites and analysis.	Sep 2016
2: Pilot reactor design construction for the application.	Jan 2017
3: Pilot study of microalgae/fungi systems to remove nutrients from different water.	Jun 2017

Activity Status as of January 1, 2016:
We start to purchase some chemicals and supplies to prepare for the activity 2.

Activity Status as of July 1 2016:
Water samples from a lake and swine manure wastewater was collected for the growth of the biofilm development. The characterization of the water is in progress.

Activity Status as of January 1, 2017:

2.1 Water sample collection from multiple sites and analysis

The lake water collected from different locations were analyzed for the nutrient and COD levels (Table 2).

Table.2 Water sample collection from multiple sites and analysis and the nutrient analysis

	Sarita Wetlands			
Test parameter	**Site 1** 	**Site 2** 	**Site 3** 	**Lake II**
pH	7.65	7.61	7.53	7.13
COD	23.7	26.2	28.5	39.1
P total	0.079	0.076	0.048	0.088
N total	0.88	0.722	1.02	1.23

	Different sources of nutrient pollution		
Test parameter	**Lagoon waste water-digested**	**Municipal waste water**	**Corn ethanol waste water**
COD	16700	500	44920
P total	93.2	21	818
N total	924	84.1	924

Batch experiments in shake flask cultures were conducted in the all the different waters with different levels of nutrients collected to test the biofilm growth and nutrient removal efficiency. The lichen biofilm formation was observed in the water samples with required amount of carbon, N/P and the samples with better light penetration for the algae to grow. For the high turbid medium like the manure lagoon water the fungal growth was predominant and the algae growth was limited as the light penetration was restricted. In the medium with very low levels of carbon, as in lake waters (Table 2) the algae growth was predominant after prolonged incubation and the fungi was not able to propagate as there was not enough carbon. So an external nutrient addition or other sources of nutrients may be required to support the fungal growth.

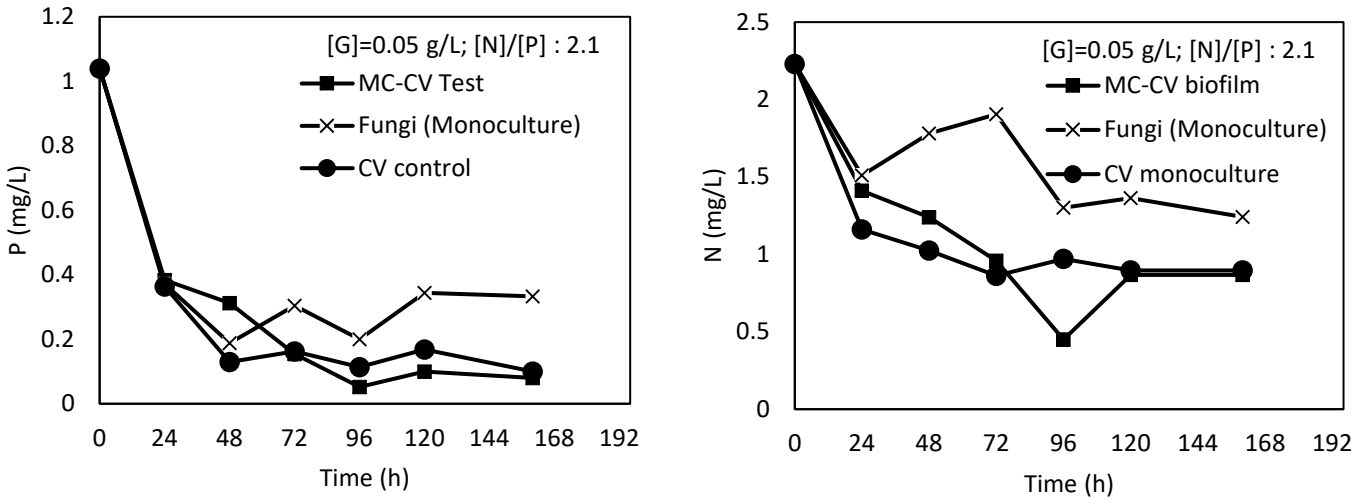


Fig. 10 Lichen biofilm growth with the simulated lake water samples

A synthetic medium was prepared to mimic the lake waters and tested the biofilm formation and nutrient removal, as the experiments with lake water turned out to have high standard deviations and results were not conclusive. As shown in Fig.10, almost 95% of phosphorus was removed in 96 h which clearly shows that the nutrient removal is by algae. Generally, at the favorable conditions of nutrient availability, the fungi dominate in the biofilm and the phosphorous removal rate was achieved at 24 h. Also, there is no significant difference in the phosphorous removal by the co-culture biofilm and the algae monoculture controls. The fungal biofilm was scarcely formed and the algae is grown in suspension than as an attached lichen biofilm. The P removal by the algae and fungal monoculture is 89% and 80% respectively. The total nitrogen removal by the lichen biofilm is 79.8 % which is significantly higher compared to 56% by algae and 41% by fungi at 96 h.

Lagoon waste water

The fungi dominate in the medium with only digested manure lagoon waste water (Fig 11a). In the lagoon waste water, the initial P concentration was 93.2 mg/L and the N concentration was 924 mg/L. The P and N removal efficiency was found to be maximum at 76.7% (10 d) and 76.4% (14 d) respectively. With the addition of external nutrients and keeping the lagoon waste water at 75%, the culture medium turned less turbid, and after the solids were attached in the fungal biomass, the algae start to grow in the biofilm as shown in Fig 11b. The initial P and N concentration increased to 425.15 mg/L and 1550.5 mg/L with the external addition of nutrients. The P and N removal efficiency was found to be maximum at 78.85% (10 d) and 80.1% (14 d) respectively. With further increase in the external nutrients (Initial P concentration of 751.1 mg/L and N concentration of 2177 mg/L) and at 50% lagoon waste water the biofilm thickness increases (Fig 11c). The P removal efficiency dropped to 63% at 10 d but the N removal efficiency remained at 80.5%. The thickness of the biofilm increases with the dilution of lagoon waste water and additional of external nutrients.

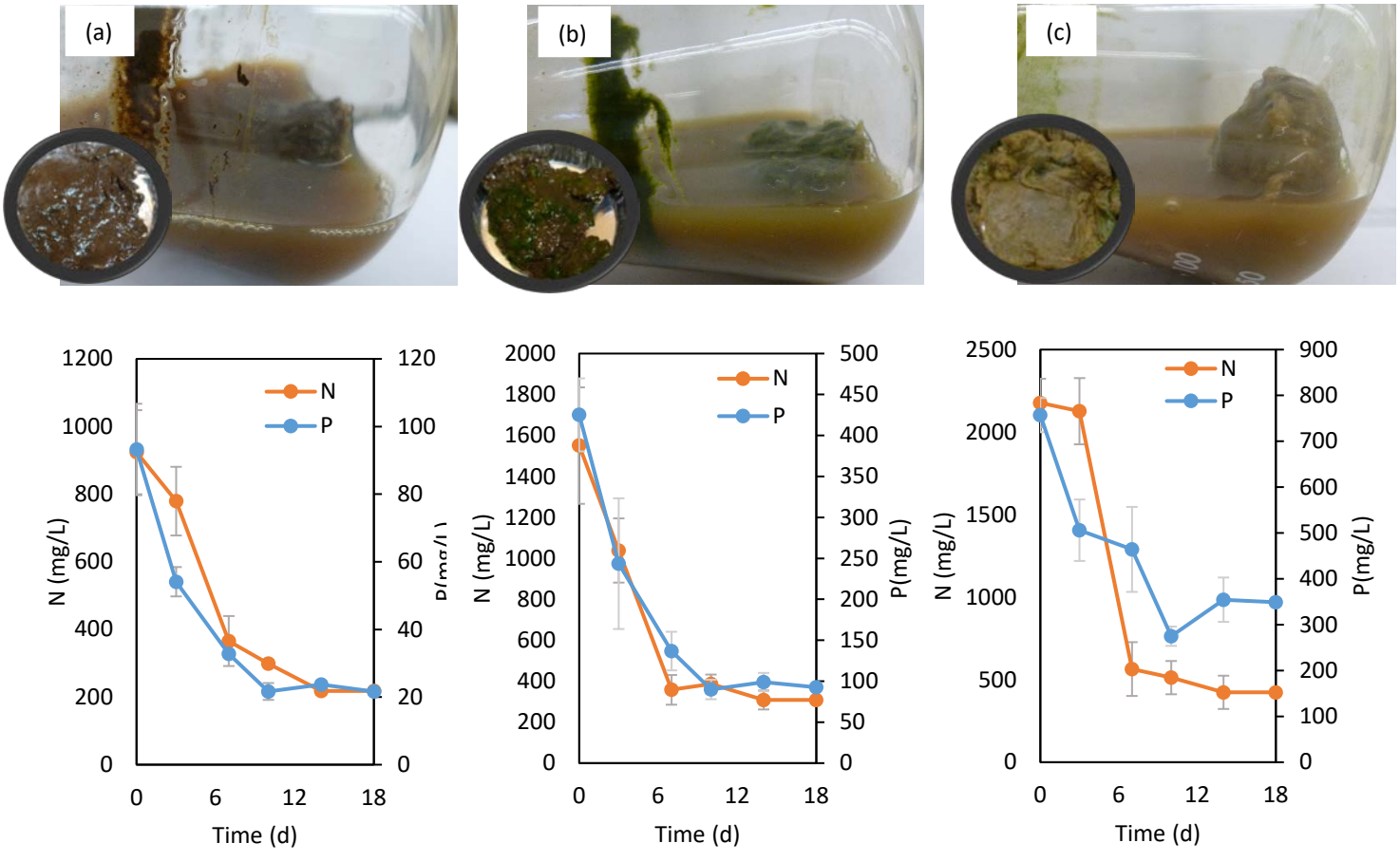
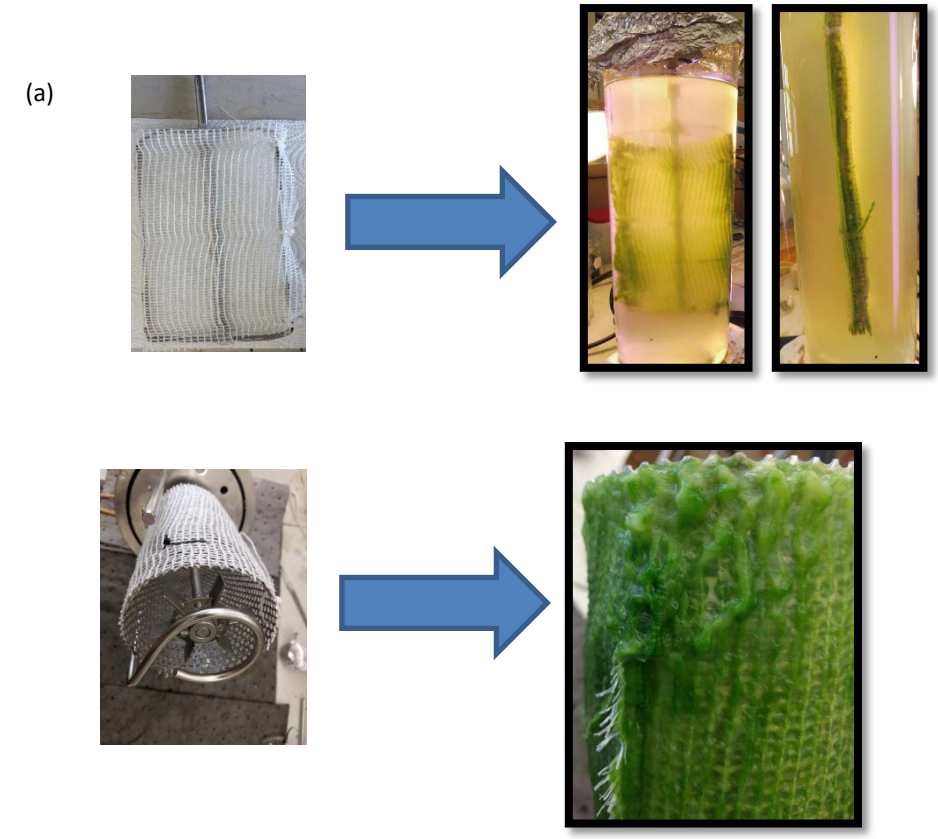


Fig. 11 Lichen Biofilm formation at various ratios of lagoon waste water and additional nutrient solution (a) 100% (b) 75% and (c) 50% lagoon waste water with additional nutrients.

2.2 Pilot reactor design construction for the application

Different design configuration for the prototype as proposed were attempted for the nutrient recovery from the different sources.

Submerged paddle wheel design



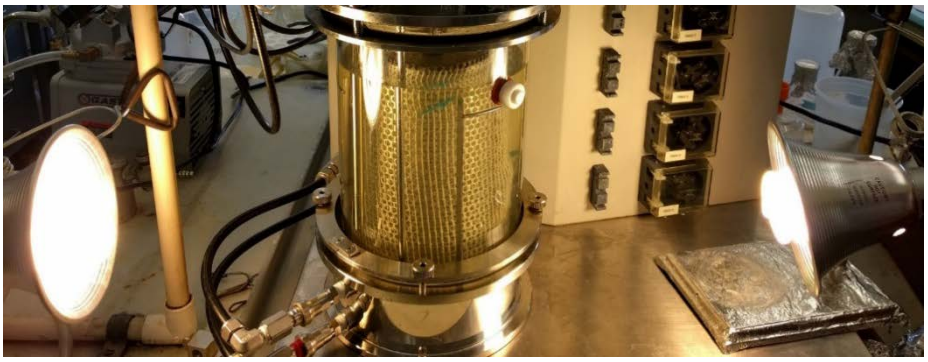


Fig. 13 Two different submerged paddle wheel designs for the lichen biofilm formation (a) flat blade and (b) rotating drum

The paddle wheel will serve dual purpose, both for mixing and as a matrix for the attachment of fungal cultures for lichen biofilm formation that will be partially immersed inside the medium. The removable paddle wheel enables harvesting the biofilm in the attached fungal cultures. The original impeller was replaced with a custom designed paddle wheel impellers as shown in figure 13. The paddle wheel impellers allow for maximized mesh

surface area in the bioreactor. The drum with the matrix attached rotates within the glass vessel under illumination which allows for the microalgae and fungi to grow attached on the matrix. Lighting is provided by two stationary 23W compact fluorescent lights which are staged for the bioreactor to receive 50 µmol m^{-2} s^{-1}. As the algae and fungi grow they remove nutrients, suspended solids, and oxygenate the wastewater. At the end of the process the algae and the fungi exhibit a high degree of attachment and are easily separated from the medium.

***Floating cascade design*:**
Cascades of the attached biofilm will be used as a float on the polluted waters and the movement of the cascade of biofilm will be mechanized in the model wetland-reactor system. Large sheets of mesh were inoculated in sterilized buckets and strapped to the shaker table for 5 days before being cut into smaller pieces and "sewn" onto larger structure. This structure was planned to be used to field test the desorption process. The frame was lifted slightly off the base with a stir bar and provided fresh media and a small re-inoculation then covered with a metal lid. Growth was very minor and after 5 days insect larvae appeared. By 7 days it was evident that the biofilm was being eaten and dying. A field test was not conducted for the floating cascade design.

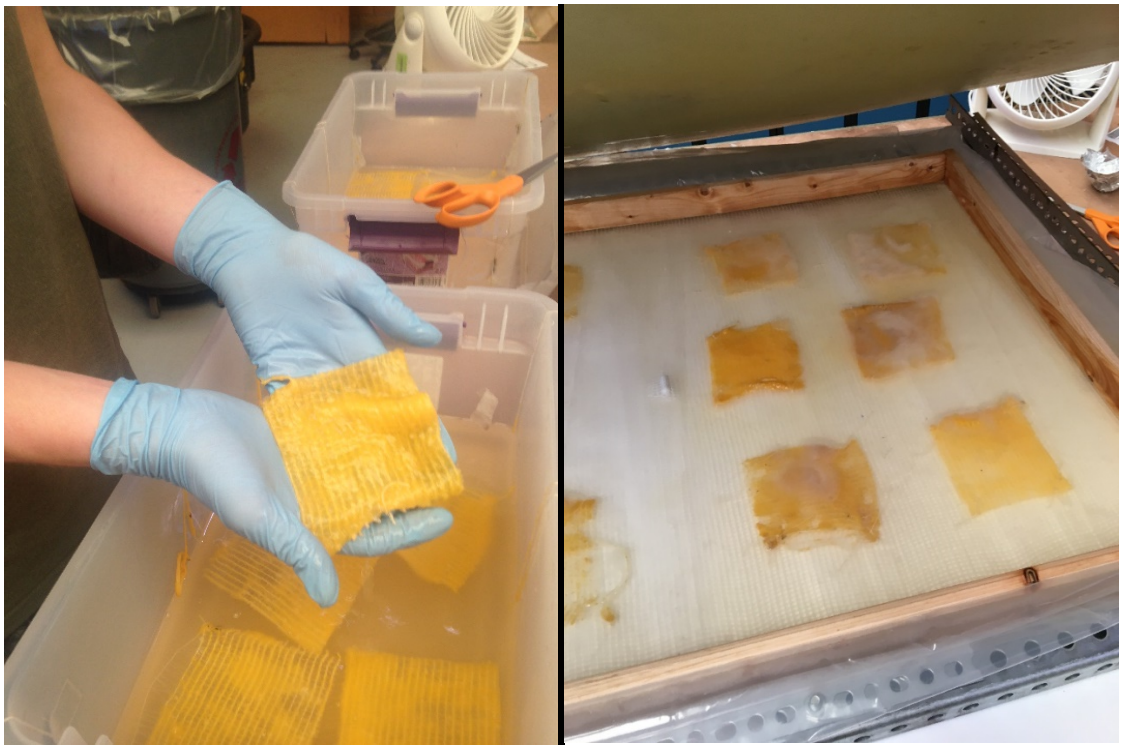


Fig. 14 Picture of the design of floating biofilm in wastewater for nutrient recovery

Flow through design:
Flow through design is an enclosed system with biofilm where the water movement will be facilitated to flow through an attached lichen biofilm continuously. This design will have the biofilm entrapped in the enclosure and will arrest the movement of cells due to the biomass settling. The reactor design is given in Figure 15, which retains the biomass and the liquid flows through the lichen biomass. The reactors are operated continuously with an initial HRT of 30 hours and with an up-flow velocity of 1 m/s in a controlled environment at 30ºC. The reactors consisted of a glass tube with approximately 1200 mL of volume. This reactor core was fed by a pump, thus providing the in-flow of wastewater. The wastewater solution was kept refrigerated and well mixed so as to avoid contamination and sedimentation. A second pump guaranteed recirculation within the reactor, extracting solution from the top part of the reactor and pumping it into the bottom part of the reactor. The reactor effluent exits from the top of the reactor and was collected in a large flask. The experimental reactor has constant illumination for photosynthetic activity of algae. The design was eventually not taken for the final demonstration because of the needs for constant pump-up of water, which can be energy intensive.

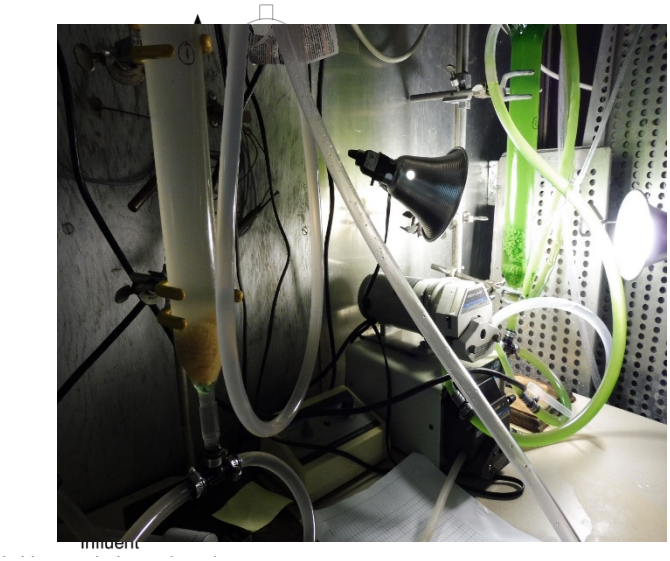


Fig. 15 Schematics and picture of the flow through design reactor for nutrient recovery

Activity Status as of January 1, 2018:

2.3 Design of a paddle wheel with fungal biofilm for Nutrient recovery in Sarita wetland

Based on the previous results from the submerged paddle wheel design in the lab scale, the prototype of the paddle wheel design was developed for installation in the Sarita wetland. The nutrient levels in the Sarita wetland was monitored for a period (Fig.16) before implementation of the paddle wheel system.

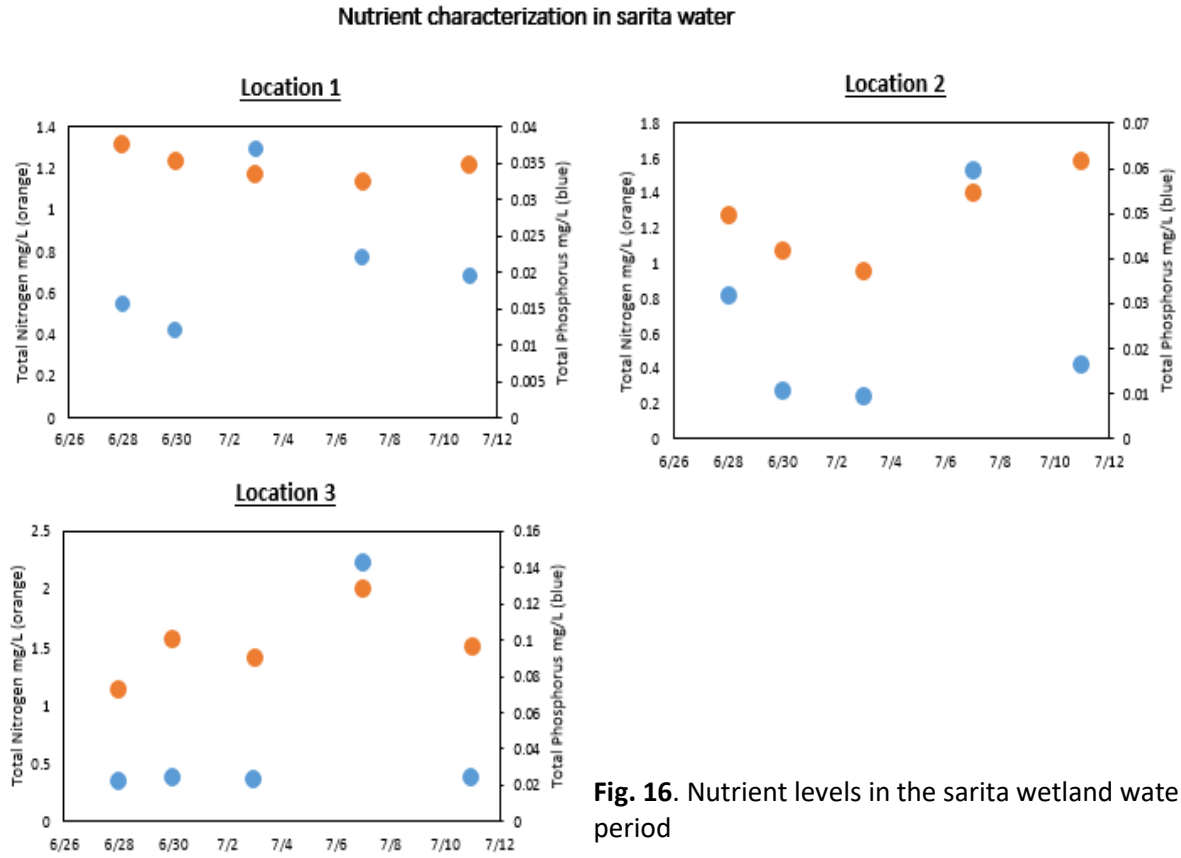


Fig. 16. Nutrient levels in the sarita wetland water over a period

The total phosphorous and total nitrogen was monitored at three different locations of the Sarita wetland. The nutrient levels fluctuate over the time maximum total phosphorous was 0.142 mg/L in location 3, and total nitrogen level was 2 mg/L in location 3 on the same day. However, on the day of installation of the paddle wheel we observed much higher initial values of the nutrients. The total phosphorous reached to high values of around 1.5 mg/L, total nitrogen of 12.1 mg/L and COD levels of 438 mg/L on some days and was highly fluctuating. On the day of installing paddle wheel prototype in the sarita wetland, nutrient levels were 0.734 mg/L TP(total phosphorous), 6.435 mg/L TN (total Nitrogen) and 344.5 mg/L COD (chemical oxygen demand) (Fig.17).

The prototype design consists of rotating paddle wheel on which the matrix was attached for the biofilm development with the working volume of 25 gallons. The biofilm was grown separately in a minimal medium, and transferred to the Sarita wetland for nutrient recovery studies (Fig.18). The paddle wheel was operated in a constant volume fed-batch mode for 7 days with 10 gal of polluted lake water fed every 24 hours. Samples were taken before and after water addition to evaluate the nutrient removal efficiency of the biofilm for each cycle.

The initial nutrient levels in the Sarita water were fluctuating over the period of prototype testing. After initiating the run, the TP in the reactor increased by 30% in the first 24 h, which might be due to the release of P from the fungal biofilm. 10 gal of water is removed and fresh water was added to maintain the liquid level constant. At 48 h, the removal of P was around 2% and it increases to 43% and 82% at 72 and 96 h. The P removal dropped after 96 h and remains constant for rest of the process duration. In this study, it was found that the initial P levels has little influence on the final concentration of P after treatment with the biofilm for 24 h. The biofilm was able to remove P from higher levels of around 1.5 mg/L to 0.26 mg/L in 24 h. After 120 h of operation, the concentration of the TP remains constant both in the inlet stream and in outlet. There is no further reduction in the P levels after 120 h. There was some biofilm loss, which was seen visually. The loss of biofilm may be due to the biomass lysis and as well as because of the bacterial action. Overall, the biofilm was durable over the period of study and in terms of the attachment of biofilm.

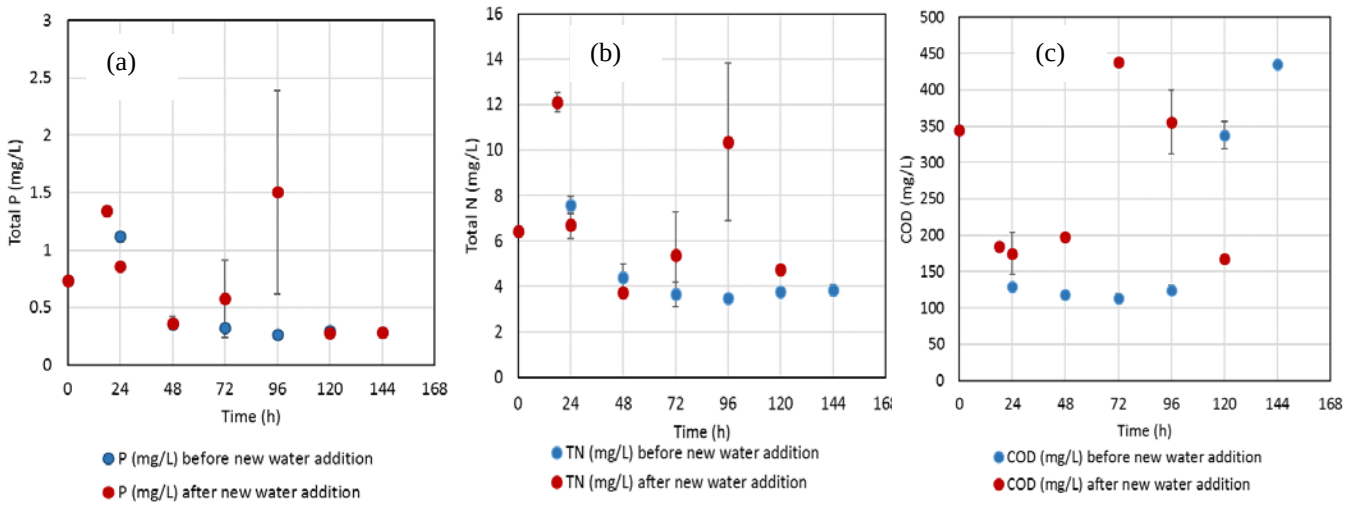


Fig. 17 (a) Location of the Sarita wetland (b) Paddle wheel reactor onsite for the nutrient recovery (c) Biofilm on the paddle recovering P,N and COD in Sarita wetland and the (d) biofilm after 7 days of operation

The total nitrogen levels in the Sarita water was between 3.65 mg/L to 12.1 mg/L over the period tested. The nitrogen level was found to increase during initial 48 h and the TN removal efficacy reaches the maximum of 66.2% at 96 h and dropped after that. The concentration of the TN in the residual waster was relatively constant over 48-144 h irrespective of the inlet TN concentration. The COD removal was increased from 26% at 24 h to 74% at 72 h and removal was consistent until 96 h. After 96 h there was an increase in COD concentration, which might be due to the cell lysis. Based on the results from the porotype model in Sarita wetland, we can conclude that the biofilm has to be operated between 96-120 h and needs replacement of biofilm (Fig. 18).

Fig. 18 (a) Location of the Sarita wetland (b) Paddle wheel reactor onsite for the nutrient recovery (c) Biofilm on the paddle recovering P,N and COD in Sarita wetland and the (d) biofilm after 7 days of

Final Report Summary:

From the previous cultures on different types of wastewater, it is obvious that the biofilm needs to have high concentration of nutrients in order to grow. We took the water samples from the Sarita site for over a month and our lab culture experiments showed that the nutrients level in the Sarita Wetland were not high enough to support our mycoalgae biofilm growth and treatment. We continued monitoring the water quality and eventually chose the end of August to install our system at the Sarita Wetland because that was the week for the State Fair and the wetland water carried very high nutrients from state fair ground. The system was running for one week and we reported our data in this report. However, we felt that the Sarita Wetland might not be a suitable site for relative long-term demonstration because the water was only heavily polluted during the State Fair period. Therefore, we decided to move to two different directions on the technology demonstration. First, we started to monitor another wetland (as shown in the photo) next to the Frank and Sims Yard Waste Collection Site on the Phalen Blvd in east St Paul. We put our system on that site since the water at that pond is heavily polluted. Second, we chose the manure wastewater to demonstrate the technology, since this type of water has very high concentration of nutrient pollutants, and our previous lab scale study showed that lagoon manure waste water supported biofilm growth and ended with good nutrient removal.

ACTIVITY 3: ONSITE EVALUATION - Floating island evaluation in Sarita wetland

Description: The Sarita wetland in University of Minnesota will be selected as a model system for tracking patterns in field parameters for the design of prototypes and implementing the scaled-up model for evaluating the effectiveness of lichen composites. A "floating island" system will be designed and constructed to use fungal and microalgae species instead of native plants. The treatment capacities of the systems will be estimated and effects of system design parameters will be evaluated. The kinetic and model parameters evaluated from the previous bench-top studies will be used to scale-up the process. Water quality characterization namely, dissolved oxygen levels at the site, salinity, and temperature effects, ammonia concentration, nitrate, nitrite, and phosphate concentration will be evaluated. Testing in the real wetland may be challenging with the unforeseen factors affecting the process, which will be realistically studied and mitigated. Water samples from different sampling point in Sarita after having the floating island design installed, will be collected at regular intervals and the physiochemical parameters will be checked for technology evaluation.

Cost estimation will be developed onto a spreadsheet calculation model in order to determine the capital investment, the useful life; and operations and maintenance costs of the system, considering size the pollution level of different water body. The research team expects that the matrix can be reused for multiple times, which will be one of the key factors to control the overall process economic feasibility.

Summary Budget Information for Activity 3:

ENRTF Budget: $100,073
Amount Spent: $ 100,073
Balance: $0

Outcome	Completion Date
1: Floating island design and construction.	Jan, 2018
2: Data collection and evaluation at Sarita.	May, 2018
3: Cost estimation	June, 2018

Activity Status as of January 1, 2016:
The activity 3 has not been started yet.

Activity Status as of July 1 2016:
The activity 3 has not been started yet.

Activity Status as of January 1, 2017:
The activity 3 has not been started yet.

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:
3.1 On-site scale up fungal treatment of manure wastewater
The first effort for the demonstration scale experimental was to set up for treating the manure wastewater in the Animal Waste Treatment Center, Waste Management Service located at the University of Minnesota-St Paul campus (Fig. 19). In batch study, Polyphosphate accumulating fungi was cultured to remove and recover the excessive phosphorus from dairy manure wastewater. The microbial treatment removed 83.9% of phosphorus in 20-fold diluted dairy manure wastewater by the fungal biomass in 12 h. At the meantime, 33.6% of chemical oxygen demand, 46.5% of total nitrogen, and 89.4% of ammonia nitrogen were removed as well. At seventh reuse batch, the cells could still remove 50.4% of phosphorus from the diluted wastewater. The average phosphorus removal efficiency in seven reuse batches was calculated as 62.1%, the nitrogen-to-phosphorus ratio in dairy manure wastewater increased from 3.6:1 to 6.3:1. With the treatment, dairy manure wastewater will be more suitable for farmland irrigation, with the better nutritional ratio to support crop growth, and it will help mitigate phosphorus pollution to water bodies caused by over-applied phosphorus in the farmland application.

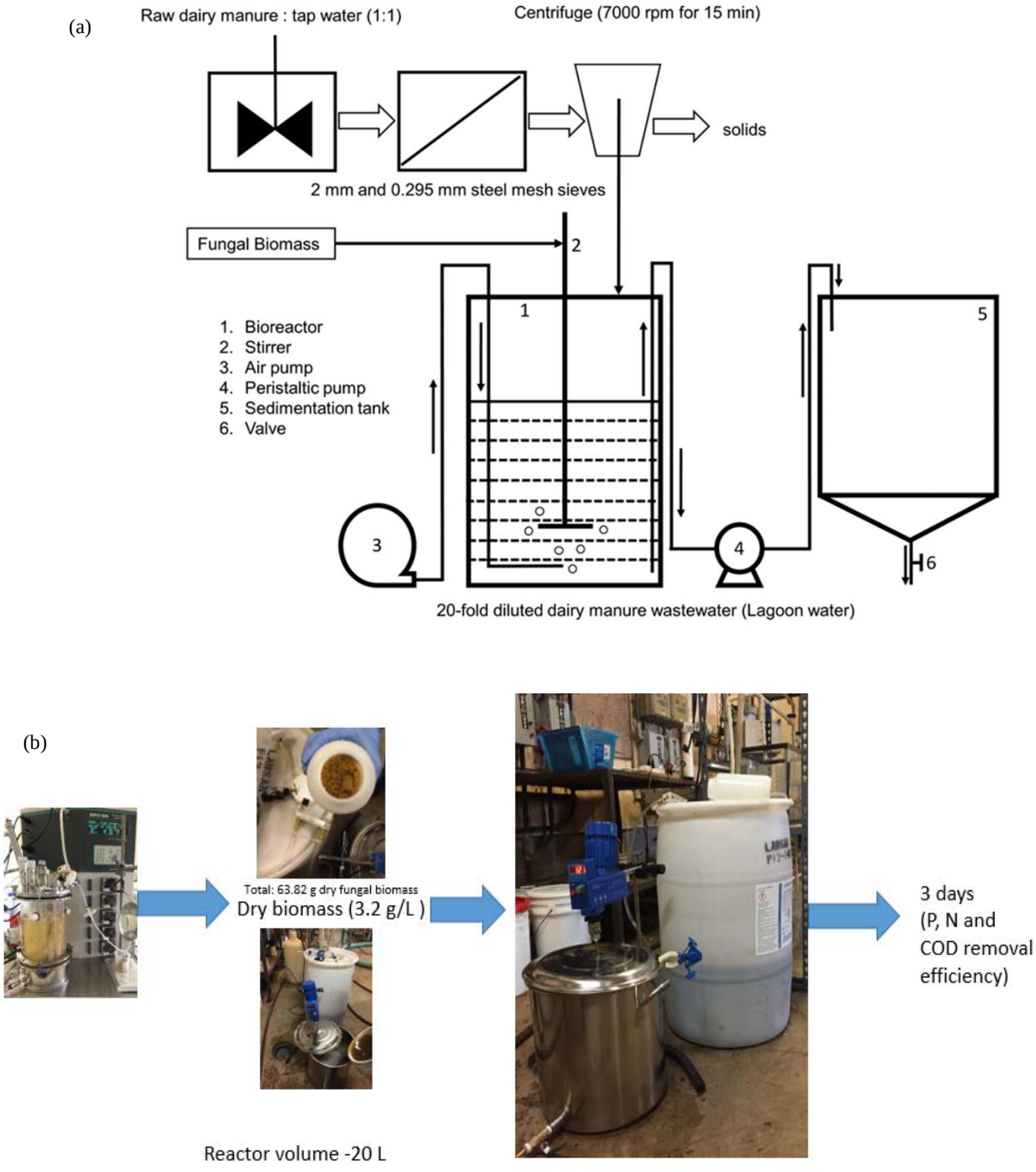


Fig. 19 (a) Schematic representation of the biofilm reactor for nutrient recovery from manure water and (b) prototype model

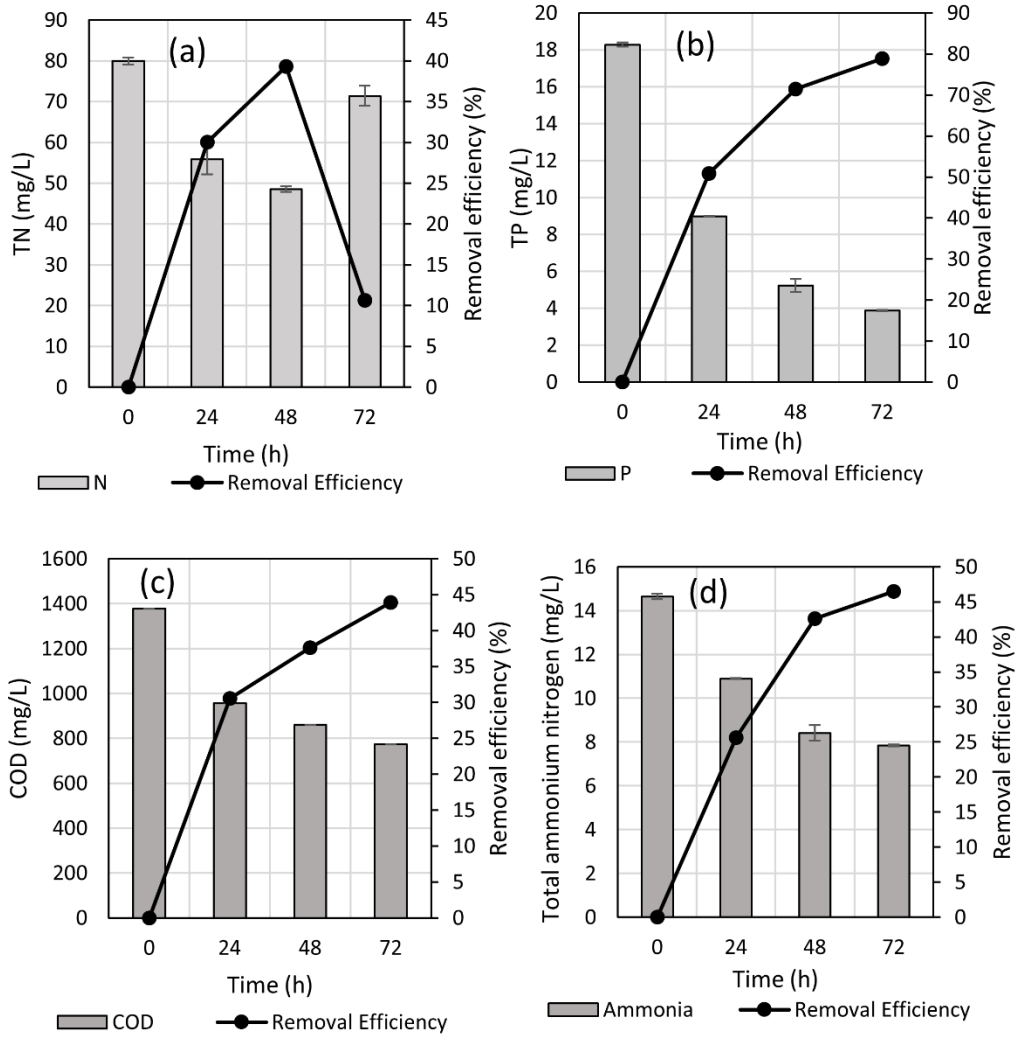


Fig. 20 (a) Time course of total nitrogen (b) Total phosphorous (c) COD and (d) total ammonium Nitrogen recovery from manure water

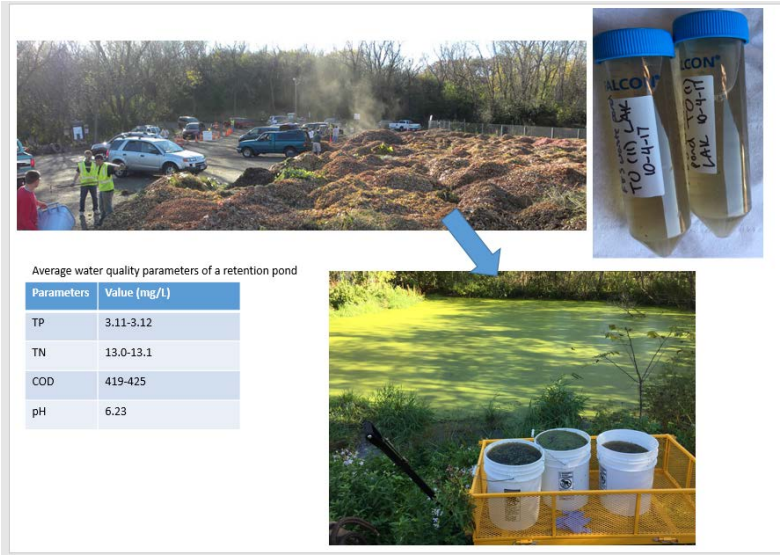
Dairy manure slurry was sampled from the dairy cattle barn in University of Minnesota (Saint Paul, MN, USA), and then uniformly mixed with tap water at a volume ratio of 1:1. The mixture was orderly sieved by two stainless steel mesh sieves with 2 mm and 0.295 mm opening sizes, respectively. The filtrate portion was centrifuged at 7000 rpm for 15 min to further remove coarse solids. The flowable layer after centrifugation was termed as DMW, stored at 4oC and used within one month after preparation. In some cases, the DMW was appropriately diluted with tap water and adjusted pH to the desirable values by 6 N HCl or 6 N NaOH before using. In pilot study, the dairy manure wastewater was prepared as described above and the fungal dry biomass of 3.2 g/L was added to 20 L diluted dairy manure wastewater in the reactor tank with aeration and stirring. After 3 d of treatment, the wastewater with biomass was allowed to stand for 12 h for the solid-liquid separation. The nutrient (P and N species) levels and the COD removal efficiency was measured every 24 h for 3 d.

The pilot-scale fungal bioreactor was tested with manure wastewater over a period of 3 d (Fig. 20). The wastewater contained 79.9 mg/L of total N, 18.28 mg/L of total P, 1379 mg/L of COD and 14.65 mg/L of Ammonium-N (Fig .20). Within 24 h, 50% of P removal was observed with fungal treatment, corresponding to an average daily uptake rate of 4.65 mg /L/ d. 78.86 % of P was removed at the end of the process, which was comparable to the observation in the batch study even though the fungal biomass addition was less than in the batch study. The total nitrogen removal was 39.3% at 48 h and the removal efficiency decreases after 48 h, which

might be due to the cell lysis. The TAN levels dropped from 14.65 to 7.84 mg/L in 3 d. Based on the result it is ideal to stop the batch at 48 h of process time. The COD levels dropped over time and the removal was 44% at 72 h, which might be due to the fungal growth or by the bacterial activity. The land application of manure is currently considered an important contributor of pollutants entering surface waters, causing eutrophication in lakes and rivers. Therefore, there are great needs to develop on-site manure treatment methods to provide environmental remediation and improve the efficiency of the land application. If P can be partially recovered from manure, it will create a cash product as a P specific fertilizer, and it will also allow the livestock industry to implement better nutrient management plans, all of which will reduce the environmental impacts of the livestock industry.

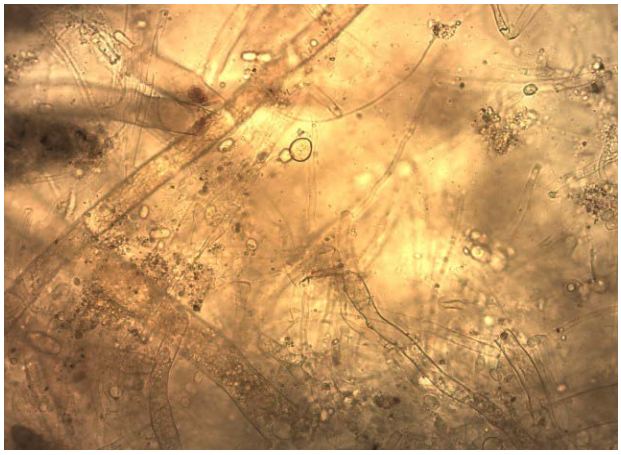
3.2 Demonstration at the Frank and Sims Yard Waste Collection Site

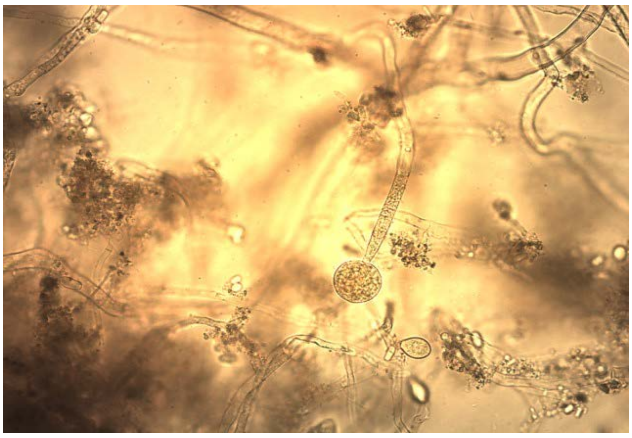
The biofilm paddle wheel reactor was tested in the retention pond located close to the Frank and Sims Yard Waste Collection Site in St Paul. This site was selected for onsite evaluation of the biofilm based on the water quality characterization as the water in the site has high levels of sediment with relatively higher levels of nutrients and was categorized as the highly nutrient polluted site. Experiments were conducted onsite to evaluate the biofilm for the nutrient removal capacity (Fig. 21). The reactor design and the process conditions was the same as tested in Sarita Wetland with the working volume of 25 gallons with 10 gallons of polluted lake water fed every 24 h. The biofilm was developed in laboratory and moved on- site for testing. The initial total P (TP) in the water was at 2.57 mg/L, and after the first 24 h there is no significant change in the total P (2.33 mg/L), which might be due to the initial biofilm acclimatization. The operation was carried out with recovering 10 gal of water and replacing it with 10 gal of fresh water from the lake. After 24 h, the TP level decreased to 0.969 mg/L (58% removal efficiency). For the next two cycle the TP removal efficiency was at 51.2% and 55%. At 96 h cycle, the P removal efficiency starts to decline with 31 % and no further P removal was observed after 120 h. This demonstrates that the biofilm reaches the maximum capacity at 120 h of operation, which is consistent with the Sarita wetland test using the same reactor configuration. During the operation the sediments attached to the biofilm and the sediment-bound P may also have been recovered together with the other forms of P. The microscopic examination of the biofilm shows that the biofilm is intact and the fungal filaments are seen attached to sediments and other biological components of the lake water.



Average water quality parameters of a retention pond

Parameters	Value (mg/L)
TP	3.11-3.12
TN	13.0-13.1
COD	419-425
pH	6.23





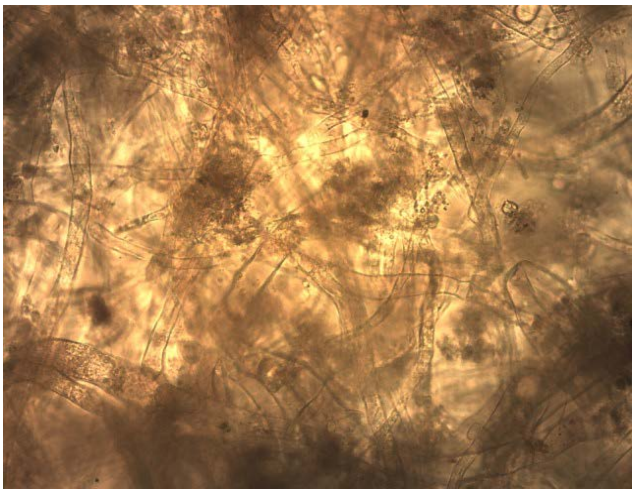
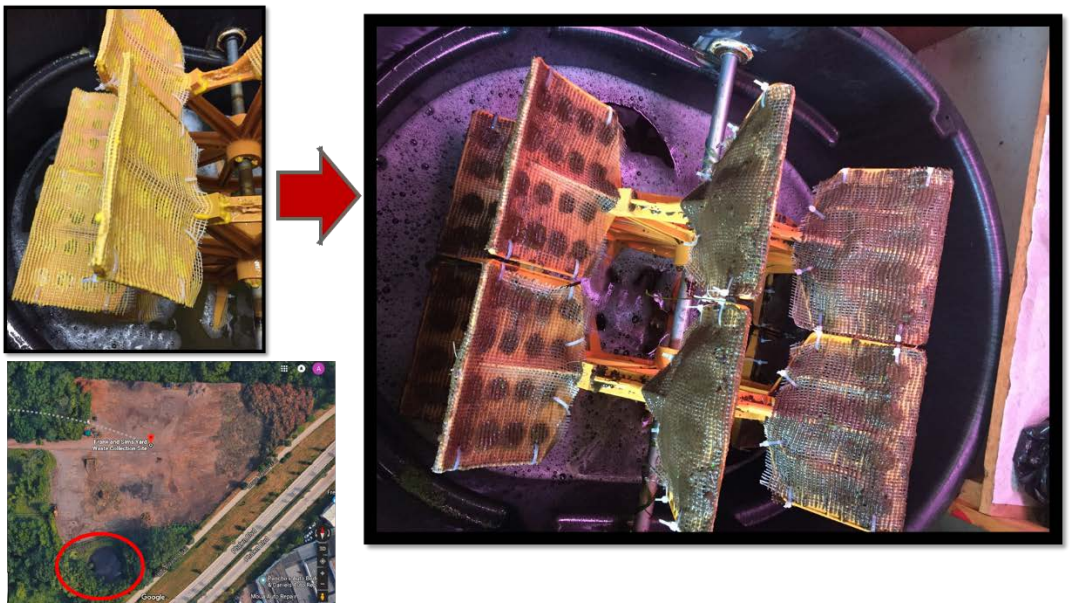


Fig .21 Microscopic images of the biofilm after 168 hours of operation



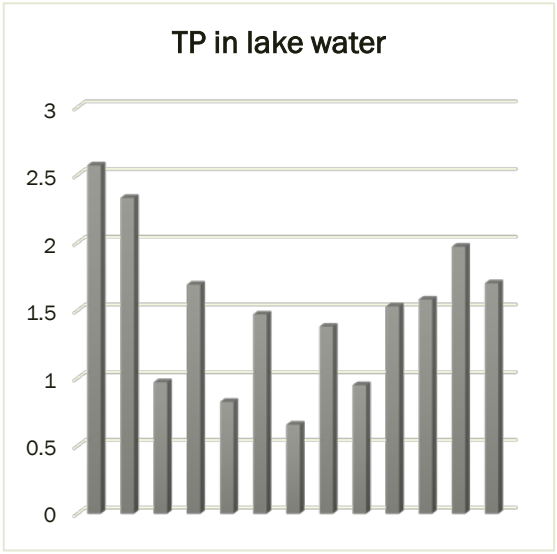
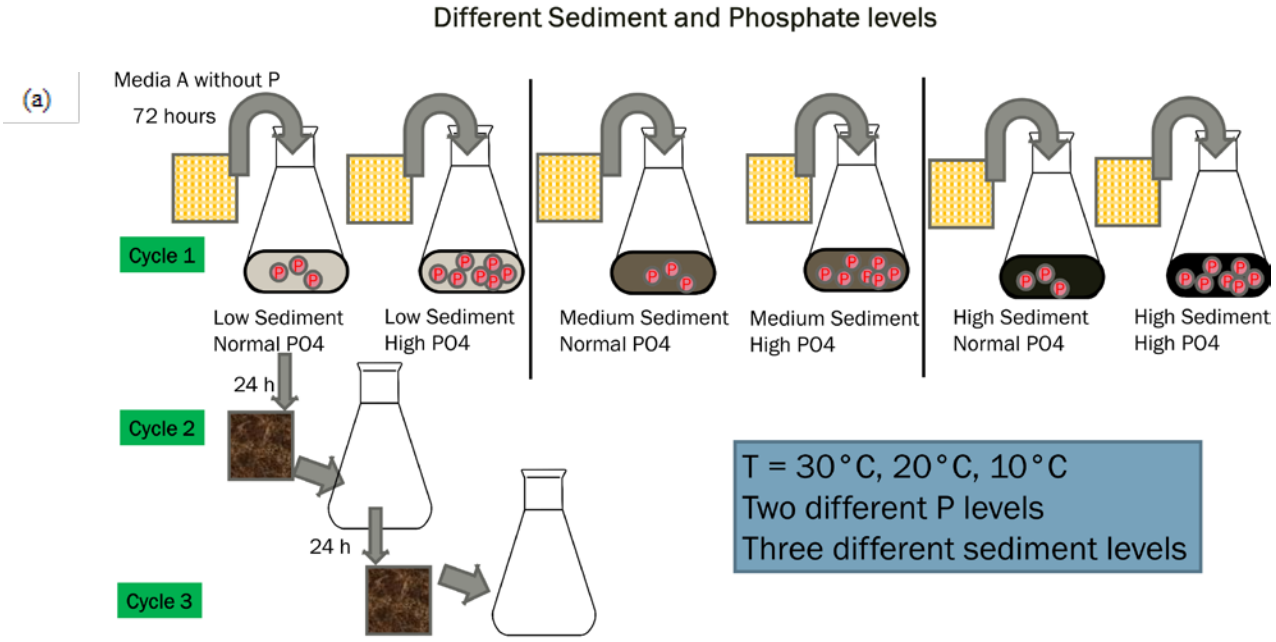


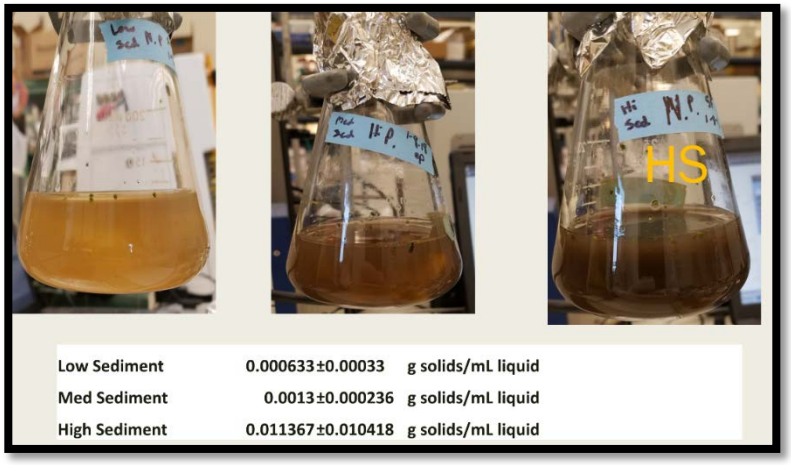
Fig.22. Testing the biofilm in the Frank and Sims Yard Waste Collection Site (a) The biofilm at the start of the test (b) the biofilm after once batch of run in a semi-continuous process (c) Arial view of the Site location (d) Plot showing the TP removal from the 24 h time period of running the reactor.

Experimental study to evaluate the mechanism and biofilm capacity in removing P or P bound to sediments

The total phosphorous in the lake waters can be free PO_4^{3-} anion, bound P-metal compound or in the organic-P form in the algae cells or other forms of inorganic P. The fugal biofilm could possibly remove all forms of P present in the water streams directly from the water streams by different mechanisms. The free phosphate will be assimilated due to the fungal metabolic pathway with the available COD in the water, and the biofilm binds physically to the other forms of phosphorous including the algae cells, which causes the reduction in total P levels in the experiments conducted. To evaluate the changes in the different forms of phosphorous in different levels of sediment containing samples, experiments were conducted to study the changes in the PO_4^{3-}anion concentration at different sediment levels and the effectiveness of biofilm in removing the free phosphate and the bound phosphate. The experiments were conducted as the semi-continuous process using the water collected

from Frank and Sims Yard Waste Collection site for three cycles using the same biofilm in laboratory scale. The experiments were mimicked the same way as done onsite. Samples containing different sediment levels (LS: low sediment samples containing 0.6 mg/ml of sediments; MS: medium sediment samples containing 1.3 mg/ml of sediments; HS: medium sediment samples containing 11.3 mg/ml of sediments) were prepared with two different levels of PO_4^{3-} concentration. The free phosphate added to the sediment containing water samples were tested for free PO_4^{3-} and total phosphorous. The fungal biofilm was used to remove P in the samples containing different levels of phosphate and sediments for three cycles as shown in the Fig. 23. The total phosphorous, total nitrogen and the total COD numbers were given in the Fig. 23(a). In the first two cycle, the total P removal was more than 90% in all the tests conducted at different levels of sediments and phosphorous. In the third cycle, the TP removal efficiency drops which might be because the biofilm binding sites have been reduced. Fig. 23 (b)shows the biofilm pictures before and after its use for the water treatment at different levels of sediments and phosphorous concentration. The resulting water after biofilm treatment after 24 h is highly clarified and free of suspended particles and total P. The biofilm has the capacity to remove free and bound P. To evaluate the type of P that is present in the sediment containing waters a comparison was made to test the nature of added free PO_4^{3-} anion. It was found that the almost more than 50% of inorganic phosphate anions added to the water samples are immediately bound to materials and minerals containing calcium or iron ions (Fig. 23b).





	Low sediment		Medium sediment		High Sediment	
TN	10.055	±2.326	24.95	±0.494	55	±1.838
TP	5.095	±0.247	6.65	±0.579	8.82	±0.042
COD	158	±20	744	±36	2133	±124

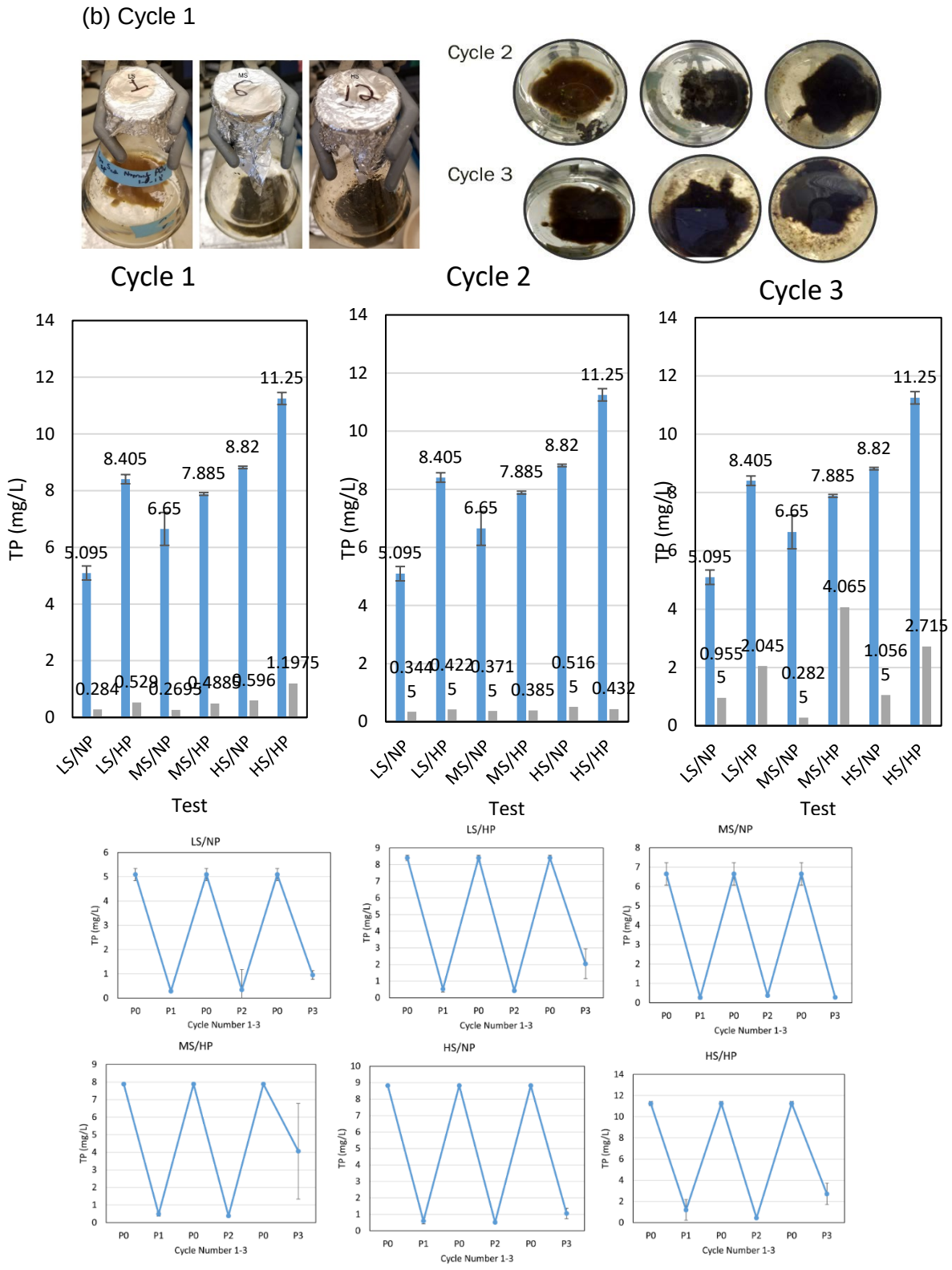


Fig. 23. Different sediment levels and phosphate levels of the Frank and Sims Yard Waste Collection Site. Three different sediment levels and two different phosphate levels

3.3 Economic Analysis

This section analyzed and summarized techno-economic results of installing and operating a biofilm remediation system (peddle wheel reactor) based on the input from the field demonstration experiments in the Sarita Wetland. The detailed cost estimate is listed in Table 3:

Table 3. Cost estimate

Items	Paddle wheel Biofilm reactor
Scale of the targeted paddle wheel biofilm, Liters	189.25
(120 h of operation - 10 gal / day)	
Capital costs	
Items	Paddle wheel Biofilm reactor
Reactor to hold rotating paddle wheel, $	89.00
Paddle wheel cost / wheel, $	52.00
Motor for driving the rotation, $	60.00
Installation cost	200.00
Battery for the motor, $	55.00
Total capital cost, $	456.00
O&M for 120 hours	
Fungal Biofilm, $5/kg, 400grams	2.00
Biodegradable Mesh Fabric / 120 h operation with ties, $	0.98
Electricity	0.60
Maintenance cost	25.00
Total O&M cost, $/120 hours	28.54
Total O&M cost, $/half year	1028.88
Total O&M cost, $/gallon	0.57

With the current setup, a rough estimation of a paddle wheel reactor will be able to handle ten gallons/day of wastewater with an annualized cost at around $2,058 per year. On each gallon level, the treatment cost is around $0.57/gallon. This annualized cost does not include the initial setup, the capital cost, which is $456 per paddle wheel reactor system. For the operation and maintenance (O&M) cost, we assume that a pre-made mesh with fungal biofilm can be directly purchased and ready to install on the paddle wheel. Besides the mesh cost, which is $0.98 per each system, the fungal biomass is estimated based on the market price for the typical fungal cell biomass. The paddle wheel system has a 1/15 HP motor, running 24 hours per day on $.10 per kilowatt-hour electricity and the cost is would cost 60 cents per 120 hours (1/15 HP x 0.7457 KW/HP x 120 hr x $.10/KW-hr = **$0.60**). The most significant factor is the labor cost, accounting to 87.6% of the O&M cost. The primary reason is that the current paddle wheel system is still a prototype reactor, and the small system is not automated nor standardized. We assumed that one-hour labor will be used to maintain 120-hour operation of a single paddle wheel. For the scale of 100 paddle wheels to run year around, we will need to spend around $180K on labor for maintenance. For the size of the paddle wheel at 10 gallons, this seems to be overestimated and very reasonable. If only half of the labor is needed, the treatment cost will decrease to $0.32/gallon. The treatment cost will drop to $0.14/gallon if no labor is included in the maintenance.

The system is still in the very early stage of development, and the demonstration serves more as a proof of concept. Different type of implementation approaches is needed for this water treatment technology to be applied to the field. Even the paddle wheel design is adopted, the location where the wheel needs to be installed should be selected so that the natural slope of the water flow can be used to drive the paddle wheel, instead of

electricity. The technology cannot be operated year around in the field, considering the seasonal climate of Minnesota and the biofilm will not be functional in the colder month.

Final Report Summary:
We built a large reactor to test the microalgae/fungal biofilm at UMN dairy center to treat dairy manure wastewater, and the research results showed great reduction of nutrient pollutions. We also designed a paddle-wheel system with the attachment matrix in order to form fungi and algae biofilm and demonstrated the system at the Frank and Sims Yard Waste Collection Site. The water pollution parameters seemed improved significantly with our treatment, and the biofilm formation was predominated by filamentous fungi. Cost estimate of the paddle wheel design was finished even though more research work is needed to implement this technology.

V. DISSEMINATION:
Description:

Part of the reactor design in the lab scale, if proved to be innovative, will be applied to the University Office for Technology Commercialization for filling the patent protection. We will publish two to three peer-reviewed manuscripts in the related journals to disseminate our results to the general public. We will also use the university extension website www.extension.umn.edu as well as PI's academic website http://bohu.cfans.umn.edu/ for dissemination of the research. If proved to techno-economically feasible, we will collaborate with UMN Office of Technology Commercialization office and actively look for commercial partners to explore the possibility for commercialization of this technology.

The primary target to disseminate our research results will be the scientific community, environmental companies as well as local community concerned with their pond health. Information obtained from the mechanism study will be directly applied to establish the cultural conditions for different strain combinations, and the lichen biofilm formation will be evaluated for the cell harvest and microalgae production. The synergistic interactions between different species will provide opportunity to extend the process to different applications. The floating island can also be re-designed based on our results to include the simulated lichen biofilm system to manage the nutrients more efficiently.

Any royalty, copyright, patent, and sales of products and assets resulting from this project will be subject to revenue sharing requirements with ENRTF according to Minnesota Statutes, section 116P.10.

Status as of January 1, 2016:
We made the following presentation and submit one manuscript to disseminate our research results and LCCMR was acknowledged at each of the presentation and paper publications.
Presentations
1. Aravindan Rajendran, Bo Hu., Simulated lichen system – A novel microalgae cultivation technology using fungi and lichen biofilm, ***Algae Biomass Summit***, September 29 - October 2, 2015, Washington, DC.
2. Aravindan Rajendran, Cristiano E. R. Reis, Yanmei Zhang, Hongjian Lin, and Bo Hu., A Novel Symbiotic Biofilm for Algae Growth and Harvesting, 2015 International Congress on Energy submission for 2015 ***American Institute of Chemical Engineers (AICHE) Annual Meeting***.
3. Aravindan Rajendran, Bo Hu, Gan, Jing; Lin, Hongjian; Zhang, Yanmei; He, Qiyang, Effect of process variables on the attached co-cultures of a fungi and algae in ethanol co-products for nutrient recovery, ***American Society of Agricultural and Biological Engineers Annual Meeting 2015***.
4. Poster presented on "Lichen biofilm" ***CFANS-UMN networking event – 2015***

Status as of July 1 2016:
Papers:
1. Aravindan Rajendran, Bo Hu. 2016. Lichen biofilm: Development of a novel platform technology using algae and fungal cultures. Biotechnology for Biofuels20169:112 DOI: 10.1186/s13068-016-0533-y.

Presentations:

1. Bo Hu, Aravindan Rajendran, Jing Gan; Deposition of manure Nutrients in microbial biofilm, US-EPA Nutrient recycling competition, The White House, Washington DC -2016

Status as of January 1, 2017:

Papers:

2. Aravindan Rajendran, Tyler Fox, Bo Hu. 2016. Nutrient recovery from ethanol co-products by a novel mycoalgae biofilm: Attached cultures of symbiotic fungi and algae", Journal of Chemical Technology & Biotechnology, doi: 10.1002/jctb.5177

Presentations:

2. Aravindan Rajendran, Bruno Hespanhol, Jason Paschke, Avi kumar, Bo Hu, Mycoalgae biofilm for Nutrient capture, BBE Showcase, UMN - Networking Reception, Research Poster session on Thursday October 27
3. Aravindan Rajendran, Bo Hu et al., Nutrient Capture by a Sustainable Symbiotic Biofilm: Simultaneous Phosphorous and Nitrogen Recovery by Attached Fungi and Microalgae Biofilm, 2016 Annual American Institute of Chemical Engineers, Nov 13-18 San Francisco (oral presentation)

Outreach:

A teaching module “Grow like a Lichen” was developed and we constructed the course for three different groups of students including (1) a Summer camp lesson for Discover STEM 2016 through College of Science and Engineering, University of Minnesota for high school students; (2) Department of Bioproducts and Biosystems Engineering freshmen undergraduate students at their department orientation course BBE 1001, and for (3) high school science teachers from Minnesota. The course has two components (A) “Grow like Lichens”- The concept of a simulated lichen system is introduced to the participants. The criteria for choosing desired microalgal and fungal combinations that grow on the surface of specific polymers to form the biofilm is discussed. Students attend the module demonstration, including a classroom lecture to introduce the concept of lichens and emphasize the importance of mitigating nutrient release events in the ecosystem and the need to recycle the nutrients. This is followed by laboratory hands-on training (B) “Synthetic Lichens for Bioremediation”: This activity introduces students to the basic concepts of autotroph, heterotroph, symbiosis, how natural lichens live, and the co-culture of microalgae and fungi in our project, and then asks students to visualize the green microalgae bio-film. The attendees were given biofilm samples from the already grown "synthetic lichen" in simulated waste water and instructed to measure the phosphorous content before and after the growth of the biofilm to calculate the nutrient removal efficiency of the biofilm and to observe the efficacy of the biofilm in removing nutrients.

We received survey response forms from the school teachers involved in the demonstration and feedback suggestions were overwhelmingly positive and constructive, which aided us in customizing the program for K-12 school students. We believe that the module can be customized based the students class level, e.g. for K-6 with a fun activity, “Lichen Biofilm–Friends forever”, for explaining lichen and how they live at extreme conditions helping each other; and 6-10th grade students with the classroom presentation and demonstration and for the 11-12 grade with the complete module including the experimental analysis of nutrients in the laboratory.

A group of school teachers of different grades from Minnesota state took this course- "grow like a lichen" presentation/experiment on December 10, 2016, and on December 14, 2016 in collaboration with College of science and engineering we organized a Science Club Field Trip for a group of about 35 high school students.

Status as of January 1, 2018:

Papers:

3. Aravindan Rajendran, Tyler Fox, Cristiano Rodrigues Reis, Bruce Wilson, Bo Hu, 2018. Deposition of manure nutrients in a novel mycoalgae biofilm for Nutrient management, *Biocatalysis and Agricultural Biotechnology,* 14: 120-128

Presentations

4. Aravindan Rajendran, Bruno Hespanhol, Tanner Barnharst, Cristiano Reis and Bo Hu., 609e Symbiotic Microbial Communities for Cleaning Agricultural Waters and Bioenergy Production, 2017 Annual American Institute of Chemical Engineers, October 29 - November 3, 2017, Minneapolis Convention Center, Minneapolis, MN

Final Report Summary:
The project generates some excitement from both the scientific community and industry. Even though a final applicable solution is still in needs of more research and development, we have presented our research in many national and local conferences, several publications either in press or in submission, and we reached to large amount of undergraduate students and high school teachers via the teaching module developed form this project.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 196,180	Bo Hu's salary: One month of salary will be charged to the project for Dr. Bo Hu's summer time on managing the grant. His one month salary is $8,956 in the first academic year and FTE with 3% salary increase for the following project years. The fringe benefit for Nine-Month B-term faculty is 33.9% based on the University regulation. Salary for Bo Hu's Postdoc Researcher: One postdoc will be hired for this project for two and half years. The postdoc researcher will be paid with $40,000 for the first year and FTE with 3% salary increase for the following project years. The rate of fringe benefits the postdoc researcher is 21.5% based on the University regulation. The postdoc researcher will work with Dr. Hu to design experiments and collect the research data on the lab scale study as well as pilot demonstration. Salary for Bruce Wilson's Postdoc Researcher: Dr. Bruce Wilson's postdoc research will be paid 100% for the third year to assist with the onsite evaluation. The annual salary for this postdoc will be $45,000 and the fringe benefit rate is 21.5%.
Professional/Technical/Service Contracts:	$	N/A
Equipment/Tools/Supplies:	$50,666	$45,666 is budgeted to purchase necessary chemicals, tools, bottles, gloves etc. Specifically it is budgeted for $13,121 for the first project year, and $6,180 and $26,365 for the following project year. Regular chemical supplies are needed to measure nitrogen, phosphorus

		content of the wastewater. For instance, Hach Chemical TOTAL NITROGEN LOW RANGE 25/PK for $79.00 (No.:TNT826), and Hach Chemical PHOSPHORUS TNTPLUS, UHR,25/PK (No.: NC9881792) for $57.23. One batch cell culture. It is estimated that around 20 boxes of each test kits are needed for us to work on the lab and pilot scale study for the first two years. For the third year, around 10 boxes of test kits for nitrogen and phosphorus are needed. A 3700 Full-Size Portable Sampler will be needed in the third year to take samples at Sarita Wetland, and the cost of this sampler is around $3K. The rest of the requested supply fund will be used to purchase other necessary chemicals, bottles and gloves etc. $5,000 is budgeted at the end of the second year to construct the floating island for the onsite evaluation at Sarita Wetland. Large scale "floating islands" will be constructed containing the supporting wood structure for around 100 square foot area, the polymer matrix and necessary fungal attachment. The large scale fungal attachment will need to be cultured at Bioconversion Resource Center at UMN using their pilot scale equipment. The cost includes the material and labor for constructing the floating island as well as the equipment rental fees to be paid to the Bioresource Center.
Capital Expenditures over $5,000:	$32,609	$22,619 is budgeted to purchase additional components for Dr. Hu's High Performance Liquid Chromatography (HPLC) for chemical analysis. Dr. Bo Hu's lab has already spent around $32K for a modular model of the HPLC, including pump, manual injection, multi-wavelength detector and software. This is a request to add an additional modular reflect index detector, an autosampler and an add-on to convert multi-wavelength detector to Photodiode Array detector. $9,990 is requested to purchase an automatic cell counter to measure the microalgae and fungi cell numbers in the lab experiments.
Travel Expenses in MN:	$1,545	Travel to the agricultural lagoon at Wasseca MN and other locations to obtain wastewaters. Two travels are planned per each project year and $250 is budgeted per travel for the first year with 3% increase for the following years.
TOTAL ENRTF BUDGET:	**$281,000**	**The total cost for the first year is $106,822, the second year $74,105, and the third year $100,073. The total project cost to ENRTF is $281,000.**

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: $30,000 is budgeted to purchase additional components for Dr. Hu's HPLC for chemical analysis. The HPLC at the lab currently has the UV detector with manual injection. An addition to this HPLC is requested to include a Reflex Index detector, an auto-sampler and an addition to convert UV detector to PDA detector. These additions will expand the analysis range of the HPLC and significantly increase the measurement efficiency so that the equipment can better serve the project. It will continue to be used throughout its useful life in Bo Hu's research group for chemical measurement o improve the nutrient removal and wastewater treatment.

$9,730 is requested to purchase an automatic cell counter. This equipment will enable us accurately and efficiently measuring the cell numbers in the culture therefore knowing the cell distributions between microalgae and fungal cells in the lichen biofilm. This capital equipment will will continue to be maintained and used throughout its useful life in the research group as well as the BBE department to analyze cell culture samples for environmental remediation types of research, even after the LCCMR project ends in 2018.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.74

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$125,461	$0	In-kind services during the project period will be provided even though UMN did not charge the indirect cost for this project. The total estimate fund $125,461 is calculated based on the University F&A rate (52% of modified base).
State			
	$	$	
TOTAL OTHER FUNDS:	$125,461	$0	

VII. PROJECT STRATEGY:

A. Project Partners: The team includes Professor Bo Hu, Dr. Aravindan Rajendran, Professor Bruce Wilson and his postdoc researcher, all from the Department of Bioproducts and Biosystems Engineering, University of Minnesota. **Bo Hu,** an assistant professor, is specialized in bioprocess development, and has extensively studied the fungal/microalgae pelletization and co-culture. He will serve as the project director to manage the project, design the experiments and write the project reports. **Aravindan Rajendran,** a post doc researcher at Bo Hu's research group specialized in bioprocessing technologies and bioprocess modeling, will execute the research activities and provide technical expertise. **Bruce Wilson,** a professor with 12 month appointment, will not receive any salary from this project. He has extensive experience in reducing nutrient loading from agricultural and urban watersheds. He will supervise his postdoc researcher to work with the onsite evaluations on Sarita wetland. Bruce Wilson's postdoc researcher is to be determined.

B. Project Impact and Long-term Strategy:

The outcomes of the project will provide a sustainable solution to capture and recycle the reusable resources from runoffs by protecting rivers, lakes, and vital landscape along with protecting terrestrial and aquatic life. The long term strategy is to transform the storm water runoff treatment facility for revenue generation by recovering and

recycling the resources, and also to incorporate social responsibility for completely eliminating the runoff pollution, decimating the adverse effects of storm water on water resources. The methods developed in this study and the results obtained will serve as necessary input on future process development for various algae-based technologies for biofuel, bioproducts, and other industrial products. The funding for this research initiative would support a comprehensive understanding of the new "lichen" type biofilms and allow exploration of new avenues in the bioproduction industry with many potential applications. The planned research will build a synthetic ecosystem to provide an innovative platform technology for any processes that involve either heterotrophic fermentation of fungi or autotrophic cultivation of microalgae.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
Funding from UMN-BTI Synthetic Ecology Program to support Bo Hu's research group working on microalgae-fungal co-culture to form cell pellets	11/2012-10/2014	$90,000
Funding from UMN Grant-in-Aid program to support Bo Hu's research group working on fungal strain screening for phosphorus accumulation and removal	7/2013-1/2015	$31,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): see attached graphic

X. RESEARCH ADDENDUM: See attached research addendum

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2016, July 1 2016, January 1, 2017, July 1, 2017 and January 1, 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.

Environment and Natural Resources Trust Fund
M.L. 2015 Project Budget

Project Title: *Biofilm Technology for Water Nutrient Removal*
Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 04b
Project Manager: *Bo Hu*
Organization: *Univeristy of Minnesota*
M.L. 2015 ENRTF Appropriation: $ *281,000*
Project Length and Completion Date: *3 Years, June 30, 2018*
Date of Report: *08/15/2018*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 01/01/2016	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget 01/01/2018	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM				***Pilot tests***			***Sarita tests***				
Personnel (Wages and Benefits)	$60,592	$60,592	$0	$62,410	$62,410	$0	$73,178	$73,178	$0	$196,180	$0
Bo Hu, Project Manager: $37,067 (74.85% salary, 25.15% benefits); 8% FTE for 3 years											
Aravindan Rajendran, PostDoc Researcher: $124,438 (83.28% salary, 16.72% benefits); 100% FTE for 2.5 years											
PostDoc Researcher: $54,675 (83.28% salary, 16.72% benefits); 100% FTE for 1 year											
Equipment/Tools/Supplies											
It is budgeted at the end of the second year to construct the floating island for the onsite evaluation at Sarita Wetland. Large scale "floating islands" will be constructed containing the supporting wood structure for around 100 square foot area, the polymer matrix and necessary fungal attachment. The large scale fungal attachment will need to be cultured at Bioconversion Resource Center at UMN using their pilot scale equipment. The cost includes the material and labor for constructing the floating island as well as the equipment rental fees to be paid to the Bioresource Center.				$5,000	$5,000	$0				$5,000	$0

Lab supplies: $18,545 is budgeted to purchase necessary chemicals, tools, bottles, gloves etc. Specifically it is budgeted for $6,000 for the first project year and FTE with 3% increase for the following project year. Regular chemical supplies are needed to measure nitrogen, phosphorus content of the wastewater. For instance, Hach Chemical TOTAL NITROGEN LOW RANGE 25/PK for $79.00 (No.:TNT826), and Hach Chemical PHOSPHORUS TNTPLUS, UHR,25/PK (No.: NC9881792) for $57.23. One batch cell culture. It is estimated that around 20 boxes of each test kits are needed for us to work on the lab and pilot scale study for the first two years. For the third year, around 10 boxes of test kits for nitrogen and phosphorus are needed. A 3700 Full-Size Portable Sampler will be needed in the third year to take samples at Sarita Wetland, and the cost of this sampler is around $3K. The rest of the requested supply fund will be used to purchase other necessary chemicals, bottles and gloves etc, and lab analysis services.	$13,121	$13,121	$0	$6,180	$6,180	$0	$26,365	$26,365	$0	$45,666	$0
Capital Expenditures Over $5,000											
It is budgeted to purchase to purchase additional components for Dr. Hu's High Performance Liquid Chromatography (HPLC) for chemical analysis. Dr. Bo Hu's lab has already spent around $32K for a modular model of the HPLC, including pump, manual injection, multi-wavelength detector and software. This is a request to add an additional modular reflect index detector, an autosampler and an add-on to convert multi-wavelength detector to Photodiode Array detector.	$22,619	$22,619	$0							$22,619	$0
It is budgeted to purchase an automatic cell counter to measure the microalgae and fungi cell numbers in the lab experiments.	$9,990	$9,990	$0							$9,990	$0
Travel expenses in Minnesota											
Mileage, lodging, meals for travels to the water site for taking water samples. U of M plan for travel expense will be used to process the travel cost	$500	$500	$0	$515	$515	$0	$530	$530	$0	$1,545	$0
COLUMN TOTAL	**$106,822**	**$106,822**	**$0**	**$74,105**	**$74,105**	**$0**	**$100,073**	**$100,073**	**$0**	**$281,000**	**$0**