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Low Temperature Biological Treatment of Chemical Oxygen Demand for Agricultural Water Reuse Application Using Robust Biocatalysts

Vedansh Gupta, Allyson Lutz, Ameen Razavi, Fatemeh Shirazi

***Abstract*—**The agriculture industry is especially vulnerable to forecasted water shortages. In the fresh and fresh-cut produce sector, conventional flume-based washing with recirculation exhibits high water demand. This leads to a large water footprint and possible cross-contamination of pathogens. These can be alleviated through advanced water reuse processes, such as membrane technologies including reverse osmosis (RO). Water reuse technologies effectively remove dissolved constituents but can easily foul without pre-treatment. Biological treatment is effective for the removal of organic compounds responsible for fouling, but not at the low temperatures encountered at most produce processing facilities. This study showed that the Microvi MicroNiche Engineering (MNE) technology effectively removes organic compounds (> 80%) at low temperatures (6-8 °C) from wash water. The MNE technology uses synthetic microorganism-material composites with negligible solids production, making it advantageously situated as an effective bio-pretreatment for RO. A preliminary technoeconomic analysis showed 60-80% savings in operation and maintenance costs (OPEX) when using the Microvi MNE technology for organics removal. This study and the accompanying economic analysis indicated that the proposed technology process will substantially reduce the cost barrier for adopting water reuse practices, thereby contributing to increased food safety and furthering sustainable water reuse processes across the agricultural industry.

***Keywords*—**Biological pre-treatment, innovative technology, vegetable processing, water reuse, agriculture, reverse osmosis, MNE biocatalysts.

I. Introduction

GLOBAL famine is a widespread scarcity of food, caused by several factors including failure of crop due to drought. The risk of famine rose 80% from 2015-2019, mainly driven by drought [1], threatening water quality [2], and increasing water insecurity [3], [4]. The United States Environmental Protection Agency (US EPA) notes that since current water supplies are insufficient for future projected water demands, water reuse technologies are vital to extending water supplies [5]. Agriculture historically requires large quantities of freshwater and is therefore vulnerable to climate change-related water shortage [6], [7]. In addition to water consumption during growing periods, the food industry supply chain also experiences high freshwater consumption rates, including for washing and packaging [8]. Among these food processing industries, the fresh and fresh-cut produce industry, in particular, is an intense water consumer [9]-[11]. One study estimated that 1.5 to 5.0 m^3 of freshwater is consumed per ton of finished fresh-cut vegetable product [12]. Considering its high-water usage and increasing demand for fresh produce [13], the fresh-cut vegetable produce industry has great potential to benefit from water reuse technologies and practices.

While water reuse provides an opportunity for increased sustainability in food processing, pathogens encountered throughout agricultural processes often pose risks to human health [14]-[17]. Therefore, proper disinfection [18] must be utilized in the water reuse processes to minimize pathogens and ensure public safety. Microbial contaminants of concern include thermotolerant coliforms, *Salmonella*, Enteric viruses, *Giardia lamblia*, and *Shigella*, which are causative agents for a variety of life-threatening diseases [19]-[21]. Integration of a pretreatment system integrated with a RO technology has been proposed for microorganism removal, with removal rates of 99.9% or greater [22].

Currently, the dominant washing method in the fresh and fresh-cut produce processing industry is flume-based washing with water [23]. This system involves three washing steps (“triple wash”): two submerged washing steps (primary and secondary tanks) using sanitized freshwater (with chlorination or peracetic acid), and a final rinsing step with potable freshwater. With this configuration, wash water in the flume tank may be recirculated over a 6–8-hour period. The washing process inherently leaves behind significant vegetable liquid and debris in the flume tank, and the constant input of organic matter consequently depreciates wash water quality over time [23]. In addition, these organic compounds have the potential to react with the free chlorine necessary for pathogen disinfection. These reactions can lead to the production of chlorinated compounds or disinfectant byproducts (DBPs), which pose serious health risks [24]-[26]. In addition, these reactions also decrease the concentration of free chlorine available for disinfection, thus limiting disinfection efficacy [24], and increasing chlorination costs.

State-of-the-art water reuse processes include RO and advanced oxidation with ozone, hydrogen peroxide, and/or UV irradiation. RO and other membrane technologies are effective for removing dissolved constituents, which is essential to water

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reuse and the removal of pathogens. However, membrane-based technologies are susceptible to membrane fouling [27]. RO pretreatment can improve membrane performance; however, significant technical challenges need to be overcome to cost-effectively apply water reuse in the produce industry:

1. Low-temperature wash water treatment using physical treatment (RO) can be expensive without any pre-treatment to tackle the high load of organics [26].
2. Use of large quantities of chlorine to disinfect wash water can lead to the formation of harmful chlorinated compounds [27]. Moreover, residual chlorine is detrimental to membranes as the amide linkages in the RO membrane are susceptible to attack by chlorine, reducing the lifespan of the membrane [28].

Soluble organic matter such as sugars, proteins/peptides, and organic acids, all varying in molecular weight, are difficult to remove using physical processes. Most organics can be easily removed biologically [29], [30]. However, conventional biological degradation is largely ineffective at low temperatures, which are common at produce processing facilities [29], and generates microbial waste, which would require stringent handling at a produce processing facility [26], [31].

The application of Microvi's patented MicroNiche™ Engineering (MNE) platform in this study offers a biological pretreatment process without microbial waste generation for enhancing RO-based water reuse processes even at low temperatures. In contrast to conventional biological technologies (suspended or fixed-film growth), the MNE technology uses synthetic microorganism-material composites [32], [33]. These composites are called "biocatalysts" since instead of growing and removing cells, the MNE technology operates on the paradigm of maintaining and controlling cells' microenvironment. The MNE technology biocatalyst composites integrate two elements: 1) highly hydrated polymer composites with engineered geometric and molecular properties; and 2) pre-grown cells (proprietary MB-Strain 17) especially selected for their suitability and effectiveness for soluble organic matter degradation at low temperatures.

The overall goal of this study was to develop an MNE-based technology process to treat fresh and fresh-cut produce wash water for the removal of organic compounds at low temperatures through the following objectives:

- To evaluate the kinetics of degrading dissolved organic compounds in wash water using MNE biocatalyst composites at low temperature.
- To optimize the MNE biocatalyst in order to maintain high specific flux in a subsequent RO membrane purification step (i.e., serial processing).

II. Materials and Methods

A. Degradation Studies

For the kinetic studies, three different types of wash waters were used: lettuce-based synthetic wash water (SWW), carrot-based SWW, and produce wash water (PWW). SWW was produced from filtrate from blended produce pulp (either lettuce or carrot). This filtrate was diluted to reflect an organics (defined as Chemical Oxygen Demand (COD)) concentration often observed in wash water facilities 800 mg/L [26]. PWW was actual wash water acquired from an agricultural cold storage facility in Modesto, CA, with COD levels of 286 ± 6 mg/L. The facility utilizes a multi-pass hydrocooler system to reduce produce temperatures before cold storage. The vegetables passed through this system are typically leafy greens or herbs. The water collected from the hydrocooler is passed over these vegetables multiple times to remove "field heat" before being wasted from the system. This waste was used as the PWW and collected on several occasions, each with variability in the type and quantity of produce that had been passed through. Upon collection, the PWW was tested for soluble COD and other water quality parameters and then stored at 4 °C until experiments were conducted.

Initial kinetic studies were conducted inside a refrigerated chamber set to 6-8 °C. Trials were conducted in autoclaved, covered 500 mL glass flasks with sterile magnetic stir bars. For each trial, the selected wash water was added to flasks containing suspended cells (MB Strain 17), MNE technology biocatalysts composites, or neither (blanks as a control). Each flask was aerated with filtered air via a sterile 10 mL serological pipette and mixed via a stir plate in the refrigerated chamber at 6-8 °C. Each flask was sampled at 0, 0.5, 1, 2, 4, 6, and 8 hours. At each sampling point, the pH and DO were measured and samples were collected for COD, ortho-Phosphate (PO_4^{3-}), and Ammonia (NH_3) analysis. Each trial was considered separately, and the rate order constants and R^2 values were averaged over a given condition. Average values are reported with standard deviations.

B. Serial Processing

From batch testing, it was determined that an 8-hour hydraulic retention time (HRT) would be sufficient for an aerobic continuously stirred tank reactor (CSTR) in a downflow configuration. The reactor was set up first on the bench at room temperature. The influent (PWW) and effluent of the reactor were sampled for one week before being placed in the refrigerated chamber to maintain a water temperature between 6-8 °C. The influent and effluent were sampled twice a day. Effluent from the CSTR was collected at the outfall and stored at 4 °C for subsequent processing in the RO system. Effluent from the CSTR was passed through the RO Purification Unit, Model EP23152, a small-scale system. Before being used for this task, the system was modified to only use RO step. A pre-RO filtration (5 µm) was added for additional pretreatment according to the treatment scheme being evaluated. Samples from the CSTR and RO system were collected to measure COD as well as turbidity using a Hach COD Kit and a Hach DR 890 Colorimeter, respectively.

Before sampling the RO system, a permeate baseline flow rate was established using tap water pumped into the system at 15 mL/min. At this continuous flowrate, the rate of soluble COD removal began to decline, that was considered a system breakthrough, and the amount of water treated by the system up to that point was taken as the baseline treatment capacity of that particular condition. Figs. 1 and 2 show the configurations tested for serial processing. The PWW was chlorinated for the relevant trials by adding 200 mg/L total chlorine as sodium hypochlorite to the PWW vessel.

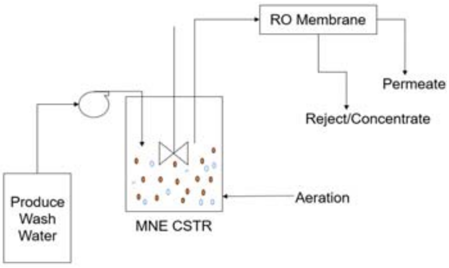

Fig. 1 Process Flow Diagram of the RO system with MNE technology pretreatment

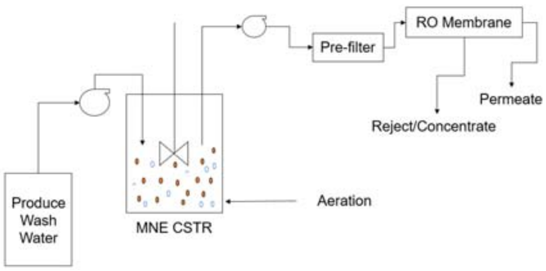

Fig. 2 Process Flow Diagram of the RO with MNE technology pretreatment and filtration

C. Techno-Economic Analysis of the MNE Technology

Based on the specific rates observed in the study, a techno-economic analysis was completed to compare COD removal using an MNE technology followed by polishing chlorination vs. chlorination only. Three scenarios were examined for produce processing facility having wash water with: 1) an initial COD of 850 mg/L COD and a flowrate of 113-126 m^3/day [26], 2) an initial COD of 850 mg/L and a flowrate of 30-34 m^3/day as observed at the cold storage facility in Modesto, CA, and 3) an initial COD of 280 mg/L and a flowrate of 30-34 m^3/day similar to the facility in Modesto, CA. Key assumptions used in the analysis included a chlorine demand ratio to COD of 4.43 mg/mg, a price of electricity of \$0.12/kWh, the price of 12% NaOCl of \$0.53/Liter, and an inflation rate of 5%.

D. Analytical Methods

Table I describes the methods and equipment used for each analysis performed in this study. All Hach methods were periodically checked for accuracy by running solutions of known standards. Standards prepared for the microplate phosphate assay were checked against the Hach methods.

III. Results and Discussion

A. Degradation Studies

Kinetic studies were performed using SWW (produced from lettuce and carrot pulp) and actual PWW. The initial water quality for each wash water is presented in Table II. The results of the kinetic study found that at cold temperatures, the MNE biocatalysts performed better than the suspended MB Strain 17 cells, removing more COD (approximately 500 mg /L vs 100-200 mg/L) as well as having a larger removal rate constant (data not shown).

TABLE I
Test Methods for Given Parameters Used over the Course of This Study

Parameter	Equipment	Method
Ammonia	Hach DR870 Colorimeter	Method 10023, 0-2.50 mg/L NH_3-N
Total Chlorine	Hach DR870 Colorimeter	Method 8167, 0-2 mg/L
Nitrate	Hach DR870 Colorimeter	Method 10020, 0-30.0 mg/L NO_3-N
Nitrite	Hach DR870 Colorimeter	Method 8507, 0-0.350 mg/L NO_2-N
Orthophosphate	BioRad iMark™ Microplate Absorbance Reader	630nm, 0.01-1 mg/L PO_4-P
Soluble COD	Hach DR870 Colorimeter	Method 8000, 3-150 mg/L
Total Suspended Solids	-	EPA Method 160.2
Turbidity	Hach DR870 Colorimeter	Method 8237, 0-1000FAU

TABLE II
Water Quality Data of Types of Wash Water Used in the Study

Type of Wash water	Average COD (mg/L)	Average NH3-N (mg/L)	Average Ortho-P (mg/L)	COD:N:P
Lettuce-based SWW	840	5.6	6.4	150:1:1
Carrot-based SWW	705	0.8	2.3	881:1:3
PWW	260	1.0	0.35	743:3:1

Similar low temperature trials using carrot SWW with only MNE biocatalysts and controls found that at low temperatures, MNE biocatalysts degraded the COD more slowly than when using lettuce SWW. While similar initial COD concentrations were used in lettuce SWW, carrot SWW proved to have much lower initial concentrations of orthophosphate and ammonia present (as shown in Table II). Differences in degradation rates could be due to either low concentrations of supporting nutrients and/or greater fractions of non-biodegradable COD as also reported by Ardley et al. [34] where they found only about 30-40% of the total COD from carrot wastewater available in the biodegradable form.

Finally, actual PWW at low temperatures had a COD of approximately 260 mg/L. While the total concentration of COD degraded was lower than the total concentration removed from the lettuce water at low temperatures, the degradation of COD in the PWW accounts for nearly 75% of the total COD. The first-order rate constant under these conditions is similar to the lettuce water trials, indicating that, despite the low concentrations of ammonia and orthophosphate as compared to the lettuce SWW (Table II) and the low temperatures, the MNE biocatalysts effectively degraded COD.

B. Serial Processing

The CSTR with an HRT of eight hours was first operated at room temperature (~25 °C) to establish a performance baseline. The reactor ran for one week at room temperature (removing

~80% of the influent COD) and was then placed in a refrigerated chamber at 6-8 °C. The influent and effluent COD levels and the COD removal rate increased from 85% to 95% over this period (Fig. 3). These results are similar to [26] where they showed about 80% COD removal at both room temperature and at 4 °C using MNE biocatalysts. The influent PWW also received a one-time spike of sodium hypochlorite to provide a total chlorine concentration of 200 mg/L. The MNE technology biocatalysts were able to achieve removal rates of 70-80% from the chlorinated water (Fig. 4). CSTR-treated water, which removed COD and decreased the turbidity (35.2 ± 16.9% turbidity reduction), was passed through the RO system.

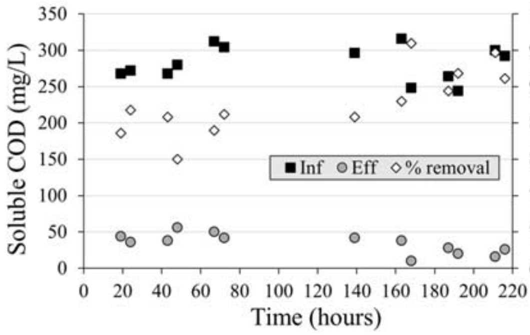

Fig. 3 Soluble COD removal over time in the MNE technology CSTR reactor at low temperature using unchlorinated PWW

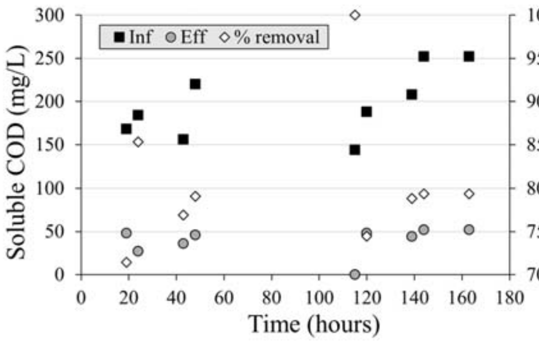

Fig. 4 Soluble COD removal over time in the MNE technology CSTR reactor at low temperature using chlorinated PWW

Due to the RO system design, which was configured for a constant outlet flow rate rather than a particular effluent water quality, the RO's performance was quantified by percent improvement in terms of amount of volume treated (Fig. 5). A treatment target of ≥ 70% COD removal was set. The performance was quantified by the volume of water processed by the RO before the COD removal dropped below 70%. Untreated and unfiltered PWW (COD of 140-220 mg/L) was passed through the RO system, treating 2300-2500 mL with ≥ 70% COD removal. Using the MNE technology treated CWW (COD of 45 mg/L), the RO system produced 3420 mL. Using MNE technology treated water where a higher level of treatment had been observed (COD of 20 mg/L), the RO produced 4650 mL. Finally, the RO was modified to include a 5 µm filter pre-treatment in addition to the MNE technology pretreatment. Under this condition, the RO system was able to produce 4800 mL. In addition, the extra filtration step was shown to reduce turbidity (Formazin Attenuation Units (FAU)) by 61.7 ± 5.8%. These results are in line with the results reported in the literature which showed less decrease in the normalized specific flux of an RO system when the influent was treated with MNE technology biocatalysts as compared to the influent water treated only with micro-filtration or ultra-filtration [26].

C. Techno-Economic Analysis of the MNE Technology

To examine the economic feasibility of the MNE technology for low temperature COD removal from PWW, a technoeconomic analysis (TEA) was completed for the three-scenarios as listed in Table II. The TEA was performed for a period of 20-years (Table III). Costs were estimated by calculating system needs based on the removal rates determined from the degradation studies. Considerations in the TEA included: 1) operational costs of the MNE technology including the cost of aeration, 2) maintenance to remove the amount of COD specified for the given scenario, 3) the cost of polishing chlorine post-MNE technology. Capital costs of the MNE technology include the cost of the biocatalysts, tanks, and major equipment (mixer, blower, pump), excluding installation.

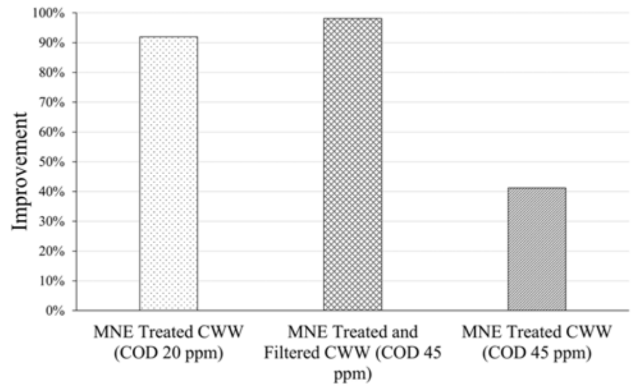

Fig. 5 Percent improvement over untreated water in volume of water treated to ≥ 70% COD removal

TABLE III
SCENARIO-SPECIFIC ASSUMPTIONS USED FOR TEA

Scenario	Flow Rate	Flow Rate	Initial COD	COD Removal by MNE (as per degradation studies)
Produce Processing Facility (850 mg/L COD)	113-12y6 m^3/day [26]	83 L/min	850 mg/L	520 mg COD/L
Produce Production Facility (850 mg/L COD)	30-34 m^3/day	23 L/min	850 mg/L	520 mg COD/L
Produce Production Facility (280 mg/L COD)	30-34 m^3/day	23 L/min	280 mg/L	245 mg COD/L

The TEA found that the most substantial savings and the fastest return on investment (ROI) occurred for a typical produce processing facility that had the largest COD load (Table IV). Even the facility with the smallest COD load considered would see a savings of $1,073,000 over 20 years and see a full ROI of an MNE technology during the second year of operation. Additional savings from the implementation of water reuse within the facility were not considered in this analysis but may be significant.

TABLE IV
ESTIMATED ECONOMIC ANALYSIS FOR FULL REMOVAL OF COD BY CHLORINATION ONLY VS MNE WITH POLISHING CHLORINATION

Scenario	Produce Processing Facility (850 mg/L COD)	Produce Production Facility (850 mg/L COD)	Produce Production Facility (280 mg/L COD)
Flow Rate	83 lpm	23 lpm	23 lpm
Full Oxidation of COD by Chlorination Only			
Chlorine cost per year	$1,227,000	$330,000	$108,000
Chlorine cost (20-year)	$16,509,000	$4,431,000	$1,450,000
Full Oxidation of COD by MNE with Polishing Chlorination			
MNE OpEx per year	$10,000	$3,000	$2,000
Chlorine cost per year	$485,000	$131,000	$16,000
MNE CapEx	$322,000	$190,000	$174,000
MNE cost (20-year)	$6,848,000	$1,941,000	$377,000
20-year cost savings	$9,661,000	$2,490,000	$1,073,000
Return on Investment	By 1st year	By 1st year	By 2nd year

IV. CONCLUSIONS

In the U.S., 40 states expect water shortages over the next 10 years [35]. Agriculture is a major consumer of water and especially vulnerable to these shortages. Water reuse is a sustainable way of reducing water demand through treatment by membrane technologies or biological treatment. However, membrane-based technologies are susceptible to fouling without pre-treatment. Biological treatment is effective for the removal of organic pollutants, but conventional biodegradation is ineffective at the low temperatures encountered at most facilities. Unlike conventional bio-treatments, the Microvi MNE technology for low temperature (6-8 °C) biological pretreatment of wash water uses synthetic microorganism-material composites. It was found that this technology can decrease COD in PWW by 80.5 ± 8.2% at low temperatures (final COD ≤ 23 mg/L) even for chlorinated water while producing negligible solids. Effluent from the CSTR was then passed through a RO system. The Microvi MNE technology pre-treatment system resulted in a 65% increase in the water treated by the RO system. A TEA using the preliminary data showed savings in excess of 60% in OPEX with using MNE technology for the removal of COD over the chlorination process alone.

This study demonstrated the feasibility of bio-treatment of PWW at low temperatures and indicated that the Microvi MNE technology is a promising RO pre-treatment for the removal of organics. By decreasing COD in wash water, this technology can decrease chlorine demand, facilitate disinfection and increase food safety, which provides both economic and public health benefits over conventional chlorination practices. Further studies are planned, including optimization of the CSTR system followed by a pilot-scale system.

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