Bioresource Technology Reports 22 (2023) 101393

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

A green approach for the treatment of oily steelworks wastewater using natural coagulant of *Moringa oleifera* seed

Edward Lester-Card [a], Graham Smith [b], Gareth Lloyd [b], Chedly Tizaoui [a,*]

[a] *Water and Resources Recovery Research Lab, Department of Chemical Engineering, Swansea University, Bay Campus, Fabian Way, Swansea SA1 8EN, UK*
[b] *Tata Steel, Port Talbot SA13 2NG, UK*

ARTICLE INFO

Keywords:
Moringa oleifera
Natural coagulant
Oil-water emulsions
Steelworks wastewater
Green processes

ABSTRACT

A new approach to treat oily steelworks wastewater using a natural coagulant is reported. *Moringa oleifera* crude extract (MOCE) was used, and its efficiency was compared to traditional alum and synthetic polymer (Nalco 9908) by optimising pH, dose and settling time. Jar tests showed that MOCE caused rapid coagulation-flocculation kinetics and higher oil removal than alum and the polymer (95 %-MOCE, 71 %-alum and 48 %-polymer). Additionally, MOCE exhibited a wider effective pH range (pH 3–11) and did not affect the initial pH of the wastewater. However, MOCE produced higher sludge volume (35 mL/L) as compared to alum (25 mL/L), and the synthetic polymer only produced a sticky sludge stuck to the walls of the vessel. Tests on steelworks wastewater combining MOCE and the polymer produced a synergistic effect exhibiting reduced settling time and increased oil removal. Overall, this study shows that MO extracts have high potential in sustainable treatment of oily wastewaters.

1. Introduction

The UK government is strengthening their stance on environmental protection due to increasing knowledge and awareness of the consequences of pollution combined with a shift toward a greener economy (DEFRA, 2019). Consequently, the steel industry is seeing an emphasis on producing "*green steel*" that meets the required quality and production volumes (Conejo et al., 2020) while at the same time meeting stringent environmental regulations such as those related to wastewater quality (Water Framework Directive, 2017).

Globally the steel industry uses a vast amount of water for cooling, de-scaling and dust-scrubbing at a water consumption rate that varies between 1 and 150 m^3/tonne of steel produced (Suvio et al., 2010). Although there is a huge variation between steelworks, due to differences in plant configuration, geographical location and local legislation (Suvio et al., 2010), the majority of the consumed water ends up as a contaminated wastewater requiring treatment before discharge into the environment. Equally, the steel production uses a vast amount of oil, which ends up as waste oil ejected to sumps and fast flowing flumes where it mixes with wastewater and other contaminants, generating emulsified oily wastewater (Ma et al., 2019). To tackle oil pollution, chemical dosing with coagulation-flocculation is commonly practiced for onsite wastewater treatment. Traditional metal salt coagulants, which are based on either aluminium or iron (i.e. aluminium sulfate (alum), polyaluminium chloride (PAC) or ferric sulfate/chloride), are often preferred. This is due to their ease of accessibility, low cost and high pollutant removal efficiencies at optimised conditions (Bratby, 2006). Synthetic cationic polymers are also commonly used to remove oil from wastewater because of their positive charge, which is effective in neutralising negative oil droplets (Bratby, 2006). Advantages of synthetic polymers over metal based coagulants include lower coagulant dosages, reduced by-product sludge volumes and potential cost savings (Bolto and Gregory, 2007). Despite being commonly used, these chemicals are hazardous, non-biodegradable, and produce voluminous sludge as well as being sensitive to pH and dosage conditions (Bratby, 2006). For example, residual aluminium in the treated water has been linked with Alzheimer's disease (Rondeau et al., 2000) and synthetic polymers contain toxic monomers, such as acrylamide (Bolto and Gregory, 2007), which could also remain as residue in the treated wastewater. Consequently, there is an increasing interest in natural coagulants due to their non-toxic, biodegradable, and safe to handle properties.

The process of coagulation-flocculation has been the subject of studies by several authors for the treatment of wastewater generated in the steel industry. Cheng and Gong (2018) treated oily Cold Mill

* Corresponding author.
E-mail address: c.tizaoui@swansea.ac.uk (C. Tizaoui).

https://doi.org/10.1016/j.biteb.2023.101393
Received 22 November 2022; Received in revised form 6 March 2023; Accepted 7 March 2023
Available online 11 March 2023

wastewater by comparing polyaluminium chloride (PAC), polyferric sulfate (PFS) and polyacrylamide (PAM), and reported oil removals of 83 %, ≈70 % and ≈45 % respectively. Kuzin and Kruchinina (2019) investigated modified alum with titanium compounds to treat steelwork wastewaters. Their results showed increased removal efficiency of suspended solids and a wider effective pH range as compared to traditional alum. You et al. (2018) modified poly-silicate-aluminium ferric sulfate (PSAFS) with manganese (Mn), zinc (Zn) and magnesium (Mg) and found that manganese was the most effective modifier for oil removal. Despite the positive removal rates observed in these studies, concerns remain about the use of synthetic metal-based coagulants. Alternatively, natural coagulants are a potential environmentally friendly solution to treat oily wastewater. For example, chitosan, which is found naturally in shells of crustaceans, has been utilised in only limited studies for oil removal. Hosny et al. (2016) showed that chitosan removed more oil than alum at 96.4 % and 85.0 % respectively. However, chitosan was found to have a limited pH range and dosage sensitivity (Hosny et al., 2016). Hence exploratory studies on other natural coagulants are mandatory. Particularly, plant based extractants are other alternatives for natural coagulants given their known reported advantages such as pH tolerance (El Bouaidi et al., 2022). However, studies concerned with their use to treat oily wastewaters are scarce. To address this knowledge gap, we have carried out this study using coagulants extracted from the seeds of the plant *Moringa oleifera* (MO).

Historically, MO has been known to have clarification properties due to observations of villagers in the Sudan using MO seed extract to clarify turbid water from the Nile (Jahn and Dirar, 1979). Over time, research interest has grown on MO, with notable studies by Gassenschmidt et al. (1995), Ndabigengesere et al. (1995), and Ghebremichael et al. (2005) who identified that the coagulating property of MO is linked to the cationic protein content of the MO seeds. In coagulation-flocculation, MO has been as effective or exceeds alum and other natural coagulants such as chitosan (Jagaba et al., 2020). *Moringa oleifera* is also financially attractive as a natural coagulant. This is because the main financial value of the MO seeds is in its oil (30–40 % by weight) commonly referred to as "Ben Oil" (Anwar et al., 2005), while the coagulating proteins are obtained from the low-value waste seed cake left after the oil is extracted. The waste seed cake currently has little industrial use and is often regarded as a waste material; except on occasions, it is used as a fertiliser or green manure (Gopalakrishnan et al., 2016). Consequently, the alternative use of the seed cake as a natural coagulant is an attractive and sustainable method for resources valorisation and meets the circular economy paradigm. Except for few studies on palm oil mill effluent (POME) (Bhatia et al., 2006; Bhatia et al., 2007a; Bhatia et al., 2007b; Jagaba et al., 2020), the use of MO to treat industrial oily wastewater is lacking. Therefore, this study aims to investigate the effectiveness of extracted coagulant from MO seed cake and compare its performance to alum and a synthetic polymer. Both model oil-water emulsions and oily steel works wastewater from Tata Steel, Port Talbot, UK were used in this study.

2. Materials and methods

2.1. Coagulant preparation

Moringa seeds (*Moringa oleifera*) were purchased from Amazon.co.uk and shipped from Beijing (China). The extraction method by Ghebremichael et al. (2005) was adapted in this study. Briefly, shells of the MO seeds were removed, and the kernels were crushed into a powder with a mortar and pestle. The obtained powder was then sieved with a 2 mm sieve; the sieved product is termed MO seed powder (MOP). The natural oil in MOP was extracted with 95 % ethanol and the solids (seed cake) recovered by centrifugation (4400 rpm, 45 min). The collected seed cake was dried in an oven (80 °C) before being cooled to room temperature in a desiccator. Samples of seed cake 50 g/L were suspended in NaCl solution 1 M, 0.2 M or 0 M (de-ionised water), stirred for 30 min, then filtered through Whatman standard filter paper and again with Whatman cellulose nitrate membrane filters (0.45 μm pore size). The obtained filtrate formed the stock solution of *Moringa oleifera* Crude Extract (MOCE), its protein content was determined via the Bradford assay using a Pierce™ Coomassie Plus Assay Kit purchased from Thermo Fisher Scientific (Loughborough, UK) at a dilution factor of 20. A stock solution of MOCE was used within 24 h from preparation.

Aluminium sulfate hexadecahydrate (Alum) ($Al_2(SO_4)_3.16H_2O$) was purchased from Fisher Scientific (Loughborough, UK) and a 100 mg Al/L stock solution was used. In addition, an industrial synthetic polymer (poly-powder Nalco 9908) was also used (stock solution at 1 g/L) for comparison. When conducting a jar test experiment, an aliquot was taken from each stock solution and dosed into the target wastewater using a pipette.

2.2. Characterization of Moringa oleifera crude extract

To investigate the charge characteristics of MOCE, zeta potential measurements were taken of the stock solution over the pH range 3–11. Zeta potential was measured using the Malvern ZetaSizer Nano ZS. Measurements were only taken with 0 M and 0.2 M NaCl extracted MOCE to avoid salt interference. Aliquots (3 mL) of MOCE were added into vials and their pH was adjusted using 0.1 M HCl or NaOH. The samples were then centrifuged (4400 rpm, 10 min) to remove any precipitate and the supernatant was collected for analysis with the zetasizer. The salting-in effect on MO protein extraction was also studied using different NaCl concentrations and the protein concentration of each extract was measured using the Bradford Assay.

The molecular mass of the proteins in MOCE (1 M) were determined using reduced SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) on NuPAGE Bis-Tris Protein Gels, 1.0 mm, 10-well (15 to 260 kDa) and a colloidal blue staining kit, all developed by Invitrogen™ and purchased from Thermo Fisher Scientific (Loughborough, UK).

2.3. Preparation of model oil-water emulsions

Model oil-water emulsions were prepared in tap water (400 mL) using a synthetic motor oil (0.3 g) (Mobile Super 3000X1 5W-40) and the surfactant sodium dodecyl sulfate (SDS) (0.025 g). The emulsion was mixed at 2000 rpm for 45 min then sonicated for 15 min to stabilise the mixture.

2.4. Steel works wastewater samples

Being the largest user of oil at the Port Talbot site, the Hot Mill was the target for wastewater sampling. Prior to collecting the sample, the wastewater was allowed to drain for 15 s to remove any stagnant liquid in the sampling pipe. The collected samples were always used on the same day of collection.

2.5. Wastewater analysis

The pH was measured with an Orion Star A211 pH meter (pH electrode: Orion, Ross, Sure-Flow-Electrode) and turbidity with a HACH 2100AN turbidimeter. The UV/Vis absorbance scans were obtained by an Agilent 8453 spectrophotometer and the removal efficiency was measured by the change in absorbance at 254 nm (Abs_{254}). Measurements of total organic carbon (TOC) were done with Shimadzu's total organic analyser (TOC-L) and particle size distribution was analysed using Malvern Zeta Sizer Nano series. Sludge analysis involved three parameters including sludge volume, total suspended solids (TSS), and sludge volume index (SVI); all determined according to the standard methods published in Clesceri et al. (1998). Analysis for oil in water was carried out with an FT-IR following a liquid-liquid extraction method described by Pisal (2009). The extraction solvent was

tetrachloroethylene, and the absorbance was measured at a wave number of 2925 cm^{-1}. Sample concentrations were measured using a pre-determined calibration line that correlated absorbance and oil concentration according to the Beer-Lambert law.

2.6. Coagulation-flocculation experiments

All jar test experiments included a 2-min rapid (flash) mix at 245 rpm as the coagulant is added, followed by a 15-min slow mix at 45 rpm before the paddles were removed and the wastewater sample was allowed to settle. The experiments on model oil emulsions evaluated the effects of pH (3−11) and coagulant dosage, varied as indicated between brackets for each coagulant: alum (2.5–100 Al mg/L), polymer (0.625–100 mg/L) and MOCE (5–100 mg/L protein dose). The MOCE dose was calculated according to its protein content of the stock solution (measured by the Bradford assay) (see Fig. S1). Experiments on Hot Mill wastewater evaluated the effect of coagulant dosage and settling time using both MOCE (1–20 mg/L) and the synthetic polymer (0.25–5 mg/L). A maximum settling time of 15 h (900 min) was used and in some experiments, samples were taken at: 0, 15, 60, 90, 120, 390 and 900 min.

To investigate the benefit of combining the MOCE and polymer as coagulant and flocculant, a dual system was proposed and tested on Hot Mill wastewater. The ratios of MOCE/Polymer were 2:1 (2 mg/L MOCE + 1 mg/L Polymer), 4:1 (2 mg/L MOCE + 0.5 mg/L polymer), 5:1 (5 mg/L MOCE + 1 mg/L Polymer) and 10:1 (5 mg/L MOCE + 0.5 mg/L Polymer). The settling time for these experiments was only 15 min to resemble more practical settling times in real industrial applications.

2.7. Statistical analysis

Each experimental data point with repeats, typically thrice, is a mean of collected data and is accompanied with error bars displaying standard error.

3. Results and discussion

3.1. Coagulation-flocculation performance on model oil-water emulsions

The model emulsions were prepared as representative oily wastewaters and evidence of effective emulsification is shown in the oil particle size (≤20 μm) (Coca et al., 2011) and their negative surface charge by the zeta potential (−32.3 mV) (Table S1). In comparison to real sources of industrial oily wastewaters, the model emulsions do not have the complexity in organics and solids that wastewaters such as steel (Cheng and Gong, 2018) or palm oil effluent (POME) (Bhatia et al., 2006) have but rather used as a basis for investigating the treatment of oil-water emulsions with MO extracts.

3.1.1. Optimisation and comparison of coagulant dosages

A critical step in the MOCE extraction is the addition of salt as it improves protein solubility. This was demonstrated by Okuda et al. (1999), who reduced the optimum MO coagulant dosage by 7.4 times when salt was used in the extraction as compared to extraction with water only. The increase of protein solubility in a salty solution was explained by the salting-in effect (i.e. increased ionic strength of the solution, caused by salt, results in stronger electrostatic repulsion between the proteins and this leads to stronger interaction between the proteins and water, thereby improved protein solubility and extraction) (Zayas, 1997). The protein concentration of the MOCE (1 M) stock solution used in this study was determined to be 8000 mg/L via the Bradford assay. The salting-in effect for MO proteins was demonstrated via increasing NaCl concentration from 0 M to 0.2 M and 1 M, which increased protein concentration by 59 % and 68.5 % respectively.

Once extracted into a liquid form, MOCE was compared to alum and the synthetic polymer for removing UV absorbance at 254 nm, turbidity, and TOC from model oil-water emulsions. The optimum coagulant dosages as shown in Fig. 1(A–C) are 2.5, 5 and 50 mg/L (6.25 mL/L) for the polymer, alum (as Al) and MOCE respectively; noting that 5 mg/L Al equates to approximately 60 mg/L of alum. The polymer had the lowest but most sensitive dosage conditions as any little deviation from the optimum (2.5 mg/L) would sharply decrease the removal rates, which implies difficult control of the polymer dose. The optimum MOCE dose of 50 mg/L (6.25 mL/L) is similar to that found by Nkurinziza et al. (2009), who treated surface water (150 NTU), removing 98.4 % turbidity with a dose of 4.2 mL/L salt extracted MO (1 M NaCl). The MOCE dose is also comparable to the aluminium dose used in this study (in terms of total mass of solids used, ~60 mg/L).

Various studies have compared MO extracted coagulants to alum, however, comparisons to synthetic polymers are limited. Salazar Gámez et al. (2015) treated natural water (47 NTU) and polluted water (3050 NTU) with MO extract (1 M NaCl) similar to MOCE, finding turbidity removals from natural and polluted waters of 90 % and 100 % for MO at dosages of 62.5 mg/L and 250 mg/L, then 96 % and 100 % for alum at 12.5 mg/L and 219 mg/L respectively. Salazar's study also reported that MO performed better on higher initial turbidity waters than lower turbidity, as also found by Muyibi and Evison (1995), making it a more attractive alternative to alum for more polluted waters. Sánchez-Martín et al. (2012) compared the treatment of surface water (123 NTU) using MO seed extract (1 M NaCl, no oil extracted), alum and several synthetic flocculants (Flocudex CS-49, AS-10, CS-21 and AS-23). Their results showed that at a dosage of 10 mg/L, turbidity removals were >75 % for alum, CS-49 and MO, with MO outperforming three of the four synthetic flocculants. Coagulation tests on POME (7720 mg/L oil) by Jagaba et al. (2020) with alum, ferric chloride, chitosan, MO (oil then water extraction for coagulating agents) and zeolite found optimum dosages for oil removal of 3000, 1000, 300, 2000 and 1200 mg/L respectively. The corresponding oil removals were alum (97.97 %), zeolite (96.54 %), ferric chloride (95.67 %), chitosan (94.86 %) and MO (87.05 %). These studies all re-enforce our results (Fig. 1A–C) that MO is a competitive coagulant that could be used as an alternative to traditional metal salts and synthetic polymers. In addition, the use of MO has no significant effect on pH change as compared to alum and its effect on the zeta potential is not as intense as alum and the polymer (Fig. 1D–E), which offers potential for MOCE blending with other flocculants/coagulants to further enhance the performance (Pise et al., 2009).

3.1.2. Optimisation and comparison of coagulant initial pH range

One of the most important parameters in coagulation-flocculation is pH of the wastewater as it can influence the coagulation mechanism and its effectiveness. From the data presented on Fig. 2(A–C), alum (Al) operates most effectively between pH 5–9. The polymer shows optimum performance at pH 8, while MOCE has the best performance across the whole pH range with consistently high removal rates at all pHs from 3 to 11; though at pHs > 9 MOCE's efficiency drops slightly.

The performance of traditional metal salt coagulants including alum is influenced by the water pH. This is linked to the formation of various metal hydrolysis products and their solubility which varies with pH (Duan and Gregory, 2001). According to Amirtharajah and Mills (1982), at our optimum alum dosage of around 5 mg/L Al (60 mg/L alum) and pH 8, the mechanism for oil removal could be predominately sweep flocculation. This is due to the amorphous aluminium hydroxide ($Al(OH)_3$) precipitates (Amirtharajah and Mills, 1982), which have been found effective for oil removal as shown in the study by Almojjly et al. (2018). Below pH 5 and above pH 9, $Al(OH)^{2+}$ and $Al(OH)_4^-$ are the aluminium species predominantly formed in solution respectively, which are less effective coagulants, thus causing a dramatic decrease in removal rates as can be seen in Fig. 2(A–C).

The synthetic polymer used in this study is cationic and is likely to operate with a charge neutralisation and bridging mechanism, both common for polymers (Bolto and Gregory, 2007). As shown in Fig. 2 (A-C), the performance of the polymer decreases below and above the

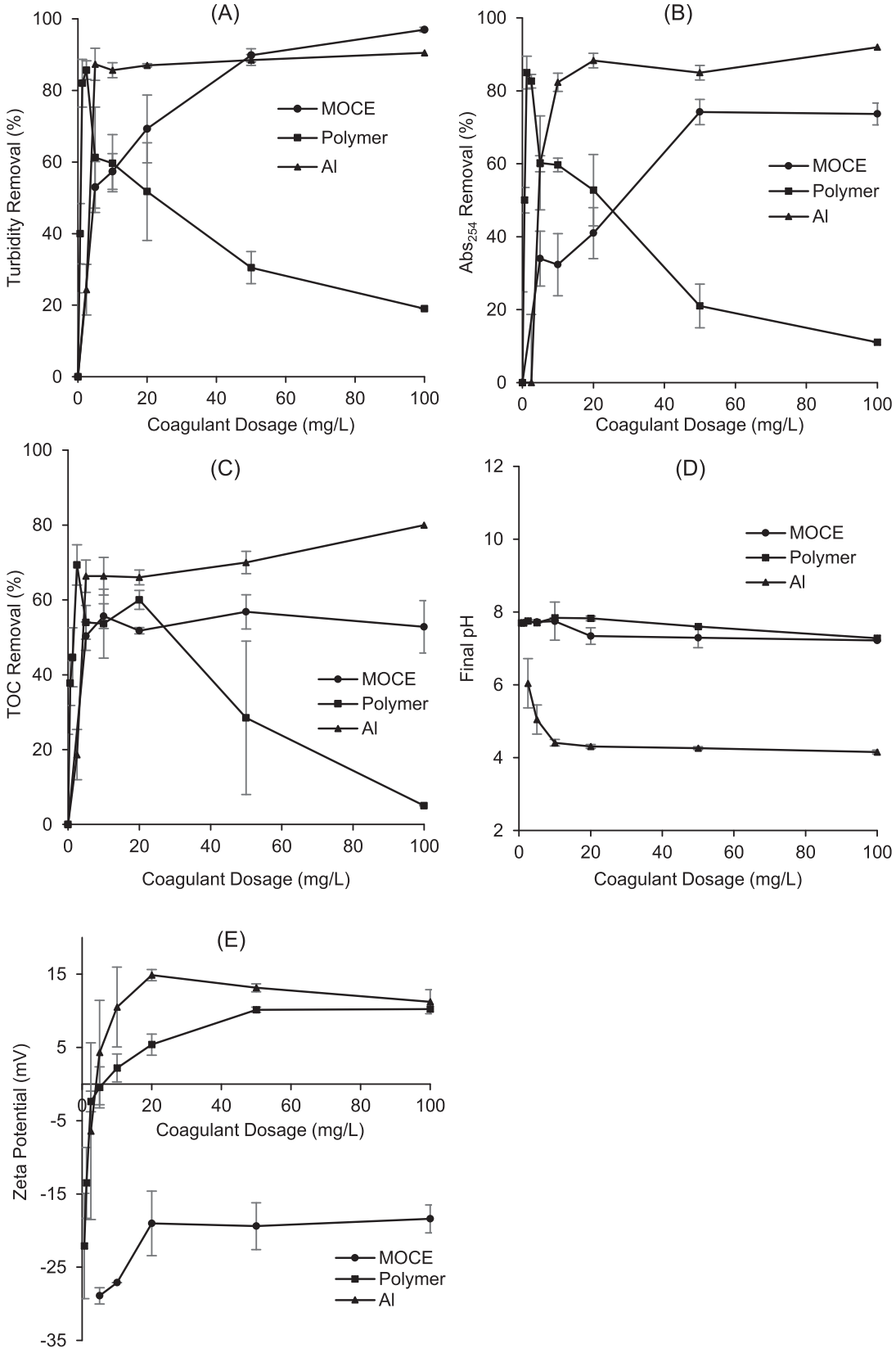

Fig. 1. Effect of coagulant dose on (A) turbidity removal, (B) absorbance removal at 254 nm (Abs_{254}), (C) TOC removal, (D) final pH and (E) zeta potential. (Initial pH 7.8 and settling time of 900 min).

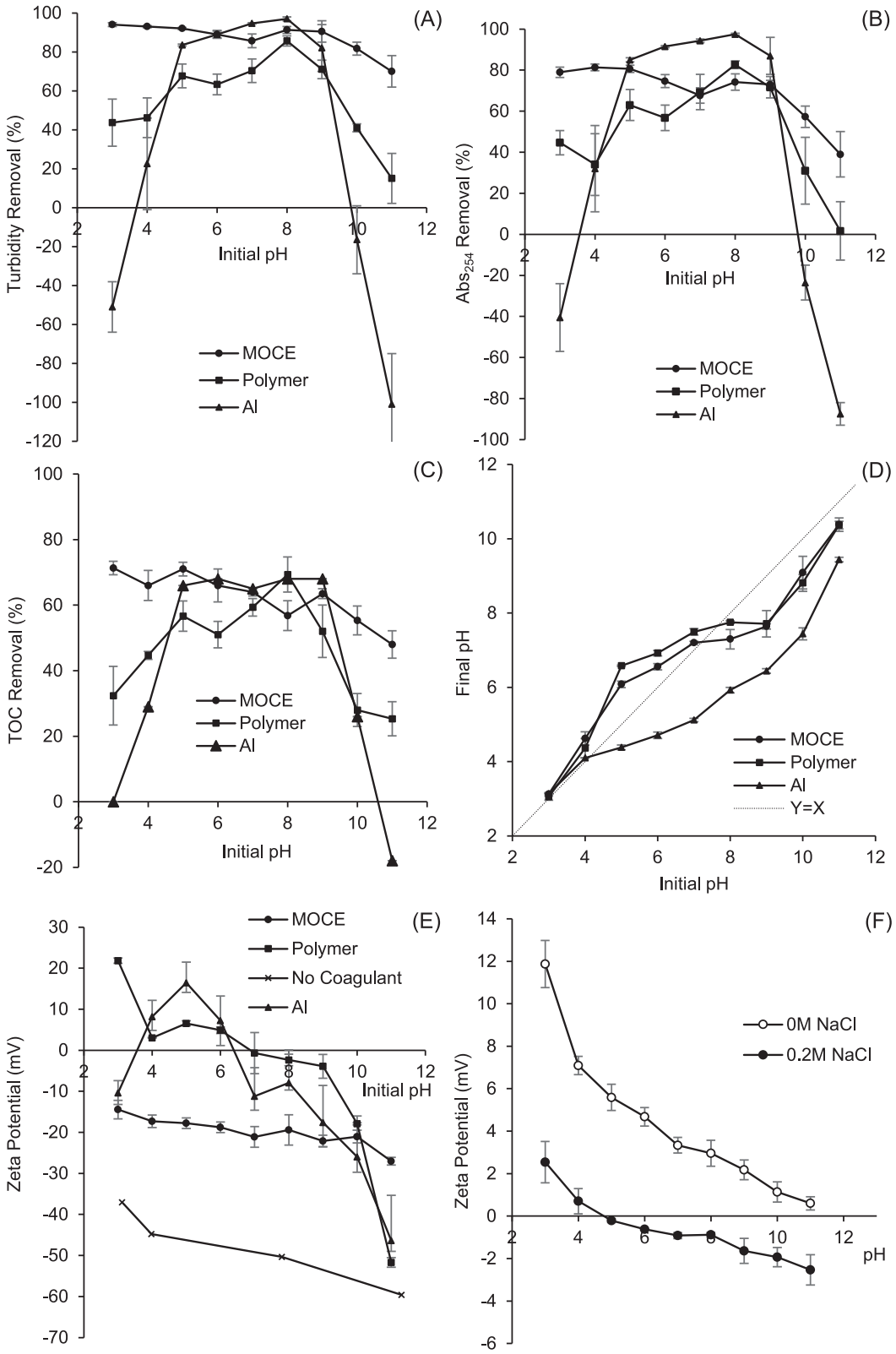

Fig. 2. Effect of initial pH on (A) turbidity removal, (B) absorbance removal at 254 nm (Abs_{254}), (C) TOC removal, (D) final pH and (E) zeta potential. (Al dosage 5 mg/L, polymer 2.5 mg/L, MOCE 50 mg/L and settling time of 900 min), (F) effect of MOCE (0 M) and MOCE (0.2 M) on zeta potential at different pHs.

emulsion pH (7.8). This is likely affected by the change in charge of the oil droplets with the addition of H^+ or OH^- ions, subsequently, hindering charge neutralisation. These results (Fig. 2A–C) are consistent with the study by Hempoonsert et al. (2010), who investigated the effect of pH on oil removal using a cationic polyelectrolyte (Cat-Floc 2953) and microscopic analysis. Their study showed that the amount of oil captured on flocs at pH 7 and 9 was greater than that at pH 5 and 11.

MOCE achieves high removal efficiencies across the pH range 3–11, which is a key advantage over alum and the polymer. Notably, the removal efficiencies are higher in the acidic pHs 3–5 with a slight

decreasing trend between pH 9 to pH 11. This was also seen visually, where the floc size increases and the clarity of the supernatant is improved with decreasing pH from 9 to 3. Beltrán-Heredia and Sánchez Martín (2008) treated textile wastewater with MO extract (1 M NaCl) and found that the dye removal was greater in the acidic pHs 4–8 (>95 %) and lower at higher pHs 8–10 (>70 %). Sánchez-Martín et al. (2012) treated surface water with MO extract (1 M NaCl) and have also reported improved performance in the acidic pHs. Bhatia et al. (2007b) treated palm oil mill effluent (POME) finding that the finest flocs were produced at its natural acidic pH (pH 4) and removal efficiency decreased with increasing pH to 9. The reason for improved coagulation-flocculation with MOCE at more acidic pHs is explained by the cationic nature (positive charge) of MO proteins (Gassenschmidt et al., 1995; Ghebremichael et al., 2005), attributed to its abundant amount of positive amino acids (e.g. arginine) (Gassenschmidt et al., 1995; Ullah et al., 2015). Upon the addition of acid (H^+), the amino acid displays a buffering effect, where the carboxyl group COO^- is suppressed to form COOH, this strengthens the positive charge of the amino group, enabling more effective neutralisation of the negatively charged oil droplets (Beltrán-Heredia and Sánchez Martín, 2008; Bhatia et al., 2007a; Sánchez-Martín et al., 2012). This ability of MOCE to operate effectively over a wide pH range indicates a highly stable coagulant. Santos et al. (2009) identified a specific coagulating-flocculating protein from MO to be a lectin, which demonstrated high thermal stability and a wide pH range via coagulating kaolin water with a similar efficiency to alum over the pH range 4 to 9. Additionally, the study of Ullah et al. (2015) attributed the high stability of isolated MO protein (MO-CBP-3) to the presence of di-sulfide bridges.

3.1.3. Effect of coagulant dosage and initial pH on final pH

While pH control for effective coagulation-flocculation is not critical for MOCE, it is for alum and the polymer as shown by the results in Fig. 2 (A–C). However, the final pH of the treated wastewater still needs to be considered so environmental standards related to pH of the discharged wastewater are met. As can be seen in Fig. 1(D), increasing the dosage of alum (Al) rapidly reduces pH. Starting from pH 7.8, the optimum dose of 5 mg/L Al (stated previously) lowers the pH to 5, this is due to the acidic nature of alum which consumes the alkalinity. Consequently, the pH has to be restored to neutral conditions by adding basic chemicals such as soda ash, lime or caustic soda generating additional costs for wastewater treatment plants (Bratby, 2006). In contrast, MOCE and the polymer show a minimal effect on the final pH. As can be seen in Fig. 1(D), both MOCE and the polymer changed the final pH by only 0.5 units at a dose of 100 mg/L and exhibited little change of the initial pH of the solution (Fig. 2D). Remarkably, when MOCE and the polymer were applied to an acidic emulsion below pH ~7, the final pH increases slightly while when they were applied to an alkaline solution, the final pH reduces slightly, thus providing a buffering effect to the solution's pH. This amphoteric property of MOCE could be a consequence of its amino and carboxyl groups being able to react with H^+ and OH^- ions. A buffering effect would be an advantage during real industrial wastewater treatment since pH adjustment, which requires the addition of chemicals, will not be required. Our results are consistent with other studies by Ndabigengesere et al. (1995) and Ali et al. (2010), who also demonstrated that MO extracts had little effect on wastewater pH, whereas, alum reduced pH to 4.2 and 5.8 respectively in each study.

3.1.4. Effect of coagulant dosage and initial pH on zeta potential

To better understand the mechanism behind MOCE's coagulation-flocculation capability, the zeta potential of MOCE's solution is recorded with changes in pH (Fig. 2E–F). Zeta potential is an important parameter in coagulation-flocculation since it indicates the net charge on the proteins in MOCE and the oil droplets in the emulsion.

Results from Fig. 2(F) show that the extraction method affects significantly MOCE's zeta potential, with MOCE extracted by 0.2 M NaCl exhibited lower zeta potential than MOCE extracted by pure water at all pHs. According to Fig. 2(F), the points of zero charge (pzc) are 4.8 and 11.5 (by extrapolation) for 0.2 M MOCE and 0 M MOCE respectively. The lower zeta potential for 0.2 M MOCE is suggested to be due to the adsorption of Cl^- ions from the NaCl solution onto the cationic proteins (Zayas, 1997). For both 0 M and 0.2 M MOCE, the more alkaline pHs lead to a decrease in their zeta potentials (for example at pH 11, their zeta potentials are 0.60 mV and − 2.53 mV, respectively) whereas, an increase is observed in the acidic pHs (for example at pH 3, 11.87 mV and 2.55 mV respectively). This confirms that the slightly improved performance of MOCE (1 M salt) in acidic pHs (Fig. 2A–C) is due to the increase in cationic charge of the proteins. The results (Fig. 2F) also display a similar trend to the data presented by Magalhães et al. (2021), however, they reported pzc at pH 9.8 compared to ours being about pH 11.5. Importantly, when 1 M MOCE is added to the emulsion, the MOCE salt concentration will be significantly diluted by about 160 times (2.5 mL/400 mL), which is equivalent to 0.006 M salt. Moringa proteins in 0 M at pH 7–8 have a positive zeta potential (≈3 mV) and 0.2 M MO proteins have a negative zeta potential (≈−1 mV) as shown in Fig. 2(F). Referring to Fig. 1(E) shows that an increase in MOCE dose increases zeta potential, demonstrating that positively charged proteins are neutralising the negative oil droplets. Therefore, the 0 M NaCl curve (Fig. 2F) could closely represent the charge effect of MOCE during use due to dilution. This is because the electrical charge of biomolecules is affected by the ionic environment and the concentration of salt present in solution as evidenced by several studies in the literature (Patel et al., 2019; Salgin et al., 2012). In addition, as shown in Fig. 2(E), the zeta potential values of oil droplets in the emulsion without coagulant addition range from −59.6 mV (pH 11) to −37.0 mV (pH 3) and oil droplets after MOCE addition range from −27.0 mV (pH 11) to −14.5 mV (pH 3). Consequently, MOCE possesses a charge neutralisation mechanism when destabilising the negative oil droplets. However, the optimum dosage for MOCE (50 mg/L) corresponds to a zeta potential of −19.4 mV (Fig. 1E). Therefore, charge neutralisation is not the sole mechanism for coagulation with MOCE.

Coagulation mechanisms for MOCE reported in the literature include adsorption and charge neutralisation along with sweep flocculation through enmeshment (Fahmi et al., 2011; Okuda et al., 2001). Ndabigengesere et al. (1995) treated kaolin wastewater (−46 mV) with water extracted MO (6 mV). At a dosage of 10 mL/L the kaolin was destabilised (0 mV) and further increase in dosage increased the zeta potential but the kaolin remained destabilised. They suggested adsorption and neutralisation of charges or adsorption and bridging of destabilised particles to explain this. In comparison to our results, 6.25 mL/L (50 mg/L) of MOCE would bring about effective demulsification but the zeta potential is not significantly altered (−19.4 mV). The mechanism of inter-particle bridging is considered unlikely as it is more common for high molecular weight polymers (>1000 kDa) (Nordmark et al., 2016; Okuda et al., 2001), whereas the molecular weights of MOCE in this study were found to range from 13.6 to 74.5 kDa (Fig. S2). Therefore, it is believed that the primary mechanism for coagulation of the model emulsions with MOCE is sweep flocculation via precipitation of the proteins. This is accompanied by secondary charge neutralisation as evident from results in Fig. 1(E) and 2 (E and F). A schematic of each mechanism is presented in Table S2.

Results from Figs. 1(E) and 2(E) could indicate that alum removes oil according to several possible coagulation mechanisms: (i) adsorption and charge neutralisation since zeta potential increases with dosages up to 20 mg/L, (ii) sweep flocculation via insoluble $Al(OH)_3 \geq 20$ mg/L as effective coagulation takes place without much change in zeta potential. Therefore, the main mechanism for coagulation at the optimum dosage of 5 mg/L Al could be a combination of charge neutralisation and sweep flocculation. The synthetic polymer exhibits a sole charge neutralisation mechanism on the model oil emulsion. As the optimum dosage (2.5 mg/L) and pH conditions (7–8) established in Figs. 1(A–C) and 2(A–C), correspond to zeta potentials of near 0 mV (Figs. 1E and 2E), which is where oil droplet aggregation and hence oil removal is maximised via

the charge neutralisation mechanism.

3.1.5. Effect of settling time

Settling time is a critical consideration, as the residence time needs to be not too long (often min) through settling tanks and canals for coagulation-flocculation to be adequate for industrial applications. The results in Fig. 3(A–C) show that oil emulsions treated by alum and MOCE rapidly settle with removals of turbidity, absorbance and oil reaching, after 15 min settling time, 94 %, 93 % and 71 % for alum and 88 %, 70 % and 95 % for MOCE, respectively. The polymer was much slower, since within the same settling time of 15 min, only 48 %, 50 % and 27 % of turbidity, absorbance and oil were removed, respectively. In addition, a near 100 % oil removal took approximately 90 min for alum and MOCE, while the polymer required >900 min to achieve near complete oil removal (Fig. 3C). In a study by Bhatia et al. (2007a), who treated POME (2658 mg/L oil) with MO (oil extracted and water active agent extraction), an optimum settling time of 114 min was reported, which is comparable to our settling time (90 min). Other studies have reported comparable or higher settling times in coagulation-flocculation with MO for other types of waters, including 120 min for surface water by Adesina et al. (2019). Our results clearly show that MOCE exhibits rapid kinetics for oil removal faster than the synthetic polymer, which would reduce the floor area of settling tanks when MOCE is used as a coagulant.

3.1.6. Coagulation by-product sludge

One of the fundamental drawbacks to coagulation-flocculation is the by-product sludge generated. Metal salts are extensively used coagulants with an estimated 107,000 and 165,000 t per year of alum and ferric, respectively, used in the UK (Keeley et al., 2014). Their frequent use is a concern due to their reported high sludge volumes, toxic components and lack of biodegradability (Bolto and Gregory, 2007; Bratby, 2006). Table 1 shows that MOCE-treated-emulsion had a sludge volume, TSS and SVI of 35 mL/L, 228 mg/L and 153 mL/g respectively, which were higher in comparison to alum's 25 mL/L, 144 mg/L, and 123 mL/g. Thus, in our study, MOCE produced about 40 % more sludge than alum, which is in agreement with Jagaba et al. (2020), who reported a 30 % greater sludge volume of MO used to treat palm oil mill effluent compared to alum. However, Ndabigengesere et al. (1995) and Bhuptawat et al. (2007) reported that MO produced lower sludge volumes than alum. The reason for the discrepancy in sludge volumes between studies could lie in the method used to extract the coagulant from MO seeds and could also be linked to the target wastewater, as our study and

Table 1
Sludge volume, TSS and SVI for alum and MOCE. Experimental conditions: initial pH 7.8, dosages Al 5 mg/L, MOCE 50 mg/L and polymer 2.5 mg/L. Settling time of 60 min.

Parameter	Units	Alum	MOCE
Sludge volume	mL/L	25	35
TSS	mg/L	144	228
SVI	mL/g	123	153

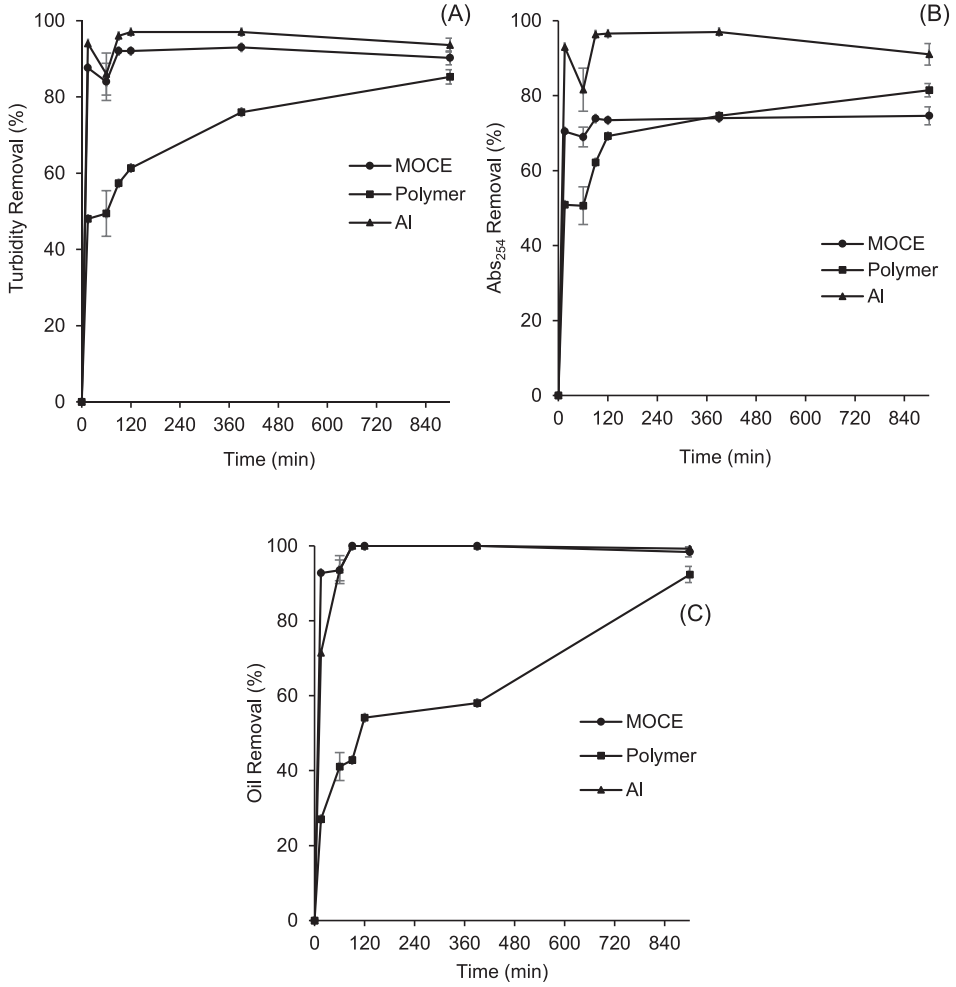

Fig. 3. Effect of settling time on the removals of (A) turbidity, (B) absorbance at 254 nm (Abs254) and (C) oil. Initial pH 7.8, dosages Al 5 mg/L, MOCE 50 mg/L and polymer 2.5 mg/L.

Jagaba et al. (2020) both treated oil based wastewaters but Ndabigengesere et al. (1995) and Bhuptawat et al. (2007) treated model kaolin and sewage wastewaters. Although the polymer exhibited no appreciable settled volume of sludge, the sticky layer on the walls of the reactor presents an important issue, since over time, this layer grows to a significant thickness that reduces the canal's cross section area for flow to pass through as observed in a real industrial system. The polymers stickiness is due to its high viscosity, which is a characteristic of polymers with a high molecular weight and charge density (Bratby, 2006).

3.2. Coagulation-flocculation performance on steelworks real wastewater

Oily wastewater was sampled from the Hot Mill of Tata Steel Port Talbot steelworks with the characteristics summarised in Table S1. In comparison to the model oil emulsion, the sample steelworks wastewater is of similar pH; however, it has a lower oil, turbidity, absorbance and TOC values (Table S1). The steelworks oil levels are lower than expected (100–150 mg/L) but are still high enough to require the wastewater being treated before discharge.

3.2.1. Optimisation and comparison of coagulant dosages

To investigate MOCE as an environmentally friendly alternative to the currently used synthetic polymer, the effect of coagulant (polymer and MOCE) dosage on the Hot Mill wastewater turbidity removal is shown on Fig. 4(A). Only turbidity removal is reported here since similar removal trends were obtained for the oil (see Fig. 3(A and C)). According to Fig. 4(A), the polymer has a slightly lower optimum dosage (1.5 mg/L) as compared to MOCE (10 mg/L equivalent to 1.25 mL/L) and both have removed 90 % turbidity at these optimum doses. In comparison to the model emulsion results (Fig. 1A), this corresponds to a decrease in the required doses by 80 % and 40 % for MOCE and the polymer, respectively. However, higher doses are likely to be required when the oil content of the Hot Mill wastewater is high. Furthermore, MOCE has a more gradual improvement in performance with increasing dosage, while the turbidity removal by the polymer was found sensitive to its dose, which is consistent with the results of the model emulsion. Indeed, high removal efficiencies were obtained for polymer doses between 1 and 2 mg/L but any further increase to >2 mg/L yielded a decrease in performance (Fig. 4A), making the control of the polymer dose difficult.

The zeta potential results (Fig. 4B) highlight the charge neutralisation effect of MOCE. This is because increasing the dosage from 0 mg/L to the optimum of 10 mg/L increases zeta potential from −8.26 mV to −4.32 mV and a further increase to 20 mg/L results in zeta potential of −1.99 mV. What was interesting in the polymer results, is that the strong charge neutralisation mechanism presented when treating the model oil emulsion (Fig. 1E) was not observed. The optimum polymer dose for the model emulsion (2.5 mg/L) increased zeta potential from −30.9 mV to −2.37 mV (Fig. 1E), whereas, the optimum dose (1.5 mg/L) in Hot Mill

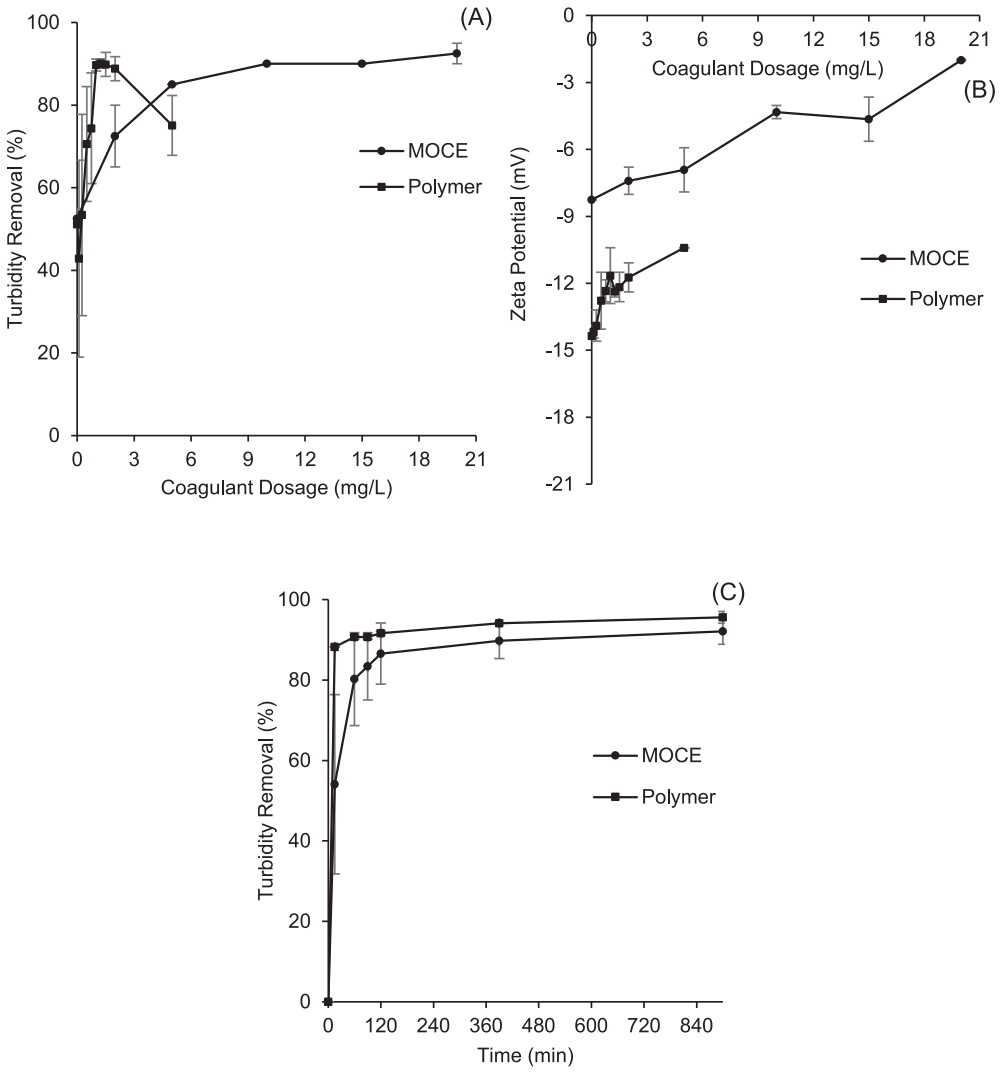

Fig. 4. Effect of coagulant dosages used in the Hot Mill wastewater on (A) turbidity removal (settling time of 900 min) and (B) zeta potential. Effect of settling time on (C) turbidity removal (dosages MOCE 10 mg/L and polymer 1.5 mg/L).

wastewater tests only slightly increased zeta potential from −14.35 mV to −12.16 mV (Fig. 4B). Therefore, the polymer demonstrates different mechanisms of oil removal that could be related to adsorption and inter-particle bridging; which are commonly reported mechanisms for synthetic polymers (Bolto and Gregory, 2007).

3.2.2. *Effect of settling time*

Fig. 4(C) demonstrates the effect of settling time on turbidity removal and shows that the polymer slightly outperforms MOCE in the treatment of Hot Mill wastewater as compared to the model emulsion. Within 60 min of settling, the polymer removed 91 % (±2 %) and MOCE 80 % (±12 %) turbidity from Hot Mill wastewater, whereas the polymer removed 49 % (±6 %) and MOCE 84 % (±5 %) turbidity from the model emulsion. The model emulsion had a higher initial turbidity, due to higher oil concentration, than the Hot Mill wastewater. This could explain the better performance of MOCE in the model emulsion in agreement with other researchers who demonstrated improved performance with MO at higher initial turbidity (Muyibi and Evison, 1995; Nkurinziza et al., 2009; Salazar Gámez et al., 2015).

3.2.3. *Dual Moringa oleifera crude extract and polymer dosing*

The use of synthetic polymers raises environmental concerns due to their toxic monomers such as acrylamide (Bratby, 2006) and un-environmentally friendly processing techniques (Renault et al., 2009). Therefore, natural coagulants such as MOCE are an attractive alternative but are not as well established. As demonstrated previously, the polymer has a lower optimum dosage, yet there is the issue of sticky layers being formed on the walls of the reactor while MOCE tends to require higher dosages but forms a clearly separable sludge. Consequently, dual coagulation-flocculation with MOCE-Polymer could be an attractive solution to exploit the benefits of both MO and the polymer. Fig. 5 compares sole MOCE and sole polymer dosing to the dual MOCE/polymer system (expressed as dosage ratios of 2, 4, 5 and 10-MOCE/polymer) with a settling time of 15 min. Results show that MOCE (10 mg/L) on its own removed turbidity (54 %), absorbance (19 %) and oil (71 %) from the Hot Mill wastewater while the polymer (1.5 mg/L) presented better results. However, when combining MOCE with the polymer, the removals depended on the ratio MOCE/polymer and have improved from MOCE alone. The removal rates increased when the polymer dose was invariant (1 or 0.5 mg/L) and MOCE dose increased (2 to 5 mg/L). In contrast, with the MOCE as invariant (2 or 5 mg/L) and the polymer dose was increased (0.5 to 1 mg/L), the removal rates reduced. Therefore, the ratio selected for the dual system was 10-MOCE/Polymer using 5 mg/L of MOCE (coagulant) followed by 0.5 mg/L polymer (flocculant) removing 69 % turbidity, 29 % absorbance and 88 % oil. Overall, the results obtained in our study are comparable or better than other studies as summarised in Table S3.

This dual coagulation-flocculation system demonstrates advantages over the sole polymer and sole MOCE dosing. At the ratio 10-MOCE/Polymer, there is a reduction in the amount of coagulant needed; the more environmentally harmful synthetic polymer has its dosage reduced by 67 % to 0.5 mg/L, and MOCE dosage reduced by 50 % to 5 mg/L (i.e. total mass of 5.5 mg/L). Furthermore, 10-MOCE/polymer achieves higher removals than MOCE on its own; increasing turbidity, absorbance and oil removals by 15 %, 10 % and 17 % respectively. This improvement could be due to the polymer reducing the settling time via its bridging mechanism, which characterises polymers as demonstrated earlier. This was also found by Bhatia et al. (2006), who carried out a pilot scale study treating POME using MO coagulant (oil extracted and water active agent extraction) and a synthetic polymer (KP 9650) as a flocculant. Highlighting that just dosing with MO produced soft and weak flocs making downstream filtration problematic. By adding the polymer as a flocculant, flocs became larger, stronger, and their settling time was reduced as also demonstrated in our study.

4. Conclusion

The *Moringa oleifera* extract (MOCE) was found an effective coagulant for model oil-water emulsions and real oily steelworks Hot Mill wastewater. The advantages of MOCE were its wider pH range and minimal effect on the final pH. MOCE exhibited sweep flocculation and charge neutralisation mechanisms for oil removal. Although MOCE could bring about effective coagulation, the settling time for Hot Mill wastewater was high. Dual coagulation-flocculation system, utilising the polymer's bridging mechanism, improved flocculation, and the settling time. Consequently, oil removal was increased, and the coagulant dosages were reduced. This study demonstrates that the *Moringa oleifera* natural coagulant could offer a green approach for the treatment of oily steelworks wastewaters.

CRediT authorship contribution statement

Edward Lester-Card: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Visualization. **Graham**

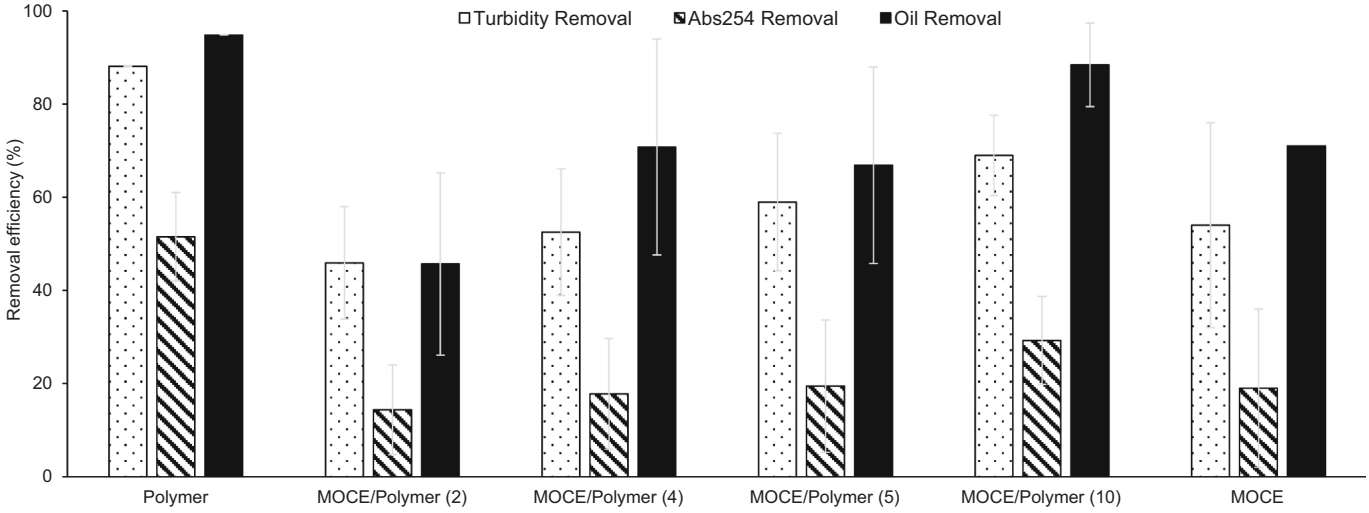

Fig. 5. Coagulation-flocculation performance of MOCE-polymer. polymer (1.5 mg/L polymer), 2-MOCE/Polymer (2 mg/L MOCE + 1 mg/L polymer), 4-MOCE/polymer (2 mg/L MOCE +0.5 mg/L Polymer), 5-MOCE/polymer (5 mg/L MOCE + 1 mg/L polymer), 10-MOCE/Polymer (5 mg/L MOCE + 0.5 mg/L polymer) and MOCE (10 mg/L). Experimental conditions, settling time of 15 min.

Smith: Conceptualization, Methodology, Writing – review & editing. **Gareth Lloyd:** Methodology, Resources, Writing – review & editing, Project administration. **Chedly Tizaoui:** Conceptualization, Methodology, Validation, Resources, Visualization, Writing – review & editing, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to acknowledge the Materials and Manufacturing Academy (M2A) funding from the European Social Fund via the Welsh Government (c80816), Swansea University, and Tata Steel that has made this research possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2023.101393.

References

Adesina, O.A., Abdulkareem, F., Yusuff, A.S., Lala, M., Okewale, A., 2019. Response surface methodology approach to optimization of process parameter for coagulation process of surface water using Moringa oleifera seed. S. Afr. J. Chem. Eng. 28, 46–51. https://doi.org/10.1016/j.sajce.2019.02.002.

Ali, E., Muyibi, S., Salleh, H., Alam, M., Salleh, M., 2010. Production of natural coagulant from Moringa oleifera seed for application in treatment of low turbidity water. J. Water Resour. Prot. 2 (3), 259–266. https://doi.org/10.4236/jwarp.2010.23030.

Almojjly, A., Johnson, D., Oatley-Radcliffe, D.L., Hilal, N., 2018. Removal of oil from oil-water emulsion by hybrid coagulation/sand filter as pre-treatment. J. Water Process Eng. 26, 17–27. https://doi.org/10.1016/j.jwpe.2018.09.004.

Amirtharajah, A., Mills, K.M., 1982. Rapid-mix design for mechanisms of alum coagulation. J. Am. Water Work. Assoc. 74 (4), 210–216. https://doi.org/10.1002/j.1551-8833.1982.tb04890.x.

Anwar, F., Ashraf, M., Bhanger, M.I., 2005. Interprovenance variation in the composition of Moringa oleifera oilseeds from Pakistan. J. Am. Oil Chem. Soc. 82, 45–51. https://doi.org/10.1007/s11746-005-1041-1.

Beltrán-Heredia, J., Sánchez Martín, J., 2008. Azo dye removal by Moringa oleifera seed extract coagulation. Color. Technol. 124 (5), 310–317. https://doi.org/10.1111/j.1478-4408.2008.00158.x.

Bhatia, S., Othman, Z., Ahmad, A.L., 2006. Palm oil mill effluent pretreatment using Moringa oleifera seeds as an environmentally friendly coagulant: laboratory and pilot plant studies. J. Chem. Technol. Biotechnol. 81 (12), 1852–1858. https://doi.org/10.1002/jctb.1619.

Bhatia, S., Othman, Z., Ahmad, A.L., 2007a. Coagulation–flocculation process for POME treatment using Moringa oleifera seeds extract: optimization studies. Chem. Eng. J. 133 (1–3), 205–212. https://doi.org/10.1016/j.cej.2007.01.034.

Bhatia, S., Othman, Z., Ahmad, A.L., 2007b. Pretreatment of palm oil mill effluent (POME) using Moringa oleifera seeds as natural coagulant. J. Hazard. Mater. 145 (1–2), 120–126. https://doi.org/10.1016/j.jhazmat.2006.11.003.

Bhuptawat, H., Folkard, G.K., Chaudhari, S., 2007. Innovative physico-chemical treatment of wastewater incorporating Moringa oleifera seed coagulant. J. Hazard. Mater. 142 (1–2), 477–482. https://doi.org/10.1016/j.jhazmat.2006.08.044.

Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Res. 41 (11), 2301–2324. https://doi.org/10.1016/j.watres.2007.03.012.

Bratby, J., 2006. Coagulation And Flocculation in Water And Wastewater Treatment, second ed. IWA, London, Seattle.

Cheng, X.-N., Gong, Y.-W., 2018. Treatment of oily wastewater from cold-rolling mill through coagulation and integrated membrane processes. Environ. Eng. Res. 23 (2), 159–163. https://doi.org/10.4491/eer.2016.134.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard Methods for the Examination of Water And Wastewater, 20th ed. APHA American Public Health Association.

Coca, J., Gutiérrez, G., Benito, J.M., 2011. Treatment of oily wastewater. In: Water Purification And Management. Springer, Oviedo,Burgos, pp. 1–55.

Conejo, A.N., Birat, J.-P., Dutta, A., 2020. A review of the current environmental challenges of the steel industry and its value chain. J. Environ. Manag. 259, 109782 https://doi.org/10.1016/j.jenvman.2019.109782.

DEFRA, 2019. In: A Green Future: Our 25 Year Plan to Improve the Environment. Department for Environment, Food & Rural Affairs, pp. 1–151.

Duan, J., Gregory, J., 2001. Hydrolyzing metal salts as coagulants. Pure Appl. Chem. 73 (12), 2017–2026. https://doi.org/10.1351/pac200173122017.

El Bouaidi, W., Libralato, G., Douma, M., Ounas, A., Yaacoubi, A., Lofrano, G., Albarano, L., Guida, M., Loudiki, M., 2022. A review of plant-based coagulants for turbidity and cyanobacteria blooms removal. Environ. Sci. Pollut. Res. 29 (28), 42601–42615. https://doi.org/10.1007/s11356-022-20036-0.

Fahmi, M.R., Najib, N.W.A.Z., Ping, P.C., Hamidin, N., 2011. Mechanism of turbidity and hardness removal in hard water sources by using Moringa oleifera. J. Appl. Sci. 11 (16), 2947–2953. https://doi.org/10.3923/jas.2011.2947.2953.

Gassenschmidt, U., Jany, K.D., Tauscher, B., Nierbergall, H., 1995. Isolation and characetization of a flocculating protein from Moringa oleifera Lam. Biochim. Biophys. Acta, Gen. Subj. 1243 (3), 477–481. https://doi.org/10.1016/0304-4165(94)00176-x.

Ghebremichael, K.A., Gunaratna, K.R., Henriksson, H.B., Dalhammar, G., 2005. A simple purification and activity assay of the coagulant protein from Moringa oleifera seed. Water Res. 39 (11), 2338–2344. https://doi.org/10.1016/j.watres.2005.04.012.

Gopalakrishnan, L., Doriya, K., Kuma, D.S., 2016. Moringa oleifera: a review on nutritive importance and its medicinal application. Food Sci. Human Wellness 5 (2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001.

Hempoonsert, J., Tansel, B., Laha, S., 2010. Effect of temperature and pH on droplet aggregation and phase separation characteristics of flocs formed in oil–water emulsions after coagulation. Colloids Surf. A: Physicochem. Eng. Asp. 353 (1), 37–42. https://doi.org/10.1016/j.colsurfa.2009.10.016.

Hosny, R., Fathy, M., Ramzi, M., Abdel Moghny, T., Desouky, S.E.M., Shama, S.A., 2016. Treatment of the oily produced water (OPW) using coagulant mixtures. Egypt. J. Pet. 25 (3), 391–396. https://doi.org/10.1016/j.ejpe.2015.09.006.

Jagaba, A.H., Kutty, S.R.M., Hayder, G., Latiff, A.A.A., Aziz, N.A.A., Umaru, I., Ghaleb, A. A.S., Abubakar, S., Lawal, I.M., Nasara, M.A., 2020. Sustainable use of natural and chemical coagulants for contaminants removal from palm oil mill effluent: a comparative analysis. Ain Shams Eng. J. 11 (4), 951–960. https://doi.org/10.1016/j.asej.2020.01.018.

Jahn, S.A.A., Dirar, H., 1979. Studies on natural water coagulants in the Sudan, with special reference to Moringa oleifera seeds. Water SA 5 (2), 90–106.

Keeley, J., Jarvis, P., Judd, S.J., 2014. Coagulant recovery from water treatment residuals: a review of applicable technologies. Crit. Rev. Environ. Sci. Technol. 44 (24), 2675–2719. https://doi.org/10.1080/10643389.2013.829766.

Kuzin, E.N., Kruchinina, N.E., 2019. Purification of circulating and waste water in metallurgical industry using complex coagulants. CIS IronSteel Rev. 18, 72–75. https://doi.org/10.17580/cisisr.2019.02.15.

Ma, N., McDowell, B.J., Houser, J.B., Andrade, M.W., Heinz, D.E., 2019. Separation of mill scale from flume wastewater using a dynamic separator toward zero wastes in the steel hot rolling process. J. Sustain. Metall. 5, 97–106. https://doi.org/10.1007/s40831-018-0203-3.

Magalhães, E.R.B., Fonseca de Menezes, N.N., Silva, F.L., Alves Garrido, J.W., dos Santos, Angélica, Bezerra Sousa, M., Santos, E.S.D., 2021. Effect of oil extraction on the composition, structure, and coagulant effect of Moringa oleifera seeds. J. Clean. Prod. 279, 123902 https://doi.org/10.1016/j.jclepro.2020.123902.

Muyibi, S.A., Evison, L.M., 1995. Optimizing physical parameters affecting coagulation of furbid water with Moringa oleifera Seeds. Water Res. 29 (12), 2689–2695. https://doi.org/10.1016/0043-1354(95)00133-6.

Ndabigengesere, A., Narasiah, K.S., Talbot, B.G., 1995. Active agent and mechanism of coagulation of turbid waters using Moringa oleifera. Water Res. 29 (2), 703–710. https://doi.org/10.1016/0043-1354(94)00161-Y.

Nkurinziza, T., Nduwayezu, J.B., Banadda, E.N., Nhapi, I., 2009. The effect of turbidity levels and Moringa oleifera concentration on the effectiveness of coagulation in water treatment. Water Sci. Technol. 59 (8), 1551–1558. https://doi.org/10.2166/wst.2009.155.

Nordmark, B.A., Przybycien, T.M., Tilton, R.D., 2016. Comparative coagulation performance study of Moringa oleifera cationic protein fractions with varying water hardness. J. Environ Chem. Eng. 4 (4 Part A), 4690–4698. https://doi.org/10.1016/j.jece.2016.10.029.

Okuda, T., Baes, A.U., Nishijima, W., Okada, M., 1999. Improvement of extraction method of coagulating active components from Moringa oleifera seed. Water Res. 33 (15), 3373–3378. https://doi.org/10.1016/S0043-1354(99)00046-9.

Okuda, T., Baes, A.U., Nishijima, W., Okada, M., 2001. Coagulation mechanism of salt solution extracted active component in Moringa oleifera seeds. Water Res. 35 (3), 830–834. https://doi.org/10.1016/S0043-1354(00)00296-7.

Patel, A., Longmore, N., Mohanan, A., Ghosh, S., 2019. Salt and pH-induced attractive interactions on the rheology of food protein-stabilized nanoemulsions. ACS Omega 4 (7), 11791–11800. https://doi.org/10.1021/acsomega.8b03360.

Pisal, A., 2009. In: Determination of Oil And Grease in Water With a Mid-infrared Spectrometer. Perkin and Elmer, Shelton, pp. 1–4.

Pise, C.P., Gidde, M.R., Bhalerao, A.R., 2009. Study of blended coagulant alum and Moringa oleifera for turbidity removal. J. Environ. Res. Dev. 4 (2), 517–527.

Renault, F., Sancey, B., Badot, P.M., Crini, G., 2009. Chitosan for coagulation/flocculation processes – an eco-friendly approach. Eur. Polym. J. 45 (5), 1337–1348. https://doi.org/10.1016/j.eurpolymj.2008.12.027.

Rondeau, V., Commenges, D., Jacqmin-Gadda, H., Dartigues, J.-F., 2000. Relation between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study. Am. J. Epidemiol. 152 (1), 59–66. https://doi.org/10.1093/aje/152.1.59.

Salazar Gámez, L.L., Luna-Del Risco, M., Cano, R.E.S., 2015. Comparative study between M. oleifera and aluminum sulfate for water treatment: case study Colombia. Environ. Monit. Assess. 187, 668–677. https://doi.org/10.1007/s10661-015-4793-y.

Salgin, S., Salgin, U., Bahadir, S., 2012. Zeta potentials and isoelectric points of biomolecules: the effects of ion types and ionic strengths. Int. J. Electrochem. Sci. 7, 12404–12414.

Sánchez-Martín, J., Beltrán-Heredia, J., Peres, J.A., 2012. Improvement of the flocculation process in water treatment by using Moringa oleifera seeds extract. Braz. J. Chem. Eng. 29 (3), 495–501. https://doi.org/10.1590/S0104-66322012000300006.

Santos, A.F.S., Luz, L.A., Argolo, A.C.C., Teixeira, J.A., Paiva, P.M.G., Coelho, L.C.B.B., 2009. Isolation of a seed coagulant Moringa oleifera lectin. Process Biochem. 44 (4), 504–508. https://doi.org/10.1016/j.procbio.2009.01.002.

Suvio, P., Van Hoorn, A., Szabo, M., 2010. In: Water Usage in Steel Making: Findings From a Worldsteel Water Management Project, 130. Stahl Eisen, pp. S39–SP50.

Ullah, A., Mariutti, R.B., Masood, R., Caruso, I.P., Costa, G.H., Millena de Freita, C., Santos, C.R., Zanphorlin, L.M., Rossini Mutton, M.J., Murakami, M.T., Arni, R.K., 2015. Crystal structure of mature 2S albumin from Moringa oleifera seeds. Biochem. Biophys. Res. Commun. 468 (1–2), 365–371. https://doi.org/10.1016/j.bbrc.2015.10.087.

Water Framework Directive, 2017. Water Environment (Water Framework Directive) (England and Wales) Regulations 2017. https://www.legislation.gov.uk/uksi/2017/407/contents/made/ accessed 01 March 2022.

You, Z., Zhang, L., Zhang, S., Sun, Y., Shah, K.J., 2018. Treatment of oil-contaminated water by modified polysilicate aluminum ferric sulfate. Processes 6 (7), 95. https://doi.org/10.3390/pr6070095.

Zayas, J.F., 1997. Solubility of proteins. In: Functionality of Proteins in Food. Springer, Berlin,Heidelberg, pp. 6–75.