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I, William K Garde, hereby submit this original work as part of the requirements for the degree of Master of Science in Environmental Engineering.

It is entitled:

Application of Moringa Oleifera Seed Extract to Treat Coffee Fermentation Wastewater

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Application of *Moringa Oleifera* Seed Extract to Treat Coffee Fermentation Wastewater

In Partial Fulfillment of the Requirement
for the Degree of

MASTER OF SCIENCE

In the Department of Biomedical, Chemical, and Environmental Engineering
Of the College of Engineering and Applied Science at the University of Cincinnati

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Abstract

Wastewater generated from wet processing of coffee beans degrades stream water quality downstream of processing mills and impacts human health. The widespread popularity of coffee as an export makes this a global problem even though the immediate impact is local. Coffee is grown in 70 countries across the globe, and is worth about \$100 billion annually—this is greater than the annual GDP of 2/3 of the 195 countries on the planet today. Approximately 40% of all coffee around the world is wet processed, producing wastewater rich in organic nutrients that can be hazardous to aquatic systems. *Moringa Oleifera* Seed Extract (MOSE) offers promise as a sustainable, local and affordable coagulation technology for aiding in the treatment of coffee wastewater. To date, its ability to reduce turbidity in coffee pulping wastewater has been established, but the reduction of total suspended solids (TSS), chemical oxygen demand (COD), nitrate, nitrite, and total nitrogen in coffee fermentation wastewater (CFW) has not been well characterized. As a result, field research was conducted at the Kauai Coffee Company in Hawaii to investigate the potential of MOSE as a viable treatment option for CFW. Coagulation tests were conducted in the field at five pH CFW levels (3, 4, 5, 6, and 7) and MOSE doses (0, 1, 2, 3, and 4 g/L) using pre-cleaned, 1-quart glass canning jars with metal lids. After settling, TSS, COD, nitrate, nitrite, total nitrogen, and pH of supernatant from each jar were measured. MOSE reduced TSS, COD, nitrate, and nitrite in CFW to varying degrees dependent on pH and dose applied. MOSE did not significantly alter the pH of CFW. TSS removal ranged from 8% to 54%. Insoluble COD removal ranged from 26% to 100% and total COD removal ranged from 1% to 25%. Although MOSE has a high organic content, only 25% is soluble and would be retained in the treated effluent. The ratio of soluble MOSE COD to potential CFW COD removal was shown to be approximately 0.04. Soluble COD is approximately 70% of the total COD in CFW and must be removed through treatment methods other than coagulation and settling. Nitrate and

nitrite reduction ranged from 20% to 100%. Optimum removal efficiency for MOSE is between 2-3 g/L and maximum TSS, COD, nitrate, and nitrite removal was observed between pH levels 5-6. Total nitrogen increased with increasing MOSE dose due to dissolved organic nitrogen in MOSE solution. MOSE shows promise as sustainable, local, and affordable coagulant for treating CFW.

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“He has told you, O man, what is good;
and what does the Lord require of you?
but to do justice,
to love kindness,
and to walk humbly with your God.”

Micah 6:8

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Introduction

Coffee. Few things in life are as common as coffee. It is both intimate and universal. It crosses geographic and cultural boundaries. Many people can remember when they had their first cup of coffee, how it tasted, where it was, whom it was with, and whether or not they ever had another cup. There is an intangible experience that surrounds coffee and the way it connects us to people and place. How many business transactions have been made over coffee? How many friendships and relationships began with that first coffee date? How many ideas have been generated during a cup of coffee? How many achievements have been fueled by coffee? It is beyond number. Yet many of us know very little about how this beverage comes to exist and the process that takes place from the coffee crop to coffee in our cup.

There are many paths coffee may take from crop to cup, but one of the most popular is where the wet processing of coffee cherries is used. Unfortunately, this process has been the source of much environmental debate in the coffee sector. Over the past few years, the Specialty Coffee Association of America (SCAA) has begun to highlight the environmental impact the coffee industry has on natural resources in countries where it is grown. Specialty coffee is coffee that receives at least an 80 out of 100 quality score by certified SCAA graders. The specialty coffee industry consists of companies such as Blue Bottle Coffee, Peet's Coffee, and Starbucks.

Leading coffee professionals and companies are beginning to take a serious look at the way their coffees are grown and processed. One area of adverse environmental impact is how coffee wet processing activity can render surface water resources unusable during coffee processing season due to discharge of untreated or poorly treated wastewater. To date, very few coffee farms and mills have adopted sound environmental policies and practices for treating their wastewater—nor has the specialty coffee industry been successful in pushing for these reforms. However, coffee

wastewater treatment technology exists, but has not been widely adopted. The reasons for lack of adoption are not completely known, but two reasons have surfaced: lack of access to treatment technology in rural coffee farming communities and economic barriers to implementation. Therefore, sustainable, local, and affordable treatment options are needed to reduce the negative environmental impact of coffee wastewater.

One such option is *Moringa Oleifera* Seed Extract (MOSE). The objective of this research was to evaluate the effectiveness of using this common coagulant to treat wastewater generated during the fermentation phase of coffee processing. Field jar tests were performed to measure the effectiveness of MOSE to treat coffee fermentation wastewater at four dosing increments (1-4 g/L), and five pH levels (3-7). The total suspended solids, total dissolved solids, chemical oxygen demand, nitrate, nitrite, and total nitrogen of the supernatant from each jar was measured. Additionally, coffee fermentation wastewater and MOSE were analyzed to determine the soluble and insoluble portion of each of the parameters measured to better evaluate the performance of MOSE.

Background

Coffee is grown in 70 countries across the globe, and is worth about \$100 billion annually (Thurston et al., 2013). Two thirds of the 195 countries in the world today have GDP lower than \$100 billion per year (World Bank, 2014). There are two primary methods for processing coffee in countries where it is grown. The two methods are dry processing and wet processing. Both methods have multiple variations, but almost all methods of coffee processing fall into one of these two categories. Approximately 40% of all coffee around the world is wet processed. This method is considered to produce superior tasting coffees, which corresponds with greater profits. In regions with abundant water resources, wet processing is a popular choice. However, pollution from wet processing activity is a growing environmental concern.

Traditional wet processing has two coffee wastewater (CWW) streams from milling activity: coffee pulping wastewater (CPW) and coffee fermentation wastewater (CFW). First, wet processed coffee is pulped. This is the step where the coffee bean is removed from the coffee fruit. Pulping wastewater is produced from this activity. Second, after pulping, the coffee beans are submerged in water in large vats. This step is referred to as fermentation and is a key step in the wet process and has a significant impact on coffee quality (Borem, 2014). Coffee is submerged for 24 to 48 hours during which enzyme activity breaks down the pectin layer encasing each bean, as shown in Figure 1. Enzyme activity is accompanied by a sharp decline in pH and a sharp increase in organic content in the fermentation water. Once fermentation has completed, the fermentation water is drained from the vats, releasing CFW. Figure 2 presents a simplified wet processing flowchart highlighting the two pollution streams of concern. Table 1 presents four stages of wet processing. Note the difference between picture B and D, the pectin layer has been removed by the fermentation process.

Figure 1: Structure of the Coffee Cherry (source: bestcoffeemaker2012.com)

Figure 2: Simplified Wet Processing Flowchart for Fully Washed, Semi-washed, and Pulped Natural Coffees

Table 1: (A) Coffee on the tree, (B) Pulped coffee with pectin (mucilage) layer, (C) Pilot scale fermentation tank with coffee, (D) Dried coffee after fermentation

(A)

(B)

(C)

(D)

Typically, both types of effluent are directly discharged into receiving water bodies with minimal to no treatment. The effluent produced from wet processing is characterized by high total suspended solids (TSS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) concentrations around 2,000 mg/L, 15,000 mg/L, and 10,000 mg/L. The World Health Organization limits for drinking water are 200 mg/L, 300 mg/L, and 100 mg/L, respectively

(Haddis and Devi, 2008). Table 2 presents typical pollutants and their concentrations gathered from the literature. Additionally, the wet processing has a high water demand, requiring approximately 10 m³ of water per tonne of processed coffee cherry (Chapagain and Hoekstra, 2007).

Table 2: Reported Values of CWW Pollution Loading in Published Literature

Authors/Date	M. Selvamurugan et al. (2010)	M. Adams and A. E. Ghaly (2007)	A. Haddis and R. Devi (2008)	Rossmann et al. (2013)	Beyene et al.* (2012)	Zayas et al. (2007)
Parameters	Concentration (mg/L unless otherwise stated)					
Color (CU)	470–640					
TDS	1130–1380				170	
TSS	2390–2820		5870	1729	598	
Total solids	3520–4200					
pH	3.88–4.11		3.57	4.7	4.6–7.4	4.6
Conductivity (dSm–1)	0.96–1.20			1.8		
DO	2.0–2.6				5.2	
BOD	3800–4780	10000	10800–14200	8005	436	
COD	6420–8480	18000	15780–25600	17244		4300
BOD:COD ratio	0.56–0.59	0.56	0.55–0.68	0.46		
TOC (%)	0.36–0.48					
Nitrogen	125.8–173.2	145–248		231.6		
Nitrate			23		6.8	
Phosphorus	4.4–6.8	7–13	7.3	23		
Potassium	20.4–45.8	71–268				

*Values from river grab samples

Beyene et al. published the first major study on CWW and its impact on an Ethiopian river system and aquatic life in 2012. The authors assessed the water quality at 44 sampling sites along 18 rivers in the Jimma Zone that received CWW from 23 coffee wet mills. The water quality was monitored during the processing season and off-season. Their goal was to determine if the river system was able to purify itself during the off-season. In order to determine this, physiochemical and biological parameters were monitored. During the peak processing season, dissolved oxygen (DO) dropped to less than 0.1 mg/L with TSS and BOD spiking to an average of 598 mg/L and

436 mg/L, respectively. This was after the CWW reached the river system and was diluted. Their findings showed significant improvement in water quality during the off-season, but noted that biodiversity of DO sensitive macroinvertebrates was severely decreased. The authors concluded that wet milling caused long-term ecological impairment of the river system monitored as a result of high organic waste being directly discharged into the waterways.

CWW has also been shown to have an adverse impact on human health. In 2008, Haddis and Devi published the results of their research on the health of a small village living near a coffee wet mill. They found highly elevated levels of organic matter in the water bodies downstream of the wet mill and that use of this water for domestic purposes resulted in an unfavorable effect on humans. Of the villagers surveyed, 89% experienced dizziness, 32% had eye irritation, 85% had skin irritation, 75% breathing problems, and various other conditions. They concluded that the WHO limits for drinking water were being far exceeded by the wet mill due to direct discharge of untreated CWW into nearby waterways. Therefore, the authors called for innovative and eco-friendly treatment techniques.

To further highlight the issue of untreated CWW, in 2013, 7,000 families were without potable water due to the activities of coffee processing (Catholic Relief Services, 2013). Matagalpa City, Nicaragua, had to fully shut down the water supply for two days and then was only able provide non-potable water for two weeks while the municipality cleaned the tanks and pipelines. This event was reported to the governing body of specialty coffee in the United States, the SCAA, and the SCAA has begun pursuing measures to improve water security and water resource management in coffee processing regions.

Although the adverse environmental impact of untreated coffee wastewaters is documented, it is estimated that only 15% of coffee wet mills treat their wastewater (Hicks, 2015). This may be

due to several factors including: lack of regulation, accessibility and cost of treatment equipment, lack of economic or social incentives for wastewater treatment, and lack of consumer awareness regarding the issue. Hence, a need exists for sustainable, local, and affordable treatment options that can mitigate the impact of CWW.

Moringa Oleifera (MO) trees may be a unique option for treating coffee wastewaters. These trees are cultivated across the entire tropical region where coffee is grown (Kansal and Kumari, 2014). MO trees have two key properties: they are highly nutritious (Mahmood et al., 2010) and their seeds can be used as a naturally occurring coagulant. *Moringa Oleifera* Seed Extract (MOSE) is derived from dried MO seeds and can be used to clarify turbid water. In rural areas lacking water infrastructure, MOSE is used to crudely treat water for drinking purposes. The application of MOSE for this purpose has been well studied and reported in the literature (Kansal and Kumari, 2014).

The mature MO seed cotyledons contain the primary mechanism of coagulation. These cotyledons contain at least one cationic protein that adsorbs and neutralizes particle charges, allowing settling of suspended solids. The cotyledons also contain other proteins that aid in coagulation (Kansal and Kumari, 2014).

Application of MOSE to treat several types of industrial wastewater has been studied. MOSE has been shown to reduce TSS and COD in palm oil mill effluent, human wastewater, textile effluent, and various other types of wastewater (Kansal and Kumari, 2014). MOSE has been shown to be as effective as aluminum salts, but unlike aluminum salts, MOSE does not significantly alter the effluent pH or produce toxic by-products (Ndabigengesere and Narasiah, 1998). To date, only MOSE's ability to reduce turbidity (Matos et al., 2007) and TSS (Mburu, 2015) in coffee pulping effluent has been studied, but not its ability to reduce key pollutants,

such as TSS, COD, BOD, nitrate, or nitrite, in CFW.

Bhuptawat et al., 2007, explored the effectiveness of MOSE to reduce COD in municipal sewage. Their experiment consisted of jar testing using varying MOSE dosage coupled with a set dosage of aluminum sulphate (alum). MOSE was prepared by grinding the seeds to a fine powder and creating a 2% stock solution (2 grams *M. Oleifera* in 100 mL water). The solution was then stirred for thirty minutes and filtered through Whatman No. 1 paper. The supernatant from the jar testing was analyzed two ways: after filtering it through Whatman No. 1 paper and unfiltered. The wastewater's initial COD value of 150 mg/L was reduced to 45 mg/L and 80 mg/L for filtered and unfiltered supernatant. The optimal dosage combination was 10 mg/L alum with 50 mg/L of MOSE. Additionally, the authors achieved 50% overall removal of COD using only MOSE and filtration.

Matos et al., (2007), performed jar tests on coffee pulping wastewater and studied five coagulants: aluminum sulfate, chlorinated ferrous sulfate, ferric chloride, and MOSE. They determined the optimal dosing and pH for each coagulant for reducing turbidity in the wastewater. Wastewater pH was the largest factor in coagulant performance. The authors determined the optimal pH for MOSE was 4.27 with a coagulant concentration of 10 ml/L. This combination yielded a 90% decrease in wastewater turbidity after a settling time of 90 minutes.

Padmapriya et al., studied the long term reduction of COD and BOD in coffee effluent after application of MOSE. Their data show a reduction of 14 mg/L to 4 mg/L BOD and 6 mg/L COD after 36 days. However, their results should be called into question as no control was established and the values for their effluent are orders of magnitude lower than previously published values. The pH values reported were also basic, which is highly irregular and not reported anywhere else in the literature. The reduction of TSS was not studied.

Joseph Mburu's PhD dissertation, 2014, contains extensive research on wet mill water usage and the comparative use of different coagulants to treat CPW. Among other items, he investigated the ability of MOSE to reduce TSS in highly polluted CPW. He found that MOSE was the best coagulant to use, as opposed to lime, because of its ability to reduce TSS in a 24-hour window, which is ideal for coffee processing. In contrast to Matos et al., MOSE took almost 24 hours before a visible difference between untreated and treated wastewater could be observed. Between hours 23 and 24, he observed almost instant formation of flocs and settling. He reports an optimum dosage is between 1-2.5 g/L MOSE for reducing TSS in CPW. This dosage resulted in a TSS reduction of 5 g/L to under 2 g/L.

The *purpose of this research* was to determine if MOSE offers promise as a sustainable, local, and affordable option for effectively treating CFW. This thesis research evaluates the effectiveness of MOSE to reduce TSS, COD, nitrate, nitrite, and total nitrogen in CFW at varying pH levels and MOSE doses.

Materials and Methods

Location

Field research was conducted on the island of Kauai, in Hawaii, at the Kauai Coffee Company (21.899662, -159.560981). The Kauai Coffee Company is located on the south shore of the island.

Coffee fermentation wastewater (CFW) generation

All CFW tested for this experiment was generated on a lab scale and not gathered from a coffee wet mill because the Kauai Coffee Company did not process coffee by fermentation at the time this research was conducted. Pulped coffee was collected from the Kauai Coffee mill before undergoing mechanical demucilage. To prepare CFW for this study, pulped *Coffea Arabica* of the varietal Yellow Catuai parchment was collected from the Kauai Coffee mill prior to mechanical demucilage. The pulped coffee was then placed in a PVC five-gallon tank and non-chlorinated irrigation water from the Kauai Coffee farm was mixed with the pulped coffee. Fermentation tanks had 3.78 liters of pulped coffee and 11.36 liters of irrigation water. Fermentation tanks were then covered, but not sealed and placed outside, under shade for 24 hours.

During the 24-hour fermentation period, pH, TDS, and temperature of the CFW were recorded each hour. Measurements were taken in the center of the tank at an approximate depth of 10 cm. Measurements between tanks were identical. TDS and temperature were measured using a HM Digital COM-100 Conductivity/TDS/Temperature probe (HM Digital, California) measuring NaCl in ppm per manufacturer's recommendation. A HM Digital PH-200 (HM Digital, California) meter was used to measure pH.

Preparation of Moringa Oleifera seed extract (MOSE)

Dried *M. Oleifera* seed pods were harvested from 2 *M. Oleifera* trees, approximately 12 meters tall and located at the Kauai company coffee site. Seed kernels were then extracted from their shells and ground to a coarse powder. After grinding, doses were weighed out using a Smart Weight PocketPro scale with a precision of 0.1 grams. Each dose was put in a 50 mL plastic conical tube and 25 mL of distilled water was mixed with the seed kernel powder. Tubes were then capped and intensely shaken by hand for 1 minute to dissolve the *M. Oleifera* coagulant. After 1 minute of shaking, the solution was filtered through cheesecloth directly into the appropriate jar containing CFW (adapted from Muyibi and Alfugara, 2003).

Jar testing

Jar testing was performed in 2 rounds (R1 and R2). The experimental design was a 2 by 2 factorial matrix. The factors analyzed were CFW pH, MOSE dose, and their interaction. The pH increments were 3, 4, 5, 6, and 7 with MOSE dose increments of 0, 1, 2, 3, and 4 g/L. R1 included all pH and MOSE levels, but R2 only included pH level 5, 6, and 7, as it was determined that MOSE was ineffective at pH levels 3, 4 in R1.

Jar testing was performed using 946 mL glass canning jars. For R1, 3 fermentation tanks were used to generate CFW. In order to reduce variability in jar testing, CFW in each fermentation tank was mixed at 200 rpm using a cordless drill with paddle bit for 30 seconds and allowed to settle for 30 seconds. After settling of coffee beans, CFW was extracted by transferring 1 liter from each of 3 fermentation tanks in an alternating fashion into a clean five-gallon bucket until 5 liters were extracted. Then the pH was adjusted using concentrated sulfuric acid or sodium hydroxide. The 5 liters were then stirred and 900 mL was added to each glass jar. Each pH increment contained 5 glass jars. The same process was followed for R2, but with only 2

fermentation tanks.

Once all jars were filled with CFW, MOSE was added to 4 of 5 jars for each pH increment with the remaining jar being a control. After MOSE was added, the CFW was briskly stirred for 2 minutes and then slowly stirred for 5 minutes. The jars were then left to settle for 24 hours. The lids were screwed onto the glass jars, but not sealed.

Supernatant testing

After 24 hours of settling, supernatant was extracted 5-10 cm from the top of the jars and tested. COD was measured using Hach HR Plus (200-15,000 mg/L) COD vials and Hach DR2700 portable spectrometer (Hach, Colorado, USA) according to Hach method 8043. TSS was measured according to Standard Methods 2540C. Nitrate was measured using Hach NitraVer® 5 Nitrate Reagent Powder Pillows Hach DR2700 portable spectrometer (Hach, Colorado, USA) according to Hach method 8039. Nitrite was measured using Hach NitriVer® 2 Nitrite Reagent Powder Pillows and Hach DR2700 portable spectrometer (Hach, Colorado, USA) according to Hach method 8153. Total nitrogen was measured using Hach Test 'N Tube HR Total Nitrogen Reagent Set and Hach DR2700 portable spectrometer (Hach, Colorado, USA) according to Hach method 10072. A HM Digital PH-200 meter was used to measure pH.

During R2, additional testing of COD was performed on the pH 5 CFW. These jars were shaken for 1 minute to resuspend settled solids. The CFW of each jar was then filtered through 1.5-micron filter paper, as used in TSS testing. The COD of the filtered solution was then measured according to previous methods. This additional analysis was performed to determine the ratio of insoluble to soluble COD. This pH level was chosen because it exhibited the best visible response of solids settling due to the addition of MOSE. Similar additional testing was not completed for other pH treatments in R2 due to limited resources in the field.

Supplemental lab analysis

To supplement fieldwork and better understand the interaction between CFW and MOSE, additional experimentation was performed at the University of Cincinnati Engineering Research Center. The nitrate, nitrite, total nitrogen, and COD content of each MOSE dose level, both total and soluble, were analyzed using the aforementioned methods.

CFW was generated from fermenting 100 mL of C. Arabica parchment coffee gathered from the Cincinnati Zoo. The coffee was pulped by hand and 100 mL of pulped coffee was fermented in 300 mL of Super-Q water at 29 C for 24 hours. Super-Q water was used to establish a baseline for COD produced from fermentation because of its purity. This eliminates potential fermentation interferences arising from other compounds in impure water. After 24 hours, the nitrite, nitrate, total nitrogen, and COD content (both total and soluble) of CFW samples were analyzed using the aforementioned methods. The filter used for both MOSE and CFW was a Whatman 934-AH filter as specified in the Standard Methods for analysis between the insoluble portion and soluble portion of a solution. Figure 3 illustrates the mass balance used to analyze both the insoluble and soluble portions of CFW and MOSE.

Additionally, Microtox bioassays were performed on the filtered portion of MOSE doses and CFW. This test was performed using a Microtox 500 analyzer with standard reagents.

Bioluminescence of saltwater bacteria is measured by the analyzer at varying concentrations of tested solution. The more toxic the solution tested, the less bioluminescence is emitted by the bacteria. An EC50 can be obtained from a dilution series of the toxin. The test is time sensitive and for this research, the EC50 at 30 minutes is reported. The results from this test are qualitative and helpful in comparing different solutions to a known toxicity of a standard solution. The standard solution chosen was 100 ppm Phenol.

Data collection and analysis

The Liquid® data collection platform was used for this study. Liquid® allows for direct digital input using an iPad, iPhone, or laptop and provides data redundancy by storing data on the device and in the “cloud.” Once a dataset has been created, collaborators/advisors can view, edit, or add data dependent on permissions given by the initial user. Statistical analysis were performed using Minitab 17 and Microsoft Excel.

Figure 3: Jar Test Mass Balance

Results

Field Results

Figure 4: pH vs. Dose after 24 hours of settling

As expected from the literature, the pH of the effluent was not significantly altered by the addition of MOSE.

Figure 5: Round 1 TSS vs. Dose

For pH levels 3 and 4, TSS was not reduced and increased with increasing MOSE dose. For pH levels 5-7, TSS was reduced. The highest TSS drop was observed in pH 6 due to no settling occurring during 24 hour settling period in control jar. Some settling was observed in the other control jars.

Figure 6: Round 1 TSS Removal as a Function of Dose

Highest removal rates were for pH levels 5 and 6. According to the second order polynomial fit, optimal TSS removal for these pH levels 5 and 6 lies between 2-3 g/L. MOSE negatively impacted CFW quality by increasing TSS concentrations for pH levels 3 and 4.

Figure 7: Round 2 TSS vs. Dose

Similar to round 1, round 2 saw TSS reduction for pH levels 5-7. Round 2 TSS results were calculated from calibration curve derived from round 1 Standard Methods TSS versus spectrometer TSS. The calibration curve had an R^2 value of 0.965 and is shown in Figure 25 in Appendix A.

Figure 8: Round 2 TSS Removal as a Function of Dose

Maximum TSS removal was 53% for pH 5 with 2 g/L dosing. Similar to round 1, optimum MOSE removal efficiency lies between 2-3 g/L for all three pH levels.

Figure 9: Round 1 & 2 TDS vs. Dose

MOSE did not reduce TDS in CFW. Overall, TDS values trended slightly upward as dose increased. Round 1 TDS values are much higher than round 2 values, this is most likely due to slightly different environmental conditions between round 1 and round 2.

Figure 10: Linear Relationship Between Dose and TDS Concentration

A strong linear relationship was observed between dose and TDS values for round 2 pH levels 5 and 6. As MOSE dose increased, so did TDS concentration at a rate of 19.7 ppm per gram of MOSE.

Figure 11: Round 1 Nitrite vs. Dose

Nitrite was significantly reduced using MOSE for all pH levels except pH 3. Results for pH 3 are not included as nitrite was undetectable. Nitrite levels were undetectable during Round 2 and are not included in the results. This may be due to changes in stream water used for fermentation as this water was gathered fresh before each round of fermentation. However, the nitrite levels of the water were tested and nitrite was not present. The reason nitrite levels were significantly higher in round 1 than round 2 is not known.

Figure 12: Round 1 Nitrite Removal as a Function of Dose

Highest removal rates were at pH levels 5 and 6. From second order polynomial, optimal MOSE dose removal efficiency lies between 2-3 g/L.

Figure 13: Round 1 & 2 Nitrate vs. Dose

Nitrate concentration decreased with increasing MOSE dose for all pH levels except pH 7. For pH 7, nitrate increased with increasing MOSE dose.

Figure 14: Round 1 & 2 Nitrate Removal as a Function of Dose

No optimal MOSE dose for nitrate removal was observed. Removal continued to increase with increasing MOSE dose except for pH 7 where negative removal rates were observed.

Figure 15: Round 1 Total Nitrogen vs. Dose

MOSE did not reduce total nitrogen in CFW. Overall, total nitrogen concentrations increased with increasing MOSE dose. The two points showing decline in total nitrogen for pH 6 are most likely due to experimental error as both MOSE and CFW have high total nitrogen content.

The increase in total nitrogen will cause an increase in nitrogenous oxygen demand downstream. This increase can be theoretically calculated if it is assumed that all MOSE nitrogen content is converted to ammonia. From the stoichiometry of the nitrification reaction, it is possible to determine how many grams of oxygen will be required to oxidize the added nitrogen content from the MOSE.

From molar ratios, 4.57 mgO₂ will be required to oxidize each mg of NH₃-N. The results of the calculations for theoretical NBOD increase due to addition of MOSE are displayed in Table 3.

Table 3: Theoretical NBOD increase due to addition of MOSE (R1)

Initial pH	MOSE (g/L)	TN (mg/L)	NBOD Increase (mg/L)
3	0	28	0
3	1	40	55
3	2	42	64
3	3	46	82
3	4	47	87
5	0	26	0
5	1	26	0
5	2	30	18
5	3	32	27
5	4	35	41
6	0	29	0
6	1	2*	-
6	2	33	18
6	3	2*	-
6	4	39	48
7	0	27	0
7	1	37	46
7	2	33	25
7	3	30	14
7	4	36	39

*Data is skewed by experimental error and therefore, omitted from the NBOD increase calculation.

Figure 16: Chemical Oxygen Demand of MOSE

MOSE has a considerable COD content and this must be considered when using it as a coagulant. A strong linear relationship exists between COD and MOSE dose for both soluble and insoluble content. Total COD increased at a rate of ~500 mg/L per gram of MOSE. However, most of the COD content was insoluble and would have settled out with only ~25% remaining as soluble COD.

Figure 17: Round 1 & 2 COD vs. Dose

COD values were measured after settling for rounds 1 and 2. Round 1 data indicate an increase in COD concentration with increasing dose except for pH levels 3 & 6. These levels show a decrease at 1 g/L dosing, but then a sharp increase for dosing above 1 g/L. The COD measurements for round 1 pH 6 control exhibited high variability. However, a similar trend was observed for pH 6 in round 2 results. Round 2 results show a measurable decrease in COD concentration at a dosing of 1 g/L and then an increase in COD as dose increases.

Figure 18: Dose vs. Round 2 COD Removal as a Function of Dose

Almost no total COD removal was observed in round 1 testing. Figure 18 displays the total COD removal rates for tested effluent in round 2. Low total COD removal rates were observed. The highest removal rate is 16% for pH 6 with dose of 1 g/L. Further investigation into why rates were low was conducted through a mass balance of round 2 pH 5 effluent.

Figure 19: Soluble and Total COD vs. Dose

Results of CFW COD concentrations after being filtered through 1.5-micron filter show that a strong linear relationship ($R^2 = 0.97$) exists between soluble COD concentration and dose. For dosing levels 1 and 2 g/L, the total COD and soluble COD values are nearly identical and then they begin to diverge as dosing increases. As mentioned previously, further investigation into soluble and insoluble COD content was conducted using the results displayed in Figure 19.

Figure 20 and Figure 21 illustrate the results of the mass balance for round 2 pH 5 effluent.

Figure 20: Round 2 pH 5 Mass Balance

Figure 21: Round 2 pH 5 Mass Balance Results

$$\text{Insoluble TSS Removal} = \frac{\text{Insoluble COD} - 597}{597} * 100\%$$

Table 4: Round 2 pH 5 Effluent Mass Balance

Sample	Dose	pH	Effluent COD _T (mg/L)	Insoluble COD (mg/L)	Soluble COD (mg/L)	Insoluble COD Removed (%)
p5d0	0	5	1960	597	1363	0
p5d1	1	5	1475	-6	1481	101%
p5d2	2	5	1665	13	1652	98%
p5d3	3	5	1940	254	1686	57%
p5d4	4	5	2310	443	1867	26%
filtered-p5d0	0	5	1363	597	765	
filtered-p5d1	1	5	1481	-6	1488	
filtered-p5d2	2	5	1652	13	1639	
filtered-p5d3	3	5	1686	254	1432	
filtered-p5d4	4	5	1867	443	1424	

Displayed in Table 4 are the results of the mass balance for pH 5 during round 2. Although COD_T removal rates were low with the highest being 16%, the results of this mass balance predict that MOSE has a high removal efficiency for insoluble COD content. The insoluble/total COD ratio of CFW was 0.30 or 30%.

Figure 22: Insoluble COD vs. TSS

When differentiating between soluble and insoluble COD levels, a clear trend appears between

insoluble COD and TSS. It was expected that lower TSS concentrations would result in lower COD concentrations and this is displayed in Figure 22.

Figure 23: Insoluble COD Removal as a Function of Dose

As shown by Figure 23, MOSE had very high removal rates for insoluble COD content with a maximum of 100%. From the trend line, MOSE peak removal lies very near 2 g/L.

Figure 24: Insoluble COD Removal vs. TSS Removal

As shown by Figure 24, a linear relationship exists between TSS removal and insoluble COD removal. This is expected as MOSE is intended to remove COD content associated with the solids portion of the CFW. A TSS removal rate of 50% achieved a minimum 80% insoluble COD removal.

Table 5: Dose vs. pH Removal Rates

MOSE Dose (g/l)	1	TSS	7.62%	-15.77%	17%	86%	-14%
		TDS	0%	-1%	-13%	-6%	-6%
		NO ₃	0%	0%	91%	30%	-209%
		NO ₂	0%	0%	13%	50%	43%
		COD _T	64%	-5%	14%	16%	14%
	2	TSS	4.84%	-12.26%	36%	87%	27%
		TDS	0%	-1%	-14%	-14%	-11%
		NO ₃	0%	0%	85%	57%	-100%
		NO ₂	0%	0%	88%	79%	33%
		COD _T	-5%	-14%	5%	6%	-3%
	3	TSS	1.47%	-23.30%	32%	87%	16%
		TDS	0%	-4%	-14%	-21%	-22%
		NO ₃	0%	0%	65%	37%	-195%
		NO ₂	0%	0%	63%	57%	38%
		COD _T	-4%	-12%	-5%	5%	1%
	4	TSS	-7.46%	-14.60%	23%	87%	25%
		TDS	0%	-4%	-19%	-37%	-29%
		NO ₃	0%	0%	76%	80%	-159%
		NO ₂	0%	0%	38%	57%	38%
		COD _T	38%	4%	-13%	-7%	-2%
			3	4	5	6	7
			pH				

Table 5 displays the highest removal rates achieved in either round 1 or 2 by MOSE at all pH and dosing levels.

Supplemental Lab Analysis

Table 6: Total, soluble, and % insoluble concentrations of nitrate, nitrite, total nitrogen, and COD in lab CFW.

	Nitrate (n=3)	Nitrite (n=3)	Total Nitrogen (n=3)	COD (n=5)
Total (mg/L)	11.1	45.7	58.5	26890
Soluble (mg/L)	1.8	29.3	28.0	18990
% Insoluble*	84%	36%	52%	30%

Table 6 presents the results of testing generated CFW in at the University of Cincinnati using 100 mL of local coffee cherries from the Cincinnati Zoo. These values are congruent with reported literature values for CFW. A large portion of nitrate is available through coagulation or filtration, up to 84%, whereas only 36% and 52% of nitrite and total nitrogen are available for removal through coagulation or filtration. For COD, only 30% is available to be removed through coagulation or filtration.

Table 7: Total, soluble, and % insoluble concentrations of total nitrogen in MOSE.

Dose (g/L)	1	2	3	4
Total (mg/L)	29	49	68	131
Soluble (mg/L)	17	34	36	37
% Insoluble	41%	31%	46%	72%

Both unfiltered and insoluble total nitrogen increased linearly with increasing dose, as shown in Table 7. After MOSE dose of 2 g/L, soluble total nitrogen content did not continue to increase with increasing MOSE dose. From these results, a threshold must exist for soluble total nitrogen content, and is approximately 36 mg/L. Nitrate and nitrite levels were also tested, but were not present in either unfiltered or filtered MOSE solutions prepared in the lab, lending support the hypothesis that the values detected in the field were associated with the water source or produced during fermentation.

Table 8: Microtox Results for CFW and MOSE

Sample	Dilution	EC50 (%)	95% Confidence Factor	95% Confidence Range	R ²	Undiluted EC50 (%)
Phenol 100 ppm	0x	14.57	1.257	11.59-18.32	0.9907	14.57
CFW	10x	20.15	1.192	16.90-24.03	0.9949	2.015
MOSE 1 g/25 mL	100x	43.02	1.367	31.48-58.79	0.9934	0.4302
MOSE 2 g/25 mL	200x	20.83	4.365	15.26-28.43	0.9845	0.1042
MOSE 3 g/25 mL	200x	15.08	1.201	12.56-18.12	0.994	0.0754
MOSE 4 g/25 mL	400x	4.308	1.2	3.589-5.171	0.9984	0.0108

Table 8 displays the results of the Microtox bioassay performed on CFW and all concentrations of MOSE. Results are relative and can only give insight to each solution's toxicity compared to each other and a standard (100 ppm phenol). Additionally, since the preparation of MOSE is somewhat crude, there can exist some variation between MOSE solutions of the same dose. However, the results in Table 8 still provide insight into general trends of CFW and MOSE. As expected, the most toxic solution was the 4 g/25 mL MOSE solution, with potency decreasing as dose decreases. Although it may appear that CFW is less toxic than MOSE, the MOSE solution tested was the 25 mL concentrate. When MOSE was used as a coagulant, this 25 mL was diluted into approximately 1 liter of CFW. Therefore, significantly diluting MOSE by approximately 40 times. The relationship may not be linear due to interactions between CFW and MOSE, but assuming it is, all but MOSE 4 g/L, would be less toxic than CFW. Whereas, CFW is not diluted and was roughly 7 times more toxic than 100 ppm phenol. It may be surprising that MOSE appears so toxic in this bioassay, but MOSE has been shown to have antibacterial properties and this likely plays a role in these results as the test relies on the bioluminescence of bacteria.

Discussion

Overall, TSS and COD of CFW measured during this research were lower than reported values in the literature. This may be due to several factors. First, fermentation may not have gone to completion and additional fermentation time may have resulted in higher TSS and COD concentrations in CFW as fermentation durations are not specified in the literature. Second, the irrigation water used for fermentation was low in TSS, nitrite, nitrate, and COD values and the purity of the water used for fermentation will have an impact on the concentrations of these values in the generated CFW. Finally, and most probable, the pulped coffee fermented was retrieved after it had been pulped by a mechanical pulper and sorted. During this time, the pulped coffee was transported throughout the mill using water and the mucilage layer would have begun to dissolve. This would result in less potent CFW due to less of the mucilage layer being present during fermentation. However, even though the reported values from this research are lower than literature reported values, the trends in the data and efficiency of removal are still valuable, as the results of this research give the first serious look at the potential of MOSE to treat CFW.

Since discharge standards can range from country to country, WHO guidelines for drinking water and industrial waste discharge will be used as a baseline for discussion. The WHO drinking water limit for TSS is 100 mg/L (Haddis and Devi, 2008). MOSE successfully reduced CFW TSS levels below this limit for all pH levels above 5. MOSE TSS dose response in CFW with a maximum TSS removal rate of 54% between 2-3 g/L and a 24-hour settling time required for MOSE to clarify CFW, are consistent with results reported by John Mburu (2014) and his research using MOSE to treat CPW.

The WHO limit for nitrate and nitrite in drinking water is 50 and 3 mg/L, respectively. MOSE was shown to reduce nitrate and nitrite levels in CFW. Nitrate levels observed in CFW during

field research did not exceed this limit, with a maximum nitrate concentration of 6.8 mg/L being recorded. The results of the additional CFW lab analysis show that the majority of the nitrate content in CFW is insoluble and available for removal by MOSE. Hence, MOSE was very efficient at reducing nitrate in CFW.

In contrast to nitrate, nitrite concentration limits were exceeded in field-tested CFW. The WHO reports that short-term, high nitrite intake may increase risk of methaemoglobinaemia, a blood disorder that can cause development delays, in infants (WHO, 2011) and these conditions could be present during peak coffee harvesting and processing seasons. Therefore, reducing nitrite to acceptable limits is crucial. Field jar tests of MOSE showed that MOSE successfully reduced nitrite, when present, in CFW to acceptable limits except for pH 7. Soluble nitrite content in lab generated CFW was 48%, 28 mg/L, which is higher than WHO limits. MOSE may not be able to reduce such high levels of soluble nitrite, but additional testing would be required to verify this.

Total nitrogen increased with increasing MOSE dose for both lab generated and field tested CFW. This increase is likely due to soluble organic nitrogen present in the MOSE (Table 6). This may lead to increased nitrate and nitrite concentrations downstream, but additional testing would be required to verify this. Overall, in the field, MOSE was effective at reducing both nitrite and nitrate concentrations in CFW to acceptable limits.

The WHO limit for effluent COD is COD 300 mg/L (Haddis and Devi, 2008). MOSE was not successful in reducing COD to this threshold. Results from this research reveal interesting trends in CFW COD and the effectiveness of MOSE to reduce these high COD levels. First, MOSE is not effective for CFW with a pH below 5. When the pH is this low, COD slightly increases with increasing MOSE dose, which is in agreement with increasing TSS levels for these pH levels.

This is expected as MOSE is a coagulant and without solids removal, COD will not decrease. Second, although TSS removal rates were up to 54%, the highest total COD reduction remained low with a maximum of 25%. It was shown that MOSE was capable of removing up to 100% of insoluble COD content, and that this removal was correlated with a reduction in TSS as expected. However, soluble COD represented 70% of the total COD content in both lab and field CFW. Therefore, the majority of the COD in CFW is soluble and is not available for removal through coagulation or filtration.

In comparison, the majority of MOSE COD is insoluble (Figure 16). Although MOSE has a high total COD content, only 25% is soluble (~120 mg COD/g MOSE). The slope of linear trendline for the soluble portion of COD in both Figure 16 and Figure 19 is identical. This supports that the increase in soluble COD, as MOSE dose increases (Figure 19), in CFW is due to soluble MOSE COD. Hence, the increase in soluble COD from the application of MOSE is insignificant compared to the insoluble COD MOSE can remove in CFW. From these results, it can be assumed that an optimum MOSE dose (2.5 g/L, ~300 mg soluble COD) can remove up to 30% of total COD in concentrated CFW (~8,000 mg/L, Table 6) resulting in a MOSE COD added to CFW COD removed ratio of ~0.04. Therefore, the COD added by MOSE is dwarfed by the exceptionally high COD present in CFW.

Settling ponds are typical treatment practice in coffee processing regions (Beyene et al., 2012 and Jacobi, 2004). In Matagalpa, Nicaragua, 90% of coffee farmers surveyed reported some type of wastewater treatment, typically in the form of settling ponds, but COD levels in tested waterways exceeded limits set by the Nicaraguan government (Jacobi, 2004). Beyene et al. (2012) report similar settling pond practices for CWW treatment in Ethiopia and that they are ineffective. Settling ponds may be good practice for suspended solids in CWW, but this research

illuminates why they are not an adequate treatment option for CFW due to the high amount of soluble COD present in CFW.

In order to be used for treating CFW, *M. Oleifera* must be easy to process into MOSE and MOSE must be in adequate supply to treat the volume of CFW generated during processing season. Preparing MOSE in small quantities for this research was time intensive and scaling this up in order to be used by a small mill could require significant time without machinery.

Dehulling is the most time intensive step for preparing MOSE, but could be sped up through the use of a press or grinder. Although not tested, the performance of MOSE may be adequate if dehulling is not performed and whole seeds are crushed and MOSE generated from both the seed hull and kernel. MOSE is also material intensive, as the optimum dose revealed in this research is 2.5 g/L.

Without water recycling, the washed process requires approximately 10 m³ of water to process 1 ton of coffee cherries (Chapagain and Hoekstra, 2007). Assuming 50% of the water is used for fermentation, 1 ton of coffee cherry CFW would require between 10-15 kg of ground *M.*

Oleifera seeds to treat. *M. Oleifera* trees yield approximately 19 kg of seed pods per year (Radovich, 2009). Therefore, it would require one full grown *M. Oleifera* per ton of coffee cherry to treat CFW produced during fermentation. This is within the realm of practicality as *M. Oleifera* trees are fast growing and can tolerate a large range of climates and soil conditions (Radovich, 2009). Although not established for coffee, *M. Oleifera* can provide shading due to its relatively open canopy (Radovich, 2009). Therefore, the trees could potentially be used as shade species during the coffee growing season, while their seed pods could be harvested for use as a coagulant during the coffee processing season.

MOSE offers several advantages over alum for treating CFW. As expected from the literature,

MOSE did not significantly alter the pH in the tested CFW during settling. In contrast, alum, a common coagulant, is highly acidic and waste treated with this coagulant must be neutralized before being discharged. However, CFW is already acidic and typically falls in the pH range of 3-5. CFW would need to be neutralized regardless of whether or not it was treated with MOSE. The largest advantage MOSE has over alum is that sludge produced from MOSE coagulation is non-hazardous and requires no additional waste management strategy (Kansal and Kumari, 2014). This sludge could potentially also be used as fertilizer for crops.

The purpose of this research was to evaluate the effectiveness of MOSE to treat CFW and to determine if it could be used as a practical treatment option for coffee mills. MOSE was shown to successfully reduce TSS, nitrite, nitrate, and COD in CFW. Additionally, cultivation of *M. Oleifera* trees may bring numerous other benefits. *M. Oleifera* trees could potentially be used as a shade species for coffee and deriving MOSE from its seeds is within the practical limits for a small coffee mill. However, further development into processing MOSE quickly and efficiently would need to be explored for it to be implemented at large coffee processing sites.

As the coffee industry seeks to reduce its environmental footprint on water resources and improve sustainability, MOSE shows promise as sustainable, local, and affordable treatment augmentation for CFW, but due to the nature of CFW, will need to be coupled with biological treatment to maximize removal of dissolved organics.

Conclusions

Original research was presented on the use *M. Oleifera* seed extract to treat coffee fermentation wastewater. MOSE was shown to reduce TSS, nitrate, nitrite, and COD levels in CFW. Total nitrogen increased with increasing MOSE dose due to dissolved organic nitrogen in MOSE solution. Optimum removal efficiency for MOSE is between 2-3 g/L and maximum removal was observed between pH levels 5-6. MOSE did not significantly alter the pH of CFW. Although MOSE has a high organic content, only 25% is soluble and would be retained in the treated effluent. The ratio of soluble MOSE COD to potential CFW COD removal was shown to be approximately 0.04. Soluble COD is approximately 70% of the total COD in CFW and must be removed through treatment methods other than coagulation and settling. MOSE was shown to remove up to 100% of the insoluble COD content in CFW. MOSE shows promise as sustainable, local, and affordable coagulant for treating CFW.

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Appendix A: Field Data

Table 9: Round 1 TSS (Gravimetric)

Dose	A (g)	B (g)	A-B (g)	mg/L	A(g)	B(g)	A-B(g)	mg/L	Average (mg/L)
pH 3									
0	0.08666	0.07840	0.00826	165.20000	0.08567	0.07768	0.00799	159.80	162.50
1	0.0851	0.07746	0.00764	152.80000	0.08554	0.07808	0.00746	149.20	151.00
2	0.08288	0.07562	0.00726	145.20000	0.08667	0.07843	0.00824	164.80	155.00
3	0.08538	0.07735	0.00803	160.60000	0.08661	0.07861	0.00800	160.00	160.30
4	0.08619	0.07742	0.00877	175.40000	0.08719	0.07840	0.00879	175.80	175.60
pH 4									
0	0.08426	0.07664	0.00762	152.40000	0.08694	0.07915	0.00779	155.80	154.10
1	0.08625	0.07680	0.00945	189.00000	0.08623	0.07784	0.00839	167.80	178.40
2	0.0856	0.07692	0.00868	173.60000	0.088	0.07938	0.00862	172.40	173.00
3	0.08781	0.07830	0.00951	190.20000	0.08566	0.07617	0.00949	189.80	190.00
4	0.08698	0.07814	0.00884	176.80000	0.08536	0.07654	0.00882	176.40	176.60
pH 5									
0	0.08546	0.07796	0.00750	150.00000	0.08698	0.07940	0.00758	151.60	150.80
1	0.08593	0.07968	0.00625	125.00000	0.08297	0.07664	0.00633	126.60	125.80
2	0.0845	0.07969	0.00481	96.20000	0.08241	0.07753	0.00488	97.60	96.90
3	0.08477	0.07968	0.00509	101.80000	0.08176	0.07660	0.00516	103.20	102.50
4	0.08472	0.07860	0.00612	122.40000	0.0824	0.07686	0.00554	110.80	116.60
pH 6									
0*	0.08395	0.07775	0.00620	688.88889	0.08544	0.07754	0.00790	718.18	703.54
1	0.08329	0.07835	0.00494	98.80000	0.08229	0.07743	0.00486	97.20	98.00
2	0.08292	0.07826	0.00466	93.20000	0.08116	0.07646	0.00470	94.00	93.60
3	0.08499	0.08039	0.00460	92.00000	0.08268	0.07795	0.00473	94.60	93.30
4	0.08352	0.07892	0.00460	92.00000	0.08306	0.07826	0.00480	96.00	94.00
pH 7									
0	0.08674	0.08019	0.00655	131.00000	0.08213	0.07647	0.00566	113.20	122.10
1	0.08481	0.07800	0.00681	162.14286	0.08198	0.07673	0.00525	116.67	139.40
2	0.0841	0.07962	0.00448	89.60000	0.08265	0.07824	0.00441	88.20	88.90
3	0.08531	0.08055	0.00476	95.20000	0.08318	0.07769	0.00549	109.80	102.50
4	0.0818	0.07727	0.00453	90.60000	0.08271	0.07805	0.00466	93.20	91.90

*only 9 mL could be filtered.

Table 10: Round 1 & 2 TSS (Spectrometer)

Dose	Round 1 TSS (mg/L)	Round 2 TSS (mg/L)
pH 3		
0	104	
1	94	
2	97	
3	102	
4	95	
pH 4		
0	98	
1	98	
2	102	
3	117	
4	109	
pH 5		
0	115	60
1	60	37
2	43	33
3	41	38
4	33	84
pH 6		
0	424	44
1	51	40
2	46	35
3	45	35
4	35	74
pH 7		
0	100	29
1	97	35
2	51	28
3	52	31
4	34	60

Figure 25: Round 1 Calibration Curve for Round 2 Gravimetric TSS

Table 11: Round 1 TDS

Dose	TDS (ppm)
pH 3	
0	1040
1	1020
2	1020
3	1010
4	979
pH 4	
0	603
1	610
2	609
3	625
4	629
pH 5	
0	315
1	315
2	336
3	341
4	354

pH 6	
0	413
1	400
2	411
3	433
4	440
pH 7	
0	512
1	507
2	529
3	531
4	546

Table 12: Round 2 TDS

Dose	TDS (ppm)
pH 5	
0	242
1	273
2	277
3	277
4	289
pH 6	
0	204
1	221
2	244
3	266
4	280
pH 7	
0	265
1	282
2	294
3	324
4	341

Table 13: Round 1 and Round 2 Nitrate

Dose	Round 1 Nitrate (mg/L)	Round 2 Nitrate (mg/L)
pH 3		
0	0.3	
1	0.1	

2	0.2	
3	0	
4	0	
pH 4		
0	0.6	
1	0	
2	0	
3	0	
4	0	
pH 5		
0	3.4	2.33
1	0.3	1.83
2	0.5	0.33
3	1.2	0.20
4	0.8	0.00
pH 6		
0	3	2.37
1	2.1	1.80
2	1.3	1.80
3	1.9	0.63
4	0.6	0.00
pH 7		
0	2.2	2.80
1	6.8	2.10
2	4.4	3.37
3	6.5	5.50
4	5.7	4.50

Table 14: Round 1 Nitrite

Dose	Nitrite (mg/L)
pH 3	
0	0.3
1	0.1
2	0.2
3	0
4	0
pH 4	
0	0.6
1	0

2	0
3	0
4	0
pH 5	
0	3.4
1	0.3
2	0.5
3	1.2
4	0.8
pH 6	
0	3
1	2.1
2	1.3
3	1.9
4	0.6
pH 7	
0	2.2
1	6.8
2	4.4
3	6.5
4	5.7

Table 15: Round 1 Total Nitrogen

Dose	Total Nitrogen (mg/L)
pH 3	
0	28
1	40
2	42
3	46
4	47
pH 5	
0	26
1	25
2	30
3	34
4	35
pH 6	
0	32
1	2

2	32
3	2
4	40
pH 7	
0	27
1	38
2	34
3	28
4	34

Table 16: Round 1 COD

Dose	Initial pH	COD (mg/L)	COD (mg/L) #2	COD (mg/L) #3	Average COD	Standard Deviation	C _v
4	7	2750	3240	2660	2883	255	8.8%
3	7	2480	2740	2790	2670	136	5.1%
2	7	2790	2290	2950	2677	281	10.5%
1	7	2630	3510	2270	2803	521	18.6%
0	7	2870	2460	2420	2583	203	7.9%
4	6	2510	3170	2990	2890	279	9.6%
3	6	2740	2310	3000	2683	285	10.6%
2	6	3290	2500	2630	2807	346	12.3%
1	6	2570	2630	2170	2457	204	8.3%
0	6	3380	5400	2350	3710	1267	34.1%
4	5	2740	2590	2450	2593	118	4.6%
3	5	2360	3080	2610	2683	298	11.1%
2	5	2330	2960	2660	2650	257	9.7%
1	5	3110	2490	2120	2573	408	15.9%
0	5	2440	2730	2070	2413	270	11.2%
4	4	3020	3460	710	2397	1206	50.3%
3	4	3640	2830	2650	3040	431	14.2%
2	4	3230	2980	2930	3047	131	4.3%
1	4	2650	2810	2690	2717	68	2.5%
0	4	2810	2670	2490	2657	131	4.9%
4	3	450	330	1550	777	549	70.7%
3	3	2880	3030	2330	2747	301	11.0%
2	3	2740	2960	2490	2730	192	7.0%
1	3	720	910	240	623	282	45.2%
0	3	2540	2590	2160	2430	192	7.9%

Table 17: Round 2 COD

Dose	Initial pH	COD (mg/L)	COD (mg/L) #2	COD (mg/L) #3	Average COD
4	7	1750	1960		1855
3	7	1860	1660		1760
2	7	1860	1870		1865
1	7	1450	1490		1470
0	7	1870	1610		1740
4	6	1940	2180		2060
3	6	1690	1570		1630
2	6	1500	1760		1630
1	6	1350	1480		1415
0	6	1390	1650		1520
4	5	2780	1840		2310
3	5	1540	2340		1940
2	5	1760	1570		1665
1	5	1350	1600		1475
0	5	2250	1670		1960
4	5	1802	1888	1910	1867
3	5	1692	1672	1694	1686
2	5	1672	1650	1634	1652
1	5	1484	1484	1476	1481
0	5	1352	1368	1368	1363

Appendix B: Fermentation Data

Table 18: Round 1 Fermentation Data

Time (hr)	pH			TDS (ppm)			Temp (F)		
	Bucket 1	Bucket 2	Bucket 3	Bucket 1	Bucket 2	Bucket 3	Bucket 1	Bucket 2	Bucket 3
0	7.32	7.33	7.32	104	98.8	106	78.4	78	77.5
1	7.17	7.19	7.18	110	105	108	81.1	79.4	79.7
2	7.05	7.09	7.08	109	101	108	79.7	79.7	79.7
3	6.91	6.95	6.92	113	103	110	78.4	78.4	78.8
4	6.72	6.77	6.75	112	103	110	77.5	77.9	78.3
5	6.5	6.54	6.52	114	106	111	77.2	77.5	77.8
6	6.28	6.32	6.34	117	107	117	76.7	76.8	77
7	6.04	6.1	6.1	120	110	117	76.2	76.5	76.6
8	5.82	5.88	5.84	143	122	144	76	76	76
9	5.68	5.7	5.73	138	132	144	76	76.7	76.2
10	5.58	5.56	5.61	146	136	148	75.8	75.8	75.8
11	5.45	5.49	5.51	148	139	153	75.9	76	76
12	5.37	5.37	5.44	153	142	159	75.6	75.2	75.4
13	5.27	5.31	5.37	156	143	159	75	75	75
14	5.24	5.29	5.3	156	146	163	74.7	74.8	74.5
15	5.19	5.21	5.26	156	145	165	74	74.2	74.4
16	5.16	5.16	5.22	158	143	168	74	73.6	73.6
17	5.02	5.03	5.06	174	162	181	74.4	74.6	74.4
18	4.95	4.94	4.97	174	160	185	74.2	74.4	74.5
19	-	-	-	-	-	-	-	-	-
20	5.06	5.07	5.1	230	246	259	76	76	75.9
21	5.02	5.04	5.07	235	251	265	76.5	76.5	76.8
22	4.94	4.99	5.02	237	251	266	77.8	77.9	78.2
23	4.93	4.96	4.96	241	253	269	78.7	79	79
24	4.88	4.91	4.89	242	254	270	80.5	80.6	81.5

Table 19: Round 2 Fermentation Data

Time	pH		TDS		Temp	
0	7.69	7.77	111	83	80.4	80
2	7.58	7.69	112	83	82.1	82
4	7.29	7.45	116	84.5	80.3	80.2
6	7.02	7.22	117	85.6	78.1	78.5
8	6.58	6.83	118	87.9	76.1	76.4
10	6.18	6.44	122	91	74.9	75.2
12	5.91	6.12	128	95.8	73.6	73.7
14	5.67	5.83	127	96.6	73.7	73.7
16	5.58	5.75	129	98.4	73.5	73.5
18	5.49	5.71	131	98.8	74	73.9
20	5.49	5.77	132	99.7	76	75.9
22	5.51	5.76	135	102	79.1	78.9
24	5.64	5.88	134	104	84.5	83.6

Appendix C: Experiment Pictures

M. Oleifera tree used in experiments.

MOSE

Fermentation tanks

CFW generated from fermentation of parchment coffee

Jar test matrix

pH 3 after 24 hours settling. MOSE dose increases left to right.

pH 4 after 24 hours settling. MOSE dose increases left to right.

pH 5 after 24 hours settling. MOSE dose increases left to right.

pH 6 after 24 hours settling. MOSE dose increases left to right.

pH 7 after 24 hours settling. MOSE dose increases left to right.

Appendix D: Trendline Coefficients and R² Values

Round 1 TSS Removal				
pH	a	b	c	R ²
3	-0.24	0.0748	0.0071	0.95811
4	0.246	-0.135	-0.0093	0.76815
5	-0.0534	0.2743	-0.0143	0.95906
6	-0.1235	0.668	0.0974	0.86155
7	-0.0049	0.0991	-0.0615	0.51874
Round 2 TSS Removal				
pH	a	b	c	R ²
5	-0.1102	0.4924	0.0294	0.96037
6	-0.762	0.3965	0.0516	0.83928
7	-0.0561	0.3059	0.0533	0.78575
Linear Relationship Between Dose and TDS Concentration				
pH	a	b		R ²
5	9.8	252		0.775
6	19.7	203.6		0.994
7	19.4	262.4		0.981
Round 1 Nitrite Removal				
pH	a	b	c	R ²
5	-0.125	0.625	-0.1	0.73171
6	-0.1071	0.55	0.0286	0.90419
7	-0.051	0.2755	0.0599	0.72503
Chemical Oxygen Demand of MOSE				
	a	b		R ²
Unfiltered	495.32	89		0.9714
Soluble	398.86	25.6		0.9527
Insoluble	100.46	63.36		0.9955
Dose vs. Round 2 COD Removal				
pH	a	b	c	R ²
5	-3.2518	8.5067	2.6557	0.85
6	-3.4088	11.098	2.3998	0.768

7	-0.8767	1.8567	3.5933	0.202
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Dose vs. Soluble and Total COD				
	a	b		R ²
	121.27	1367.2		0.972

TSS vs. Insoluble COD				
pH	a	b		R ²
5	7.2473	-429.41		0.832

Insoluble COD Removal				
pH	a	b	c	R ²
5	-0.2349	0.9737	0	0.817

TSS Removal vs. Insoluble COD Removal				
pH	a	b		R ²
5	1.6453	0		0.825