

# Final Abstract

Final Report Approved on January 11, 2025

## M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

**Project Title:** Assessing Membrane Bioreactor Wastewater Treatment Efficacy

**Project Manager:** Heiko Schoenfuss

**Affiliation:** Minnesota State Colleges and Universities - St. Cloud State University

**Mailing Address:** 720 4th Avenue South St Cloud State University, WSB-273

**City/State/Zip:** St. Cloud, MN 56301

**Phone:** (320) 308-3130

**E-mail:** hschoenfuss@stcloudstate.edu

**Website:** <https://www.stcloudstate.edu/>

**Funding Source:**

**Fiscal Year:**

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04f

**Appropriation Amount:** \$419,000

**Amount Spent:** \$372,868

**Amount Remaining:** \$46,132

### Sound bite of Project Outcomes and Results

By comparing traditional secondary treatment of municipal wastewater with membrane bioreactor treatment, we have determined that both are excellent options for reducing the overall contaminant load of wastewater. Membrane bioreactor treatment has the added benefit of requiring a smaller physical footprint while reducing microbial loads in the final discharged effluent.

### Overall Project Outcome and Results

Treated municipal wastewater effluent is an important pathway for contaminants to enter aquatic ecosystems. As the US aging wastewater infrastructure requires upgrades or replacement, assessing new treatment technologies, such as membrane bioreactors, in the context of adverse contaminant effects may provide additional support for science-based resource management. Membrane bioreactors require a smaller physical footprint than traditional secondary treatment and may eliminate the need for follow-up disinfection of effluents, thus reducing the addition of disinfectant chemicals and potentially harmful disinfection byproducts in the discharged final effluent. In 2022, we used three lines of evidence, analytical chemistry, fish exposure experiments, and microbiome analysis, to assess the effectiveness of membrane bioreactor treatment to replace traditional secondary activated sludge treatment. We analyzed treated wastewater

samples from a Minnesota Wastewater Treatment Plant with a split treatment stream (approximately half the primary effluent is treated with activated sludge, the rest with membrane bioreactor filtration) taken across nine months for alkylphenolic surfactants (known endocrine disruptors), bisphenol analogues (including BPA, a likely endocrine disruptor), hormones, pharmaceuticals, pesticides; heterotrophic bacteria counts; and respective effluent microbiome. We also exposed fish twice for three weeks each in an onsite controlled laboratory environment. We found that except for pesticides, total sample concentrations of contaminant classes were lower in traditional secondary treated wastewater or membrane bioreactor treated wastewater, but similar between the two secondary treatment methods. These reductions were consistent across the duration of the study. Fish remained healthy in both secondary treatment waters with few signs of stress. Bacterial diversity and bacterial loads were reduced by both secondary treatments but to a greater degree by the membrane bioreactor. Results from our study indicate that membrane bioreactor treatment is as effective as activated sludge treatment at producing high-quality effluent and offers a viable alternative to conventional secondary treatment methods.

### **Project Results Use and Dissemination**

This project produced a range of information that has been disseminated through several meeting presentations (for example MN Water Resources Conference), a St. Cloud State University Master's Thesis, USGS data reports, and two peer-reviewed manuscripts (Environmental Toxicology & Chemistry; Environmental Research) that are currently in final revision and should be accepted for publication shortly. All dissemination products have referenced support from the MN Environment and Natural Resources Trust Fund and will be shared upon publication.



## Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

### General Information

**Date:** January 13, 2025

**ID Number:** 2021-358

**Staff Lead:** Mike Campana

**Project Title:** Assessing Membrane Bioreactor Wastewater Treatment Efficacy

**Project Budget:** \$419,000

### Project Manager Information

**Name:** Heiko Schoenfuss

**Organization:** Minnesota State Colleges and Universities - St. Cloud State University

**Office Telephone:** (320) 308-3130

**Email:** [hschoenfuss@stcloudstate.edu](mailto:hschoenfuss@stcloudstate.edu)

**Web Address:** <https://www.stcloudstate.edu/>

### Project Reporting

**Final Report Approved:** January 11, 2025

**Reporting Status:** Project Completed

**Date of Last Action:** January 11, 2025

**Project Completion:** June 30, 2024

### Legal Information

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04f

**Appropriation Language:** \$419,000 the first year is from the trust fund to the Board of Trustees of the Minnesota State Colleges and Universities system for St. Cloud State University to conduct a comprehensive assessment of membrane bioreactor treatment of wastewater to inform managers of options for updating or replacing aging wastewater infrastructure.

**Appropriation End Date:** June 30, 2024

## Narrative

**Project Summary:** A comprehensive assessment of membrane bioreactor efficacy will provide the best options and information to the wastewater treatment plant and natural resource managers to update or replace aging wastewater infrastructure.

**Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Much of the rural wastewater treatment infrastructure in Minnesota has reached or exceeded its designed lifespan. Replacing existing aging infrastructure or adding new treatment components is expensive and often constrained by factors such as wastewater consistency, on-site logistics, operating expenses, and current and future permitting requirements. Membrane bioreactors have become an interesting alternative to standard secondary and tertiary treatment of wastewater due to their small footprints, low operating costs, and disinfection of effluent. However, information is currently lacking to inform Minnesota water resource managers to the treatment benefits of membrane bioreactors. This information is critically needed to provide Minnesota water resource managers for their decision making based on data. A comprehensive assessment of membrane bioreactor efficacy in Minnesota is required to inform the wastewater treatment plant (WWTP) operators and water resource managers of the best options for update and replace aging wastewater infrastructure in rural Minnesota.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

The proposed study will:

- (i) Monitor a wide range of common and emerging contaminants in treated wastewater outflows of traditional tertiary or membrane bioreactor effluent using the same wastewater inflow stream.
- (ii) Measure microorganisms in wastewater effluent treated either with tertiary treatment or a membrane bioreactor using effluent from the same inflow source.
- (iii) Measure the biological responses to traditional tertiary and membrane bioreactor effluent in real-time flow-through exposure experiments with native fish.

**What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

This study will provide a comprehensive assessment of membrane bioreactors vs. traditional tertiary treatment performances using a common source and a split-stream of wastewater flow at the Hutchinson WWTP. In general, membrane bioreactor technology is expected to efficiently remove pathogens and other bacterial community disruption common to streams receiving secondary treated effluents. These disruptions include fish microbiomes - an understudied cause of biological degradation in effluent dominated streams. The proposed project will provide critical information to renew or replace the aging infrastructure statewide.

## Project Location

**What is the best scale for describing where your work will take place?**

Watershed(s): Crow Wing River

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

In the Future

## Activities and Milestones

### Activity 1: Monitor a wide range of common and emerging contaminants in two effluents

**Activity Budget:** \$188,500

**Activity Description:**

Designed to treat an average of 3.67 million gallons per day (MGD) of wastewater, the WWTP (Hutchinson, MN) will meet the wastewater treatment needs of the city through the year 2028. Hutchinson WWTP uses Biological (Oxidation) and Membrane Bioreactor (MBR) processes to remove impurities from the wastewater.

We will collect time-integrated inflow and outflow samples from both the traditional tertiary treatment and the membrane bioreactor treatment pathways within the Hutchinson, MN, wastewater treatment plant (WWTP). Both process paths in the plant will be sampled approximately monthly during the summer season and weekly during three weeks twice coincident with fish exposures outlined in Activity 3 and analyzed for over 200 trace-organic compounds of concern, including pharmaceuticals and surfactants. Comparison of detections and concentrations of compounds between the two types of wastewater treatment technology will assess the ability of membrane bioreactor treatment to remove or reduce a wide range of trace organic contaminants. A total of 57 environmental and quality assurance samples will be collected from these sites and analyzed by the USGS National Water Quality Laboratory (NWQL) and AXYS Laboratories. We will coordinate data analysis of all laboratory results with samples collected and analyzed under Activity 2.

**Activity Milestones:**

Description	Approximate Completion Date
Publish the data in a publicly-available, web-accessible database	June 30, 2023
Water sample correction and chemical analysis	June 30, 2023
Produce a final report summarizing the results of Activity-1	June 30, 2024

### Activity 2: Measure the biological responses to traditional tertiary and membrane bioreactor effluent in real-time flow-through exposure experiments with native fish

**Activity Budget:** \$230,500

**Activity Description:**

We will expose native fathead minnows for 21 days to either treated wastewater following secondary treatment, wastewater treated with traditional tertiary disinfection, wastewater treated with a membrane bioreactor, or a control water source on-site utilizing our existing mobile exposure laboratory trailer (MELT). We will analyze essential biological endpoints such as physiological condition, reproductive ability, the gut microbiome community in fathead minnow. This approach will provide a holistic assessment of the totality of biological responses and health in native fish, and leading to a better understanding of aquatic environmental health in downstream of WWTP.

We will also characterize the bacteria communities found in the influent and effluents from the two treatment technologies using genetic-based methods that detect microbial genes associated with bacteria. A total of 40 samples will be analyzed using genetic material analysis of both raw water samples and viable, cultured organisms from the same samples. The sample collection will correspond to the same three weeks twice schedule under Activity 1. Results from the analysis will provide a microbial signature based on the viable bacterial community found in each effluent from the two treatment technologies. This is critical information in aquatic environmental health.

**Activity Milestones:**

Description	Approximate Completion Date
-------------	-----------------------------

. Conduct repeat exposure experiments on-site	June 30, 2023
Measure the microbial community in water samples and fathead minnow intestines	December 31, 2023
Produce a final report summarizing the results of Activity-2	June 30, 2024

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Satomi Kohno	Saint Cloud State University	Running exposure experiments and assessing biological activity.	Yes
Sarah Elliott	U.S. Geological Survey	Contaminants of emerging concern data analysis, including data QA/QC and public database support	Yes
Richard Kiesling	U.S. Geological Survey	Water quality sampling and statistical analysis	Yes

## Dissemination

**Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.**

The target audience for results from this research will be professionals in the areas of wastewater treatment and natural resource management. Specific targets will be wastewater and environmental engineers. In addition, scientists in academia, industry, state agencies such as the DNR and MPCA, and environmental consultants will be briefed on the findings from the proposed study. The regular meetings of the Contaminant Roundtable of MN State and federal agencies will provide another ready outlet for results from the current study. Results will be disseminated through scholarly publications in peer-reviewed journals such as Environmental Science and Technology. Results from the research project will also be presented at regional conferences such as the annual meeting of the Midwest Chapter of the Society for Environmental Toxicology & Chemistry (SETAC) and the Minnesota Water conference and if possible, at targeted seminars at the DNR and MPCA. Results will be used to determine whether membrane bioreactors provide adequate disinfection, nutrient removal and pollutant when compared to traditional activated sludge treatments.

## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?**

The proposed research supports a statewide research agenda focused on sources of contaminants of emerging concern and the protection of aquatic environments in Minnesota. The proposed research complements current and prior research that, to date, did not previously compare contaminants of emerging concern and their biological effect in effluents between Biological (Oxidation) and Membrane Bioreactor (MBR) processes at the same WWTP. This study will assess the efficacy of novel treatment technology to remove harmful contaminants from wastewater indirect chemical measurements and direct biological responses in rural municipalities in Minnesota.

## Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Biological Consequences of Septic Pollution in Minnesota Lakes	M.L. 2015, Chp. 76, Sec. 2, Subd. 04c	\$364,000
Protect Water Quality with Efficient Removal of Contaminants in Treatment Ponds for Storm Water	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04d	\$325,000

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>Personnel</b>										
Graduate student		To conduct MELT fish exposures and assist in analysis (Activity 2)			7.65%	0.5		\$8,612	-	-
Co-investigator		To conduct all activities and organizing data			40%	0.75		\$153,000	-	-
Principal Investigator		overall project oversight			40%	12		\$22,188	-	-
Undergraduate summer help		Assist in fish exposure experiments and water collections			0%	0.25		\$3,500	-	-
							<b>Sub Total</b>	<b>\$187,300</b>	<b>\$167,346</b>	<b>\$19,954</b>
<b>Contracts and Services</b>										
US Geological Survey	Professional or Technical Service Contract	2 USGS personnel (0.3FTE/ea.; 30% benefit; 1.5 years @\$55,000/ea.) \$110,000; Microbial analysis (Upper Midwest Science Center) \$11,000; National Water Quality Lab Analysis \$675/ea.x44 samples; shipping, mileage,supplies) \$30,000; Laboratory supplies \$14,000. AXYS analysis (525/ea.x40samples; setup) \$23,500				0.9		\$188,500	\$165,166	\$23,334
							<b>Sub Total</b>	<b>\$188,500</b>	<b>\$165,166</b>	<b>\$23,334</b>
<b>Equipment, Tools, and Supplies</b>										
	Tools and Supplies	Supplies for Biological Analysis. Egg your protein assay, \$15 x 400 fish =\$6,000; Hormone analysis, \$20 x 400 fish = \$8,000; Miscellaneous cost = \$1,000; Subtotal \$15,000 x 2 repeats = \$30,000.	Assess biological responses in the Lab.					\$30,000	\$29,633	\$367
	Tools and Supplies	Fish \$5 x 400 = \$2,000; Fish maintenance = \$500; exposure supplies = \$2,000; subtotal \$4,500 x 2 repeats = \$9,000.	Expose native fish, fathead minnow to effluents at the site.					\$9,000	\$9,000	-



							<b>Sub Total</b>	<b>\$39,000</b>	<b>\$38,633</b>	<b>\$367</b>
<b>Capital Expenditures</b>										
							<b>Sub Total</b>	-	-	-
<b>Acquisitions and Stewardship</b>										
							<b>Sub Total</b>	-	-	-
<b>Travel In Minnesota</b>										
	Miles/ Meals/ Lodging	50 miles each way x \$0.55 x 20 trips x 2 repeat = \$2,200; AirBnB rental in Hutchinson during fish exposures; \$1,000 for three weeks x 2 = \$2,000	Drive between St Cloud and Hutchinson					\$4,200	\$1,723	\$2,477
							<b>Sub Total</b>	<b>\$4,200</b>	<b>\$1,723</b>	<b>\$2,477</b>
<b>Travel Outside Minnesota</b>										
							<b>Sub Total</b>	-	-	-
<b>Printing and Publication</b>										
							<b>Sub Total</b>	-	-	-
<b>Other Expenses</b>										
							<b>Sub Total</b>	-	-	-
							<b>Grand Total</b>	<b>\$419,000</b>	<b>\$372,868</b>	<b>\$46,132</b>

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
---------------	---------------------	-------------	--

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>State</b>						
			<b>State Sub Total</b>	-	-	-
<b>Non-State</b>						
Cash	US Geological Survey Cooperative Matching Funds	This will support part of chemical and microbiome analyses in the USGS laboratory as matching funds.	Pending	\$55,000	-	\$55,000
			<b>Non State Sub Total</b>	<b>\$55,000</b>	-	<b>\$55,000</b>
			<b>Funds Total</b>	<b>\$55,000</b>	-	<b>\$55,000</b>

## Attachments

### Required Attachments

#### *Visual Component*

File: [180dccbb-ea9.pdf](#)

#### *Alternate Text for Visual Component*

An assessment of efficacy in membrane bioreactor wastewater treatment. Wastewater Treatment Plant at Hutchinson is uniquely running both ordinary and novel membrane bioreactor treatments. We will assess if the membrane bioreactor treatment has any advantages in the removal of harmful contaminants, their biological effects, and microbial health in downstream using on-site mobile exposure Lab trailer. This project will provide the best options and information to the wastewater treatment plan...

### Supplemental Attachments

#### *Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other*

Title	File
Quotation for AXYS Chemical Analysis	<a href="#">c786b021-2c3.pdf</a>
Research Addendum finalized 10.26.2020	<a href="#">07dde4e5-84c.docx</a>
Background check form	<a href="#">97770e59-ea7.pdf</a>
Evaluating Membrane Bioreactors for Removal of Contaminants of Emerging Concern using Analytical Chemistry and Fathead Minnow ( <i>Pimephales promelas</i> ) Exposure (Master's Thesis)	<a href="#">1de2f50a-a8a.pdf</a>
Activity 1 Abstract	<a href="#">3e7f613d-857.docx</a>
Activity 2 Abstract	<a href="#">b132158f-911.docx</a>
MS Thesis Abstract	<a href="#">706f6b5a-f9d.docx</a>

## Difference between Proposal and Work Plan

### *Describe changes from Proposal to Work Plan Stage*

We have made two substantive changes in developing the workplan from the original proposal. (i) We eliminated fall/spring semester funding for the prospective graduate student at St. Cloud State University. The selected student will still receive summer salary to allow her/his full attention to the exposure experiments to be conducted at the Hutchinson Wastewater Treatment Plant. To provide tuition and salary support during the academic year, the graduate student will be expected to serve as a graduate teaching assistant in the Department of Biological Sciences at SCSU during his participation in this research. This change is consistent with the mission of the university to provide graduate students with a broad set of applicable skills through their graduate education. (ii) We replaced the analysis of hormones through AXYS Laboratories with an in-house analysis of estrogenicity in collected water samples. This change will not affect our ability to assess the overall efficacy of membrane bioreactor treatments when compared to traditional activated sludge treatment. To fill any gap through this reduced analytical schedule we will rely on our previous data sets to estimate likely presence of key hormonal compounds in the effluent as a part of the total estrogenicity of the effluent. These two changes were made to accommodate the recommended funding level for our proposal. All proposal objectives will remain intact despite these changes.

## Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

**Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?**

N/A

**Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?**

Yes, I understand the Commissioner's Plan applies.

**Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?**

No

**Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?**

N/A

**Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?**

N/A

**Does your project include original, hypothesis-driven research?**

Yes

**Does the organization have a fiscal agent for this project?**

No

## Work Plan Amendments

No Amendments Entered

# Status Update Reporting

## Final Status Update August 14, 2024

**Date Submitted:** September 26, 2024

**Date Approved:** October 7, 2024

### Overall Update

Our study set out to provide a comprehensive assessment of membrane bioreactor efficacy in removing contaminants from municipal wastewater and comparing this efficacy with that achieved by traditional tertiary (activated sludge) treatment in the split-stream Hutchinson Wastewater Treatment Plant. We completed all project activities, milestones, and outcomes as described in the original project proposal. Specifically, we monitored a wide range of contaminants in primary effluent as well as membrane bioreactor and activated sludge effluent. We also measured the presence and diversity of microorganisms in these effluents and conducted two replicate fish exposure experiments to measure the biological responses of fish to these effluents. Milestones associated with these activities have been reached, the data collected through these activities are available through USGS data bases and in two manuscripts submitted for publication. Several presentations to natural resource managers, wastewater treatment plant operators, and the scientific community have been given to broadly disseminate the results of this project in MN and beyond. A graduate student was supported through this project with a Master's thesis published as a result. The experience allowed the student to obtain a federal position in this area of study as a result.

### Activity 1

To comprehensively assess the efficacy of membrane bioreactor and activated sludge-treated effluents, we collected time-integrated inflow and outflow samples from both the traditional tertiary treatment and the membrane bioreactor treatment pathways within the Hutchinson, MN, wastewater treatment plant (WWTP). Both process paths in the plant were sampled approximately monthly during the summer season and weekly during three weeks twice coincident with fish exposure experiments. The samples were analyzed for a diverse range of trace-organic compounds of concern, including pharmaceuticals and surfactants. Laboratory results were subjected to extensive quality assurance and quality control measures and published through the publicly accessible USGS data base system. Analytical data were then integrated with results of the microorganisms monitoring and the replicate fish exposure experiments to gain a comprehensive understanding of how effluent treatment drives contaminant presence and concentrations and affects biota in receiving waters. Results of this activity are incorporated into one published Master's thesis and two manuscripts currently in peer-review. Shortened abstracts (to meet the 200 word limit) for all three written dissemination products are added as attachments.

*(This activity marked as complete as of this status update)*

### Activity 2

The purpose of Activity 2 was to assess the biological responses to effluent treated by either membrane bioreactor or traditional tertiary treatment. To do so, we exposed native fathead minnows for 21 days to either treated process, or an on-site reference water source utilizing our existing mobile exposure laboratory trailer (April-July, 2022). We analyzed essential biological endpoints such as physiological condition, reproductive ability, and the gut microbiome community in fathead minnow. We also characterized microorganism communities in the influent and effluents from the two treatment technologies using genetic-based methods that detect microbial genes associated with bacteria. Both exposure experiments and the microorganism sampling were completed successfully. Resultant data were subjected to rigorous quality assurance and quality control measures and integrated with the analytical chemistry data obtained through Activity 1. Information gleaned through this activity were incorporated into one published Master's thesis and two manuscripts currently in peer-review. Shortened abstracts (to meet the 200 word limit) for all three written

dissemination products are added as attachments.

*(This activity marked as complete as of this status update)*

### **Dissemination**

Since the last update, two manuscripts have been submitted to colleague- and peer-review, respectively:

1. Currently in peer-review at the Journal for Environmental Toxicology and Chemistry:

Christen CH, Elliott SM, Kiesling RL, VanDenBoom A, Kohno S, Givens CE, Schoenfuss HL. In review. A comprehensive assessment of membrane bioreactor contaminant removal efficacy through analytical chemistry, fish exposures, and microbiome characterization.

2. Currently in peer-review at the journal Environmental Science: Water Research & Technology:

Givens CE, Elliott SM, Kiesling RL, Christen CH, Schoenfuss HL. In review. Comparison of trace organic contaminant and microbial signatures of wastewater treated by membrane bioreactor and oxidation ditch methods.

Once published, copies of the papers will be attached to the work plan/final report or provided electronically to the LCCMR.



# Status Update Reporting

## Status Update June 1, 2024

**Date Submitted:** September 26, 2024

**Date Approved:** October 7, 2024

### **Overall Update**

All fieldwork, analytical work, and data processing have been completed. One manuscript, focusing on the two on-site fish exposure experiments at the Hutchinson Wastewater Treatment Plant, was submitted for colleague review (as required by the USGS), revised by the authors based on the colleague review, and is now readied for submission to the peer-reviewed journal *Environmental Toxicology & Chemistry*. A second manuscript, detailing water chemistry and the microbiome analysis has been advanced to the internal review stage. We have completed all outcomes as described in our original application.

### **Activity 1**

All activities have been completed. Water samples were collected, analyzed for a suite of compounds, and quality controlled. The data have been integrated into two manuscripts, one focusing on the period bracketed by the fish exposure experiments and a second describing water chemistry and microbiome changes throughout the entire sampling year.

### **Activity 2**

All proposed components of this activity were completed. Fish were exposed in two flow-through exposure experiments, tissues were analyzed for a host of environmentally relevant endpoints. Biological results were integrated with relevant water chemistry and microbiome data and submitted in a manuscript for colleague review (a USGS required step prior to journal peer-review submission). The revisions suggested by the colleague reviewer were integrated into the manuscript and the manuscript is now being readied for submission.

### **Dissemination**

A first manuscript is currently readied for journal submission. No additional updates since the last progress report.

# Status Update Reporting

## Status Update December 1, 2023

**Date Submitted:** November 17, 2023

**Date Approved:** January 25, 2024

### Overall Update

Our project to assess the relative removal efficacy of membrane bioreactors as an alternative secondary treatment is nearing completion. The focus of our work has shifted from sample analysis to data integration, quality control, and manuscript preparation. Our three-pronged approach of assessing water quality, fish health, and microbiome characteristics in activated sludge secondary effluent and membrane bioreactor treated effluent indicates that both methods have good efficacy in removing many traditional and emerging contaminants. Subtle differences in fish health outcomes and microbiome characteristics are currently being evaluated as we only recently received the last batch of hormone data from the USGS National Water Quality Laboratory. Nevertheless, we are on track to complete the project within its allotted timeline and budget and with completion of all activities and project outcomes. Results from this project will provide Minnesota's water treatment managers with information that can be used to make informed decisions regarding plant modifications and/or upgrades.

### Activity 1

All time-integrated inflow and outflow samples from the Hutchinson Wastewater Treatment Plant (WWTP) have been analyzed. Final results for hormone analysis (the last outstanding data set) were made available by the USGS National Water Quality Laboratory this summer and their integration into the overall data set is ongoing. USGS personnel has been working with personnel at St. Cloud State University to integrate chemical and biological data into one comprehensive manuscript describing the results of the fish exposure experiments (Activity 2) conducted in April-July 2022. A second manuscript is being outlined to include all results from analytical chemistry.

### Activity 2

All biological results have been analyzed and integrated into a thesis that was published in spring 2023 (Christen, C. Thesis. St. Cloud State University, May 2023). The biological data are being integrated with concurrent water chemistry (Activity 1) and fish microbiome data into one comprehensive manuscripts. The principal investigator (Schoenfuss) has been advancing this manuscript with his collaborators during the current reporting period, with a complete draft for co-author review expected by the end of 2023. Individual data sets are being quality controlled with some final comparisons between biological, microbiological, and cell-assay derived data. In addition, a second manuscript covering the entire sampling period is being prepared with input from SCSU collaborators.

### Dissemination

Elliott et al., October 2023. Trace Organic Contaminants in Wastewater Effluent: Comparison of Oxidation and Membrane Bioreactor Treatment Technologies. Minnesota Water Resources Conference, St. Paul, MN.

Elliott et al., November 2023. Trace Organic Contaminants in Wastewater Effluent: Comparison of Oxidation and Membrane Bioreactor Treatment Technologies. Minnesota Pollution Control Agency Wastewater Conference, Brooklyn Park, MN.

# Status Update Reporting

## Status Update June 1, 2023

**Date Submitted:** May 29, 2023

**Date Approved:** May 31, 2023

### Overall Update

This project evaluates the efficacy of membrane bioreactors to remove contaminants of emerging concerns when compared to traditional oxidative tertiary treatment. To accomplish this outcome, we combined three related but independent lines of investigation: (i) in-depth water chemistry analysis to compare the composition of the two effluents; (ii) biological effects assessment through 21-day exposures of fish in controlled on-site laboratory settings; and (iii) an analysis of the microbiome of the two effluents and of the gut of exposed fishes. All three approaches have advanced considerably since the last project update. Chemical analysis has been completed for most compounds (except pharmaceuticals and hormones) and these data have been integrated with the biological effects analysis, which is complete. Microbiome data have been generated and a first-pass analysis has been completed. Results have been presented at a regional toxicology meeting (April 22, Milwaukee, WI) and during a Master's Thesis defense (April 23). A comprehensive summary of the biological effects data and their integration with the available water chemistry has been published as a Master's Thesis (May 23).

### Activity 1

The successful sample collection was described in the last progress report. Since then, we have received data on plasticizers, surfactants, and pesticides from two analytical chemistry labs. These data have been subjected to quality analysis and integrated with the biological data. We are working actively with our partners at the USGS National Water Quality Laboratory (Denver, CO) to obtain the remaining suites of data detailing pharmaceutical and hormone concentrations in the two effluents. We anticipate to receive all remaining water chemistry data during the next reporting period and will immediately integrate those data with the biological effects data.

### Activity 2

All biological data have been analyzed as proposed in the original proposal. These include molecular gene expression data, concentrations of various bio-molecules in the blood of exposed fish, microscopic analysis of fish tissues including reproductive organs, livers, and gills, and data on reproduction and reproductive behaviors. These analysis have been integrated into a common mode-of-action framework and published as a Master's Thesis in May 23. These data have also been integrated with the available water chemistry to identify compounds that may be causally linked to the observed biological effects.

Concurrent to the biological effects analysis, the microbiome data for the two effluents and guts of fish maintained in the two effluents has been analyzed. A first-pass analysis of these data has been completed and the data are now being integrated with the available water chemistry and the biological effects data.

### Dissemination

Three presentations on the results of this study were given during the current reporting period:

1. An oral presentation at the Annual Midwest Meeting of the Society for Environmental Toxicology & Chemistry (C. Christen, April 23 Milwaukee, WI)
2. A poster presentation at the Annual Midwest Meeting of the Society for Environmental Toxicology & Chemistry (Fleck & Xiong, April 23 Milwaukee, WI)
3. A thesis defense seminar was held at St. Cloud State University (C. Christen, April 3, 23 St. Cloud, MN)

In addition, a comprehensive analysis of the biological effects data, integrated with the available water chemistry was published as a Master's Thesis (C. Christen, St. Cloud State University, May 23)



# Status Update Reporting

## Status Update December 1, 2022

**Date Submitted:** November 11, 2022

**Date Approved:** November 22, 2022

### Overall Update

We have made excellent progress in comprehensively assessing the potential of membrane bioreactors to remove common and emerging contaminants from wastewater and to compare the efficacy of this advanced treatment with traditional tertiary treatment. We collected inflow and treatment water samples from the membrane bioreactor and tertiary effluent of the Hutchinson Wastewater Treatment Plant (WWTP) between April and September 2022 and submitted those for detailed chemical analysis. Microbial samples were taken concurrently to ascertain the potential of membrane bioreactors to disinfect effluent without the need for chemical treatment. We successfully completed two replicate on-site fish exposure experiments at the Hutchinson WWTP between May and July 2022. Samples collected for these fish have been archived and analysis is ongoing. Two presentations were given during this reporting period to share preliminary results with stakeholders on the regional and national level. A short write-up of the ongoing study was prepared in collaboration with staff at the Hutchinson WWTP to be published by the Minnesota Wastewater Operators Association.

### Activity 1

To monitor a wide range of common and emerging contaminants in wastewater and to assess the relative efficacy of their removal by membrane bioreactor or tertiary treatment, we collected water samples at the Hutchinson Wastewater Treatment Plant (WWTP). Samples were taken monthly from April to September (while the membrane bioreactor is in use) and weekly during two replicate fish exposure experiments between May and July 2022. All samples have been processed and shipped to the analytical laboratories for analysis (National Water Quality Laboratory, Denver, CO; AXYS Laboratory, Vancouver, CA). Preliminary data for surfactants (alkylphenols) and plasticisers (Bisphenol A) indicate greater removal efficacy by membrane bioreactors than tertiary treatment. Both forms of treatment, however, reduce concentrations of these compound classes when compared to influent wastewater. Analysis is ongoing and more data are expected to arrive during the next reporting period.

### Activity 2

To measure the biological response to wastewater treated either by membrane bioreactor filtration or tertiary treatment, two replicate fish exposure experiments were conducted between May and July 2022. In both experiments breeding pairs of fathead minnows were placed in spawning tanks and randomly assigned to either a control, membrane bioreactor, or tertiary flow-through treatment. Exposures lasted 21 days and were concurrent with water and microbial sampling. Following the collection of several reproduction related endpoints, fish were sacrificed to assess molecular, cellular, and organismal responses to the exposures. Samples from both experiments and all fish have been archived and are currently being analyzed. Analysis for reproductive hormones has been completed as has the analysis of blood-borne parameters such as egg-yolk and blood glucose concentrations. Molecular and histological analyses are ongoing. Most of these analyses are expected to be completed in the next reporting period. Microbiological samples have been submitted for analysis.

### Dissemination

Two presentations were given during this reporting period to share preliminary results of these investigations with regional and national water resource managers and scientists:

(i) MN Water Resources Conference, St. Paul, MN, October 2022: Charles Christen "Assessing the efficacy of Contaminant of Emerging Concern Removal by Membrane Bioreactor in Wastewater Treatment Utilizing Fathead Minnow (*Pimephales promelas*) Exposure". Oral presentation.

(ii) Society for Environmental Toxicology, North America annual meeting, Pittsburgh, PA, November 2022. "Assessing the Efficacy of Contaminant if Emerging Concern Removal by Membrane Bioreactors." Poster presentation.

In addition, we collaborated with staff at the Hutchinson WWTP on a short write-up on this study for "Wastewatcher", the publication published by the Minnesota Wastewater Operators Association. This publication is forthcoming.

# Status Update Reporting

## Status Update June 1, 2022

**Date Submitted:** June 2, 2022

**Date Approved:** July 8, 2022

### Overall Update

The overall goal of this project is to provide a comprehensive assessment of membrane bioreactors vs. traditional tertiary treatment performances using a common source and a split-stream of wastewater flow at the Hutchinson Wastewater Treatment Plant (WWTP). To accomplish this outcome, we proposed to conduct repeated water sampling of wastewater inflow, as well as outflows from the membrane bioreactor and the traditional tertiary treatment. This analytical approach was to be augmented by replicate 21-day fish exposures to these water sources (and a negative control) to determine the overall impact of treatment technologies on the downstream biota. To-date, we have conducted four rounds of water sampling and send these samples to the respective analytical laboratories. We also designed and executed the first of two replicate 21-day fish exposure studies. Samples from the first exposure experiment have been archived and will be analyzed after the second exposure experiment (scheduled for June 2022) has been completed.

### Activity 1

To assess the efficacy of membrane bioreactors vs. traditional tertiary treatment, we proposed to sample the wastewater inflow prior to its split and the outflows from the membrane bioreactor and the traditional tertiary treatment. We begun the monthly sampling regime in spring 2022 and intensified it to weekly sampling during our first 21-day fish exposure experiment in May'22. All collected samples have been logged and shipped to the respective analytical laboratories for analysis. We anticipate early data to be provided from these laboratories in fall 2022.

### Activity 2

The key objective of this activity was to measure the biological responses of fathead minnows to traditional tertiary and membrane bioreactor effluent in real-time flow-through 21-day exposure experiments. We developed the necessary experimental protocols and exposure infrastructure during winter/spring 22, placed the Mobile Exposure Laboratory Trailer (MELT) on-site at the Hutchinson WWTP in April, and conducted a complete 21-day exposure experiment in May 2022. This first experiment was recently completed successfully and all samples collected during this experiment have been archived. Following a replicate exposure experiment scheduled for June 2022, all samples will be analyzed with preliminary data becoming available in fall 2022.

### Dissemination

An abstract (Christen et al.) describing the experimental design and objectives of this study was submitted and accepted for the North American Annual Meeting of the Society for Environmental Toxicology & Chemistry (SETAC) in November 2022 in Pittsburgh, PA. The timing of the conference will allow us to present a range of biological and preliminary chemical results from this study.