



Application of *Moringa Oleifera* seed extract to treat coffee fermentation wastewater



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HIGHLIGHTS

- The use *Moringa Oleifera* Seed Extract to treat coffee wastewater is investigated.
- Coffee fermentation wastewater has high soluble COD content.
- *Moringa Oleifera* Seed Extract can remove insoluble coffee wastewater COD.
- Settling ponds are not an adequate treatment method for coffee wastewater.

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ABSTRACT

Wastewater generated from wet processing of coffee cherries 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, although the immediate impact is local. 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 local and affordable “appropriate” coagulation technology for aiding in the treatment of coffee wastewater. Field research was conducted at the Kauai Coffee Company to investigate the application of MOSE to treat coffee fermentation wastewater (CFW). Coagulation tests were conducted at five pH CFW levels (3–7) and MOSE doses (0–4 g/L). After settling, TSS, COD, nitrate, nitrite, total nitrogen, and pH of supernatant from each test were measured. MOSE reduced TSS, COD, nitrate, and nitrite in CFW to varying degrees dependent on pH and dose applied. TSS removal ranged from 8% to 54%. Insoluble COD removal ranged from 26% to 100% and total COD removal ranged from 1% to 25%. Nitrate and nitrite reduction ranged from 20% to 100%.

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1. Introduction

Coffee is grown in 70 countries across the globe, and is worth about \$100 billion annually [19]. Two thirds of the 195 countries in the world today have GDP lower than \$100 billion per year [20]. There are two primary methods for processing coffee, wet and dry, and approximately 40% of all coffee around the world is wet processed. The wet method is considered to produce superior tasting

coffees, which corresponds to greater profits for farmers and cooperatives. 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 (Fig. 1): coffee pulping wastewater (CPW) and coffee fermentation wastewater (CFW). First, wet processed coffee is pulped to remove the coffee fruit. After pulping, the coffee beans are submerged in large water-filled open-air tanks for the fermentative removal of the pectin layer encasing the bean. This step is an essential part of the wet process and has a significant impact on coffee quality [4]. Coffee is submerged for 24–48 h during which enzyme activity produces a significant increase in

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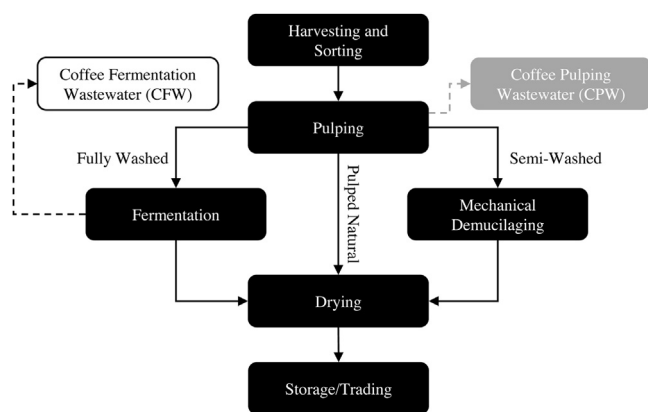


Fig. 1. Simplified wet processing flowchart for fully washed, semi-washed, and pulped natural coffees that identifies the source of CFW.

dissolved organics accompanied by a sharp decline in pH due to the dissolving of the pectin layer. Once fermentation has completed, the fermentation water is drained from the tanks, releasing CFW.

Typically, coffee processors discharge both CPW and CFW directly into surface water 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 as shown in Table 1.

In a major study on CWW involving 23 coffee mills and 18 river systems, Beyene et al. [2] concluded that wet milling caused long-term ecological impairment of the river systems monitored as a result of high organic waste being directly discharged into the waterways. Haddis and Devi [7] determined CWW has an adverse impact on human health. They found highly elevated levels of organic matter in the water bodies downstream of the wet mill studied and noted that use of this water for domestic purposes resulted in unfavorable, but nonspecific health effects such as dizziness, eye and skin irritation and breathing problems. They concluded that the WHO limits for drinking water (200 mg TSS/L, 300 mg COD/L, and 100 mg BOD/L) 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. In another report by Catholic Relief Services [5], 7000 families in Nicaragua were documented to be without potable water for two weeks due to the impacts of wet coffee processing wastewater on the Matagalpa City water treatment facility. The water treatment facility itself was inoperable for

two days and system cleaning took two weeks before water service was restored.

It is estimated that only 15% of coffee wet mills treat their wastewater [8]. This may be due to several factors including lack of regulation, difficult accessibility to mill sites and high cost of treatment equipment, as well as a scarcity of economic or social incentives for wastewater treatment [8]. There is a need for local, and affordable “appropriate” technology treatment options that can mitigate the impact of CWW.

Moringa Oleifera (MO) trees may be a viable option for treating CWW. These trees are cultivated across the entire equatorial region where coffee is grown [10]. MO trees have two key properties: they are highly nutritious [11] 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 treatment infrastructure, MOSE is used as a primitive coagulant to remove solids and improve potability. The application of MOSE for this purpose has been well studied and reported in the literature [10].

Although the application of MOSE to CFW has not been reported in the literature to date, application of MOSE to reduce turbidity in CPW has been previously studied [12]. Jar tests on CPW were conducted using five coagulants: aluminum sulfate, chlorinated ferrous sulfate, ferric chloride, and MOSE. The objective of research was to find the optimal dosing and pH for each coagulant for reducing turbidity in CPW. 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 min.

In Kenya, the application of MOSE to reduce TSS in CPW has been previously studied [13]. The study concluded that MOSE was an ideal coagulant because it could reduce TSS in a 24-h window, which is ideal for coffee processing in Kenya. In contrast to the above study [12], MOSE required almost 24 h before a visible difference between untreated and treated wastewater was observed. Between hours 23 and 24, an almost instant formation of flocs and settling was observed. The optimum dosage in the study was reported to be between 1 and 2.5 g/L.

In addition to CPW, MOSE has been shown to reduce TSS and COD in palm oil mill effluent, human wastewater, textile effluent, and various other types of wastewater [3,10]. It 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 [15]. The ability of MOSE to reduce TSS, COD, BOD, nitrate, and nitrite, in CFW is the subject of the study reported here.

Table 1
Coffee wastewater pollution loading reported in the literature.

Authors/Date	Selvamurugan et al. [18]	Adams and Ghaly [1]	Haddis and Devi [7]	Rossmann et al. [17]	Beyene et al. ^a [2]	Zayas Pérez et al. [22]
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	13–Jul	7.3	23		
Potassium	20.4–45.8	71–268				

^a River grab samples.

2. Materials and methods

2.1. Location

Field research was conducted on the island of Kauai, in Hawaii, at the Kauai Coffee Company (21.899662, –159.560981).

2.2. 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 during the time this research was conducted. Pulped coffee (varietal Yellow Catuai) was collected from the Kauai Coffee mill before undergoing 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 L of pulped coffee and 11.36 L of irrigation water. Fermentation tanks were then covered, but not sealed, and placed in a shaded area for 24 h.

During the 24-h 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. 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.

2.3. Preparation of Moringa Oleifera seed extract (MOSE)

Dried *M. Oleifera* seed pods were harvested from two *M. Oleifera* trees, approximately 12 m tall and located at the Kauai Coffee Company. Seed kernels 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 g. 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 capped and intensely shaken by hand for 1 min to dissolve the *M. Oleifera* coagulant. After one minute of shaking, the solution was filtered through cheesecloth into a test sample jar containing CFW, with this filtration step adapted from Muyibi and Alfugara [14].

2.4. Jar testing

Jar testing was performed in two 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 3–7 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 5–7, 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, three fermentation tanks were used to generate CFW. CFW in each fermentation tank was mixed at 200 rpm using a cordless drill with paddle bit for 30 s and allowed to settle for 30 s. After settling of coffee beans, CFW was extracted by transferring 1 L from each of 3 fermentation tanks in an alternating fashion into a clean five-gallon bucket until 5 L were collected. Then the pH was adjusted using concentrated sulfuric acid or sodium hydroxide. The pH-adjusted 5 L samples were stirred and 900 mL aliquots were added to each glass jar. Each pH level was transferred to 5 replicate glass jars. The same process was followed for R2, but with only two fermentation tanks.

Once all jars were filled with CFW, MOSE was added to four of five jars for each pH level, with the fifth jar as a MOSE-free control. The CFW was briskly stirred for two minutes and then slowly stirred

for five minutes. This stir rate and time was adapted from a previous study [13]. Initially, a settling time of 90 min was chosen, but similar to the results in application of MOSE to reduce TSS in CPW [13], no observable settling had occurred in the jar. Therefore, the settling time was extended to 24 h. After stirring, lids were screwed onto the glass jars, but not sealed during the settling phase.

2.5. Supernatant testing

After 24 h of settling, supernatant was pipetted 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 a 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 and the same portable spectrometer according to Hach Method 8039. Nitrite was measured using Hach NitriVer[®] 2 Nitrite Reagent Powder Pillows and the portable spectrometer according to Hach Method 8153. Total nitrogen was measured using Hach Test 'N Tube HR Total Nitrogen Reagent Set and the portable spectrometer according to Hach Method 10072. A HM Digital PH-200 m was used to measure pH.

During R2, additional testing of COD was performed on the pH 5 CFW. These jars were shaken for 1 min to re-suspend settled solids. The CFW of each jar was then filtered through 1.5- μ m 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.

2.6. Supplemental lab analyses

Supplemental lab analyses were performed at the University of Cincinnati Engineering Research Center to answer several questions about insoluble vs soluble fractions and MOSE nitrogen content that arose during analysis of the field data. This was done to better understand the interaction between CFW and MOSE. The nitrate, nitrite, total nitrogen, and COD content of each MOSE dose level, both total and soluble, were analyzed using the aforementioned methods.

In the laboratory, a small amount of 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 29C for 24 h. Because of its purity, Super-Q water was used to establish a baseline for COD produced from fermentation. This eliminated potential fermentation interferences arising from other compounds in impure water. After 24 h, 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.

3. Results

Overall, TSS and COD of CFW measured during this research were lower than other reported values in the literature (See Table 1). This may be due to several factors. First, fermentation may not have gone to completion in 24 h and additional fermentation time may have resulted in higher TSS and COD concentrations in CFW. Fermentation durations are not specified in the literature, so it is possible that the values reported in Table 1 correspond to longer

Table 2

Average TSS, COD, Nitrate, and Nitrite concentrations (mg/L) in supernatant for both rounds of jar testing measured after 24 h of settling.

		pH							
		3 (R1)	4 (R1)	5 (R1)	6 (R1)	7 (R1)	5 (R2)	6 (R2)	7 (R2)
MOSE Dose (g/L)		TSS (n = 2)							
	0	163	154	151	704 ^a	122	147	132	110
	1	151	178	126	98	139	76	71	65
	2	155	173	97	94	89	68	71	61
	3	160	190	103	93	103	75	79	71
	4	176	177	117	94	92	110	85	62
		COD (n = 3)							
	0	2430	2657	2413	3710	2583	1960	1520	1740
	1	623 ^b	2717	2573	2457	2803	1475	1415	1470
	2	2730	3047	2650	2807	2677	1665	1630	1865
	3	2747	3040	2683	2683	2670	1940	1630	1760
	4	777 ^b	2397	2593	2890	2883	2310	2060	1855
		Nitrate (n = 3)							
	0	<0.3	0.6	3.4	3	2.2	2.8	2	2.2
	1	<0.3	<0.3	0.3	2.1	6.8	1.5	2.2	2.1
	2	<0.3	<0.3	0.5	1.3	4.4	0.4	1.9	3
	3	<0.3	<0.3	1.2	1.9	6.5	0.6	0.8	5.3
	4	<0.3	<0.3	0.8	0.6	5.7	<0.3	<0.3	5.7
		Nitrite (n = 3)							
	0	<1	5	8	14	21			
	1	3	<1	7	7	12			
	2	<1	<1	1	3	14			
	3	<1	<1	3	6	13			
	4	<1	<1	5	6	13			

^a No settling occurred during 24 h settling period resulting in an abnormally high TSS concentration.^b Values exhibited high variability and are inconsistent with expected results.

fermentation times. Second, the irrigation water used for fermentation in the current study 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. No water source data were provided with the values reported by others in Table 1 so the contribution of water used for fermentation in the other cases is impossible to judge. Finally, the pulped coffee used in this study experienced transit times in the processing plant and some fermentation may have occurred during this time, partially dissolving the mucilage layer before the pulped coffee was added to the experimental fermentation tanks. Only the pulped coffee and not the transport water was retrieved for the study, and since the mucilage layer is the main contributor of COD in CFW, this would have resulted in less potent CFW due to less of the mucilage layer being present during fermentation. Although the CFW generated was weaker than anticipated, these first reports of MOSE impact on CFW are still instructive.

3.1. Field jar tests

Results from the field jar testing show that MOSE was ineffective for pH levels below 5 as shown in Table 2. Fig. 2 displays the visual results of jar testing after 24 h of settling.

Using 2-way ANOVA, pH, MOSE dose, and their interaction had statistically significant effects on the final 24-h concentration of TSS ($p < 0.05$). For pH levels 3 and 4, TSS was not reduced over a settling period of 24 h. Instead, TSS levels increased with increasing MOSE dose and no settling was observed for these jar tests. However, for pH level 5 and above, settling of solids can be observed with the minimum dose of 1 g/L. TSS removal ranged from 16% to 54% relative to TSS in the control jar. One removal rate of 86% was observed, but this is considered an outlier due to abnormal settling in the control jar. Maximum TSS removal was observed in R2 at pH 5. Fig. 3 displays the results of this jar test with 95% confidence intervals. From the polynomial trend-line, the optimum dose is 2.25 g/L MOSE.

The data for total COD in the supernatant after 24 h of settling are summarized in Table 2. Using 2-way ANOVA, pH, MOSE dose, and their interaction had statistically significant effects on the final 24-h concentration of COD ($p < 0.05$). Unlike TSS removal, COD removal remained low for both rounds of jar testing with a maximum removal of 25% observed for pH 5 in R2. For pH 3 and 4, COD increased with increasing MOSE dose.

Additional analyses were done on the highest COD removal rate jar test, R2 pH 5, to explore the reason for the observed low COD removal rates. This was done in the field because it was suspected that a large portion of the COD content in CFW was soluble and additional analyses could confirm this premise. The raw CFW supernatant obtained after 24 h of settling was filtered through a 1.5- μ m filter and the soluble COD remaining was measured. Fig. 4 displays the COD values of the raw supernatant (total COD) and COD remaining after filtering CFW (soluble COD). A strong linear relationship 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 due to the contribution of soluble COD from MOSE. The insoluble CFW COD concentration for R2 pH 5 was 597 mg/L (see difference in control at 0 mg/L), or 30% of the total COD. Insoluble COD removal rates can be calculated from these data and correlated to TSS removal rates as shown in Fig. 5. One-way ANOVA testing confirms that TSS removal is a statistically significant factor for insoluble COD removal ($p < 0.05$).

The COD content of MOSE was explored in supplemental laboratory analyses to clarify and confirm the results noted in the field data. The data from these analyses are shown in Fig. 6 plots of total and soluble COD with MOSE concentration. A strong linear relationship exists between COD and MOSE dose for both total and soluble COD. Total COD increased at a rate of ~ 500 mg/L per gram of MOSE, and soluble COD increased at a rate of ~ 120 mg/L per gram of MOSE, which is identical to the increase in soluble COD in Fig. 4. Fortunately, most of the COD content of the MOSE solution ($\sim 75\%$

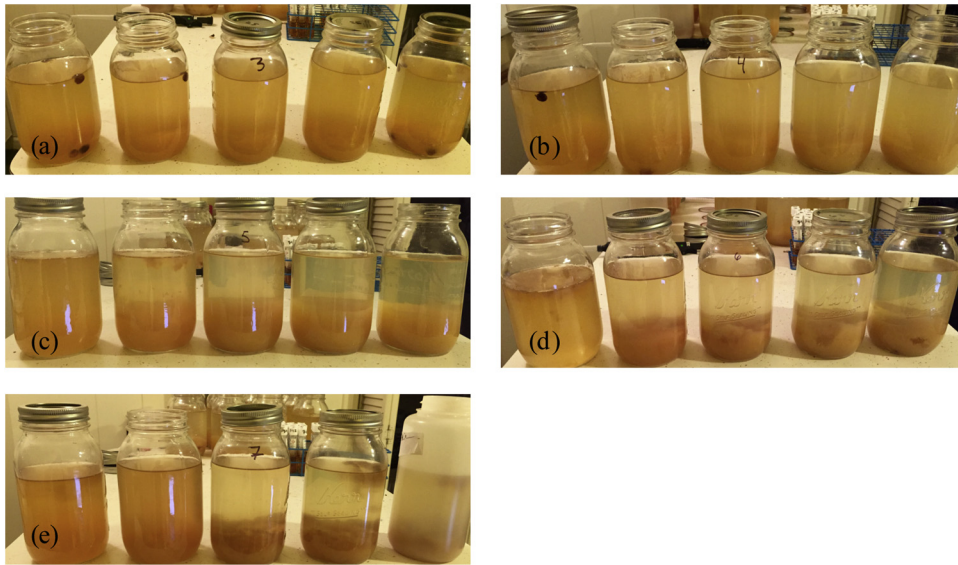


Fig. 2. R1 jar tests for all pH levels. Dose increases from left to right with 0 g/L MOSE on the left and 4 g/L on the right. Photographs taken by William Garde on 10/28/2015. (a) pH 3 (b) pH 4 (c) pH 5 (d) pH 6 (e) pH 7.

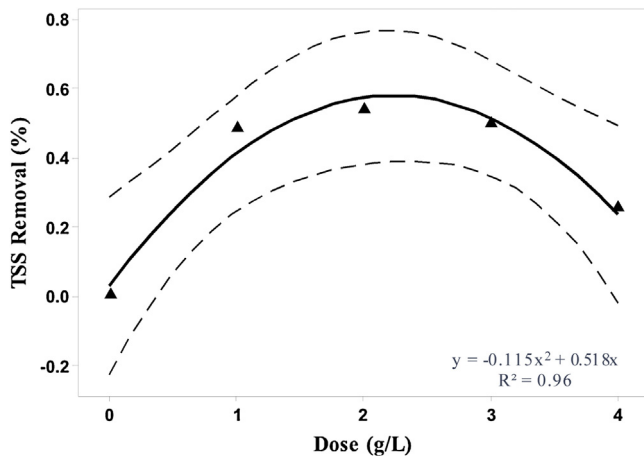


Fig. 3. R2 pH 5 TSS removal with 95% confidence intervals. Maximum TSS removal occurs at MOSE dose of 2.25 g/L.

from the data shown in Fig. 6) is insoluble and would settle with the solids during treatment.

Nitrite was significantly reduced using MOSE for all pH levels except pH 3 in R1. Results for pH 3 are not included as nitrite was undetectable. Nitrite levels were undetectable during R2 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 R1 than R2 is not known.

Nitrate concentration decreased with increasing MOSE dose for all pH levels except pH 7 in R1 and R2. For pH 7, nitrate increased with increasing MOSE dose.

Total nitrogen increased with increasing MOSE dose in tested supernatant. Its increase was dependent on pH, MOSE dose, and their interaction ($p < 0.05$) with a maximum increase of 68% from 28 mg/L to 48 mg/L at pH 3. Minimum increase occurred at pH 6 from 32 mg/L to 40 mg/L, or 25%.

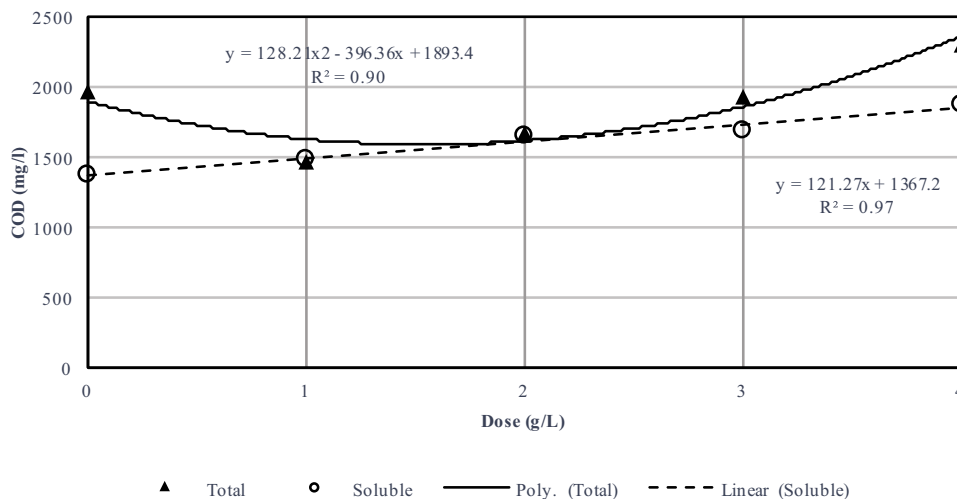


Fig. 4. COD results of R2 pH 5 jar test. Filtered CFW exhibited a linear soluble COD increase with increasing MOSE dose. Whereas, raw supernatant COD was reduced at doses 1 and 2 g/L and then began increasing at doses 3 and 4 g/L. Maximum COD removal occurs where the 2 trendlines intersect.

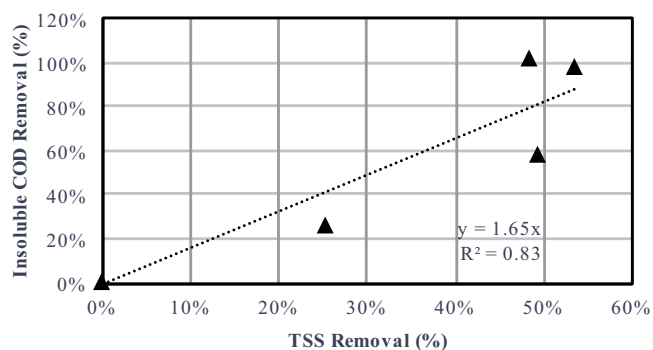


Fig. 5. Insoluble COD removal vs. TSS removal for R2 pH 5. A linear relationship exists between TSS removal and insoluble COD removal. This is expected as MOSE is intended to remove COD content associated with the solids portion of the CFW.

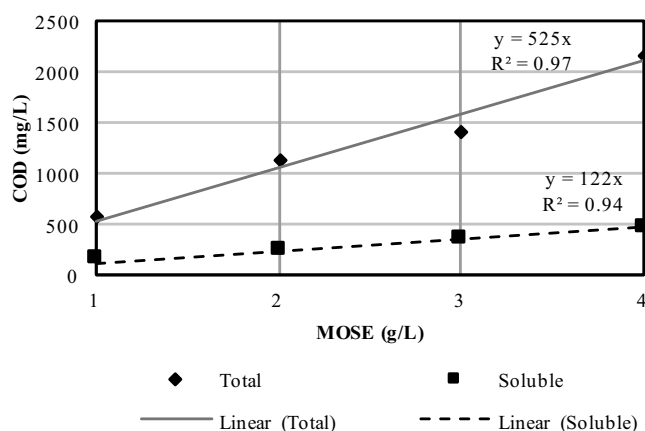


Fig. 6. COD concentrations of total and soluble MOSE. The majority of MOSE COD is insoluble and settles out. Approximately 120 mg/L soluble COD is added per gram of MOSE dose.

3.2. Supplemental lab analyses

In addition to the previously discussed supplemental COD data shown in Fig. 6, Tables 3 and 4 present the results of other supplemental testing in the laboratory at the University of Cincinnati. The nitrogen and COD levels in the laboratory CFW shown in Table 3 are congruent with reported literature values given in Table 1, lending support to the observation that the lower levels in the field generated CFW may have been due to mucilage layer losses during

Table 3

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	18990
% Insoluble*	84%	36%	52%	30%

* Insoluble = (Total – Soluble)/Total*100%.

Table 4

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%

* Insoluble = (Total – Soluble)/Total*100%.

transport before the coffee pulp was collected for fermentation. While not directly comparable to the field samples, the laboratory results do provide insight into some of the questions regarding distribution between soluble and insoluble fractions, showing that a large portion of nitrate is insoluble (84%) and therefore available for removal through coagulation or filtration, whereas only 36% and 52% of nitrite and total nitrogen are insoluble. For COD, only 30% is insoluble and therefore available to be removed through coagulation or filtration. These observations support the removal trends in the field samples.

The soluble and insoluble total nitrogen content of MOSE alone was measured with respect to dose and the data are shown in Table 4. Total nitrogen increased linearly with increasing dose, but after a MOSE dose of 2 g/L, soluble nitrogen concentration did not continue to increase with increasing MOSE dose. From these results, a threshold for soluble nitrogen concentration 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 to the speculation that the values detected in the field were associated with the water source and/or produced during fermentation.

4. Discussion

Since discharge standards can range from country to country, WHO guidelines will be used as a baseline for discussion. MOSE successfully reduced CFW TSS levels below 100 mg/L, the more stringent WHO drinking water limit [7], for all pH levels above 5. MOSE TSS dose response in CFW with a maximum TSS removal rate of 54% between 2 and 3 g/L and a 24-h settling time required for MOSE to clarify CFW, are consistent with results reported by Joseph Mburu [13] and his research using MOSE to treat CPW.

MOSE was shown to reduce nitrate and nitrite levels in CFW. Nitrate levels observed in CFW during field research did not exceed the WHO 50 mg/L limit for drinking water [7], 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, WHO drinking water nitrite concentration limits of 3 mg/L [7] were exceeded in field-tested CFW. The WHO reports that short-term, high nitrite intake may increase risk of methaemoglobinemia, a blood disorder that can cause development delays, in infants [21]. 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, 29.3 mg/L, 74% of the total nitrite content, 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 4). 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 limits below WHO drinking water standards.

The WHO COD limit for industrial wastewater discharge is 300 mg/L [7]. 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 (Fig. 6). Although MOSE has a high total COD content, only 25% is soluble (~120 mg COD/g MOSE). The slope of linear trend-line for the soluble portion of COD in both Figs. 4 and 6 is identical. This supports that the increase in soluble COD, as MOSE dose increases (Fig. 4), 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 that 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 (~8000 mg/L, Table 3) resulting in a MOSE COD added to CFW COD removed ratio of ~0.04. Therefore, the soluble COD added by MOSE is dwarfed by the exceptionally high COD present in CFW.

Settling ponds are typical treatment practice in coffee processing regions [2]. 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 [9]. Beyene et al. [2] report similar settling pond practices for CWW treatment in Ethiopia, concluding 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 CWW due to the high amount of soluble COD present in CFW.

To be a practical treatment approach, *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 for use in a small coffee 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 [6]. Assuming 50% of the water is used for fermentation, 1 ton of coffee cherry CFW would require between 10 and 15 kg of ground *M. Oleifera* seeds for treatment at the optimal dose. *M. Oleifera* trees yield approximately 19 kg of seed pods per year [16]. 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 [16]. Although not established for coffee, *M. Oleifera* can provide shading due to its relatively open canopy [16] and 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.

When compared to alum, MOSE may offer several advantages for treating the insoluble pollutants in CFW. MOSE has been shown to be as effective at treating modeled wastewater as alum without producing hazardous sludge that would require an additional waste management strategy [15]. Although not explored in this research, a comparison between the efficiencies between MOSE and alum

when treating CFW would be a valuable line of research. MOSE did not significantly alter the pH in the tested CFW during settling, as expected [15]. In contrast, alum, a common coagulant, is highly acidic and waste treated with this coagulant must be neutralized before being discharged. From the results of this experiment, CFW must be neutralized before adding MOSE as the optimum pH for pollutant removal is between 5 and 6. Regardless of which coagulant is used, CFW would need to be neutralized due to its acidity and this would need to be done either after adding alum or before adding MOSE. The CFW could be neutralized with lime.

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 insoluble 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, methods for producing 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 a local and affordable “appropriate” treatment technology augmentation for CFW. However, MOSE will not stand alone for effective treatment of CFW and will need to be coupled with another treatment technology that can remove the dissolved organic content present in CFW.

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